Developments in Applied Microbiology and Biotechnology

Microbial Biomolecules

Emerging Approach in Agriculture, Pharmaceuticals, and Environmental Management

Edited by
Ajay Kumar, Muhammad Bilal,
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Emerging Approach in Agriculture, Pharmaceuticals, and Environmental Management

Edited by

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CHAPTER 1

Rhizobacterial biomolecules for sustainable crop production and environmental management: plausible functions and molecular mechanisms

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1.1 Introduction

The rhizosphere is a complex environment with dynamic signaling mechanisms between plant roots and the root-inhabiting microbes in a continuous flux of biochemical and physiological processes which collectively contribute to the cumulative effects on plant nutrition and bioprotection (Kumari, Meena, Gupta, et al., 2018; Srivastava & Sarethy, 2021). Consequently, the rhizosphere has been the center of attention for agricultural research for several years owing to its role in soil and crop health and sustainable agriculture.

Rhizospheric bacteria are among the most studied microbiota as natural inhabitants of plant roots. These bacteria interact with plant roots either externally or internally as rhizoplane (on plant root surfaces), rhizosphere (in the soil adjacent to plant roots), and endophytic (within the plant root tissues) forms. The intimate relations formed with plant roots, alongside their plant beneficial processes, make them important players in soil and plant health. Rhizobacteria are involved in not only plant nutrient solubilization (Aloo, Mbega, Makumba, et al., 2021; Chaiharn & Lumyong, 2011; Gupta et al., 2021) but also nitrogen (N) fixation (Gopalakrishnan et al., 2017; Hara et al., 2020; Sutariati et al., 2020) and phytopathogen control (Chenniappan et al., 2019; Gowtham et al., 2016; Qaiser et al., 2015; Samaras et al., 2018). These functions are actualized through the secretion of biomolecules like plant growth-regulating hormones, antibiotics, volatile organic compounds (VOCs), and organic acid. A schematic illustration of the different rhizobacterial biomolecules is shown in Fig. 1.1.

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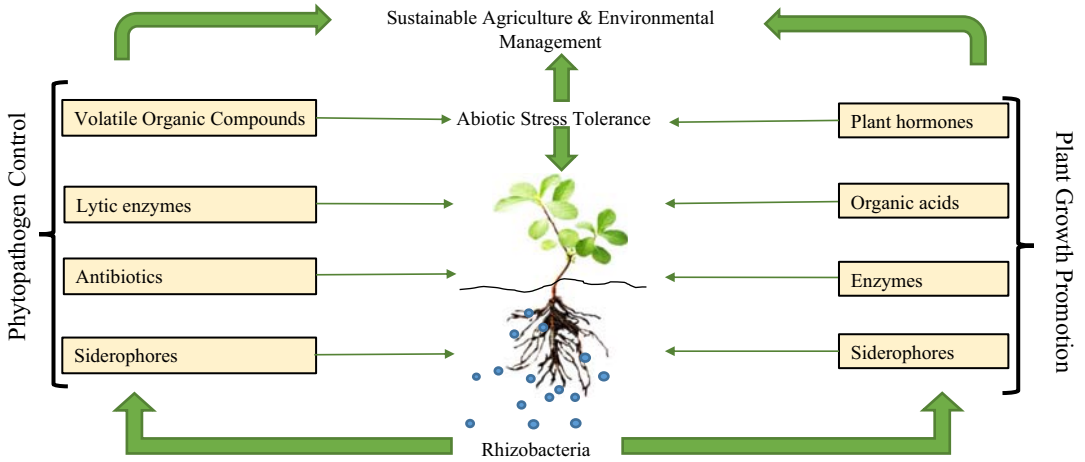

Figure 1.1
A schematic representation of various rhizobacterial biomolecules produced in plant rhizospheres.

Despite decades of research, the link between rhizobacterial biomolecules and agricultural sustainability is not yet fully comprehended. Our understanding of the molecular mechanisms underlying their production is similarly limited. This chapter explores the types and functions of rhizobacterial biomolecules and assesses emerging issues and perspectives relative to their roles and applications in PGP to shed light on their importance in the sustainability of agricultural production systems and for environmental management. The chapter further attempts to provide an account of the molecular mechanisms underlying rhizobacterial biosynthesis of the different agriculturally important biomolecules. Such information can ultimately increase the utilization of rhizobacteria and their biomolecules in agroecosystems as alternatives to agrochemicals for environmental sustainability.

1.2 Types and functions of rhizobacterial biomolecules

1.2.1 Enzymes

Rhizobacterial enzymes include chitinases, glucanases, and proteinases that are lethal to phytopathogens (Bhagwat et al., 2019; Jadhav et al., 2017). The biocontrol potential of rhizobacterial enzymes against fungal plant pathogens has been demonstrated in several studies. A study on the biocontrol efficiency of native plant growth-promoting rhizobacteria (PGPR) against the rhizome rot disease of turmeric in India recently established the expression of various genes encoding different lytic enzymes in several bacilli (Chenniappan et al., 2019). Similarly, several rhizobacterial isolates of Date palm (*Phoenix dactylifera*) with biocontrol activities against its wilt-causing *Fusarium* were recently shown to produce various hydrolytic enzymes like amylases, cellulases, chitinases, and proteases (Bouamri, 2021).

The fungicidal action of *Pseudomonas* and *Bacillus* against *Fusarium udum* causing *Fusarium* wilt in pigeon pea was recently established to ensue from the production of numerous biocidal biomolecules like chitinolytic enzymes (endochitinases, exochitinases, and chitobiases) and other lytic enzymes like proteinases, cellulases, amylases, pectinases, and lipases (Dukare & Paul, 2021). Likewise, studies have shown *Bacillus amyloliquefaciens* that synthesize proteases and other lytic enzymes also inhibit *F. oxysporum* through cell wall lysis (Gowtham et al., 2016; Guleria et al., 2016; Passari et al., 2018; Qaiser et al., 2015; Samaras et al., 2018). Rhizobacterial enzymes, therefore, represent functional biomolecules with crucial capabilities of controlling fungal pathogens and indirectly promoting plant growth.

Chitinases, whose target substrates are chitin, are especially important in the biocontrol of fungal palnt pathogens whose cell walls are predominantly composed of chitin which is their target substrate. They catalyze the hydrolysis of the β-1, 4-linkages in chitin and directly inhibit hyphal growth in many fungal pathogens (Oyeleyeo and Normi 2018). Likewise, cellulases and endoglucanases are responsible for the degradation of cellulose and other cell wall polymers, and are, therefore, important in phytopathogen control and the colonization of plant endospheres to form more complex interactions with plants (López et al., 2018; Reinhold-Hurek et al., 2006). Owing to the potential of chitinases in phytopathogen control, researchers have attempted to explore the genetic capactiy of their production for chitin hydrolysis. The presence of chitinase (*chi*) genes in tomato-endophytic *Bacillus* sp. and their involvement in the suppression of *Fusarium* wilt has recently been demonstrated (Abdallah et al., 2017). The glycoside hydrolase family 18 (GH18) Group A bacterial *chi* gene has also been discovered in the production of chitinolytic metabolites (Dutta & Thakur, 2017; Kobayashi et al., 2002).

The genetic capacity of rhizobacterial production of other lytic enzymes has similarly been elucidated. Very recently, the presence of *htrBC*, *eglS*/*bglS*, and *lipAC* encoding proteases, glucanases, and lipases, respectively, were established in the genome of *B. subtilis* in Pakistan (Iqbal et al., 2021). Increased expression of the superoxide dismutase (*sod*), catalase (*cat*), phenylalanine ammonia-lyase (*pal*), chitinase (*chi*), and β-1, 3-glucanase (*glu*) genes has been observed upon artificial inoculation of PGP strains in sugarcane (Singh et al., 2021). The pattern of enzyme activity and expression differ based on the environmental stimuli faced by these rhizobacteria. This is because gene expression is controlled at many stages and in many different ways, such as transcriptional and posttranscriptional regulations. A thorough understanding of gene expression involved in the production of these enzymes can aid in the identification of novel strains that would revolutionize their use in crop production for environmental sustainability.

Rhizobacterial enzymes are also involved in the solubilization of soil nutrients. Since most plant nutrients are always available in complex and inaccessible forms, which limits their

availability in soils, nutrient solubilizing rhizobacterial enzymes are critical players in plant nutrition. For instance, rhizobacterial phytases and phosphatases are key enzymes in the mineralization of organic P. The former facilitates the degradation of phytates which is the key soil organic P (Pradhan et al., 2017). Phosphatases are nonspecific enzymes that may be classified as acid or alkaline based on their pH optima, phosphatases (Jorquera et al., 2011), and function by dephosphorylating the phosphoester or phosphoranhydride bonds in organic matter (Iqbal et al., 2021). Studies have established that rhizobacterial phosphatases and phytases mediate the solubilization of organic and inorganic P forms in soil (Aloo, Mbega, & Makumba, 2021; Gupta et al., 2021; Prakash & Arora, 2019; Santos-Torres et al., 2021). Recently, the characterization of chili (*Capsicum annuum)* PGPR showed that several P-solubilizing Pseudomonads produced alkaline phosphatases (Han et al., 2021).

The production of both acid and alkaline phosphatases has also been reported in *Rhizobium* and *Herbaspirillum* spp. and confirmed through in silico genome analyses (Santos-Torres et al., 2021). The complete genetic machinery for phosphate solubilization has recently been established in *B. subtilis* by Iqbal (2021), who confirmed that the production of phosphatases involves the *phoAD* gene and the genes involved in phosphate transport (*pstACS*) (Iqbal et al., 2021). The expression of the glucose dehydrogenase (*gdh*) gene *gdhB*, and the pyrroloquinoline quinone (*pqq*) synthesis protein series genes (*pqqA*, *pqqB*, *pqqC*, *pqqD*, and *pqqE)* in phosphate solubilizing bacteria (PSB) is elevated in the presence of insoluble-P forms (Ding et al., 2021). According to a study by Zeng et al. (2017), the genes involved in glucose metabolism are continually downregulated in the presence of insoluble P to channel glucose towards the phosphorylative pathway. The identification of a specific protein network in PSB is critical for improved PGP traits. With the emergence of transcription-activator-like effector nucleases, targeted mutagenesis, and genome-editing tools like CRISPR/Cas systems, the discovery of novel traits, trait development, and site-specific genome modifications can be achieved for several PSB for improved P solubilization.

1.2.2 Plant growth-promoting hormones

Rhizobacterial PGP hormones like abscisic acid (ABA), gibberellic acids (GA), cytokinins (CK), and auxins are critical biomolecules in plant rhizospheres. The functions of various rhizobacterial PGP hormones are schematically depicted in Fig. 1.2 and examples of rhizobacterial producers of PGP hormones from various studies are presented in Table 1.1.

Indole-3-acetic acid (IAA) is a physiologically active auxin with important functions in root elongation and proliferation of root hairs that together improve the uptake of water and mineral nutrients from the soil (Godbole et al., 2021; Kumari, Meena, & Upadhyay, 2018). In a recent investigation, a positive correlation was established between IAA synthesis and the root length of rice seedlings (Sutariati et al., 2020). These results have also been

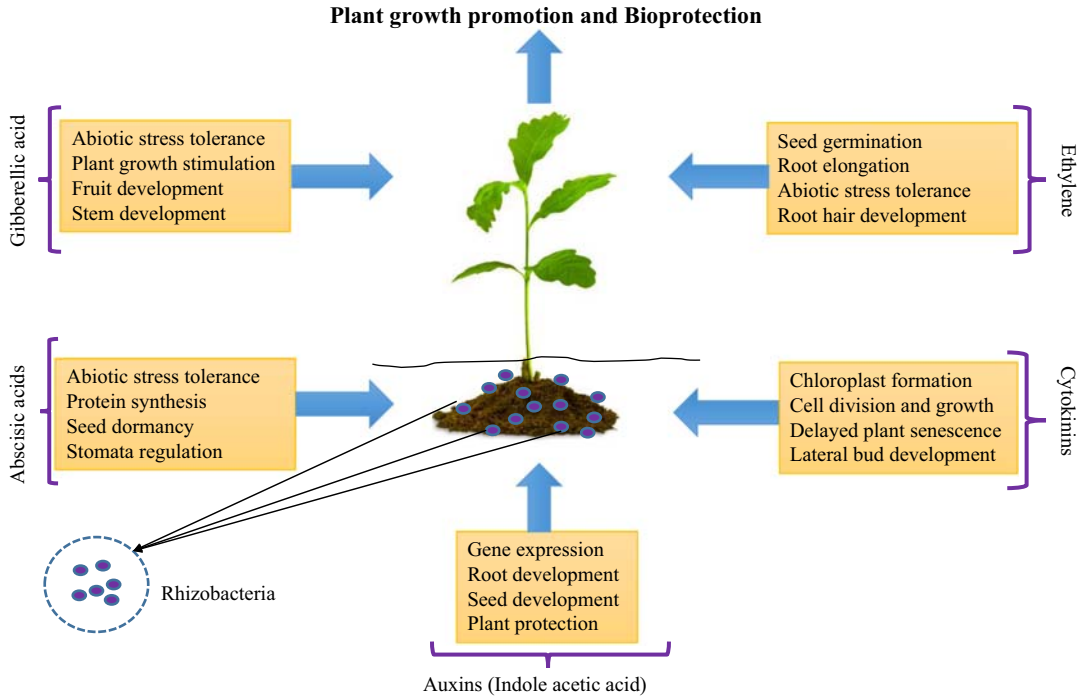

Figure 1.2
Functions of various rhizobacterial plant growth-promoting hormones.

replicated in tomato (Dashti, 2021; Kalimuthu et al., 2019), sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum)* (Chandra et al., 2020), groundnuts *(Arachis hypogea*), and rice (*Oryza sativa*) (Panigrahi et al., 2020). Similarly, GA are rhizobacterial PGP hormones that modulate several physiological processes like stem elongation, germination, dormancy, and fruit senescence in plants (Sharma et al., 2018). GA mediate several plant developmental processes like root, flower, and fruit development (Yamaguchi, 2008).

ABA also have vital roles in several physiological processes in plants and are important in plant drought stress resistance (Waghmode et al., 2019). Similarly, CK are involved in cell division, leaf senescence, apical dominance, and shoot differentiation and are an active precursor of auxin phytohormones (Iqbal et al., 2021).

Most rhizobacterial hormones are critical regulatory components of plant responses to abiotic stresses. For instance, given water-deficit conditions, rhizobacterial PGP hormones can be critical components in enhancing plant growth. In a recent study by Ahmed et al. (2021), IAA and other *Enterobacter* biomolecules were evidenced to enhance the biological attributes and drought tolerance of *V. radiata* in water-limiting conditions. In the same way, some rhizobacteria have been shown to produce IAA at elevated temperatures

Table 1.1: Rhizobacterial producers of plant growth-promoting hormones.

Hormone	Host plant	Rhizobacteria	Quantity (μg/mL)	References
IAA	*Cucumber* (*Cucumis sativus*)	*P. fluorescens, B. subtilis*	35.3–66.3	Khabbaz et al. (2015)
	Chili (*Capsicum annuum*)	*Pseudomonas, Bacillus* spp.	34.97–92.15	Narayanan & Madhavan (2020)
		Not identified	Not established	Kesharwani & Singh (2020)
		Pseudomonas sp.	5.73	Han et al. (2021)
	Chickpea (*Cicer arietinum*)	Not identified	11.12–68.46	Maheshwari et al. (2019)
	Coleus (*Coleus scutellarioides*)	*P. stutzeri, Stenotrophomonas maltophilia, P. putida*	233–240	Patel & Archana (2017)
	Maize (*Zea mays*)	*Burkholderia* spp.	47.5	Batista et al. (2018)
		B. altitudinis, B. aryabhattai B. megaterium	1.39–15.74	Javoreková et al. (2021)
		Not identified	0.16–4.51	Kesaulya et al. (2021)
	Date Palm (*Phoenix dactylifera*)	Not identified	3.8–46.8	Bouamri (2021)
	Groundnuts (*Arachis hypogea*)	Not identified	0.43–10.43	Thakur & Parikh (2018)
	Mungbean (*Vigna radiata*)	*Pseudomonas, Bacillus, Acinetobacter* sp.	45.66–111.94	Kumari, Meena, & Upadhyay (2018)
		Rhizobium sp.	23.0–30.0	Mahmood et al. (2021)
		Bacillus, Pseudomonas, Rhizobium spp.	6.06–32.13	Routray & Khanna (2018)
	Cardamom (*Elettaria cardamomum*)	*B. subtilis, P. putida*	10.1–43.25	Panchami et al. (2020)
	Arabidopsis (*Arabidopsis thaliana*)	*P. putida*	1.0.5	Ghosh et al. (2019)
	Basil (*Ocimum basilicum*)	*P. alcaliphila, P. hunanensis, Streptomyces laurentii, Sinorhizobium* sp., *B. safensis*	0.30–6.92	AlAli et al. (2021)
	Pea (*Pisum sativum*)	Not identified	11.12–68.46	Maheshwari et al. (2019)
	Pigeon pea (*Cajanus cajan*)	*Pseudomonas, Serratia, Bacillus, Rhizobium*	0.16–34.82	Modi & Khanna (2018)
	Potato (*Solanum tuberosum*)	*Enterobacter, Serratia, Citrobacter, Klebsiella* spp.	5.74–14.98	Aloo, Mbega, Makumba, et al. (2021)
	Rice (*Oryza sativa*)	*B. cereus, Alacligenes faecalis, Stenotrophomonas maltophilia, S. pavanii, Ochrobactrum cicero*	333.75–50.51	Rahma & Kristina (2019)
		Not identified	0.316–1835	Haerani et al. (2021)
		Pseudomonas spp., *Bacillus* spp.	47.27–71.27	Sutariati et al. (2020)
		B. megaterium, B. aryabhattai, B. subtilis	55.66–75.89	Liu et al. (2022)
		B. subtilis, P. fluorescens	7.36–28.0	Sivasakthi et al. (2013)
		Klebsiella sp.	2.55–291.97	Chaiharn & Lumyong (2011)
		P. brassicacearum, P. chengduensis, P. plecoglossicida, resinovorans, P. straminea	2.0–92.3	Habibi et al. (2019)
	Soybean (*Glycine max*)	*Burkholderia* spp.	47.5	Batista et al. (2018)
	Sorghum (Sorghum *bicolor*)	*Azospirillum* sp.	9–17	Godbole et al. (2021)
	Sugarcane (*Saccharum officinarum*)	*Azospirillum* sp.	9–17	Godbole et al. (2021)
	Turmeric (*Curcuma longa*)	*B. amyloliquefaciens*	16.8–60.5	Passari et al. (2018)
	Walnut (*Juglans* sp.)	*Micrococcus yunnanensis*	28–30	Dar et al. (2018)
	Wheat (*Triticum aestivum*)	*Bacillus, Azospirillum brasilense, A. lipoferum, P. stutzeri*	0.5–2.1	Ilyas et al. (2020)
		Azospirillum sp.	9–17	Godbole et al. (2021)

GA	Apple (*Malus pumila*)	*Pseudomonas* sp.	485.8–419.2	Sharma et al. (2018)
	Banana (*Musca* spp.)	*Bacillus licheniformis*	22.6–72.4	Silpa et al. (2018)
	Cabbage (*Brassica rapa*)	*Pseudomonas P. korreensis*	1.92–9.82	Kang et al. (2021)
	Cardamom (*Elettaria cardamomum*)	*B. subtilis, P. putida*	7.28–36.25	Panchami et al. (2020)
	Chili (*Capsicum annuum*)	*Pseudomonas, Bacillus*	1.21–35.74	Narayanan & Madhavan (2020)
	Coleus (*Coleus scutellarioides*)	*P. stutzeri, Stenotrophomonas maltophilia, P. putida*	27–34	Patel & Archana (2017)
	Lettuce (*Lactuca sativa*)	*Pseudomonas P. korreensis*	1.92–9.82	Kang et al. (2021)
	Maize (*Zea mays*)	Not identified	4.21–6.89	Kesaulya et al. (2021)
	Mungbean (*Vigna radiata*)	*Rhizobium* sp.	111.92–131.04	Mahmood et al. (2021)
		Bacillus, Pseudomonas, Rhizobium spp.	109.8–126.3	Routray & Khanna (2018)
	Basil (*Ocimum basilicum*)	*P. malcaliphila, P. hunanensis, Streptomyces laurentii, Sinorhizobium* sp., *B. safensis*	1.37–2.16	AlAli et al. (2021)
	Pasture plants (*Lolium* sp., *Festuca* sp., *Dactylis* sp.)	*Paenibacillus* sp., *Bacillus* sp.	32–37	Acuña et al. (2011)
	Pear (*Pyrus communis*)	*Pseudomonas* sp.	485.8–419.2	Sharma et al. (2018)
	Potato (*Solanum tuberosum*)	*Enterobacter, Serratia, Citrobacter, Klebsiella*	0.39–0.45	Aloo, Mbega, Makumba, et al. (2021)
	Rice (*Oryza sativa*)	*B. subtilis, P. fluorescens*	2.89–5.96	Sivasakthi et al. (2013)
		B. megaterium, B. aryabhattai, B. subtilis	16.33–23.58	Liu et al. (2022)
	Sugarcane (*Saccharum officinarum*)	*Pseudomonas* sp., *Azotobacter* sp.	10–22	Desai (2017)
	Walnut (*Juglans regia*)	*B. licheniformis, Micrococcus luteus*	28–30	Dar et al. (2018)
	Wheat (*Triticum aestivum*)	*P. monteilii*	7.5–93.93	Pandya and Desai (2014)
	Arabidopsis (*Arabidopsis thaliana*)	*P. putida*	3.0	Ghosh et al. (2019)
ABA	Wheat (*Triticum aestivum*)	*B. subtilis, P. mandelii*	Not mentioned	Arkhipova et al. (2020)
		Bacillus sp., *Azospirillum brasilense, A. lipoferum, P stutzeri*.	1.9–3.4	Ilyas et al. (2020)
Cytokinins	Coleus(*Coleus scutellarioides*)	*P. stutzeri, Stenotrophomonas maltophilia, P. putida*	7.5–13	Patel & Archana (2017)
	Wheat (*Triticum aestivum*)	*Bacillus* sp., *A. brasilense, A. lipoferum, P. stutzeri*	0.39–0.64	Ilyas et al. (2020)

(Modi & Khanna, 2018). ABA supports plant root growth under osmotic stress and promotes their water-uptake abilities (Arkhipova et al., 2020). According to Timmusk et al. (2014), the mechanism of drought mitigation by rhizobacteria for plants might be a collective outcome of the synthesis of IAA, ABA, GA, and CK, the presence of aminocyclopropane-1-carboxylate (ACC)-deaminase that reduces the ethylene levels in roots and promotes the secretion of exopolysaccharides, and induced systemic resistance of plants to diseases. Since drought stress is a major challenge to agricultural systems, such functions place rhizobacteria and their biomolecules at the center of efforts to mitigate and manage the effects of drought stress and climate change in agricultural ecosystems.

While comparing the PGP activities of potato rhizobacteria in Tanzania, Aloo et al. (2021) established that the endophytic isolates produced significantly ($P < .05$) more IAA (7.86 μg/mL) and GA (0.45 μg/mL) on average than their external counterparts which produced 5.75 and 0.39 μg/mL, respectively. It is hypothesized that endophytic rhizobacteria may be better placed at PGP than their external counterparts owing to PGP's more intricate and intimate relations with plant root tissues (Aloo, Tripathi, Mbega, et al., 2021). Cognizant of this, the PGP hormones produced by endophytic rhizobacteria may have more effects on plant growth compared to those produced by external rhizobacteria. Such potential differences should be considered for better exploitation of these metabolites for PGP since biomolecules produced by endophytic PGP diffuse quickly and more directly into host root tissues. It should, however, be noted that PGP hormones are physiologically active biomolecules that can facilitate plant root development and nutrient uptake even at minimal concentrations (Hayat et al., 2010).

The production of PGP hormones is dependent on specific genes in rhizobacterial genomes. Similar to plants, rhizobacterial biosynthesis of IAA occurs through different tryptophan-dependent pathways, namely, indole-3-pyruvate, indole-3-acatamide (IAM), indole-3-acetonitrile (IAN), and tryptamine (TPM), and tryptophan side-chain oxidase (Ona et al., 2005; Spaepen et al., 2007). In the indole-3-pyruvica acid pathway, indole-3-pyruvate decarboxylase that is encoded by *ppdC/ipdC* mediates the conversion of indole-3-pyruvate into indole-3-acetaldehyde as has been identified in *Azospirillum brasiliense* and *Pseudomonas putida* (Baudoin et al., 2010; Patten & Glick, 2002). The IAM pathway involves the decarboxylation of tryptophan into IAM by tryptophan monooxygenase (*iaaM*) and the hydrolysis of IAM into IAA by an indole acetamide hydrolase (*iaaH*) (Spaepen et al., 2007). Some bacteria, such as *V. boronicumulans* CGMCC 4969, have two enzyme systems: nitrilase and nitrile hydratase/amidase that metabolize IAN to IAA (Sun et al., 2018), and these two systems harbor different regulatory mechanisms, affecting the synthesis rate and duration. However, this has been extensively studied in plants as opposed to rhizobacteria. In the TPM pathway, tryptophan is primarily converted into TPM by a decarboxylase and is directly converted to indole-3-acetaldehyde by an amine oxidase (Zhang et al., 2019a,b) and, subsequently, transformed to IAA via the action of dehydrogenases. Tryptophan can be directly converted into indole-3-acetaldehyde by a

tryptophan side-chain monooxygenase enzyme, which is known as tryptophan side-chain oxidase (Suzuki et al., 2003). The *ysnE* gene, which encodes a putative tryptophan acetyltransferase, is also known to contribute to IAA production in *Bacillus* (Shao et al., 2021). The tryptophan-independent pathway is also suggested, although no enzyme involved in this pathway has been characterized (Shao et al., 2015). Nevertheless, this demonstrates that rhizobacteria use different biosynthetic pathways, which entail different molecular capabilities to produce IAA.

1.2.3 Siderophores

Siderophores are Fe-chelating rhizobacterial biomolecules produced under iron (Fe)-deficient conditions (Trapet et al., 2016). Although Fe is among the most abundant elements on earth, it is not readily assimilated by bacteria or plants because it is sparingly soluble (Godbole et al., 2021). Siderophore production thus increases the concentration of bioavailable Fe in the rhizosphere and helps plants in Fe sequestration (Dimkpa, 2016).

Various studies have demonstrated the functions of rhizobacterial siderophores in different plants (Table 1.2). Apart from aiding plant Fe acquisition in Fe-limiting soils, rhizobacterial siderophores are also involved in plant bioprotection (Table 1.2). The principal mechanism behind phytopathogen control by siderophores involves nutritional competition by limiting Fe availability to pathogens and inhibiting their growth (Ahmed & Holmström, 2015). Likewise, siderophores have also been implicated in salt-tolerant rhizobacteria that support plant growth in saline soils (Sultana et al., 2021).

Salinity being a widespread agricultural problem (Kumar et al., 2020), salt-tolerant, siderophore-producing PGPR can be an eco-friendly innovation for climate-smart agriculture in Fe-deficient saline soils (Sultana et al., 2021). Although siderophores are produced by rhizobacteria as part of their normal metabolism under Fe-limiting conditions to improve Fe acquisition, it is hypothesized that once the siderophores chelate ferric ions, plants acquire the bound Fe by degrading the complexes (Rajkumar et al., 2009). As such, siderophores aid in plant Fe-nutrition. According to Sarwar (2020), over 100 siderophore-producing rhizobacteria isolated from groundnut in Chakwal, Pakistan, were shown to increase Fe availability in soil. Comparable findings have also been established by Patel et al. (2018) for *Pantoea dispersa* and *P. putida* in mungbean.

Whereas rhizobacterial siderophore production is largely driven by soil Fe deficiency, the process also involves complex ecological interactions in the rhizosphere. Like phosphatases, the production of siderophores is largely dependent on soil pH, which also dictates the redox state of the available Fe (Dimkpa, 2016). Besides, the presence of other metals in the rhizosphere affects the rate of siderophore production; production can be up- or downregulated depending on the metal and the microorganism (Dimkpa et al., 2012a,b; Gaonkar & Bhosle, 2013;

Table 1.2: Siderophore-producing rhizobacteria of various plants and their functions.

Host/test plant	Rhizobacteria	Tested functions	References
Plant growth promotion			
Chickpea (*Cicer arietinum*)	Not identified	Plant growth Promotion	Maheshwari et al. (2019)
Groundnut (*Arachis hypogea*)	*B. subtilis, B. halotolerans*, *B. safensis* *Achromobacter* sp.	Iron release in soil Not mentioned	Sarwar et al. (2020) Sayyed et al. (2019)
Pea (*Pisum sativum*)	Not identified	Plant growth promotion	Maheshwari et al. (2019)
Pigeon pea (*Cajanus cajan*)	*Pseudomonas, Serratia, Bacillus, Rhizobium*	Not mentioned	Modi & Khanna (2018)
Potato (*Solanum tuberosum*)	*Providencia alcalifaciens, P. syringae, Serratia marcescens, S. liquefaciens, P. putida, Serratia ficaria*	Plant growth promotion compared to the negative control	Salinas et al. (2021)
	Klebsiella, Enterobacter, Citrobacter, Serratia	Increased Iron release in soil	Aloo, Mbega, & Makumba (2021)
	Klebsiella, Citrobacter, Serratia	Plant growth promotion	Aloo, Mbega, Makumba, et al. (2021)
Rice (*Oryza sativa*)	*P. brassicacearum, P. chengduensis, P. plecoglossicida, resinovorans, P. straminea*	Plant growth promotion	Habibi et al. (2019)
Walnut (*Juglans regia*)	*B. licheniformis, B. tequilesnis, B. cereus, B. subtilis, Micrococcus luteus, M. yunnanensis*	Plant growth promotion	Dar et al. (2018)
Biocontrol activities			
Chili (*Capsicum annuum*)	Not established	Biocontrol of *Ralstonia solanacearum*	Kesharwani & Singh (2020)
Cocoa (*Theobroma cacao*)	*P. chlororaphis*	Antagonistic against *Phytophthora palmivora*	Acebo-Guerrero et al. (2015)
Common bean (*P. vulgaris*)	*Bacillus* sp.	Biocontrol of *F. oxysporum*, *Macrophomina phaseolina*, *F. solani*, *Rhizoctonia solani*, *Colletotrichum* sp.	Kumar et al. (2012)
Cucumber (*C. sativus*)	*Pseudomonas* sp., *Bacillus* sp.	Biocontrol of *Pythium* root rot and damping-off diseases	Khabbaz et al. (2015)

Date Palm (*Phoenix dactylifera*)	*B. cereus, Alcaligenes faecalis, Stenotrophomonas malthopilia, S. pavanii, Ochrabactrum cicero*	Biocontrol of *Xanthomonas oryzae*	Rahma & Kristina (2019)
Maize (*zea mays*)	*B. altitudinis, B. aryabhattai, B. megaterium*	Inhibition of growth of *Sclerotinia sclerotiorum* and *Rhizoctonia solani*	Javoreková et al. (2021)
Pea (*Pisum sativum*)	*Pseudomonas* sp. *Bacillus* spp.	Biocontrol of *F. udum* causing *Fusarium* wilt	Dukare & Paul (2021)
Peanut (*Arachis hypogea*)	*B. velezensis*	Inhibition of *Aspergillus flavus*	Chen et al. (2019)
Pigeon Pea (*Cajanus cajan*)	*B. amyloliquefaciens*	Antagonism of *F. oxysporum*	Gowtham et al. (2016)
Several vegetable plants	*B. amyloliquefaciens*	Biocontrol of *Fusarium, Rhizoctonia* and *Colletotrichum* spp.	Passari et al. (2018)
Strawberry (*Fragaria ananassa*), potato (*Solanum tuberosum*)	*Pseudomonas, P. putida, Serratia* spp., *Pantoea agglomerans*	Biocontrol of *Verticillium dahliae causing rhizome rot*	Berg et al. (2002)
Turmeric (*C. longa*)	*B. cereus*, *P. aeruginosa*, *B. amyloliquefaciens*, *B. tequilesnis, B. subtilis*	Biocontrol of *Rhizoctonia solani*, *Schizophyllum commune*, *Macrophomina phaseolina*, *F. graminearum*, *F. solani*	Chenniappan et al. (2019)
	Not identified	Biocontrol of *Fusarium* wilt	Bouamri (2021)

Sayyed & Chincholkar, 2010). In line with this, a recent review by Dimkpa (2014) has demonstrated that metallic nanoparticles in the rhizosphere can affect the production of siderophores in fluorescent Pseudomonads. Over 500 biomolecules are categorized as siderophores; thus, several genes and regulators are involved in their biosynthesis and transport (Dimkpa, 2016). The major siderophore groups are hydroxamates, catecholates, and carboxylates, depending on the functional group that acts as the sequestrant (Rungin et al., 2012).

While hydroxamates are linear and cyclic biomolecules of Gram-positive and Gram-negative bacterial genera like *Streptomyces, Pseudomonas*, and *Staphylococcus* (Maheshwari et al., 2019), carboxylates include staphyloferrin-A and rhizobactin that are predominantly produced by *Staphylococcus* and *Rhizobium*, respectively. The catecholates (or phenolates) are siderophores that are characterized by high stability and Fe-binding abilities even at very low concentrations. Rhizobacteria can produce one or more siderophores depending on the amount and accessibility of nutrients (Maheshwari et al., 2019). However, the predominant siderophores include enterobactin, enterochelin, mycobactin, and agrobactin (Saha et al., 2016).

The genome mining of several rhizobacterial strains has shown the presence of siderophore-associated genes (Nouioui et al., 2019). Rhizobacterial biosynthesis of siderophores is dictated by the expression of *Fur* protein which in turn regulates siderophore synthesis and iron transport. The iron uptake chelate (*iuc*) genes are involved in siderophore production in *Haloferax volcanii* (Niessen & Soppa, 2020). More recently, Iqbal et al. (2021) identified two genes: *yclNOPQ* and *dhbABCF* involved in siderophore biosynthesis and transport in the genome of *B. subtilis* isolated from *Cynodon dactylon* in a study conducted in Pakistan.

1.2.4 Volatile organic compounds

These are low molecular weight biomolecules of microbial primary and secondary metabolism (Santoro et al., 2011). The origin, structure, biosynthesis, and biological activities of rhizobacterial VOCs have recently been discussed by Veselova et al. (2019), who assert that the functional and ecological roles of these biomolecules are presently the subjects of interest owing to their potential in promoting sustainable agricultural systems and environmental management. Aspects of sampling, detection, identification, and analysis of rhizobacterial VOCs have also been elucidated by Kai et al. (2020).

There has been increasing evidence that rhizobacterial VOCs play important functions in microbe–plant interactions. Rhizobacterial VOCs are largely known for their antimicrobial properties and plant bioprotection against phytopathogens, as has been established in several studies (Table 1.3). A more updated and comprehensive list of rhizobacterial VOCs and their activities has also been made available by Poveda (2021). The main chemical classes of rhizobacterial VOCs are ketones, alcohols, esters, terpenes, alkanes, organic acids, aldehydes,

Table 1.3: Rhizobacterial volatile organic compounds that inhibit various phytopathogens.

Genus	Species	Host plant	Volatile organic compounds	Inhibited pathogen	References
Bacillus	*B. amyloliquefaciens*, *B. thuringensis*,	Bambara groundnut	Dimethylfuvene, tridecane, acetic acid butyl ester, Formic acid 2-methylpropyl ester, paraldehyde, tropone, phthalan	*B. cereus*, *P. aeruginosa*, *Micrococcus cryophilus*, *Enterococcus feacalis*	Ajilogba & Babalola (2019)
	B. axarquiensis, *B. subtilis*, *B. licheniformis*	Sugarcane	Dodecane, methoxy-phenyl-oxime, acetic acid, 5,7-dimethyl-undecane, octamethyl-cyclotetrasiloxane, hexamethyl-cyclotrisiloxane	*Colletotrichum falcatum*	Jayakumar et al. (2021)
	Bacillus sp.	Avocado	Alcohols, carboxylic acids, aldehydes, esters, ketones	*Phytophthora capsici*	Syed-Ab-Rahman et al. (2019)
	Bacillus sp.	Avocado	3-amino-1,3-oxazolidin-2-one, 2,3,5-trimethylpyrazine, 9-undecadien-2-one, 6,10-dimethyl-5	*P. cinnamomi*	Méndez-Bravo et al. (2018)
	B. amyloliquefaciens	Avocado (*Persea americana*)	2,5-Dimethyl pyrazine, 2-Tetradecanone, 2-Dodecanone	*Colletotrichum gloeosporioides, Fusarium* sp.	Guevara-Avendaño et al. (2019)
	B. amyloliquefaciens	*Arabidopsis thaliana*	2,3-Butanedione, Acetoin	*Alternaria brassicicola, Botrytis cinerea, A. brassicae, Scletotinia sclerotiorum*	Asari et al. (2016)
	B. pseudomycoides, Brevibacillus brevis	Not mentioned	Hydrogen cyanide	*Ralstonia solanacearum*	Hassan et al. (2018)
	B. amyloliquefaciens	Peanuts	1,3-methanopentalene, 3,5-octadiyne, N-ethyl-hexahydro-1H-azepine, cyclopentasiloxane, oxirane, decane	*B. cinerea*	Nakkeeran et al. (2020)
	B. subtilis	Rice	nonanal, benzothiazole, acetophenone	*Clavibacter michiganensis*	Rajer et al. (2017)
	B. velezensis	Maize	5,5'-tetramethyl-1,1'-biphenyl, 2,4-dimethyl-6-tert-butylphenol, 5-undecene, decyl formate, dodecanenitrile, 2-methylpentadecane	*Verticillium dahliae, F. oxysporum*	Li et al. (2020)
	B. subtilis	Cucumber	Hydrogen cyanide	*Pythium, Phytophthora capsici, Rhizoctonia solani*	Khabbaz et al. (2015)
	Bacillus sp.	Turmeric	Hydrogen cyanide	*Rhizoctonia solani*, *Schizophyllum commune*, *Macrophomina phaseolina*, *F. graminearum*, *F. solani*	Chenniappan et al. (2019)
	B. subtilis	Cardamon	Hydrogen cyanide	*Not mentioned*	Panchami et al. (2020)
	Bacillus sp.	Chili (*Capsicum annuum*)	Ammonia, Hydrogen cyanide	*R. solanacearum*	Kesharwani & Singh (2020)

(*Continued*)

Table 1.3: (Continued)

Genus	Species	Host plant	Volatile organic compounds	Inhibited pathogen	References
	Bacillus sp.	Groundnuts	Ammonia, Hydrogen cyanide	*Sclerotium rolfsii, Aspergillus niger*	Thakur & Parikh (2018)
	B. velezensis	Not mentioned	2-undecanone, 2-nonanone, 2-heptanone, acetoin, benzaldehyde, butyl formate, 1-butanol, diacetyl, nonane, or pyrazine	*Monilinia fructicola, Botrytis cinerea, Penicillium italicum, M. laxa, P. digitatum, P. expansum*.	Calvo et al. (2020)
	Bacillus sp.	Rice	6-methyl-5-hepten-2-one, 3-methyl-butanoic acid, 2-ethyl-1-hexanol, Benzoic acid ethyl ester, 3-methyl-1-butanol	*Rhizoctonia solani*	Wang et al. (2021)
Pseudomonas	*Pseudomonas* sp.	Avocado	3-amino-1,3-oxazolidin-2-one, 6,10-dimethyl-5,9-undecadien-2-one, 2,3,5-trimethylpyrazine	*Phytophthora cinnamomi*	Méndez-Bravo et al. (2018)
	P. putida	Cardamon	Hydrogen cyanide	*Not mentioned*	Panchami et al. (2020)
	P. chlororaphis	Not mentioned	2-methyl-1-butanol, 3-methyl-1-butanol, phenylethyl alcohol	*Ceratocystis fimbriata*	Zhang et al. (2019a)
	P. fluorescens	Cucumber	Hydrogen cyanide	*Pythium, P. capsici, R. solani*	Khabbaz et al. (2015)
	P. putida	Black pepper	2-methyl pyrazine; 2, 5-dimethyl pyrazine; dimethyl trisulfide; 2-ethyl 3, 6-dimethyl pyrazine, 2-ethyl 5-methyl pyrazine	*Colletotrichum gloeosporioides*, *Athelia rolfsii*, *P. capsici Gibberella moniliformis*, *R. solani*, *Magnaporthe oryzae*, *R. pseudosolanacearum*, *Pythium myriotylum*, *Radopholus similis*	Agisha et al. (2019)
	Pseudomonas sp.	Rice	6-methyl-5-hepten-2-one, Benzoic acid ethyl ester, 2-ethyl-1-hexanol, 3-methyl-1-butanol, 3-methyl-butanoic acid	*R. solani*	Wang et al. (2021)
Others	*Arthrobacter*	Avocado	6,10-dimethyl-5,9-undecadien-2-one, 2,3,5-trimethylpyrazine, 3-amino-1,3-oxazolidin-2-one	*P. cinnamomi*	Méndez-Bravo et al. (2018)
	Enterobacter, Ralstonia, Arthrobacter, Brevibacillus, Paenisporosarcina	Rice	3-methyl-butanoic acid, Benzoic acid ethyl ester, 2-ethyl-1-hexanol, 6-methyl-5-hepten-2-one, 3-methyl-1-butanol	*R. solani*	Wang et al. (2021)
	Stenotrophomonas maltophilia, Streptomyces toxytricini	Not mentioned	Hydrogen cyanide	*R. solanacearum*	Hassan et al. (2018)

and nitrogen and sulfur compounds (Schenkel et al., 2015). Most rhizobacterial VOCs are metabolic products of glucose oxidation from various intermediates.

There are now attempts to detect the genes underlying the production of rhizobacterial VOCs. Iqbal et al. (2021) recently established the presence of genes like acetolactate decarboxylase (*budA*), acetolactate synthase (*alsS*), and acetoin dehydrogenase (*acoABCR*), which are associated with the synthesis of acetoin and 2, 3-butanediol in *B. subtilis* genome. The global regulation of most antimicrobial compounds, VOCs included, is governed by *gacS/gacA* genes which encode a two-component regulatory system (Bloemberg & Lugtenberg, 2001).

At the transcriptional level, the *hcnABC* genes are regulated by the anaerobic regulator (*ANR*) (Bloemberg & Lugtenberg, 2001). Ammonia and hydrogen cyanide (HCN) are some of the most common rhizobacterial VOCs that inhibit various phytopathogens. While characterizing chili PGPR in India, the production of ammonia and HCN was established as one of the mechanisms of biocontrol and antagonistic activities against *Ralstonia solanacearum* (Kesharwani & Singh, 2020). Similar results have also been reported by Verma & Pal (2020) for *Pseudomonas, Bacillus, Rhizobium*, *Mesorhizobium*, and *Azotobacter* spp. isolated from various plants in India. The fungicidal action of *Pseudomonas sp*. and *Bacillus* sp. against *F. udum* causing *Fusarium* wilt in pigeon pea was also recently shown to be due to the presence of ammonia and HCN (Dukare & Paul, 2021).

Rhizobacterial VOCs have also been implicated in plant stress tolerance. For instance, salinity stress can be alleviated by rhizobacterial VOCs (Cappellari et al., 2020; Li et al., 2021). According to Ayuso-Calles et al. (2021), the mechanisms through which PGPR ameliorate saline stress involve hormonal balance changes, synthesis and release of extracellular osmoprotectant biomolecules, and chemical signals that improve soil conditions. In a previous study, the mode of plant stress tolerance exhibited by lettuce (*Lactuca sativa*) was also established to be due to the production of phenolic compounds by *R. laguerreae* (Ayuso-Calles et al., 2020). There is also evidence that these volatile compounds can stimulate plant immunity and positively improve the rhizosphere conditions for plant development (Rojas-Solís et al., 2018; Yi et al., 2016).

1.2.5 Organic acids

Besides enzymatic degradation, the solubilization of P, Zn, and K into plant-accessible forms can also ensue through organic acids 2-ketogluconic acid, citric acid, succinic acid, and lactic acid (Hussain et al., 2015; Muleta et al., 2013; Ramesh et al., 2014). Organic acids commonly originate from the oxidation of glucose as a source of energy and carbon (Macias-Benitez et al., 2020). Different microbes produce different quantities and types of organic acids, which may be dependent on the carbon type available for their metabolism

(Patel et al., 2008). Subsequently, rhizobacteria are bound to differ extensively in their nutrient solubilization efficiencies.

During P solubilization, the hydroxl and carboxyl ions lower the soil pH, and chelate cations like Fe^{3+}, Ca^{2+}, and Al^{3+} complexed to P compete with P for the sites of adsorption in soil and/or form soluble compounds with the P-associated metal ions (Pradhan et al., 2017; Sharma et al., 2013). Some researchers have shown that inoculation of PGPR that produces organic acids and dissolves P can increase the quantity of soil P and improve crop yields (El-Sayed & Hagab, 2020; Israr et al., 2016; Yazdani et al., 2009). During the characterization of PGPR of *C. annuum* in China, several Pseudomonads involved in the solubilization of tri-calcium phosphate and tri-magnesium phosphate were found to produce 2-ketogluconic acid, α-ketoglutaric acid, and succinic acid in addition to alkaline phosphatases (Han et al., 2021). Similarly, phytate-mineralizing rhizobacterial isolates of *Cajanus cajan* have previously been shown to include gluconic and acetic acids (Patel et al., 2010). Kang et al. (2021) also recently established the production of malic acid (112.6 ug/mL), tartaric acid (87.6 ug/mL), and citric acid (308.4 ug/mL) by rice rhizospheric *Psudomonas koreensis* in South Korea.

Besides nutrient solubilization, organic acids may also be involved in the degradation of organic matter by acidogenesis, hydrolysis, methanogenesis, and acetogenesis (Adeleke et al., 2017). Organic P can also be released as a by-product of soil organic matter mineralization. While investigating the response of groundnut (*Arachis hypogea*) to PGPR, Kausar et al. (2018) observed that the increased availability of plant nutrients was correlated to the quantities of bacterial organic acids. Similar findings have also been reported by Wu et al. (2017). The degradation of organic matter mediated by organic acids also contributes to the solubilization or liberalization of the nutrient elements held within the organic matter for use by plants. These biomolecules are, therefore, important in the maintenance of soil fertility and nutrient levels for sustained production.

Previous studies have shown that pyruvic lactic, succinic, and citric acids are the main organic acids secreted when inoculated with P-solubilizing *Burkholderia multivorans* and *Enterobacter cloacae* (Lee et al., 2019; Zeng et al., 2017). These findings indicate that different PSB activate different mechanisms leading to the production of varied types and concentrations of organic acids to solubilize insoluble phosphate. Transcriptome analysis has recently revealed higher expression of genes associated with tricarboxylic acid cycles such as citrate synthase (*cs*), aconitic hydratase (*aco*), isocitrate dehydrogenase (*idh*), α-ketoglutarate dehydrogenase (*ogdh*), succinyl-CoA synthetase (*suc*), succinate dehydrogenase (*sdh*), and fumarate hydratase (*fh*) genes in insoluble P medium (Ding et al., 2021).

1.2.6 Antibiotics

The bioprotection of plants against several phytopathogens is commonly attributed to the synthesis of antibiotics, which are low molecular weight characterized rhizobacterial

biomolecules. Various studies have shown antibiotics-mediated biocontrol of plant pathogens (e.g., Cossus et al., 2021; Jin et al., 2020). Similarly, several antibiotics have successfully been used to control plant pathogens and increase crop yields due to their biocontrol activities. For instance, the root rot of pepper (Ezziyyani et al., 2007), the anthracnose disease in mungbean (Keerthana et al., 2018), and the leaf blight/ seedling blight of rice, *Fusarium* root rot, and wilt in tomato (Minuto et al., 2006) have all been shown to be controlled with rhizobacterial antibiotics.

Antibiotics are generally produced by *Bacillus, Pseudomonas, Stenotrophomonas*, and *Streptomyces* sp. as active biomolecules against plant pathogens. Bacilli have especially found massive applications as biocontrol agents because of not only antibiotic production but also their catabolic diversity, production of endospores, and other PGP biomolecules (Aloo et al., 2019). The production of endospores especially makes bacilli promising PGPB since they can survive in diverse habitats (Setlow, 2006). A recent study in India on the biocontrol efficiency of native turmeric PGPR against rhizome rot disease established the presence of various genes encoding for 4-diacetyl-phloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, bacillomycin D, and fengycin in several bacilli (Chenniappan et al., 2019). These results have been replicated in other studies on rhizospheric bacilli (Arfaoui et al., 2019; Crovadore et al., 2020). According to Stein (2005), between 4% and 5% of *B. subtilis* genome is devoted to the synthesis of antibiotics. Fairly recently, lipopeptides were also established in the genomes of endophytic bacilli of wild Solanaceous plants with antagonistic potential against *F. oxysporum* f. sp. *lycopersici* (Abdallah et al., 2017). The antagonistic potential of cotton-endophytic *Bacillus* spp. *Verticillium* wilt has also been linked to the existence of genes encoding for bacillomycin, surfactin, and fengycin antibiotics (Hasan et al., 2020). Also recently, molecular studies revealed the occurrence of biosynthetic genes encoding for lipopeptides in common-bean-endophytic *B. amyloliquefaciens*, *B. velezensis*, *B. halotolerans*, *Agrobacterium fabrum*, and *Pseudomonas lini* with antifungal activities against *Fusarium* sp., *Alternaria* sp., and *Macrophomina* sp. causing the root rot disease in beans (Sendi et al., 2020).

Antibiotics are diverse and their regulation is complex. The different pathways of antibiotic production are discussed in a recent review (Hou & Kolodkin-Gal, 2020). Antibiotics can be classified according to their biosynthetic pathways as nonribosomal peptides (NRPs), ribosomally synthesized posttranslationally modified peptides (RiPPs), and polyketides. The NRPs include surfactins and fengycins, which are synthesized by large enzymes called nonribosomal peptide synthetases (NRPSs) (Iqbal et al., 2021). The synthesis of antibiotics in Pseudomonads and other rhizobacteria involves the NRPS that contains domains for selecting, loading, and synthesizing amino acids and secreting the antibiotics (Strieker et al., 2010). The synthesis of antibiotics is also modulated by the histidine protein kinase/response *GacA*/*GacS* regulatory system (Zhang et al., 2020), cell density-dependent regulation via N-acyl homoserine lactones, and sigma factors (Srivastava et al., 2012). In Gram-negative

rhizobacteria, it is postulated that acyl-homoserine lactones are synthesized and perceived by *LuxI*/*LuxR* homologs, respectively (Brodhagen et al., 2004). In *Pseudomonas fluorescens*, the *GacS-GacA* system regulates the expression of the *phlACBD* locus, which is responsible for DAPG production by inducing the transcription of the small non-coding RNAs; *rsmX*, *rsmY*, and *rsmZ* (Zhang et al., 2020).

Antibiotics are probably some of the most important rhizobacterial biomolecules in the context of plant bioprotection. Antibiotic-producing plant-endophytic rhizobacteria especially hold immense potential in plant defense against phytopathogens because of the intimate interaction of endophytes and their plant hosts, which can allow for more efficient and direct antibiosis on phytopathogens. Besides, some antibiotics have a broad spectrum of activities and can be effective against several phytopathogens. Owing to the current concerns regarding the continued application of chemical pesticides and the immense environmental repercussions associated with them, antibiotics, alongside other rhizobacterial biomolecules with established biocontrol potential, should be explored further and exploited to sustainably control plant pathogens.

1.3 Emerging gaps and perspectives

Rhizobacteria undoubtedly produce multifarious biomolecules with important PGP functions in plant rhizospheres. Unraveling the biomolecules or chemical agents as actors in the complex rhizosphere ecosystem can pave the way in mapping several functional aspects existing between microbes and plants. Recent technological advances in meta-omics and high-throughput sequencing tools present opportunities for the discovery of novel rhizobacterial biomolecules and overcoming the challenges of conventional or cultural methods. Metabolomics of plant rhizospheres has facilitated the profiling and annotation of rhizobacterial biomolecules (exo/endo) involved in various biochemical systems in soil and plants (Srivastava & Sarethy, 2021). There are also prospects for molecular and physiological manipulations of rhizobacteria for improved production of efficient biomolecules. Advances in omics and gene editing tools continue to ease the process of gene manipulation and could allow the engineering of non-PGPR strains to work as PGPR inoculants for crop production. These engineering methods can facilitate the utilization of plant beneficial rhizobacterial mechanisms and biomolecules for improved functioning and sustainable crop production.

The hunt for rhizobacterial secondary metabolites and biomolecules has long generated immense interest due to their unique functionalities. However, the biosynthetic genes involved in their synthesis and secretion have received less attention globally. Genomic evaluations of as many rhizobacteria as possible to comprehend their genetic constitutions for the production of plant-important biomolecules can facilitate their exploitation in this regard. Similarly, whole-genome sequences and advanced genomic studies can be useful. Similarly, a lot of attention seems to revolve around rhizobacterial auxins with very little focus on other rhizobacterial PGP hormones like GA, ABA, and CK, which are equally

important in agricultural systems. More investigations into these hormones and other rhizobacterial biomolecules, which have so far received less attention, could open up new avenues for PGP or improve the existing ones on a greater scale. The comprehension of genes involved in the rhizobacterial synthesis of PGP biomolecules creates the opportunity to improve the performance of biocontrol strains and/or construct novel biocontrol strains through genetic modification (Bloemberg & Lugtenberg, 2001).

The ecological functions of microbial VOCs are not yet understood in detail. Rhizobacterial VOCs exhibit dynamic control of plant rhizosphere functions and complex changes across microbial growth phases, which result in varied composition and emission rates of species-specific compounds (Misztal et al., 2018). These VOCs may not only be involved in the suppression and antagonisms of plant pathogens but are also capable of modulating plant hormonal and physiological pathways and increasing plant biomass and yield (Sharifi & Ryu, 2018, 2020; Tyagi et al., 2018).

Presently, global warming and climate change are key areas of concern for agricultural systems. These phenomena not only result in increased droughts or water-limiting conditions but also increased temperatures that cause evaporation and enhance the salinity of agricultural soils. The tolerance of rhizosphere bacteria to drought conditions can be used as an effective tool for promoting plant-microbe interactions under drought and or water stress. It is now increasingly evident that various rhizobacterial biomolecules are involved in improving the tolerance of plants to drought and salinity stresses. It cannot be emphasized enough that these biomolecules will soon form a critical component of agricultural ecosystems. However, it is important to evaluate the salinity tolerance mechanisms of PGPR associated with various plants to better exploit them for these purposes. According to de Boer et al. (2019), the abiotic and biotic complexity of plant rhizosphere soils hinders the exploitation of VOCs as pathogen-suppressing rhizobacterial biomolecules. Additionally, numerous pathogen-suppressive VOCs produced by artificially-manipulated cultures also occur in soil. Therefore an integration of laboratory and field studies regarding the production of various rhizobacterial VOCs is needed to understand and predict the composition and dynamics of these biomolecules in plant rhizospheres.

1.4 Concluding remarks

The anthropogenic activities in agricultural fields continue to fuel global warming and climate change in many ways, especially through the indiscriminate use of agrochemicals. The exploitation of rhizobacterial PGP biomolecules is a plausible environmentally friendly approach for the sustainability of agricultural systems and environmental management. This chapter contains a synthesis of the plausible functions of various rhizobacterial biomolecules that can each be pursued in this regard. It has explored the roles of enzymes, hormones, antibiotics, organic acids, siderophores, and volatile organic acids produced by various rhizobacteria in PGP in the context of enhanced plant nutrition as well as phytopathogen biocontrol. The information

contained here can direct and enhance the exploitation of these biomolecules and promote the development of sustainable agricultural systems and the management of the environment.

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CHAPTER 2

Microbial biomolecules: reducing viral loads in agriculture

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2.1 Introduction

Agriculture in the 21st century is facing the challenge of producing more food to accomplish the demand of the growing population, along with multiple complications caused by various invading pathogens. Minimizing the risk of crop disease is crucial as it impacts food security, farming systems, and the health of human livestock worldwide (Jones, 2009). Among all the pathogens affecting agriculture, almost 50% of the microbes are viruses, and out of them, the most vigorous are the plant viruses threatening cultivated plants by reducing their growth and viability but also reducing gross yield by decreasing the quality of plants (Anderson et al., 2004). Such crop losses lead to economic consequences that mostly affect the people's livelihood that completely rely on agriculture. The emerging and reemerging of viruses and their multiple host ability has increased the viral problem to a greater extent (Savary et al., 2019).

Rhizobacteria form a symbiotic relationship with the host plant and enhance the growth and development of the plant. They exhibit a significant relationship with the roots of the host plants and have positive effects on plant growth directly or indirectly, and also play an important role in the reduction of biotic and abiotic stresses (Gouda et al., 2018). Farmers have spent plenty of time, money, and energy on managing viral diseases, resulting in poor disease management and an adverse result from chemicals used in the field. Crop losses tend to be more in the tropical region as many viruses have been known to have multiple hosts. Much great emphasis must be put on addressing the management of viral loads on economically important crops. This chapter is focused on reducing viral loads in agriculture by using potential environment-friendly alternatives to chemical fertilizers (Jones, 2021).

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2.2 Virus impact on plants and economy

Over the last few decades, virologists have discovered a large number of viruses using different advanced techniques, which led to the expansion of our knowledge on the diversity of viruses in nature and also about their multiple hosts, particularly where unraveling the virome of many economically important crops is concerned. The staple food crops, along with grain legumes, oilseeds, vegetables, and fruits, are greatly affected by invading viruses' affinity at increasing the risk to food security, genetic erosion, and extinction of plant species (Bos, 1981).

Numerous plant viruses are known to destroy crops. They are classified based on characteristics that describe the virus-like property, their molecular composition and structure, host range, pathogenicity, and similarity in sequences. For the first time in 1882, viral diseased tobacco was investigated by scientist Adolf Mayer, and the disease was termed Mosaic; and, then in 1898, M.W. Beijerinck established the causal determinant of tobacco mosaic disease. Later various virologists focused on understanding the plant diseases and viruses infecting them. According to ICTV 2020 report, 189 families and 9110 species of plant viruses are known to exist, including both DNA and RNA viruses (International Committee on Taxonomy of Viruses ICTV, 2021). Both RNA and DNA viruses are known to cause destruction globally. Interaction in virus in mixed infection between unrelated viruses and new encounters of viruses that facilitate viral host species jumps to new crops and weeds has increased the severity of agricultural and economic damage. Virus belonging to the economically important DNA virus family includes the double-stranded DNA virus pararetroviruses (Caulimoviridae) and the single-stranded DNA virus Geminivirdae and Nanoviridae (Hohn, 2009). Geminivirus is the largest family of DNA viruses divided into nine different genera on the basis of genome organization, host range, and vectors. + ve RNA viruses are Bromoviridae, Closteroviridae, Luteoviridae, and Potyviridae. Some −ve strand RNA plant viruses include families Bunyaviridae and Rhabdoviridae, which are all RNA viruses that are economically most destructive of plant viruses (Gergerich and Dolja, 2006). Some of the plant viruses are banana bunchy top virus (BBTV), bean common mosaic virus, cowpea mosaic, bean yellow mosaic virus, bitter gourd yellow mosaic virus, cucumber green mottle mosaic virus, cucumber mosaic virus (CMV), papaya ringspot virus, pepper mild mottle virus, potato virus X, potato virus Y, sunflower necrosis virus, tobacco mosaic virus, tobacco necrosis virus, tomato chlorotic spot virus (TCSV), tomato mosaic virus, tomato leaf curl Palampur virus, tomato mottle virus, tomato yellow leaf curl virus (TYLCV), urdbean leaf crinkle virus, watermelon mosaic virus, soybean mosaic virus, rice stripe virus and many more (Savary et al., 2019; Agnihotri et al., 2018; Sofy et al., 2019; Jones, 2021; Srivastava et al., 2016; Kumar et al., 2016; Raj et al., 2015).

Once the plants get infected by the virus, curing plants is not feasible as the disease gets transmitted from one plant to another rapidly, and it's unlikely that the plants survive the

disease as they cannot be treated using antibacterial or antifungal chemicals. Some insecticides are being used to control the vector transmitting the virus, but these have their adverse effects too. So, viral disease management should be based on either blocking the entry of the virus or making the plant resistant to viral infection. Various plant growth-promoting rhizobacteria (PGPR) strains are being used to develop resistance in plants.

2.3 Plant growth-promoting rhizobacteria—the redeemer of plants

A varied population of soil bacteria usually resides in plant rhizospheres. A group of these microorganisms is advantageous to the plants in terms of endorsing their growth. These bacteria, therefore, are very commonly stated as PGPR or plant growth-promoting microbes that aid potential environment-friendly alternatives to chemical fertilizers. Plant roots are surrounded by a thin layer of soil known as the rhizosphere, which acts as a primary location for nutrient uptake and prime resident for rhizobacteria. They are capable of forming stress-tolerant spores and secret metabolites that stimulate plant growth and restrict viral infection. PGPR contributes to the welfare of plants either directly or indirectly. In the direct response, PGPR encourages plant growth by fixing atmospheric nitrogen, solubilizing the soil insoluble to phosphates and potassium, providing iron available for the plant, and also producing different phytohormones like indole acetic acid, which have direct involvement in cell differentiation and division, and also in the plants' elongation (Bhardwaj et al., 2014; Mishra et al., 2010), gibberellic acid (Narula et al., 2006), cytokinins (Ortíz-Castro et al., 2008), and accumulation of ethylene in response to stress that may increase plant tolerance or exacerbate stress-response symptoms and senescence (López-Bucio et al., 2007; Morgan and Drew, 1997). PGPRs also reveal antagonistic action against viruses by producing siderophores, antibiotics, cyanide, soluble phosphorus, biofilm, biotic elicitors derived from plant cell wall components or the chemicals released from the plant and abiotic elicitors like metal ions or inorganic compounds (Akhtar and Siddiqui, 2009). Siderophores are low molecular weight, iron-binding protein compounds involved in the process of chelating ferric iron (Fe (iii)) from the environment. When Fe is limited, microbial siderophores provide plants with Fe, enhancing their growth. Flores-Felix showed that a siderophore-producing Phyllobacterium strain promotes the growth and quality of strawberries (Flores-Félix et al., 2013).

PGPR produces volatile organic compounds that play a vital role in improving plant growth, inducing systemic resistance toward invading viruses, and improving stress tolerance. For example, a strain of *Bacillus megaterium* secretes polyamine, spermidine, acetoin, and 2,3-butanediol that are responsible for significant improvements in plant growth (Bailly and Laure, 2012; Ruzzi and Aroca, 2015).

As the biotic stress in plants increases, the disease resistance mechanism gets activated by PGPR that involves specific gene and hormone expression, like the degradation of

1-aminocyclopropane-1-carboxylate deaminase (ACC), which is a precursor of ethylene that induces stress relief and maintains normal growth (Glick, 2005). Some PGPR secretes exopolysaccharides that inhibit toxic ion movement along with the growth and movement of invading viruses (Radhakrishnan et al., 2017). Some of these reactions of PGPR are locally restricted, but others systemically give cause to the initiation of an array of responses resulting in the accumulation of plant defensive bioactive compounds in the entire plant (Van Loon et al., 1998; Verbon and Liberman, 2016). Bacillus amyloliquefaciens enhances defense responses in plants by producing secondary metabolites by means of maintaining a balance of reactive oxygen species (ROS) and ROS scavengers (Srivastava et al., 2016).

2.4 Nutrients availability by plant growth-promoting rhizobacteria for plants

PGPR has the capability of increasing the availability of nutrient concentration in the rhizosphere (Choudhary et al., 2011) by fixing nutrients and preventing them from purging out. For instance, Phosphorus (P) is essential for crop growth and productivity, and its deficiency in plants is due to the abundant number of insoluble forms. Only two water-soluble forms, that is, ($H2PO4^{-}$) and ($HPO4^{-}$), are available to plants (Vessey, 2003). Various phosphate solubilizing bacteria can solubilize the inorganic insoluble soil P into soluble plant P form through organic acid production, secretion of protons, and acidification which increases the availability of nutrients for plant growth promotion. PGPRs have the ability to solubilize precipitated phosphates and increase their availability for crop growth promotion under field trials (Verma, 2001).

Nitrogen is important for the synthesis of amino acids and proteins that are the most limiting nutrients for plants. The mechanisms by which atmospheric nitrogen is added into organic forms that can be assimilated by plants are sole to prokaryotes (Lloret and Martínez-Romero, 2005; Raymond et al., 2004). PGPR also fixes atmospheric nitrogen in two ways, that is, either symbiotically or nonsymbiotically. *Azotobacter* and *Bacillus* species are the most effective PGPR with the potential to fix atmospheric nitrogen in a symbiotic way when inoculated to the leguminous crop plants (Esitken et al., 2006). *Azospirillum* is a free-living nitrogen-fixing organism often associated with cereals in temperate zones and is also reported to be able to improve rice crop yields (Tejera et al., 2005; Bashan and De-Bashan, 2010). Development of commercially available free-living N-fixing bacteria such as *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter* sp., *Diazotrophicus* sp., *Herbaspirillum* sp., *Azotobacter* sp., *Bacillus polymyxa*, and especially *Azospirillum* sp. (Vessey, 2003) is worth noting. However, in spite of having adequate amounts of essential micronutrients in soil, plants still show deficiencies due to the inadequacy of these mineral nutrients. In calcareous soils, the availability of plant nutrients such as Fe, Mn, Cu, B, and Zn are generally low. These nutrients play vital roles as enzyme

activators, electron carriers, or Osmo-regulators and serve in the regulation of metabolism, reproduction, and protection against abiotic and biotic stresses (Riaz et al., 2021). Fe deficiency-induced chlorosis is the main limiting factor restricting plants' growth. Various PGPR increase the Fe availability in soil by decreasing the pH via releasing organic acids or synthesizing low molecular weight, iron-chelating agents (siderophores) (İpek and Eşitken, 2017).

2.5 Plant growth-promoting rhizobacteria itself as a biofertilizer

PGPRs as biofertilizers are attractive as well as an economical approach for sustainable agriculture (Riaz et al., 2021). Biofertilizers are termed as products that contain living microorganisms that colonize the rhizosphere or interior of the plant and promote nitrogen fixation, siderophore production, phytohormone synthesis, phosphate solubilization, regulation of ethylene level, and biosynthesis of vitamins and cyanides by enhancing the availability of primary nutrients that can then be easily assimilated and absorbed by the plants (Vessey, 2003; Mishra et al., 2013; Vessey, 2003). Researchers reported the use of PGPR strains like *Rhizobium*, *Mesorhizobium*, *Azorhizobium*, *Bradyrhizobium*, as biofertilizers.

Earlier studies stated that a biofertilizer prepared by combining PGPR with composts could improve growth-promoting effects and biocontrol of plants (Chen et al., 2011). *Bacillus* spp. (Gong et al., 2006) and *Pseudomonas* spp. (La Fuente et al., 2006) are two PGPR that have been reported to be effective biocontrol agents. Adequate amounts of PGPR in biofertilizer retains a beneficial role in creating a proper rhizosphere for plant growth and recycling nutritionally important elements through a biological process, for example, enhancing the availability of N, P, K, as well as hindering pathogen growth (Vessey, 2003; Waddington, 1998). The high availability of N, P, and K could enhance soil fertility, improve antagonistic isolates' biocontrol effects, and extend microorganisms' survival rates in soil (Yang et al., 2011).

PGPR are the eco-friendly source of sustainable nutrients required for balancing soil health and biology (Sun et al., 2020; Raklami et al., 2019; Bhardwaj et al., 2014). Further, they exhibit antagonistic activity against several agricultural pathogens and overcome abiotic stresses (Timmusk et al., 2014, 2015; Ilangumaran and Smith, 2017). Various microbial taxa have been commercially used as efficient biofertilizers based on their ability to obtain nutrients from the soil, fix atmospheric N_2, stimulate the solubilization of nutrients, and act as biocontrol agents (Basu et al., 2021).

2.6 Strains of plant growth-promoting rhizobacteria play a role in empowering plants to fight against virus stress

Viruses are the main causal agents that undermine the production of grains and infect more than 80% of the crop. They are spreading in nonpersistent, semipersistent, and persistent

manners by insect vectors, that is, aphids, leafhoppers, and mites, and through soil and seeds. Traditional biological and chemical agents are not very effective to control these viruses. The alternative approach is PGPR to control these viruses. The results from a study showed that applying a mixture of PGPR strains is more efficient to control *Papaya ringspot virus* (PRSV-W) and *TCSV* compared to individual PGPR strains only (Abdalla et al., 2017). *Bacillus amyloliquefaciens* has the ability to draw a broad range of induced systemic resistance in several crops that is phenotypically similar to pathogen-induced systemic acquired resistance. (Ahn et al., 2002). *Enterobacter asburiae* BQ9 induced resistance against TYLCV by enhancing the expression levels of defense-related genes and antioxidant enzymes, including phenylalanine ammonia-lyase, peroxidase, catalase, and superoxide dismutase (Li et al., 2016). Kumar analyzed and found that *Nicotiana tabacum* cv soil inoculated with *Peanibacillus lentimorbus* B-30488 showed decreased CMV RNA accumulation. This was associated with an increase in stress and pathogenesis-related gene expression and antioxidant enzyme activity suggesting induced resistance against the virus (Kumar et al., 2016).

Besides PGPR's commercial use against fungal and bacterial diseases, there is an increasing number of studies regarding their potential action against viruses. *Bacillus* spp. has been reported to induce antiviral responses against *CMV* in tomato (Zehnder et al., 2000), pepper (Lee and Ryu, 2016) and Arabidopsis (Ryu et al., 2004), and against PVY and *Potato virus X* (PVX) in potato plants (Park et al., 2006). Moreover, *Pseudomonas* spp. reduced disease severity of *Tomato spotted wilt virus* (Kandan et al., 2005) and *tomato mottle virus* (ToMoV) (Murphy et al., 2000) in tomato plants and of *BBTV* (Harish et al., 2009) in banana plants. The defense mechanism triggered by PGPR against viruses depends on the complicated interactions among PGPR, host plant, and virus, involving mostly the salicylate (SA) signaling pathway and, in some cases, both SA and jasmonate (JA) pathways (Ryu et al., 2004; Gkizi et al., 2016; Lee et al., 2017).

2.7 Conclusion

Viral stress is becoming a foremost in the worrying problems faced by people in agriculture, due to which the productivity of economically important crops is declining exponentially. Our dependence on chemical-based fertilizers and insecticides has increased the use of life-threatening chemicals, which not only are harmful to human life but have also disturbed the ecological balance in the ecosystem.

The present chapter gives an overview of the development and formulations of PGPR in the promotion of the growth and defense mechanism of plants along with the enhancement in agriculture productivity through a different mechanism. The successful applications of PGPR provide a model for enhancing the biocontrol of plant pathogens and abiotic stress management, etc. The characteristics exhibited by the PGPR strains like biological nitrogen

fixation, solubilization of nutrients like phosphorus, iron, and potassium, also add significance to enhancing plant growth and yield. The implication of PGPR can save a significant number of chemical fertilizers, besides contributing toward sustainable agriculture. This knowledge will aid in the advancement of technology that will harness a promising plant-microbe interaction for sustainable crop production.

Conflict of interest

The authors declare that they have no conflict of interest.

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CHAPTER 3

Quorum quenching in marine bacteria and its applications

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Bacterial cells often communicate in a collective manner by a mechanism termed Quorum sensing (QS) (Gestel et al., 2021). QS allows bacteria to occupy themselves in complex ecological niches by regulating the expression of certain genes, including the production of antibiotics or virulence factors, toxins, extracellular polysaccharides, secondary metabolites, pathogenesis, bioluminescence, biofilm development, sporulation, conjugal DNA transfer, and other physiological activities (Gantner et al., 2006; Leipert et al., 2017; Rehman and Leiknes, 2018; Borges and Simões, 2019). This intra- and inter-species communication is achieved by bacteria by the production, release, and detection of small chemical signaling molecules known as auto-inducers. Bacteria produce different types of QS signals that differ in chemical structure and specific functions. Broadly, three major classes of auto-inducers are identified: (1) acyl homoserine lactones (AHLs) or auto-inducers-1 (AI-1) are quorum sensing molecules consisting of a homoserine lactone moiety and acyl side chain that regulate QS primarily in Gram-negative bacteria, (2) the auto-inducer peptides (AIPs) are short peptide chains that mediate QS in Gram-positive bacteria, and (3) auto-inducer-2 (AI-2) are furanone-derived molecules mediating QS in both Gram-positive and Gram-negative bacteria. In addition, other signaling molecules have also been described in many previous studies (Sperandio et al., 2003; Hughes and Sperandio, 2008; Deng et al., 2010; Ryan and Dow, 2011). Generally, these auto-inducers that exceed a threshold concentration known as "quorum level" are required to trigger the transcription of QS-regulated genes and, hence, QS is considered a population-dependent ability of bacteria. At the threshold concentration, the auto-inducers will be recognized by the specialized receptors located either in the membrane (Gram-positive bacteria) or in the cytoplasm (Gram-negative bacteria). The detection of auto-inducers by the specific receptors will then initiate group-beneficial behaviors by the responsive bacterial population (Borges and Simões, 2019; Vadakkan et al., 2018; Rehman and Leiknes, 2018; Kawaguchi et al., 2008). The QS communication system was first observed in a Gram-negative marine bacterium, *Vibrio fischeri*, in which the QS machinery involved the regulation of bioluminescence (Vadakkan et al., 2018). The impact of QS-phenomenon in

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marine microbial system has been well demonstrated and still receiving increasing research interest. QS-controlled behaviors are found in both free-living and associated forms of marine bacterial communities and play a substantial role in ecologically and biogeochemically significant processes (Borges and Simões, 2019). The predominant classes of QS signals in the marine microbial environments include AI-1 (e.g., AHLs, α-hydroxy ketones) and AI-2 (e.g., furanosyl-borate diesters). As a matter of fact, Proteobacteria are widely prevalent in marine habitats in which AHLs are the major class of auto-inducers. Hence, AHLs mediated QS is intensively studied in marine microbial systems (Hmelo, 2017). The AHLs consist of a core lactone ring and a fatty acid side chain with varying numbers of carbon (4−18). So far, various approaches have been developed for qualitative and quantitative detection of AHLs (e.g., biosensor assay, high-performance liquid chromatography, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, thin layer chromatography, and isotopic labeling) (Kawaguchi et al., 2008; Kumar et al., 2016). A general scheme of AHLs and AI-2 mediated QS is depicted in Fig. 3.1.

3.1 Quorum quenching

QS plays a pivotal role in the regulation of various bacterial functions, including pathogenesis and biofilm formation. Hence, QS blockade can offer new avenues to control bacterial infections and biofouling in various domains such as medical, aquaculture, wastewater treatment plants, ship hulls, and other industries. Quorum quenching (QQ) denotes the mechanism by which bacterial QS can be inhibited (Rehman and Leiknes, 2018). Many living organisms such as plants, animals, bacteria, and fungus are reported to produce various compounds with QQ activity. Indeed, QQ activity in diverse bacterial groups (e.g., α-proteobacteria, β-proteobacteria, some Gram-positive bacteria) is well-documented. Hence, bacterial metabolites with QQ activity

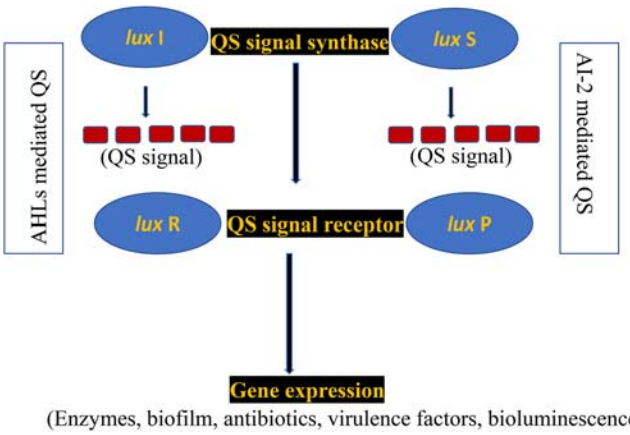

Figure 3.1
A schematic diagram showing the main QS mechanisms found in marine environments.

are one of the common strategies to inhibit the QS mechanism (Borges and Simões, 2019). QQ and QS bacteria are found to co-exist in different environments. It clearly indicates that the QS signal interference mechanism provides a competitive advantage for nutrients and ecological niches to the producer (Zhang and Dong, 2004; Chen et al., 2013; Borges and Simões, 2019). Three main steps in the QS circuit can be targeted to achieve QQ: (1) signal themselves-degradation/deactivation of auto-inducers, (2) signal synthase-inhibition of auto-inducers biosynthesis, and (3) signal receptor-interfering auto-inducer detection by a signal receptor. Quorum quenching strategies are generally classified as QQ enzymes (enzymatic methods) and QS inhibitors (non-enzymatic methods). In general, QS inhibitors block the QS circuit by structural modification or competitive inhibition, whereas QQ enzymes degrade or modify QS signals. Three major categories of QQ enzymes are observed in bacteria—(1) AHL lactonase: that hydrolyzes lactone moiety of AHL; (2) AHL Acylase: that cleaves amide bonds between lactone ring and the fatty acid side chain; and (3) AHL Oxidoreductase: that modify AHL chemical structure by oxidation or reduction of a third carbon of the fatty acid side chain (Natrah et al., 2011; Rehman and Leiknes, 2018; Vadakkan et al., 2018; Borges and Simões, 2019). Examples of QQ enzymes and QS inhibitors produced by bacteria are shown in Table 3.1.

3.2 Quorum quenching in marine bacteria

The marine environment represents a plethora of bioactive resources. As mentioned in the previous section, QS plays a key role in regulating a wide range of bacterial behaviors in the ocean. QS signals released into the marine environment face a variety of abiotic and biotic challenges that could attack their structural integrity and prevent their accumulation to required quorum levels (Hmelo, 2017). In fact, biological QQ activity was first observed in the marine environment (Kjelleberg et al., 1997). To date, several groups of marine organisms, including bacteria, algae, corals, and sponges that could produce QS inhibitory metabolites, are reported (Borges and Simões, 2019). Previous studies suggest that bacterial interference with QS is quite common in the marine environment, which signifies its importance in marine microbial processes (Dobretsov et al., 2009; Romero et al., 2012). These antagonistic strategies will provide a competitive advantage to the bacterial producers in their ecological niches, such as biofilm and eukaryotic hosts (Borges and Simões, 2019). Interestingly, Romero et al., 2012 suggest that AHL degradation might be one of their functions since some QQ enzymes display homology to the enzymes with other metabolic activities. Thus, further investigations on the ability of QQ enzymes to degrade AHLs and other structures similar to AHLs would be helpful to completely elucidate the ecological significance of QQ activities. Many marine bacteria are known to produce both QQ enzymes and QS inhibitors to confound QS-regulated phenotypes. The bacteria that exhibit QQ activity have been isolated from pelagic environments, eukaryotic hosts, dense microbial biofilms, particle-free seawater, and sea sediments. The isolated bacterial groups dominantly belong to α-proteobacteria, β-proteobacteria, Firmicutes, Actinobacteria, and

Table 3.1: QQ enzymes and QS inhibitors produced by bacteria.

QQ mechanism	Mode of action	Quorum quenching component	Bacterial producer	References
Enzymatic	QS signal degradation/ modification↓Switch off signal transmission	AHL lactonase	*Bacillus* sp., *Geobacillus stearothermophilus*, *Geobacillus caldoxylosilyticus*, *Geobacillus kaustophilus*	Dong et al. (2000); Chow et al. (2010); Seo et al. (2011)
		AHL Acylase	*Streptomyces* Sp., *Pseudomonassyringae*, *Tenacibaculum maritimum*	Park et al. (2005); Shepherd and Lindow (2009); Romero et al. (2010)
		AHL Oxidoreductase	*Rhodococcus erythropolis*, *Bacillus megaterium*	Uroz et al. (2005); Chowdhary et al. (2007)
Non-enzymatic	Competitive binding/ structural modification↓Inactivation of AI synthases or receptors	Cis-9-octadecenoic acid	*Stenotrophomonas maltophilia*	Singh et al. (2013)
		Cyclic dipeptides	*Lactobacillus reuteri*	Li et al. (2011)
		Isobutyramide	*Halobacillus salinus*	Teasdale et al. (2009)
		3-methyl-N-(2-phenylethl)-butyramide	*Halobacillus salinus*	Teasdale et al. (2009)
		Yayurea A and B	*Staphylococcus delphini*	Chu et al. (2013)

Bacteroidetes (Dong and Zhang, 2005; Hmelo and Van Mooy, 2009; Romero et al., 2011; Van Mooy et al., 2012; Rehman and Leiknes, 2018). Some examples of QQ activity of cultivable bacteria isolated from various marine sources are shown in Table 3.2. In addition, a study by Romero et al., 2012 reported a relatively high frequency of QQ acylase and lactonase genes in marine metagenomes.

Altogether, marine bacteria could definitely be a promising resource for potential QQ molecules with a wide range of applications.

3.3 Applications of quorum quenching

QQ has now emerged as a powerful strategy against various bacterial misfortunes. In the current scenario of the growing emergence of antimicrobial resistance and the harmful environmental impact of antibiotics, it is the need of the hour to find nonlethal alternatives to treat bacterial infections. QS system plays a critical role in the virulence gene expression of many bacterial pathogens that are important to clinical, agriculture, and aquaculture sectors. Hence, QQ has been developed as an effective alternative to conventional antibiotics (Chen et al., 2013). Generally, QS disruptions are more versatile because they inhibit virulent gene expression rather than produce bactericidal substances. Thus, they provide long-term, sustainable effectiveness against microbial infections with reduced occurrence of bacterial resistance. Nevertheless, QS inhibition could induce some selective pressure in bacteria, as it is commonly spread slowly and more moderately than antibiotics (Dobretsov et al., 2009; Borges and Simões, 2019). Numerous previous studies reported successful attenuation of virulence in microbial pathogens by bacterial quorum quenchers. For instance, reduction of virulence by AHL lactonase AiiA from *Bacillus* sp. in phytopathogen, *Erwinia carotovora* (Dong et al., 2000). Similarly, *Aeromonas hydrophila* infection in zebrafish was significantly attenuated by AHL lactonase activity of *Bacillus* sp. (Cao et al., 2012). A study by Torres et al., 2016 observed reduced pathogenicity of *Vibrio mediterranei* in the presence of AHL degrading strain, *Alteromonas stellipolaris*. QQ strategies could also be used for combatting biofilm infections which are difficult to eradicate by antibiotics (Chen et al., 2013). Furthermore, the application of quorum quenchers as adjuvants to antibiotics could increase the bacterial susceptibility to antibiotics. Consequently, the use of a higher dose of broad-spectrum antibiotics could be reduced (Borges and Simões, 2019). Another promising application of quorum quenchers is in control of biofilm formation. Within a biofilm, bacterial cells are protected by a matrix of EPS (extracellular polymeric substances) and have increased resistance to antimicrobial compounds, UV exposure, temperature fluctuations, nutrient depletion, changes in salinity, and predation (de Carvalho, 2018). Biofilm formation on artificial substrates leads to metal corrosion, biofouling, acid production, and oxygen removal. Hence, biofilm eradication is a major concern in various industries such as aquaculture, maritime transport, desalination

Table 3.2: QQ activity of marine bacteria.

QQ Metabolite	Source	Bacterial Producer	QS Inhibitory Activity	References
AHL analogs	Water	*Rhizobium* sp.	Biofilm formation and production of virulence factors by *Pseudomonas aeruginosa*	Chang et al. (2017)
Lactonases andAcylases	Sediment	*Labrenzia* sp.	Biofilm formation by *P. aeruginosa*	Rehman and Leiknes (2018)
Phenethylamide, (N-(2′-phenylethyl)-isobutyramide & 3-methyl-N-(2′-phenylethyl)-butyramide)	Seagrass	*Halobacillus salinus*	Bioluminescence by *Vibrio harveyi*	Teasdale et al. (2009)
QQ Enymes	Water	*Salinicola salariusOlleya marilimosaMaribacter ulvicola*	Violacein production in *Chromobacterium violaceum*	Romero et al. (2012)
Phenolic groups and C—H stretches with amine groups	Sediment	*Bacillus pumilus*	Biofilm formation by *P. aeruginosa*	Nithya et al. (2010)
Lactones	Sediment	*Streptomycetes* spp.	Virulence factors by *Candida albicans*	Cho et al. (2001)
Lactones	Sediment	*Actinomycetes*	Biofilm formation in *Vibrio* species	You et al. (2007)
Cinnamic acid, lineardipeptides proline—glycine & N-amido- α-proline	Marine sponge	*Actinomycetes*	Violacein production in *Chromobacterium violaceum* and production of virulence factors by *P. aeruginosa*	Naik et al. (2013)
Actinomycin D & cyclic (4-hydroxy-Pro-Phe)	Water	*Streptomyces parvulus*	Biofilm formation by *P. aeruginosa*, *Staphylococcus aureus, Micrococcus luteus*, and *Ruegeria* sp.	Miao et al. (2017)
DL-homocysteinethiolactone	Coral	*Staphylococcus hominis*	Violacein production in *Chromobacterium violaceum* and biofilm formation by *P. aeruginosa*	Dobretsov et al. (2013)
Cyclo(L-leucyl-l-prolyl)	Mangrove	*B. amyloliquefaciens*	Prodigiosin production in *Serratia marcescens*	Gowrishankar et al. (2019)

plants, and oil and gas industries (Dobretsov et al., 2013). QQ can be used as an environmentally friendly strategy to reduce biofouling in aquaculture, medical, and other industrial domains. A study by Jo et al. (2016) has demonstrated that biofouling in membrane bioreactors could be considerably reduced by QQ. QQ bacteria were further explored to understand how bacteria mitigate biofouling in a laboratory-scale RO system in a study by Oh et al. (2017). In sum, metabolites from bacteria that disrupt the QS mechanism in microbial pathogens or in biofouling causing microorganisms could be explored to understand their function as an efficient and eco-friendly alternative to antibiotics and other biocides. However, QQ alone may not be useful for solving biofouling and infections, but combinatorial therapies can be used to achieve desired outcomes (Rehman and Leiknes, 2018).

3.4 Future perspectives

Most studies on QS and QQ have been carried out under laboratory conditions that limits the understanding of these bacterial communication processes under natural conditions. Hence, more field studies that mimic real infections or biofouling are needed. Likewise, scale-up of bioreactors are also crucial for industrial-scale utilization of QQ metabolites. Furthermore, bacteria can develop resistance to QS inhibitors by efflux pumps or mutations in QS circuits. The application of noncompetitive inhibitors with manifold biological activities should have been given priority in overcoming such resistance (Kalia et al., 2014; Borges and Simões, 2019). Finally, prior to broader commercial applications, their effectiveness, side effects, including toxicity and impact on humans, beneficial microbes and other organisms, cost, stability, comparability with antibiotics and other biocides, and mode of application of QQ metabolites also need to be addressed (Dobretsov et al., 2009; Chen et al., 2013; Dobretsov et al., 2013; Borges and Simões, 2019). Overall, QQ in marine bacteria opens up new avenues to control bacterial infections and biofouling in various sectors.

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CHAPTER 4

Microbially synthesized nanoparticles: application in health-care management

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4.1 Introduction

Tiny particles with diameters the size of 100 nanometers or less have drawn a lot of attention because of their odd and fascinating features, as well as their advantages over their bulk counterparts in terms of applications and efficiency. It is possible to create several types of nanoparticles by using different methods, including chemical, physical, biological, and other hybrid processes. Nanoparticles can be synthesized via chemical and physical processes; however, the use of harmful substances severely limits their biomedical applications, especially in clinical sectors. To expand nanoparticles' biomedical applications, developing reliable, nontoxic, and eco-friendly methods for their production becomes crucial. In this context, microorganisms emerge as a viable alternative to synthesize nanoparticles. Compared to chemically produced nanoparticles, biogenic enzymatic nanoparticles have several advantages.

Firstly, microorganisms can produce huge quantities of nanoparticles having proper sizes and shapes in a short period of time, but these microorganisms are complex and not very efficient apart from their producing perilous toxic wastes that are detrimental to the environment as well as human health. Secondly, in contrast to the chemical method, an enzymatic process does not require the use of costly chemicals, is slighter energy-intensive, and is more environmentally friendly. Furthermore, the "biogenic" approach is supported by the fact that most bacteria live in environments with varying temperatures, pH, and pressures (such as oceans), and the resulting particles are more catalytic, have a better

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region, and with better interaction between both the enzyme and the metal salt because of the bacterial carrier matrix. Lastly, by capturing target ions from their surroundings and subsequently converting metallic ions into element metal, the microorganisms make nanomaterials by enzymes generated by cell operation.

Nanoparticles have received a lot of attention lately because of their excessive surface-to-volume ratio and other interesting, innovative, and noteworthy properties. Given that these properties have emerged with the development of nanotechnology and nanoscience in the last few decades, the traditional methods for making nanoparticles are either chemical or physical, both of which use hazardous chemicals and require a lot of energy, making them expensive, while the use of microbes to synthesize nanoparticles is biocompatible, cost-effective, environmentally friendly, and energy-intensive. Bacteria, viruses, fungi, and algae all produce metallic and nonmetallic nanoparticles, as well as metal oxide and sulfide nanoparticles. These nanoparticles operate as adsorbents for the treatment of water and wastewater contaminants due to their physicochemical features, nano size, controlling growth, and surface functionalization. As a result, fewer hazardous chemical surfactants are needed because the enzymes, proteins, and carbohydrates found in these microbes act as capping agents and surfactants. Dye remediation, heavy metal remediation, microbial remediation, and pesticide remediation are all areas in which nanoparticles have found application in wastewater treatment. Iron oxide nanoparticles, titanium oxide, alumina, oxide of zinc, etc., are among the most commonly used adsorbents today. In this chapter, we'll look at how bacteria, algae, fungi, and viruses can be used to synthesize nanoparticles and how they can be used in health management.

4.2 Characteristics of nanoparticles

Nanodevices and nanosystems for industrial, consumer, and biomedical applications are being developed by modern science and technology. Richard Feynman, a physicist, first envisioned the theoretical application of nanotechnology in 1959. Nanotechnology is the potential to manipulate and manage matter at the atomic and molecular levels. Nanoparticles have been synthesized as a result of recent advances in nanotechnology. Optoelectronic, electronic, and various chemical and biochemical sensors are on the list to be developed using these nanoparticles (Narayanan and Sakthivel, 2010). It is known that nanomaterials can be found in biotic systems such as bacterial cells, spider silk, and insect tentacles, as well as human bones (Calabia et al., 2010).

4.3 Classification of nanoparticles

Generally, nanoparticles are classified according to their size and agglomeration. The toxic effects of nanoparticles found in the environment on human health are affected by their

shape and anatomy (Buzea et al., 2008). According to their dimensions, nanoparticles can be categorized into one, two, or even three-dimensional. Thin film circuits and sensor devices comprise one-dimensional nanoparticles. When it comes to the two-dimensional nanoparticles, which include carbon nanotubes, they have excellent adsorption and stability. Quantum dots, dendrimers, and other three-dimensional nanoparticles make up the last-named in the list (Pal et al., 2011).

Nanoparticle's shape can be flat, spherical, or crystallized. In addition, they can be found in a single form or in a composite form. Nanoparticle's formation can be categorized as intracellular or extracellular synthesis. Ions are carried inside the cell to produce nanoparticles in intracellular production. While in extracellular synthesis, it is possible to make nanoparticles outside the cell by trapping metal ions and reducing them in the presence of enzymes. The different uses of nanoparticles in human health management, like drug carriers for gene therapy, cancer treatment, targeted delivery, and DNA analysis, have been made with biosynthesized nanoparticles. There has been a constant exchange of information between living organisms and inorganic matter since the beginning of time. As a result of this regular interaction, there occurs a comprehensive array and placement of many types of minerals to support life on Earth. Interaction between biological species and inorganic molecules has been a growing area of research for scientists in recent years. Many microorganisms can generate inorganic nanoparticles either intracellularly or extracellularly (Li et al., 2011).

4.4 Intracellular synthesis of nanoparticles by bacteria

An ultrasound conducted towards a cleanser or a reaction with a cleanser is required in order to release the intracellular nanoparticles. When it comes to mining wastes and their leachate, this method of releasing intracellular nanoparticles can be used to recover precious metals from these sources. Moreover, organic metal nanoparticles in many chemical processes might be used for catalytic purposes. As a result, the nanoparticles can be stored indefinitely in bioreactors. For some time now, the deposition of minerals has been linked to the activity of bacteria. According to new research, an Alaskan placer containing Pedomicrobium-like budding bacteria has been found to accumulate gold. To reduce water-soluble Au + 3, *Bacillus subtilis* 168 produced an octahedral arrangement in the cell walls with dimensions of 5−25 nm. H_2S was released from the bacterial envelope when gold(I)-thiosulfate complexes (Au(S2O3)2 3) were destabilized by the heterotrophic (SRB). Gold was intracellularly precipitated in the periplasmic region as part of the reduction in Fe(III). At 25°C, mesophilic cell iron(III) reduction and Shewanella algae decreased Au + 3 ions at 10−20 nm in the periplasmic region (pH7.0) and at 15−200 nm at the anaerobic surface, even in the existence of hydrogen gas (pH2.8).

4.5 Extracellular synthesis of nanoparticles by bacteria

The position of the reductive constituent in the cell determines the microbial production of metal nanoparticles. There is no doubt that metal nanoparticles can be found extracellularly when cell wall reducing enzymes or secreted enzymes are intricated in the reduction of metal ions. Compared to intracellular accumulation, extracellular production of nanoparticles has a wider range of applications in optoelectronics, sensor technologies, bioimaging, and electronics. *Rhodobacter capsulata* reduces Au^{+3} to Au^{0} at room temperature, according to a study on Rhodopseudomonas. For example, when pH was set to 7, TEM analysis revealed that at pH7, most of the particles were 10−20 nm round. A summary biosynthesis of nanoparticles from microbes is given Table 4.1.

However, with the pH of the suspension having changed, and as a result, nanoparticles of different sizes are formed. When the pH level reaches 4.0, triangular nanoparticles are shown alongside spherical nanoparticles for the first time. In addition to triangular nanoparticles, spherical particles with a size of 10−50 nm are also found. The circumstances for the synthesis of anisotropic gold nanostructures with varying amounts of gold ions were also optimized, according to the study. Cell-free extract of *R. capsulata* was combined with an insignificant amount of gold ions to produce spherical-shaped gold nanoparticles with a diameter of 10−20 nm. It was possible to synthesize 50−60 nm gold nanowires that were highly networked using higher gold ion concentrations. One or even more proteins (14−98 kDa) were implicated in the bioreduction and also in the capping of gold nanoparticles, according to SDS-PAGE analysis (Hulkoti and Taranath, 2014).

4.6 Virus-mediated biosynthesis of nanoparticles

Nanocrystals can be grown using biological molecules such as amino acids, polyphates, and some amino acids as templates. CdSe, Cdte, and CdS nanocrystals can be shaped by altering the ratio of various fatty acids (chain lengths). For the environmentally friendly synthesis of inorganic materials, there are a number of other biological methods that could be used. For the template-mediated synthesis of inorganic nanoparticles and microstructures, biotic materials such as DNA and protein cages have been used. The tobacco mosaic virus (TMV), which is a positive-sense single-stranded RNA virus, was used as a template for the oxidative hydrolysis of oxides of iron, the co-crystallization of PbS and CdS, and the sol−gel condensation of SiO_2. It happened because of the aspartate and glutamate groups on the virus's surface. A genetically engineered virus' self-assembling viral capsids have been used to build quantum dot nanowires. M13 bacteriophages contain pVIII fusion proteins that contain peptides like A7 and J140 that can nucleate ZnS and CdS nanocrystals. They were then exposed to semiconductor precursor solutions and selected using a phage display using pIII peptides as templates. Zirconium wurtzite nanoparticles of

Table 4.1: Biosynthesis of nanoparticles from microbes.

Source	Nanoparticles	Method of biosynthesis	Substrate	Size	Shape	References
Bacillus licheniformis	Silver	Extracellular	Silver nitrate	50	Unknown	Kalishwaralal et al. (2008)
Enterobacteria	Silver	Extracellular	Silver nitrate	52.5	unknown	Shahverdi et al. (2007)
Bacillus sp.	Silver	Extracellular	Silver nitrate	42–92	Spherical	Das et al. (2014)
Lactobacillus sporogens	Zinc oxide	Intracellular	Zinc chloride	5–15	Hexagonal	Prasad & Jha (2009)
Morganella sp.	Copper	Intracellular	Copper sulfate	15–20	Unknown	Ramanathan et al. (2011)
Pseudomonas fluorescens	Copper	Extracellular	Copper sulfate	49	Spherical	Srinivasan & Rani (2014)
Lactobacillus crispatus	Titanium dioxide	Extracellular	Titanium dioxide	70–114	Oval, spherical	Abdul et al. (2020)
Verticillium luteoalbum	Gold	Intracellular Extracellular	Hydrogen tetracholoroaurate	100	Hexagonal Spherical triangular	Gericke & Pinches (2006)
Fusarium oxysporum	Silver	Extracellular	Silver nitrate	5–15	Unknown	Ahmad et al. (2003)
Trichoderma viridae	Silver	Extracellular	Silver nitrate	2–4	Spherical	Amanulla et al. (2009)
Geobacillus sp.	Gold	Intracellular	Hydrogen tetracholoroaurate	5–50	Quasi-hexagonal	Correa et al. (2013)
Streptomyces sp.	Silver	Extracellular	Silver nitrate	10–100	Spherical	Zonooz & Salouti (2011)
Cladosporium	Silver	Extracellular	Silver nitrate	10–100	Spherical	Balaji et al. (2008)
Penicillium	Silver	Extracellular	Silver nitrate	5–25	spherical	Kandasamy et al. (2009)
Stereum hirsutum	Copper oxide	Extracellular	Copper chloride	5–20	Spherical	Cuevas et al. (2015)
Salmonella	Copper	Extracellular	Copper nitrate	40–60	unknown	Ghorbani (2013)
Salmonella typhirium	Silver	Extracellular	Silver sulfate	50–150	unknown	Ghorbani (2013)
Planomicrobium sp.	Titanium dioxide	Extracellular	Titanium dioxide	8.89	Spherical	Chelladurai et al. (2013)
Stenotrophomonas	Gold	Intracellular	Gold chloride	40	unknown	Nangia et al. (2009)

5 nanometers were found on the viral capsid, as were CdS nanowires of 3"−5". The study was performed by using dual peptide virus engineered to show A7 and J140 within the same viral capsid to create hybrid nanowires (ZnS−CdS).

4.7 Metallic nanoparticles

4.7.1 Gold nanoparticles

As far back in time as ancient Rome, gold nanoparticles were being used to mark glass for decorative purposes. In ancient times, AuNPs (gold nanoparticles) were used to treat a wide range of diseases. More than 1 and 50 years ago, Michael Faraday was the first to perceive that colloidal gold solutions have characteristics that show the difference from the bulk gold. This led to the modern synthesis of AuNPs. Nanoparticle biosynthesis, a new bionanotechnology (at the nexus between biology and nanotechnology), has attracted a lot of attention as a result of the growing need to develop environmentally friendly materials synthesis methods. *Fusarium oxysporum* and actinomycete *Thermomonospora sp*. were used by Sastry and coworkers to synthesize gold nanoparticles extracellularly. *Verticillium* sp. was found to generate gold nanoparticles within its cells. When bacteria are incubated with Au^{3+} ions, nanoscale gold particles can be precipitated within the cells, as Southam and Beveridge demonstrated. *Rhodococcus* sp., an alkali-tolerant bacterium, was used to synthesize monodisperse gold nanoparticles. According to Lengke et al., filamentous cyanobacteria were able to synthesize gold nanostructures in a variety of shapes (octahedral, cubic, and spherical) from Au(I)-thiosulfate and Au(III)-chloride complexes and analyzed their formation processes. Lactobacillus was used by Nair and Pradeep to grow nanocrystals and nano alloys (Lekhak et al., 2014). Table 4.2 lists a summary of metallic nanoparticles prepared from different microbial sources.

Table 4.2: Metallic nanoparticles.

Microorganisms used	Products obtained	Temperature required for culturing (°C)	Size (in nm)	Shape	Location	References
Fusarium oxysporum	$PbCO_3$, $CdCO_3$	At 27	120 to 200	Spherical in shape	Extracellular	Sanyal et al. (2005)
Fusarium oxysporum	$SrCO_3$	At 27	10 to 50	Resemblance to needle	Extracellular	Rautaray et al. (2004)
Brevibacterium casei	PHB	At 37	100 to 125	Not known	Outside the cell	Srk et al. (2009)
Yeasts	$Zn_3(PO_4)_2$	At 25	10 -to 80 * 80 to 200	Shown as rectangle	Outside the cell	Yan et al. (2009)
Fusarium Oxysporum	CdSe	At 10	9 to 15	Spherical	Extracellular	Kumar et al. (2007)

4.7.2 Silver nanoparticles

Antimicrobial undertaking against Gram-negative and Gram-positive bacteria is demonstrated by silver nanoparticles, as well as highly multiresistant strains such as methicillin-resistant *Staphylococcus aureus*. Discovering nature's secrets has led to the expansion of biomimetics, proceeding to the production of advanced nanomaterials. Nanoparticles can be synthesized from microorganisms in an environmentally friendly manner. $Ag+$ is reduced by various microbes into silver nanoparticles, the maximum of which is found to be sphere-shaped. When added to a concentrated aqueous solution of silver nitrate, the silver mine bacterium *Pseudomonas stutzeri* AG259 played a significant role in the reduction of silver ($Ag+$) ions and the formation of silver nanoparticles (AgNPs) of distinct topography and proper shape within the bacteria's periplasmic region. Researchers found that fungi, such as *Verticillium* and *Fusarium oxysporum*, produce AgNPs in film form or in solution or accumulate them on the surface of their cells (Natarajan et al., 2010).

4.7.3 Alloy nanoparticles

For their implementation in catalysis purposes and as electronic devices, optical materials, and coatings, alloy nanoparticles are highly sought after. According to researchers, the cofactor nicotinamide adenine dinucleotide (NADH) plays a key role in the composition of Au-Ag alloy nanoparticles. It was discovered that yeast cells can synthesize Au-Ag alloy nanoparticles. Gold-silver (Au-Ag) alloy nanoparticle characterization by fluorescence microscopy and transmission electron microscopy revealed that they were primarily generated through an extracellular method and generally existed as irregular polygonal nanoparticles. This sensor, which uses Au-Ag alloy nanoparticles, causes glassy carbon electrodes to change and was found to be able to increase the electrochemical response of vanillin by as much as five times, according to electrochemical studies. On the other hand, Sawle, a researcher, showed that fungi strain *Fusarium semitectum* can be used to produce core-shell Au-Ag alloy nanoparticle suspensions that remain stable for many weeks.

4.7.4 Other metallic nanoparticles

It is well-known that the presence of heavy metals in the environment can prove toxic to microorganisms. Biochemical removal of these toxins—which is also known as detoxification—and the energy-required ion efflux from the cell by proteins present in the membrane that can be either an ATPase or an antitransporters for proton or cation are responsible for the heaviest metal resistance in nature. Changes in solubility also play a part in the development of microbial immunity. *Shewanella algae*, a metal ion-reducing bacterium, was used by Konishi and coworkers to produce platinum nanoparticles. When lactate was used

for a donation of electrons, resting cells of *S. algae* became capable of reducing soaking hexacholoroplatinate PtCl6 2 ions into elemental platinum within 60 minutes. In the periplasm, platinum nanoparticles, which are about 5 nm in size, have been found to exist. They showed that *Enterobacter* sp. cells can generate nanoparticles of mercury. Phosphoric acid concentrations at pH8.0 and lower mercury concentrations in the culture media promote the production and distribution of 2−5 nm-sized and monodispersed intracellular mercury particles within the cells. When *Pyrobaculum* is used with hydrogen as an electron donor, *Pyrobaculum islandicum* is able to reduce many heavy metals such as U(VI) and Tc (II) as well as Cr, Co, and Mn (IV). In order to produce palladium nanoparticles, bacteria that reduce sulfur dioxide, *Desulfovibrio desulfuricans*, and ion-reducing bacteria, *Shewanella oneidensis*, were used.

4.8 Oxide nanoparticles

Another important class of nanoparticles synthesized by microbes is the oxide nanoparticle. Magnetic oxide (MON) nanoparticles and nonmagnetic oxide nanoparticles have been discussed in this section. Ancient Egyptians discovered antibacterial properties in metals like silver (Ag) and copper, gold (Au), and titanium (Ti) in the 1500 s BP [14e16]. They have recently been studied for their potency, as well as their broad spectrum of activity against a variety of resistant bacteria. Due to differences in metal transport systems and metalloproteinase, metals can distinguish between mammalian and bacterial cells. They can be used as antibacterial materials for a long time because of this advantage over conventional antibiotics. This is because metal nanoparticles are so small that when they are placed in contact with bacteria, they have a very strong interaction. *En outre*, these metals can be combined with oxygen to produce metal oxide nanoparticles (MONPs) (Narayanan and Sakthivel, 2010).

4.8.1 Magnetic oxide nanoparticles

In recent years, magnetic nanoparticles have been developed as a new material because of their novel micro configuration and some attractive properties, such as super paramagnetic, as well as their potential for multiple applications in biomedicine and also in biological separation. Nanoparticles of iron oxide (magnetite) and iron trioxide (maghemite) have been shown to be polymeric. In the past few years, research on these has been active with the following materials: magnetized hyperthermia, stem cell sorting, handlebarrier medication delivery, gene editing, DNA testing, and magnetic resonance imaging (MRI). Internal magnetic particles made of iron oxide, sulfides of iron, or both, are produced by magnetotactic bacteria. These particles are referred to as magnetic particles of bacteria in order to differentiate them from artificially synthesized magnetic particles (AMPs) (BacMPs). Under the effect of the geomagnetic field, bacterial magnetic particles (BacMPs) are thought to act as (biological) compass needles that allow bacteria to migrate along

oxygen gradients in water, an environment in which, according to numerous reports since 1975, magnetotactic bacteria have been found to exist. As a result of their organic membranes, BacMPs can be easily dispersed in aqueous solutions. The organic membranes are primarily composed of phospholipids and proteins. Moreover, each BacMP compromises a single magnetic domain that gives it higher magnetic properties. It has been found that magnetotactic cocci, for example, have a wide range of species and have been detected many times on the surface of aquatic sediment. These bacteria, as well as the only magnetotactic coccus strain cultured, MC-1, have been found to be microaerophilic.

It has been found that three types of marine facultative anaerobic marine vibrio bacteria have been isolated from estuarine salt marshes: strains MV-1, MV-2, and MV-4. According to classification, these bacteria are members of the genus Proteobacteria and may come from the Rhodospirillaceae family. They synthesize BacMPs with an irregular hexa-octahedron shape and grow chemolithoautotrophically as well. Magnestospirillaceae, on the other hand, is a family of bacteria that lives in freshwater sediment. Many magnetotactic bacteria have been found to be members of this family after a growth medium and magnetic isolation techniques were developed. As the first member of the family to be isolated, Magnetospirillum magnetotacticum strain Ms1 has been extensively studied, as has Magnetospirillum gryphiswaldense strain MSR-1. Anaerobic magnetotactic spirilla Magnetospirillum magneticum AMB-1 was isolated by Arakaki and colleagues.

Diverse aquatic environments have been found to harbor new magnetotactic bacteria since 2000. Magnetotactic bacteria that have not been cultured have been found in a variety of habitats. Magnetic bacteria are generally mesophile and do not grow well above 30°C (86°F). At 30°C and below, magnetotactic bacteria that had not been cultured predominately grew. Es gibt nur wenige reports on thermophilic magnetotactic bacteria (TMCBs). For example, in springs that ranged from 32°F to 63°F in temperature, the magnetotactic bacteria HSMV-1 has been found to exist. TEM images of HSMV-1 cells without staining revealed a polar flagellum and a single chain of magnetosomes in bullet shapes on the cell surface. 113 nm × 34 nm × 5 nm is the average size of magnetosome crystals per cell. Many magnetotactic bacteria were found to be at least moderately thermophilic based on the study's findings. In environments where magnetotactic bacteria are present and are likely to grow (63°F) and where magnetosome magnetite is sedimented, they increase at the upper temperature limit. When yeast cells were used as templates, researchers found magnetic Fe_3O_4 materials along a mesoporous construction.

4.8.2 Nonmagnetic oxide nanoparticles

Other nanoparticles of oxide, such as TiO_2, SIo2 Sb_2O_3, $BaTiO_3$, and ZrO_2 nanoparticles, have also been observed. Scientists have developed a low-cost and reproducible way to make Sb_2O_3 nanoparticles using *Saccharomyces cerevisiae* (Bansal et al., 2006).

The synthesis was carried out at a temperature close to that of ambient air. Nanoparticle Sb_2O_3 was found to be a 2−10 nanometer aggregate. SiF6 2 and TiF6 2 anionic complexes were synthesized by Bansal et al. using *F. oxysporum* (fungus) (Jha et al., 2009). These nanoparticles were made from *F. oxysporum* in size within the range of 4−5 nm and some 3−11 nm, respectively, and were made from BaTiO3 and ZrO2 (Fasiku et al., 2020).

4.9 Sulfide nanoparticles

Sulfide nanoparticles have gained considerable attention as strengthening the basis of research, as well as making possible practical applications such as quantum dot fluorescens biomarkers or cell labeling agents because of their interesting and unique electronic properties. A typical sulfide nanoparticle is CdS nanocrystal, which has been produced by microorganisms. It was found by Cunningham and Lundie that *Clostridium thermoaceticum* can precipitate CdS on the cell surface and in the culture broth from CdCl2 in the addition of cysteine hydrochloride which is the likely origin of sulfide in the growth medium. Researchers found that *Klebsiella pneumoniae*, when treated with Cd2 + ions, formed 20−200 nm CdS on the cell's surface. After incubation with CdCl2 and Na_2SO_4, *Escherichia coli* forms cadmium sulfide nanocrystals, which have a polymorph form of crystal phase. When *E. coli* was grown in the stationary phase, nanocrystal formation gets larger by about 20-fold compared to *E. coli* which was grown in the late logarithmic stage. CdS nanoparticles were produced in yeast cells by Dameron et al. using S. pombe and *Candida galbrata* (Bazylinski et al., 1995). Biological systems have successfully synthesized nanoparticles of ZnS and PbS. Scientists became capable of obtaining ZnS nanoparticles with an average diameter range between 8 nm and 2−5 nm by using *Rhodobacter*. *Rhodobacter sphaeroides* was also used to make PbS nanoparticles, whose diameters were controlled by the culture time.

Microbial eukaryotes such as mushrooms are good candidates for the extracellular synthesis of metal sulfide nanoparticles, according to Ahmad and colleagues. The fungus *F. oxysporum* can produce stable metal sulfide nanoparticles such as Cds, PbS, MoS2, and ZnS when exposed to aqueous solutions of metal sulfate. To create quantum dots, it's Cd2 + ions that reacted with sulfur ions, which were created by enzymatic reduction of sulfuric acid into sulfuric acid. Fe3S4 or FeS nanoparticles are another type of sulfide nanoparticles. It was reported that uncultured magnetotactic bacteria can produce Fe3S4. There were approximately 1105 magnetotactic bacteria per cm^3 in the sediment sample, and after purification by the procedure of racetrack, they obtained approximately 105 cells. Magnetosomes in cells that are uncultured are rectangular in shape and of elongated length. Magnetic bodies were found in large groups within cells, with an average of 40 magnetosomes per cell. Along with the large cluster, some aligned structures of magnetosomes forming a chain-like composition were also

observed. Bacteria capable of reducing sulfate were observed to produce FeS nanoparticles that were magnetic.

4.10 Mechanisms of nanoparticle formation by microorganisms

To form nanoparticles, it's different microorganisms that have different mechanisms that are either extracellular or intracellular. Metal ions are trapped on the surface (extracellular) or inside microbial cells(intracellular) before being transformed into nanoparticles. When enzymes are present, metal ions are converted to nanoparticles in this process.

In general, microorganisms have a twofold effect on mineralization. As a result of this, the solution can become more or less supersaturated in relation to a given stage. When microorganisms produce organic polymers, they can influence mineral formation by favoring (or preventing) the initial stabilization of minerals. Some typical nanoparticles were discussed in this section, including gold and silver nanoparticles, as well as magnetic and sulfide particles. How *Verticillium* sp. or algal biomass produces gold and silver nanoparticles by which specific mechanism is still unknown. As a consequence of electrostatic interactions between the gold or silver ions and negatively charged cell walls from enzyme carboxylate groups, nanoparticles were formed on the surface of mycelia rather than in solution. The enzymes then proceed to reduce and accumulate gold or silver nuclei even further. Researchers believe that the nitrate reductase enzyme is important in producing silver nanoparticles in Licheniformis. When silver ions are exposed to nitrate ions, this enzyme reduces them to metallic silver. One method that could entail the reduction of silver ions is the electron shuttle enzymatic metal reduction process. For biosynthesis of metal nanoparticles, NADH and NADH-dependent nitrate reductase enzymes play a key role. When *Bacillus licheniformis* secretes the cofactor NADH and NADH-dependent enzymes, particularly nitrate reductase, the bioreduction of Ag + to Ag0, and the subsequent production of silver nanoparticles, is thought to occur.

Heavy metallic nanoparticles are formed as a result of the genetic and proteomic responses of metallophilic microorganisms to hazardous environments. When microorganisms are exposed to heavy metal ions like Hg2, Cd2 + , Ag2, Co2 + , CrO4, Ni2 + , and Pb2 + , their survival is threatened. A variety of genetic and proteomic responses are used by microbes to counteract these effects. Microbial cells contain a large number of metal resistance genes, which enable cell detoxification through a variety of mechanisms, for instance, complexation and efflux or reductive precipitation, respectively. A high concentration of mobile heavy metals, such as mine waste, metal processing plant effluent streams, and naturally mineralized zones, is required for metallophilic bacteria to thrive. BacMP biomineralization is hypothesized to be a multi-step process at the molecular level. As the cytoplasmic membrane invaginates, a vesicle is formed that serves as the precursor to BacMP membrane. However, the exact mechanism of envelope formation is still

unknown. A GTPase-mediated invagination is most likely the mechanism for magnetotactic bacteria. It was then necessary to link the formed vesicles together with cytoskeletal filaments to form a chain. By using transmembrane iron transporters, BacMP biomineralization progresses to the buildup of ferrous ions into the vesicles in the second step. Siderophores and transport proteins bind iron from the environment, allowing it to be absorbed. An oxidation-reduction system always keeps the body's iron in check. Finally, BacMP proteins that are firmly bound can cause the nucleation of magnetite crystals or regulate their shape.

In magnetite generation, several proteins correlated with the BacMP membrane take part in the process. To name a few: ferrihydrite reduction and dehydration, iron oxidation to induce mineralization, and the accumulating of iron concentrations that are supersaturating. Using Shewanella oneidensis, Perez-Gonzalez and his fellows have recently presented a new procedure for the production of magnetites that includes both passive and active mechanisms. When bacteria use ferrihydrite as a terminal electron acceptor to create Fe2, the pH around their cells rises, which is likely owing to bacterial amino acid metabolism. The magnetite phase precipitates as a result of the passive mechanism when the localized concentration of Fe2 + and Fe3 + at the net negatively charged cell wall, cell structures, and/or cell debris produces a local spike in super saturation with regard to magnetite. As proposed by Sanghi and Verma, CdS nanoparticles are formed by cleaving the S−H bond and forming a new bond, the Cd−S−CH_2COOH (Cd−S−CH_2COOH) complex on the surface of nanoparticles. As opposed to the −NH_2 groups of proteins, the −COOH groups of the cadmium-thiolate complexes interact with hydrogen bonds. Because of this, CdS nanoparticles that have been caped are hydrogen-bonded to −NH_2 groups. Cd^{2+} ions are held together by one of the carboxylic group's oxygen atoms (−COOH), which competes with Thiol group for the ability to attach to CdS nanoparticle surfaces. Fig. 4.1 explains the synthesis of nanoparticles from different microbial sources.

4.11 Control of size and morphology of nanoparticles

The electrical and optical properties of nanoparticles are influenced by their size and form. As a result, nanoparticles have gotten a lot of press attention recently in terms of size, shape, and surrounding media. For the past few years, researchers have placed a lot of emphasis on shaping particles because it let them be fine-tuned in many ways, giving them a unique nature. These methods can create vast numbers of nanoparticles with precise size and form in a short amount of time; however, they are complicated and have downsides, such as the generation of hazardous toxic waste that is harmful to the environment and human health. To control the size and shape of biological nanoparticles, microbes, regarded

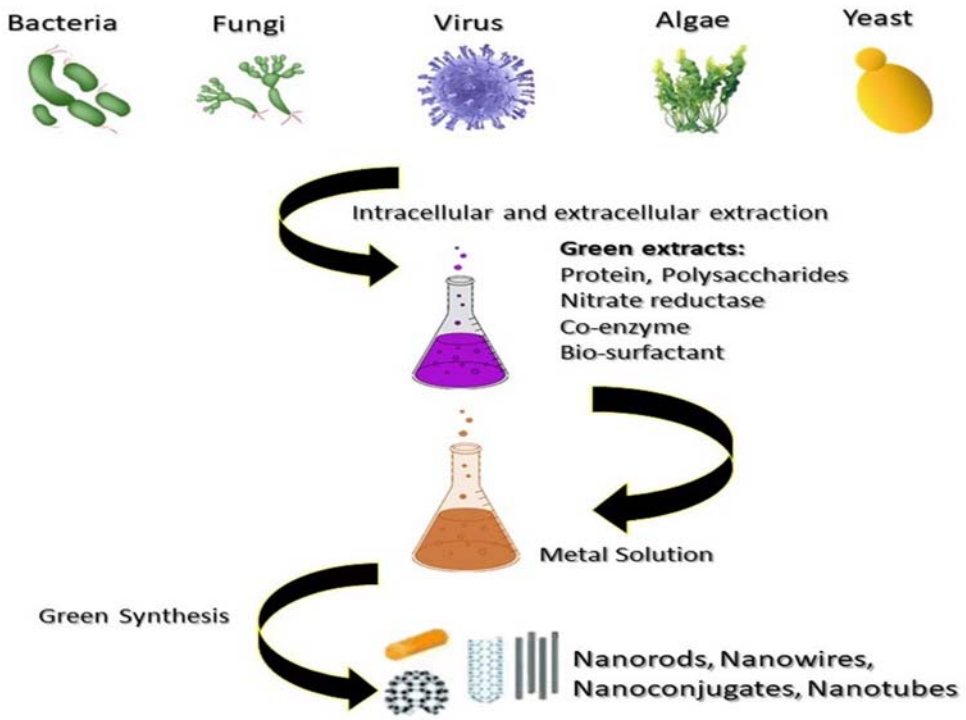

Figure 4.1
Biosynthesis of nanoparticles from different microbial sources.

as potent green nano factories, can be used as an eco-friendly alternative. According to a study, gold nanoparticles having numerous shapes and sizes can be generated in two fungal cultures, luteoalbum and Isolate. To impact the rate of particle formation and particle size, the control of variables such as pH, temperature, gold concentration, and AuCl4 exposure duration is necessary. Scanning electron microscopy revealed a variety of particle shapes: spherical, triangular, hexagonal, and other shapes are among them. It was found that particle sizes ranged from just a few nanometers to about 100 nm. Scientists' results also revealed hexagonal-shaped and triangular-shaped particles, which were generally larger in size than spheres.

Some of the microorganisms examined in the study were able to make small, uniform-sized gold nanoparticles within their cells. The particles in the cytoplasm of the cell were generally spherical in shape. According to (Gurunathan et al., 2009), the best reaction circumstances for maximizing the output of AgNPs as well as particle size reduction have been determined. AgNPs were synthesized in a variety of mediums with varying AgNO3 concentrations, reaction temperatures, and pH values in order to get the optimal conditions.

This maximum synthesis was observed in a nitrate medium with 5 mM AgNO3, 60°C reaction temperature, and a pH of 10. By using the culture supernatant of bacteria, i.e., *E. coli*, it took only 30 minutes to achieve a 95% conversion rate. For similar particles synthesized chemically, it's on par with or faster than what's possible with this method. Variations in $AgNO_3$ concentration, reaction temperature, and pH could be used to tune the particle size from 10 to 90 nm on average. In the absence of the cell wall's spatial constraints, Riddin and colleagues claim that cell-soluble extract (CSE) can convert Pt(IV) to create nanoparticles that are stabilized in solution by binding proteins and have both geometric and irregular shapes. When Pt(IV) concentrations were high, it appeared that the particles were more regular and geometric. Since Hydrochloride was produced in greater quantities (pH4 in this case) in the system, the nanoparticle−protein bioconjugates precipitated, and a decrease in soluble particles was observed in the colloid as a result. They also demonstrated that protein-stabilized biogenic Pt (0) nanoparticles of various sizes and shapes may be produced without cellular constraints.

Bacteria with magnetotactic properties manufacture uniformly sized and uniformly shaped iron oxide magnetic particles. In addition to cuboids and bullets, magnetites can also take the form of rhombic and rectangular shapes. Species- and strain-dependent crystal morphologies and compositions have been observed. These particles show biological control as high. The researchers found that AMB-1 Mms6 from Magnetospirillum magneticum is a dominant protein that binds to the surface of bacterial magnetites. It was discovered that the protein has a role in the production of cubo-octahedral magnetite crystals. Mms6 protein-like peptides were used to study magnetite formation in a laboratory setting. As with bacterial magnetites and Mms6 protein, particles that are produced with short peptides harbor the C-terminal acidic region showing similar circularities of 0.70−0.80 spherical shape. With the use of different types of synthetic peptides (circularities of 0.60−0.85), a rectangular morphology was obtained instead. Magnetite crystals were synthesized using recombinant magnetotactic bacterium protein Mms6 in aqueous solution at low temperatures, which was developed by the same group. Analyses of magnetic bacteria-like magnetite crystallization indicate that Mms6 is involved in the synthesis of magnetite particles with an unusual distribution of shape and size. Mms6 has a strong affinity for iron and has a sequence motif with numerous other biomineralization scaffold proteins identified in other animals. Mms6 is found in bacteria as well as plants. Mms6 is present in both bacteria and plants. This is in contrast to crystals formed in the absence of Mms6, which are smaller (20 nm) and morphologically different (cubo-octahedral). As a result, Mms6 has a strong influence on nanoparticle size and shape during synthesis. Other nanoparticles have also been shown to be able to be controlled in terms of particle size. Yan and colleagues discovered that inducing yeasts is a good technique to get zinc-phosphate powders with a limited diameter distribution. As part of their method, the yeasts in the reaction system were utilized to prevent Zn3(PO4)2

crystals from clumping together, allowing them to effectively manage particle size and dispersion.

4.12 Applications of nanoparticles

4.12.1 Nanomedicine

Research in this area is in its infancy, but it holds great promise for making better the detection and treatment of human diseases. Biomedical applications of nanoparticle dispersion include drugs, fluorescent biological labels, and gene delivery agents, as well as biodetection of microbes involved in causing disease, tissue engineering, destruction of tumors formed via hyperthermia (heating), MRI, and phagokinetic studies. More than one hundred reviews and research papers have been issued on the use of nanoparticles in the biomedical field. However, despite the fact that biosynthesized nanoparticles are a relatively new field, researchers have begun studying further the use of these particles for medical purposes such as drug delivery procedures, treatment of cancer, gene therapy (including DNA analysis), and antibacterial or antimicrobial agents. Fig. 4.2 and Table 4.3 represent the medical application of microbially synthesized nanoparticles.

4.12.2 Drug delivery

Uniquely designed drug delivery systems must deliver drugs in a correct way which means delivering them accurately and safely to the target sites keeping in mind to deliver them at the right time to have a controlled release and to gain the maximum medical or therapeutic effect, which is a critical issue. It is necessary for targeted nanocarriers to cross blood-tissue

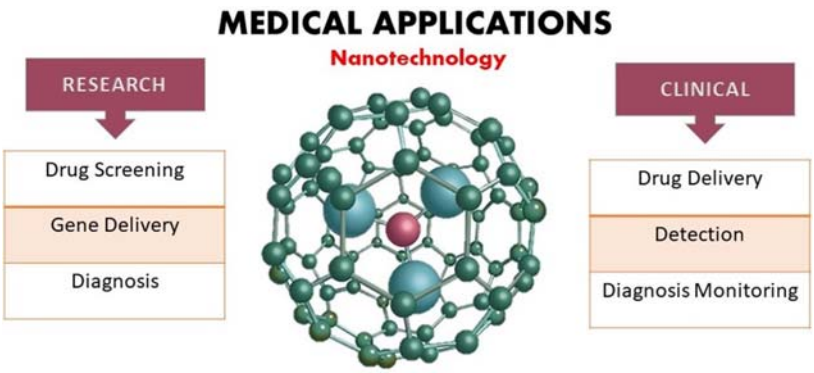

Figure 4.2
Medical application of microbially synthesized nanoparticles.

Table 4.3: Application of technology of nanoparticles.

Applications	Role of technology of nanoparticles	References
Cancer therapy	Diagnosis of precancerous and also malignant lesions; Testing of patients who might have solid tumors	Sanna et al. (2014)
Respiratory medicine	Help in the therapy of lung cancer, pulmonary fibrosis, and tuberculosis; Gene therapy required for cystic fibrosis	Omlor et al. (2015)
Gastroenterology	Helps in treating colorectal and gastric cancer	Brakmane et al. (2012)
Reproductive medicine	Help in treatment for ectopic pregnancy; Treats genital infections; Treats reproductive diseases	Barkalina et al. (2014)
Dermatology	Imaging of skin cancer Immunomodulation Vaccine delivery through the skin Wound healing Acts as antimicrobials	Delouise (2012)
Antimicrobial activity	Bacterial activity Bacteriostatic action	Seil and Webster (2012)

barriers in order to arrive at their target cells. Their cytoplasmic targets must be accessed via specific endocytotic and other transcytotic transport mechanisms that allow them to enter target cells and reach the site awaiting drug dose administration. They can bypass the barrier of blood-brain and tight epithelial junctions of skin because of their small size. Due to their high surface area to volume ratios, nanocarriers exhibit improved pharmacokinetics and biodistribution of healing agents and thus minimize harmfulness by preferential gathering at the target site. For parenteral administration, they increase the solubility of hydrophobic compounds. Aside from that, they improve the stability of peptides and nucleotides.

In addition, some magnetic nanoparticles like ferrosoferric oxide (Fe_3O_4)—which is an iron oxide NP—and Fe_2O_3—which is a ferric oxide NP—are biocompatible. MRI, cancer treatment (via magnetic hyperthermia), stem cell arrangement and framing, guided delivery of drugs, gene therapy and DNA analysis, and MRI have all been actively investigated. It was determined that magnetosomes from Magnetospirillum gryphiswaldense have been proved nontoxic to mouse fibroblasts in vitro (Wei et al., 2007). A study of Sprague-Dawley (SD) rat BM biocompatibility and pharmacokinetics was conducted by Sun et al. Their method for purification and disinfection of BMs yielded BMs with more purity and small size distribution. Following intravenous administration of the drug for 72 h, the researchers found only liver-bound BMs and no evidence that BMs were present in the dejecta or urine.

When it comes to drug delivery, MTB-MC-1 (Mycobacterium tuberculosis) with magnetosomes has been used. MTB embedded with nanoparticle magnetite and flagella

were steered using magnetotaxis, developed by Felfoul and colleagues. A medical imaging modality must be able to make an image of these MTBs in vivo to guide them in the direction of a target. Due to the magnetosomes' effect on T1 and T2 relaxation times when imaging with MRI system, it has been shown that the bacteria can be tracked by using an MRI system. The study's T1- and T2-weighted images, along with T2 relaxivity of MTB, were used for the purpose of checking the possibility of monitoring MTB drug delivery operations by using a clinical Mr scanner, according to the study. An analysis of T1 and T2 relaxation rates revealed that MTB is more effective as a negative contrast agent. To detect bacteria at 2.2×10^7 cells/mL, it has T2-weighted images that were used. It was reported that MTB-NPs can be used for gene delivery, which were successful in delivering -galactosidase genes in vitro and also in vivo using PEI-associated MTB-NPs. According to their research, MTB-PEI-NP systems are much more efficient and also are less toxic than PEI alone. There is a long tradition of using gold and its compounds as medicinal agents, dating back to at least 5000 years in Egypt. AuNPs have distinctive optical and electronic characteristics that rely on their size and shape, as well as on theirs having a large surface-to-volume ratio. However, the surfaces of gold nanoparticles could be easily changed by attaching functional groups such as amines, phosphines, and thiols to them. For drug and gene delivery, gold nanoparticles came into view as an encouraging alternative to more traditional delivery vehicles.

Toxicologically inert compounds with large surface area and the function tunability should also allow new delivery methods. Before, chemically produced AuNPs were studied for their biomedical applications. When used as a unique therapeutic or medicinal agent, silver nanoparticles have proved effective against many bacteria, yeast, viruses, and inflammation. Silver nanoparticles generated from *Bacillus licheniformis* have the ability to reduce the growth of new blood vessels termed antiangiogenic potential, according to Kalishwaralal et al. In the presence and absence of VEGF, bovine retinal endothelial cells (BRECs) were exposed with varying doses of nanoparticles for 24hrs, and a 500 nM (IC50) silver nanoparticle solution was capable of stopping the proliferation and movement of BRECs. As a result of the apoptosis-inducing caspase-3 activation and DNA ladder formation, the cells showed clear signs of death (Bazylinski et al., 1995). A PI3K/Akt-dependent pathway was found to be the mechanism by which silver nanoparticles inhibit cell survival in BRECs. On the other hand, the delivery of drugs with the help of nanoparticles is expected to significantly lessen the dosage or amount of anticancer drugs while improving their specificity, effectiveness, and toxicity. Our prediction is that nanotechnology-based medicinal, therapeutics, and detections will be increasingly used in clinics over the next few years. A second important area where nanotechnology could play a unique role is individualized medicine. Each type of treatment/targeted therapy might not be operative for every patient population because of cancer heterogeneity and the evolution of resistance. Besides, magnetic nanoparticles could also be used to treat hyperthermia cancer. As part of the hyperthermia cancer treatment, magnetic

nanoparticles are injected directly inside the body at cancerous tissue sites. A magnetic field from the outside enables local heating at specific locations (Bruschi and Toledo, 2019).

4.12.3 Antibacterial agent

Silver-based antiseptics have become increasingly popular in the last few years because of the prevalence and growth of microorganisms that are resistant to many antibiotics. For the biosynthesis of silver nanoparticles, *Trichoderma viridae* was used. There was a reduction in the concentration of silver ions in solution when exposed to *T. viridae* filtrate, causing the formation of strongly stable gold nanoparticle (AgNPs) with a size of 5−40 nm. For Gram-positive and Gram-negative bacteria, these nanoparticles were also tested for their increased antimicrobial activity. Erythromycin, kanamycin, ampicillin, and chloramphenicol's antibacterial activities were enhanced in the existence of AgNPs against test strains, according to the study. Ampicillin had the greatest effect on test strains. AgNPs combined with antimicrobial drugs showed superior efficacy, and the study provided valuable information for developing new antimicrobial agents. Duran and coworkers demonstrated that extracellular silver nanoparticles with Fusarium oxysporum can be included in fabrics to avoid diseases with harmful germs or to decrease them (Fariq et al., 2017).

4.12.4 Biosensor

As a result of their novel electronic and optical features, nanoparticles could also be used as biosensors. It has been reported that *B. subtilis* can produce nanoparticles of selenium with diameters ranging from 50 to 400 nm. Once the Se nanoparticles have been at room temperature for one day, they can be modified into one-dimensional (1D) trigonal structures that are highly anisotropic. A biosensor based on horseradish peroxidase was constructed using Se nanomaterial crystals with a large surface-to-volume ratio, fine adhesive ability, and also biocompatibility. Because of Se nanomaterials' excellent adhesion and biocompatibility, these sensors had a large electrocatalytic activity for the reduction of H_2O_2. Biosensors for H_2O_2 with an 8×10^8 M detection limit showed much sensitivity along with an affinity for hydrogen peroxide (H_2O_2). Their results also showed that the electrochemical application of different crystals of Se nanomaterials was not significantly different. A wide range of applications, including clinical, pharmaceutical, food, industrial, and ecological analyses, could benefit from the Se nanomaterials-modified electrode. According to research, yeast-produced Au-Ag alloy nanoparticles were being used to fabricate an electrochemical vanillin sensor. This sensor, which uses Au-Ag alloy nanoparticles modified glassy carbon electrode, was found to be able to significantly increase the electrochemical response of vanillin. Vanillin's oxidation peak current at the sensor increased linearly with its amount in the span of 0.2−50 M with a low detection

limit of 40 nM under optimal working conditions. There is some evidence that this vanillin sensor could be useful for vanillin monitoring systems. An AuNP (Silver nanoparticle) based glucose oxidase biosensor was also produced based on the observation that AuNPs can enhance GOx enzyme activity. According to the manufacturer, this biosensor can detect glucose concentrations as low as 20 micrograms (mg) and as high as 0.80 milligrams (mg) (S/N = 3). Commercial glucose injections can be accurately measured using this type of biosensor.

4.12.5 Reaction rate enhancement agent

Since their much accommodative surface area and specific characteristics, nanoparticles have been used extensively as catalysts and reductants to improve various chemical reactions, such as the oxidation of organic compounds. Improved microbiological reaction rates are achieved by using magnetic nanoparticles (MNP). Aside from their catalytic function, MNPs were also used for their good dispersibility. According to researchers, magnetic Fe_3O_4 nanoparticle-coated *Pseudomonas delafieldii* was used to desulfurize dibenzothiophene. The more adsorption of nanoparticle on the cells was caused by their more surface energies. Without mixing, the cells were well dispersed by an external magnetic field. This made it easier to collect cells for reuse. Desulfurization efficiency of *P. delafieldii* remained nonaffected, and, according to the results of the study, the cells can be reused many times.

4.12.6 Magnetic separation and detection

Biological labels have been proposed using magnetic particles joined with biotic molecules. To rapidly and sensitively detect molecules of small size, like pollutants in the environment, hormones, and harmful detergents, chemiluminescence enzyme immunoassays have been developed. On the basis of the competitive reaction of xenoestrogens, monoclonal antibodies immobilized onto BacMPs were able to detect alkyl phenyl ethylates, biphenol A (BPA), and linear alkylbenzene sulfonates (LAS). The entire procedure took only 15 minutes, compared to typical plate methods that can take up to 2.5 hours. Because the same antibodies were used for comparison, this method was more sensitive and had a lower detection limit than the enzyme linked immunosorbent assay method. As an exciting new research area, surface modification of magnetic nanoparticles has a variety of potential applications. For DNA extraction, the surface of the BacMP can be changed with aminosilane compounds. For DNA extraction, magnetic particles are an ideal solid-phase adsorbent because they can be easily manipulated by applying an applied magnet.

4.12.7 In diagnostics

Infectious and prevailing diseases are among the leading reasons of morbidity (rate of disease) and mortality (rate of death) in developing countries because of their high prevalence rates. They begin in one place, but they could move swiftly from one place to another and emerge as a global pandemic in no time at all. In order to detect diseases as quickly as possible, we need new diagnostic technologies (Hauck et al., 2010). The culture of microorganisms, biochemical tests, immunoassays, and also molecular diagnostics are some of the conventional methods of disease detection. But these methods are time-consuming and laborious and require a great deal of effort and patience. An easy-to-use diagnostic tool for the detection of a pathogen can be created using nanomaterials. Many pathogens have been imaged, tracked, and identified using metallic nanoparticles and fluorescent nanoparticles, as well as MNPs (Tallury et al., 2010). What we know about biosynthesis nanomaterials for diagnostic purposes is limited. Biosynthesized gold nanoparticles mediated by *Candida albicans* were evaluated for their potential as biosensors of liver cancer cells through their attachment with liver cancer cell surface-specific antibodies in one research project. Because the antibody-conjugated gold particles attach to the antigen present on the surface of the affected cell, they are able to distinguish between normal and cancerous cells with absolute certainty. We are just getting started with the use of microbially synthesized nanomaterials for diagnostics.

4.13 Conclusion

It is becoming more and more popular to use environmentally friendly chemistry-typical and biological processes for the production of nanoparticles that are not harmful to the surroundings. The microbial production of nanoparticles is a low-cost and eco-friendly alternative to other processes in chemical and also in physical methods that use harmful chemicals. Due to its importance in nanobiotechnology, microorganism synthesis of nanoparticles has emerged. Since microbes have such a wide range of genetic variations, they may be thought of as biofactories for the production of nanoparticles due to their capability of synthesizing them. Although factors such as downstream processing procedures must be improved, the method of photobiological processes may be used to increase the rate of nanoparticle synthesis and monodispersity. Nanoparticle biosynthesis also requires the presence of certain genes and enzymes, as well as their characterization. Determining the size and crystallinity of nanoparticles rely on understanding the molecular mechanisms that intercede microbial nanoparticle production. The potential of nanotechnology to advance biotechnology and medical research has been extensively studied. In order to keep up with this new technology, regulatory agencies such as the FDA have decided to rely on the existing legal framework. We believe that future research into the synthesis of unique optical, physicochemical, and electronic characteristics in nanoparticles with the help of microbes will be critical for applications in chemistry, electronics, medicine, and agriculture.

4.14 Future prospects

Over the last decade, nanoparticles generated from microorganisms and their applications have seen tremendous progress. There is still a lot of work to be done to enhance the efficiency of the production, as well as the control of the size of the particle and its morphology, among other things. Comparatively to physical and chemical techniques, the synthesis of nanoparticles using microorganisms is known to take several hours or even a few days. Reduced biosynthesis time will make this route more appealing. To evaluate nanoparticle synthesis, the size of the particle and its monodispersity are critical. There should be extensive research into proper control over particle size and monodispersity. The nanoparticles formed by microorganisms can decompose over time, according to several studies. As a result, the firmness of nanoparticles generated by biological procedures should be further studied and improved. Chemical and physical nanoparticle syntheses are still working on controlling particle morphology. Particle size and monodispersity may be sufficiently controlled by a variety of parameters such as type of microorganism, its growth stage (phase) of microbial cells, medium required for proper growth, synthesizing conditions, pH, and amount of substrate, as well as reaction time, the addition of nontarget ions and temperature. Objects could harvest energy from their environment in the future, thanks to nanotechnology. For high-efficiency energy production from movement, light, temperature variations, glucose, and other sources, new nanomaterials and concepts are currently being developed.

When nanoparticles are coated with a thin sheet of lipid, which confers solubility and stability, it becomes crucial for many biomedical applications. Cells are being manipulated at the genomic and also at proteomic levels as part of the research being conducted. An improved cell- and molecular-level understanding of the nanoparticle reduction mechanism is expected to lead to shorter reaction times as well as higher synthesis efficiencies. The field of nanomedicine is in its infancy, but it has tremendous potential for making the diagnosis better and improving the treatment of human diseases. Environmentally friendly “green chemistry” procedures include the biosynthesis of nanoparticles by microbes. If you want to create nanoparticles with the use of microorganisms such as yeast, fungi, bacteria, or actinomycetes you have two options: an intracellular or an extracellular synthesis. Some factors such as pH and temperature, as well as the concentration of substrate and exposure time, can be controlled so that intracellular particle formation can be slowed down or accelerated as needed. Microorganisms are currently being manipulated at the genomic along with proteomic levels in research. Recent advancements and ongoing work to improve the efficiency of particle synthesis and to explore their role in biomedical applications have given hope that these methods will be implemented on a large scale and used commercially in medicinal and health care within the next few years.

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CHAPTER 5

Microbial biofilm approaches in phytopathogen management

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5.1 Introduction

Global food security is a burning topic in countries embattled by food insecurity and the need for hunger management while achieving food security is one of humanity's greatest challenges in the twenty-first century (Flemming and Wuertz, 2019). In recent years, fungal diseases have had a devastating impact on crop output and quality and have become a worrying constraint in the development of sustainable agriculture (Marín-Menguiano et al., 2019). Infection of phytopathogens in crops is an important limitation for agricultural areas while carrying out harvesting of crops; an estimated 20%–40% of crops harvested are annually lost due to phytopathogen infection (Rana et al., 2020). Therefore, the use of pesticides, harvesting resistant seed varieties, application of chemical fungicides, solar radiation, and good water management are agronomic approaches for the management of phytopathogens. However, excess use of chemical fungicides can have a diverse effect on human health and the environment, including the incidence of chemical fungicide resistance (Angus and Hirsch, 2013). Nowadays, plant biotechnology also emphasizes the introduction of desirable features in the novel crop types with biotic and abiotic resistance and also promotes the effective use of beneficial microbes in agriculture (Ahmed et al., 2018). Around 40%–80% of microbial cells on earth can form biofilms (Flemming and Wuertz, 2019) which could be an alternative approach to crop management away from chemical fertilizers. Biofilms are surface-attached colonies of microbes bound together by self-produced polymer matrices mostly composed of polymers, secreted proteins, and

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extracellular DNA (Tremblay et al., 2013). A biofilm can be made up of a single microbial species or a variety of microbes that create a strong relationship with one another, such as bacteria, protozoa, archaea, algae, filamentous fungi, and yeast through quorum sensing (Silva et al., 2014; Costa-Orlandi et al., 2017). Quorum sensing is a bacterial cell-to-cell communication mechanism that encourages collective activity in a population. This cooperative behavior is based on signal molecule generation, detection, and response in a cell density-dependent manner (Baltenneck et al., 2021). In low cell density, bacteria produce a low-level signal molecule and diffuse it to the extracellular environment. Signaling molecules are detected by bacteria after they reach a certain threshold; thereafter, a cascade of biological actions takes place (Raghupathi et al., 2018).

Microbial interactions influence plant health to proliferate in the rhizosphere. Different microorganism species populate the rhizosphere's endorhizosphere, ectorhizosphere, and rhizoplane levels (Hassani et al., 2018). The rhizodeposition of carbohydrate-rich mucilage, sloughed cells, and root exudates by plant roots have an impact on the surrounding soil. The secreted material contains sugars, fatty acids, amino acids, phytohormones, vitamins, and antibacterial substances, which aid the plant with nutrient intake and protect the roots from pathogenic attacks (Dhawi et al., 2016). Microbes can attach to both biotic and abiotic surfaces and exist as single cells, clumps, or biofilms (Armbruster and Parsek, 2018). Biofilms have also been shown to degrade a variety of substances containing complex nutrients like nitrogen and phosphorus (Ikuma et al., 2013). They can capture pathogens in contaminated water before releasing it into the environment or using it for agriculture (Sehar and Naz, 2016). Many studies have been conducted on biofilm interactions with biotic surfaces, which has fueled the usage of microbial biofilms in agriculture (Singh and Chauhan, 2017). Fungal−bacterial biofilms, which are bacterial biofilms adhering to fungal surfaces, have been demonstrated to improve nutrient uptake, plant development, and tolerance to environmental stress when compared to mono or mixed cultures without biofilm formation (Hassani et al., 2018). These naturally occurring biofilms, which are connected with plant roots, boost crop output while also protecting the host plant from various environmental challenges. *Bacillus* spp. are widely reported for controlling the fungal pathogen in tomato plants such as *Bacillus tequilensis* against Fusarium wilt (Bhattacharya et al., 2019). Understanding the consequences of such agriculturally significant cross-kingdom biofilms on plant protection, bioremediation, and increasing plant nutrition and soil quality in more depth would bring new insights. Biofilms are important in different sectors, such as biofilm-forming bacteria could be useful as plant growth promoters and biocontrol agents in agriculture, have a hand in plant growth promotion and soil health, and might be helpful in abiotic stress management, wastewater treatment, biofortification, bioremediation, etc. (Kour et al., 2020).

This chapter highlights the mechanism behind the biofilm formation and possible signaling during the interaction of biofilm with plants to mitigate the biotic stress condition (Fig. 5.1).

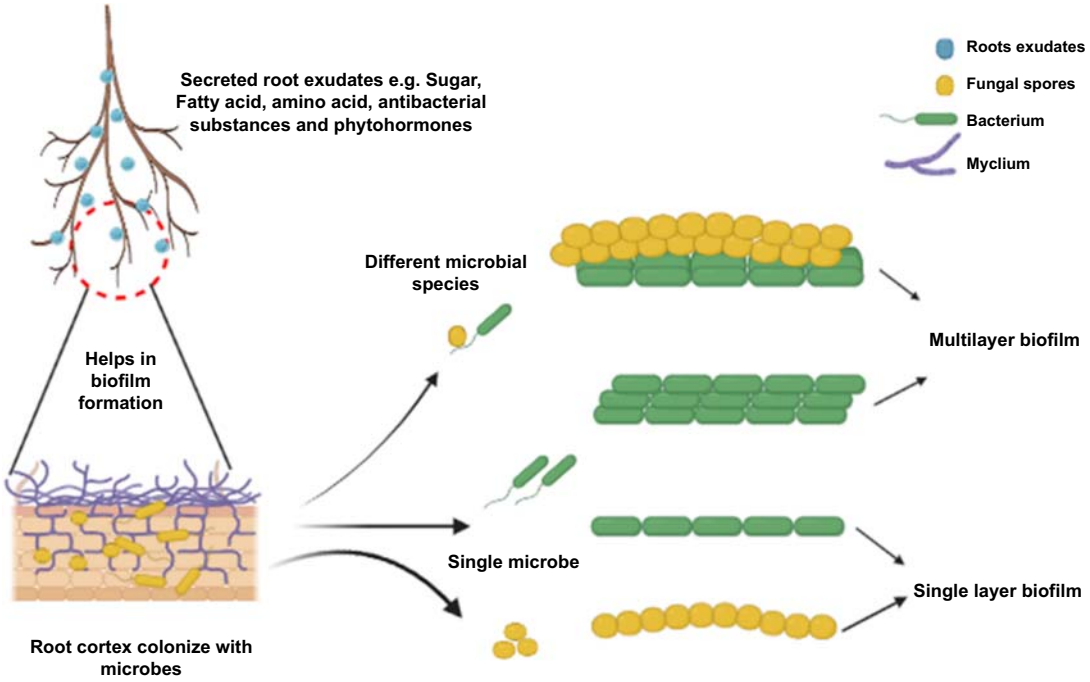

Figure 5.1
Role of root exudates in the formation of different types of biofilms. *Created by biorender.com.*

5.2 Role of microbial species in biofilm formation

Biofilm formation includes a collection of several microorganisms rooted in a matrix of polysaccharides. The formation of biofilm generally takes place on solid biological or non-biological surfaces, which are medically important, covering 80% of microbial infections in the body (Jaggessar et al., 2017). The various factors affecting the biofilm formation are initial adhesion of the microbes such as polarity; and the London-Van der Waals forces along hydrophobic interactions. Therefore, numerous bacterial species are fused to the protein surface resulting in primary adhesion and biofilm formation. There are two types of aggregation that have been reported: autoaggregation and coaggregation. Autoaggregation involves the adherence between similar strains of bacteria, whereas aggregation of two or more different species is known as coaggregation. The surface-associated molecules of bacteria include proteins (adhesins, lectin/nonlectin adhesins) found on the bacterial outer membrane, cell wall, or fimbriae and sugars, which assist in the process of aggregation in addition to multispecies biofilm formation (Karched et al., 2015). The role of fungi and bacteria has been thoroughly studied for biofilm development (Alsteens et al., 2013). Agriculturally important numerous genera of fungi and bacteria are known to form single or multispecies biofilms such as *Agrobacterium* spp. (Matthysse et al., 2005), *Cyanobacterium* spp. (Prasanna et al., 2011),

Table 5.1: Role of agriculturally important microbes in biofilm formation.

S.N.	Microbes	Beneficial role in agriculture as well as biofilm formation	References
1.	*Anabaena-Azotobacter chroococcum; Trichoderma-Azotobacter chroococcum*	Promotion of Nitrogen(N) fixation, plant growth promotion	Prasanna et al. (2015)
2.	*Bacillus subtilis*	Plant growth promotion and biocontrol activity	Rudrappa et al. (2008a,b)
3.	*Trichoderma-Bacillus* sp.; *Anabaena- Bacillus* sp.	Promotion of nitrogen fixation, plant growth promotion	Triveni et al. (2013)
4.	*Bradyrhizobium japonicum*	Promotion of N fixation, plant growth promotion	Lee et al. (2010)
5.	*Clavibacter michiganensis*	Plant pathogen, canker	Ramey et al. (2004)
6.	*Erwinia amylovora*	Plant pathogen, blight	Koczan et al. (2009)
7.	*Gluconacetobacter diazotrophicus*	It acts as an endophyte, N fixer, and in plant growth promotion	Wang et al. (2008)
8.	*Herbaspirillum seropedicae*	It acts as an endophyte, N fixer, and in plant growth promotion	Balsanelli et al. (2014)
9.	*Ralstonia solanacearum*	Plant pathogen, wilt disease	Sorroche et al. (2012)
10.	*Rhizobium leguminosarum*	Promotion of N fixation, plant growth promotion	Fujishige et al. (2006)
11.	*Sinorhizobium meliloti*	Promotion of N fixation, plant growth promotion	Sorroche et al. (2012)
12.	*Xanthomonas campestris*	Plant pathogen, black rot formation	Malamud et al. (2013)
13.	*Xanthomonas axonopodis*	Plant pathogen, canker disease	Malamud et al. (2013)
14.	*Xanthomonas oryzae*	Plant pathogen, blight disease	Killiny et al. (2013)

Paenibacillus spp. (Yegorenkova et al., 2021), *Pseudomonas* spp. (Toyofuku et al., 2012), *Trichoderma* spp. (Triveni et al., 2012; Mishra et al., 2019), and *Xanthomonas* spp. (Malamud et al., 2013). There are some filamentous fungi known to form biofilm sporadically (Chandrasekar and Manavathu, 2008). *Pseudomonas aeruginosa* forms polymicrobial biofilms on food contact surfaces which assist in food safety and public health concerns (Bai et al., 2021). The role of microbes has also been seen in the rhizospheric region of the plants, where it assists in the protection of roots during several natural processes taking place in the soil (Davies and Whitbread, 1989). Some of the agriculturally important biofilm-forming microbes have been listed in Table 5.1.

5.3 Mechanism of biofilm formation

Biofilm formation is a complex process and is classified into five stages, which are (1) initial attachment to the surface, (2) irreversible attachment, (3) colony formation, (4) maturation, and (5) detachment (Fig. 5.2). During biofilm formation, a collection of bacterial cells are enclosed by an adjacent matrix or substrates of extracellular polymers (O'Toole et al., 2000).

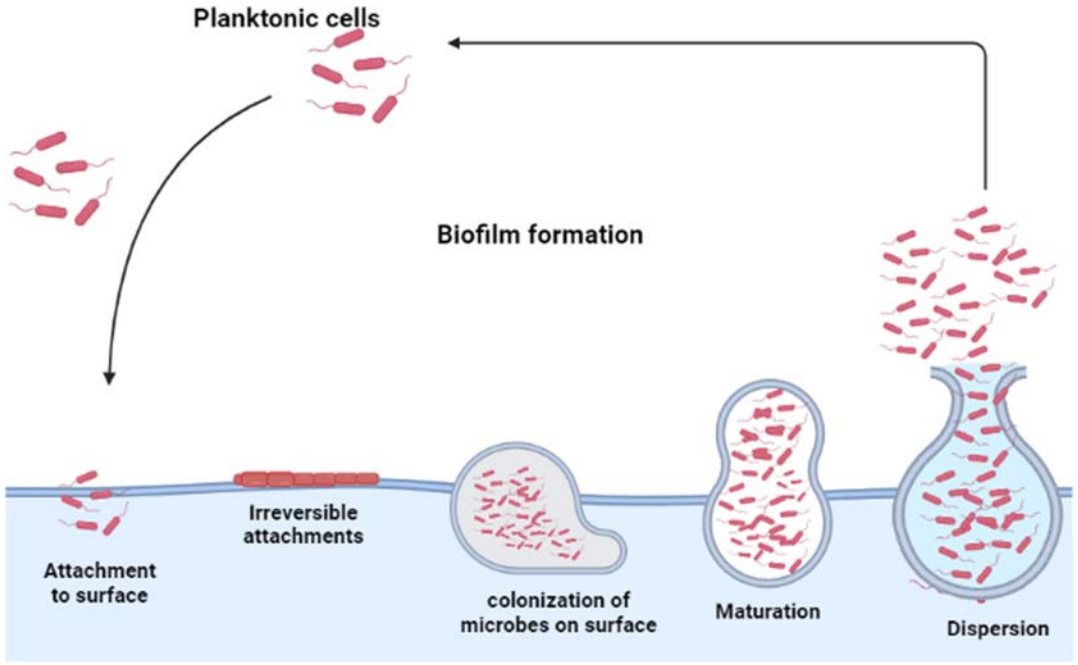

Figure 5.2
Mechanism of biofilm formation by microbes. *Created by biorender.com.*

5.3.1 Initial attachment of microbial cells to the surface

Initial attachment of microbial cells to the surface facilitates through the van der Waals force and the electrostatic forces (Tuson and Weibel, 2013). The process may be either passive or active (Rana et al., 2020). The cell attachment is reversible during this stage; subsequently, attached bacteria undergo a variety of morphological changes, resulting in biofilm development (Hall-Stoodley et al., 2004). After, bacterial cells are enclosed by a matrix of extracellular polymers that usually contain stronger adhesive materials. Microbial cell attachment is mediated by pili, fimbriae, Exopolysaccharide (EPS), and flagella. Additionally, the adhesion of microbial cells can be increased by the nature of the surface, i.e., an organic substance containing a surface enhanced by the forces of adhesion of microbial cells (Vlamakis et al., 2013).

5.3.2 Irreversible attachment

In this stage, microbial cells affix themselves more securely by forming exopolymeric compounds and stabilized irreversible attachment to the surface. Microbial cells adhere permanently to the biofilm due to the presence of exopolymeric compounds, rendering it resistant to treatments such as shear force and chemical treatment with enzymes, detergents, and surfactants (Hall-Stoodley et al., 2004; Sinde and Carballo, 2000).

5.3.3 Colony formation

Colony formation takes place after the irreversible attachment of microbial cells to the surface, which leads to microcolony formation. Chemical signaling triggers the multiplication of microbes in the biofilm (Jamal et al., 2015). Microcolonies are regarded to be successful because they allow microbial cells to exchange substrates and eliminate communal end-products (Madsen et al., 2012).

5.3.4 Maturation

After the colony formation stage, the biofilm begins to mature and becomes a three-dimensional structure containing cells packed in clusters (Berhe et al., 2017). Surface contact causes changes in gene expression levels throughout the maturation phase. It leads to upregulating the matrix-supporting characteristics, which are involved in the formation of the extracellular matrix (Kostakioti et al., 2013). It is reported that bacterial adhesion has been shown to stimulate the production of extracellular matrices on its own. The development of a matrix is promoted by the production of water-filled channels for the transportation of nutrients within the biofilm. According to researchers, these water channels act as circulatory systems, transporting nutrients and eliminating waste items from the communities of microcolonies in biofilm (Parsek and Singh, 2003).

5.3.5 Detachment

The last stage of biofilm formation is detachment. Some researchers have often noticed that after the formation of the biofilm, microbial cells leave the biofilm by themselves on a regular basis. As a result of this, microbes will be able to multiply and scatter quickly. Dispersion of microbial cells from the biofilm is a natural and programed process. Microbial cells are periodically separated from the colony and released into the environment due to mechanical stress. However, in the majority of situations, microbes stop producing EPS and are released into the environment. Biofilm cells disperse due to the dissociation of newly produced cells from developing cells, as well as dispersion of biofilm aggregates due to flowing effects or quorum sensing (QS) (Solano et al., 2014). Cells are eliminated from the biofilm due to an enzyme activity that triggers alginate breakdown. The disseminated cells that develop the biofilm as a result of proliferation may soon revert to their original planktonic nature (Vlamakis et al., 2013).

5.4 Limiting factor for biofilm formation

Several factors affect the production of biofilm in microbial cells, including nutritional factors, environmental factors, deterrence posed by antimicrobials, and enzymes. Different

environmental factors such as temperature, ionic strength, and pH availability of nutrients have been observed to influence the microbial cell surface attachment process (Kostakioti et al., 2013). The structure and physiology of microbes, the intervention of QS, production of EPS, formation of root exudates, and soil organic matters can influence the colonization and growth of microbial cells (Haldar and Sengupta, 2015). Both harmful and beneficial microbes interact with the root in the rhizosphere, and the synthesis of numerous microbial compounds impacts biofilm development. According to Rudrappa et al. (2008a,b), extracellular metabolites produced by microbes can initiate the growth of a plant's roots. Hydrophobic bacteria have more ability to attach to the surface than hydrophilic, through surfaces that are rough, hydrophobic, and covered with surface conditioning films are favorable for biofilm adhesion (Pagán and García-Gonzalo, 2015; Donlan, 2002). Environmental variables, such as pH, temperature, and nutrition levels, can alter the physicochemical parameters of the substratum, such as texture (rough or smooth), hydrophobicity, and charge (Pagán and García-Gonzalo, 2015). In aquatic environments, increasing the velocity of the flow, water temperature, or nutrient content can enhance the rate of microbial attachment, as long as these parameters do not reach critical values (Prakash et al., 2003).

5.5 The molecular mechanism involved in biofilm formation

It's essential to understand the molecular pathways that microorganisms use to form biofilm.

Some researchers have done a study on genes that perform multiple functions at various phases of biofilm development, as well as the processes that occur inside a biofilm. The conversion from a planktonic to a stationary biofilm habitat requires the coordination of genes involved in biofilm development (Prüß et al., 2006). For the analysis of microbial biofilms, various molecular techniques such as microarray analysis, whole-genome sequencing, fluorescent labeling techniques, nanoscale analysis (tip-enhanced Raman spectroscopy and mass spectroscopy), and nanoscale imaging (electron microscopy, confocal laser scanning microscopy, epifluorescence microscopy, and atomic force microscopy) have been used (Davey and O'toole, 2000; Lawrence et al., 2003). These approaches facilitate the understanding of microbial physiology and metabolisms, as well as microbial genetics, in an analysis of microbial biofilm development. Several studies supported the understanding that microbial cell surface is one of the vital structures for adherence during the development of biofilm. Biofilm production requires both extracellular QS and intracellular cyclic dinucleotide signaling cascades. These two cascades can intersect and control each other during biofilm development, resulting in enhanced biofilm development synergistically (Camilli and Bassler, 2006). In general, host environmental signals or factors such as pH, temperature, insulin, steroid hormones, monoamines, and vitamin K that resemble and serve as exogenous quorum signaling substances are required for early microbial adherence and proliferation within the host (Feraco et al., 2016). QS is a

type of intercellular communication that detects cellular density using signaling molecules such as N-acyl homoserine lactones (AHL), auto-inducing peptide (AIP), and autoinducer-2 (AI-2) in both Gram-negative and Gram-positive bacteria (Kalia, 2013). *las, rhl*, PQS, and integrated QS (IQS) are four QS systems, which are reported in *P. aeruginosa* and these cascades control each other through QS-related genes and other transcription factors by following a hierarchical order (Lee and Yoon, 2017). Johnson et al. (2005) reported that in *Thermotoga maritima* QS regulates c-di-GMP level by influencing the gene coding proteins', such as phosphodiesterase's A (PDEA) and diguanylate cyclase (DGC), activities; similarly, c-di-GMP regulate QS by affecting the expression of autoinducer synthases. In another study, it was reported that glycolysis and gluconeogenesis are two important pathways in biofilm formation. During attachment of microbial cells, the genes of glycolysis pathways were downregulated while those of the gluconeogenesis pathways were upregulated. Moreover, the same pattern of upregulation and downregulation was followed during the maturation stage in biofilm development (Li et al., 2015). Besides, in *Saccharomyces cerevisiae*, Mitogen-activated protein kinase (MAPK) signaling, cAMP-PKA pathways, and *Phd1, Mga1, Ash1*, and *Sak2* genes are involved in biofilm formation. MAP kinase, such as *SakA, MpkC*, and *MpkA*, in the signal transduction pathway is important for biofilm formation by enhancing the production of extracellular matrix and adhesion in *Aspergillus fumigatus* (Manfiolli et al., 2018). In vivo expression technology system (IVET) confirmed that biofilm formation was downregulated in *P. aeruginosa* if PA3782-encoding putative AraC-like transcriptional regulator, PA3710-encoding putative alcohol dehydrogenase, and PA0240-encoding putative porin proteins mutated (Finelli et al., 2003). Additionally, from a null mutation in phosphatase, *ΔsitA*, and *ΔptcB* establishing cell wall degradation, it concluded that phosphatases regulate *MpkA* and *SakA* phosphorylation and maintain cellular integrity. Phosphoenolpyruvate and PTS were shown to enhance biofilm formation in *K. pneumonia*. Enzyme II complex, a PTS homolog: KPN00353−KPN00352−KPN00351, and the expression of KPN00353−KPN00352−KPN00351 genes in PTS promote the production of capsular polysaccharide and eDNA, which are required for biofilm formation (Horng et al., 2018). Blake et al. (2021) demonstrate that in *Bacillus subtilis* chemotaxis occurs when the cells detect a gradient of root exudates which work as attractants and migrate toward the root in response. In this process, several genes have been involved, such as chemoreceptor CheW/CheV, and the kinases CheA and CheY. When bacteria reach the root, the Spo0A pathway regulates the transition from individual motile cells to biofilm formation. Recognition of plant signals by the sensor kinase KinCD triggers a phosphorylation cascade that includes Spo0F, Spo0B, and finally Spo0A. Phosphorylated Spo0AP causes the synthesis of SinI, which represses SinR, resulting in slrR derepression. SlrR is expressed and forms a complex with SinR, which maintains free SinR levels low and thereby derepresses matrix genes. Furthermore, the produced SlrR-SinR complex inhibits the expression of hag, *lytABC*, and *lytF*, resulting in sessile, cohesive matrix-producing cell chains. Besides that, phosphorylated SpOA inhibits AbrB, a second matrix repressor.

According to Xu et al. (2019), an sfp mutant *Bacilus velezensis* SQR9 a Plant growth-promoting rhizobacteria (PGPR) was unable to form normal biofilm. GgaA, a minor wall teichoic acid (WTA) synthesizing protein, was reduced by approximately fourfold in the Δsfp, and the ggaA gene deficiency delayed biofilm formation and reduced cucumber root colonization ability. Furthermore, they also found that *gtaB* a major WTA biosynthetic enzyme is engaged in both biofilm production and root colonization. They suggested that Δ*gtaB* inability to produce biofilms might be due to a scarcity of UDP-glucose, which is required for the production of biofilm matrix exopolysaccharides (EPS).

Zheng et al. (2015), explored the change of *A. fumigatus* from vegetative growth to conidiation by phenazine-derived compounds generated by *P. aeruginosa* through the NapA oxidative stress signaling cascade in a polymicrobial interaction of *A. fumigatus* and *P. aeruginosa*. According to Pérez-Montaño et al. (2014), the symbiosis and root colonization of *Sinorhizobium fredii* with *Glycine max* cv Osumi require biofilm formation. They suggested that NodD1 protein and nod gene that induces flavonoids are necessary for the change of biofilm structure. Transcriptome analysis revealed that under different conditions including biofilm formation and planktonic growth *B. amyloliquefaciens* (FZB42) transcriptional profiles consisting of a broad group of highly transcribed genes and *lci* gene encode an antibacterial peptide (Kröber et al., 2016). In *B. subtilis*, *Fusarium culmorum* impacts the expression of critical genes involved in biofilm formation. Finally, the study found that a considerable rise in the expression of *tasA* and a decrease in the expression of sinR confirm that when the pathogen is present, it induces the development of a biofilm in the antagonistic bacteria (Khezri et al., 2016). The function of genes involved in the formation of biofilm by microbes is given in Table 5.2.

5.6 Phytopathogen management approaches through biofilm

Plants not only form positive relationships with microbial communities but also deal with illnesses caused by a variety of pathogenic microbes. Microbial root colonization, as well as their growth and survival, is regulated by microbial cells. The colonization, growth, and survival of microbes on roots are influenced by the structure and physiology of microbial cells, QS interference, exopolysaccharide synthesis, synthesis of various root exudates, and the physical and chemical characteristics of soil and various organic matter present in the soil (Haldar and Sengupta, 2015). The production of biofilms is regulated by the creation of a variety of microbial chemicals. The significance of plant microbiomes in protecting their hosts from phytopathogens has long been investigated, and the word "biocontrol" refers to the processes that eradicate disease-causing organisms (Ritpitakphong et al., 2016). Beneficial microorganisms usually defend plants against pathogen attacks either directly (by interacting with pathogens) or indirectly (by stimulating the host plant's innate immune responses). Microbial species produce

Table 5.2: Function of genes or proteins involved in the formation of biofilm by microbes.

S.N.	Microbes	Gene/protein	Function	Reference
1.	*Bacillus subtilis*	*c-di-AMP*	Biofilm formation	Townsley et al. (2018)
2.	*Xanthomonas axonpodis*	*hfq*	Biofilm formation	Liu et al. (2019)
3.	*Pseudomonas aeruginosa*	*Psl* (polysaccharide)	Activate the diguanylate Cyclases	Silva et al. (2014)
4.	*Pseudomonas fluorescens*	*LapA* Protein	Biofilm formation	Collins AJ et al. (2020)
5.	*Salmonella enterica sv. typhimurium*	*BapA* protein	BapA, a large cell-surface protein required for biofilm formation by S. Enteritidis	Taglialegna et al. (2020)
7.	*Bacillus velezensis*	*ftsE*	Biofilm formation	Li et al. (2018)
8.	*Pseudomonas fluorescens*	*map A*	Biofilm formation	Collins AJ et al. (2020)
9.	*Rhizobium etli*	*cheRCh2*	Chemotaxis	Reyes-Pérez et al. (2016)
10.	*Bacillus cereus*	*calY*	Biofilm formation	Caro-Astorga et al. (2015)
11.	*Herbaspirillum seropedicae*	*espB*	Origination of EPS and biofilms	Balsanelli et al. (2014)
12.	*Sinorhizobium meliloti*	*fadD*	Surface motility and biofilm formation	Amaya-Gómez et al. (2015)
13.	*Bacillus cereus*	*sipW-tasA*	Biofilm formation	Caro-Astorga et al. (2015)
14.	*Bacillus subtilis*	*yqxM*	Formation of biofilm	Sharma et al. (2020)
15.	*Rhizobium*	*celABC*	Cellulose biosynthesis operon involved in biofilm formation	Robledo et al. (2012)
16.	*Rhizobium etli CFN42*	*raiR, rpoA, rpoE4*	Increase QS signaling during the development of early biofilms	Reyes-Pérez et al. (2016)
17.	*Sinorhizobium fredii*	*nodD1*	NodD1 together with flavonoids activates certain QS systems implicit in the development of the symbiotic biofilm	Pérez-Montaño et al. (2014)
18.	*Kloeckera apiculata*	*KaFlo1* to *KaFlo9*, which are required for biofilm growth	Biofilm formation	Rana et al. (2020)
19.	*Herbaspirillum seropedicae*	*espB*	Encodes glucosyltransferase during biofilm formation	Balsanelli et al. (2014)

exo metabolism, which stimulates root growth (Rudrappa et al., 2008a). Rudrappa et al. (2008c) demonstrated the role of cyanide production in *pseudomonad* pathogenicity, as it affects plant root development and other rhizosphere activities. A biofilm is a microbial colony that is adhered to a plant surface and embedded in a self-produced polymeric matrix. According to the

current investigation, plant pathogens associated with the plant's surfaces have morphological and physiological characteristics that are compatible with biofilm.

5.6.1 Cross talk between pathogenic and beneficial microbes with plants

In order to have a strong pathogenic influence on plants, pathogens must interact with the rhizosphere's diversity of microbial population (Barelli et al., 2020). Pathogens harm plants by competing for resources and space with beneficial bacteria and producing antimicrobial chemicals. For instance, *Pseudomonas* spp. can inhibit the growth of plant pathogens through competition and antibiosis; however, the overall disease suppression in the soil is influenced by a variety of factors, including the genetic background of hosts and pathogens, pathogen population dynamics, plant microbiota diversity and composition, and biotic and abiotic conditions (Durán et al., 2018). Plant strength is the most noteworthy for survival. To enhance the crop quality, it's imminent that there be a suitable deal with microbial biofilms, which play a vital role in plant health maintenance (Ahmed et al., 2018). Plants that have an immunological response initiated by a microbe are more resistant to pathogen attack and have higher disease-fighting efficacy. Biofilms connected with plants, whether above or below ground, can work cooperatively or antagonistically with the plants. Many microorganisms wreak havoc on plant health and cause diseases (Ahmed et al., 2018). Positive interactions include PGPR with those microbes that protect plants from infections while also stimulating their growth. Bacterial antagonists from the genera *Achromobacter, Comamonas, Curtobacterium, Enterobacter, Leclercia, Microbacterium, Pantoea, Sphingobacterium*, and *Stenotrophomonas* showed great biocontrol potential against *M. oryzae* and triggered the expression of defense genes like *OsCEBiP, OsCERK1, OsEDS1*, and *OsPAD4* in rice seedlings to protect them from rice blast disease (Sahu et al., 2021). Similarly, disease suppression was produced in *M. oryzae* challenged rice plants by root-associated *Pseudomonas* sp. EA105 and *Pantoea sp*. EA106, which triggered jasmonate- and ethylene-dependent induced systemic resistance (ISR) responses (Stringlis et al., 2018). Plants' ISR responses can also be induced when they come into direct contact with beneficial bacteria's released chemicals, such as volatile 2,3-butanediol, DAPG, and cyclic lipopeptide surfactants (Stringlis et al., 2018).

In *A. thaliana*, the transcription factor *MYB72* is implicated in the control of ISR. Beneficial microorganisms decrease plant immune responses by mimicking pathogens. *Rhizophagus intraradices*, an ISR-inducing *arbuscular mycorrhizal* (AM) *fungus*, produces an SP7 effector, which reduces plant immune responses and promotes root colonization. *Laccaria bicolor*, a symbiotic ectomycorrhizal fungus, colonizes plant tissues successfully by inhibiting salicylic acid-mediated immune responses *via* the MiSSP7 effector (Noman et al., 2021). *Trichoderma, Bacillus subtilis* (Lakshmanan et al., 2013), and *Pseudomonas fluorescens* WCS417r have all been found to colonize in *A. thaliana*, resulting in impaired

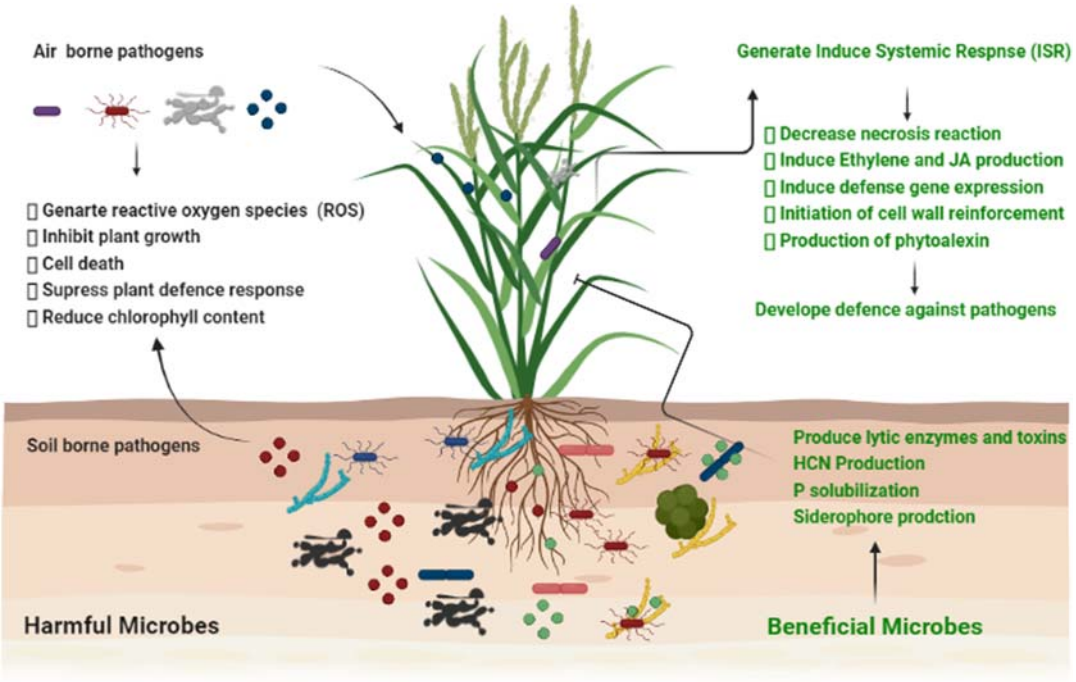

Figure 5.3
Cross talk relationship mechanism between pathogens and beneficial microbes with plants.
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immunological responses. Plant-beneficial bacteria create elicitors/signals that trigger systemic immune responses in addition to downregulating local immune responses to enable colonization. The cross-talk between microbes and plants is given in Fig. 5.3.

5.6.2 Role of biofilm as in biocontrol

Biofilm on plant roots can protect the colonization site and prevent infections from accessing nutrients in root exudates (Altaf and Ahmad, 2016; Haggag and Timmusk, 2008). The PGPR protects plants through a variety of methods, including the synthesis of antibiotics that are effective against harmful bacteria (Gupta et al., 2017; Maksimova et al., 2016). Antibiotics, for example, amphisin, 2, 4-diacetylphloroglucino, cyclic lipopeptides, tropolone, pyrrolnitrin, pyoluteorin, phenazine, and neomycin A are known widely to be synthesized by *pseudomonads* (Loper and Gross, 2007; Gupta et al., 2017). *Bacillus, Streptomyces, and Stenotrophomonas* species have also been shown to produce zwittermicin-A, xanthobaccin, oligomycin A, and kanosamine, which inhibit the propagation of phytopathogens (Gupta et al., 2017). Microbes form a biofilm to inhibit the growth of phytopathogenic microbes by the QS mechanism. In aquatic and other environments, biofilm production is a primary

bacterial adaptation mechanism to changing environmental conditions. Surfactin and iturin A, which are lipopeptides containing a hydroxy fatty acid coupled by an ester peptide bond to a cyclic heptapeptide, have been found to be responsible for *B. subtilis* biocontrol abilities against the fungal pathogen *Rhizoctonia solani* (Saharan and Nehra, 2011).

5.6.3 Role of biofilms in mitigating stress

The theory of biofilm plays a new eco-friendly agricultural strategy against plant pathogenic fungus. Interfering with the essential steps that govern the genesis of biofilm (e.g., attachment, cell-to-cell communication, dispersion) could lead to new preventive measures that don't necessarily kill cells but disturb their proclivity for a biofilm lifestyle (Villa et al., 2015). Because these compounds do not destroy cells, they should not create an evolutionary pressure that causes resistance to develop.

Sub-lethal concentrations of zosteric acid (ZA), a secondary metabolite produced by the seagrass Zostera marina, inhibit fungal adherence and help define the thickness and structure of fungal biofilms (Villa et al., 2017). Although the cells are metabolically active, they are still unable to produce filamentous structures. ZA also promotes antimicrobial agent efficiency in *Daphnia magna* by displaying cytocompatibility with soft and hard tissues, minimal bioaccumulation potential, and absence of toxicity (Villa et al., 2021; Polo et al., 2014). The relevance of reactive oxygen species (ROS) in fungal proliferation and pathogenicity suggests that ZA's involvement in the oxidative stress response could be a promising alternative to existing control approaches (Mir et al., 2015).

Antibiosis, or the prevention of microbial growth by diffusible antibiotics, volatile organic compounds (VOCs), toxins, and biosurfactants, is a process that occurs when microbes emit antagonistic metabolites. Parasitism may also involve the production of extracellular cell wall-degrading enzymes such as chitinases and 1,3-glucanase. *Bacillus subtilis* strains produce a variety of antifungal metabolites from the fengycin, iturin, and surfactin families, including kanosamine, lipopeptides, and zwittermicin-A, which demonstrated that overproduction of extracellular protease in mutant strains of *Stenotrophomonas maltophilia* W81 improves *Pythium ultimum* biocontrol (Saharan and Nehra, 2011).

5.6.4 Microbial approaches in the agriculture sector

Microbial biofilms that are significant in agriculture have a variety of biotechnological applications. Biofilms operate as biofertilizers and have tremendous promise for plants in the agriculture industry (Fig. 5.4). Such eco-friendly fertilizers have the ability to overcome the drawbacks of regular chemical fertilizers. Microbial biomass, which can improve root exudation response in the presence of biofilm in the rhizosphere when the plant is stressed, could be useful in improving water stability (Kasim et al., 2016). Because of the presence

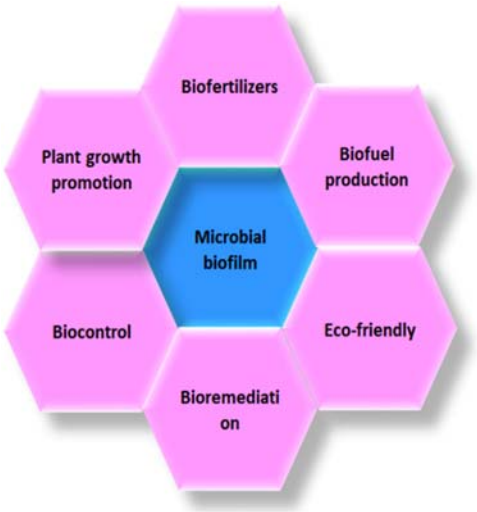

Figure 5.4
Biotechnological application of biofilm in the agriculture field.

of biofilm, biodegradation of environmental contaminants and heavy metals in the soil might be possible since such structures can easily withstand harsh environments (Gkorezis et al., 2016). Due to the use of fungal exudates for bacterial adhesion to the soil's surface, metabolic cooperation is vital in the formation of novel bacterial niches in the presence of fungi. The biofilm's constituent microorganisms, particularly the bacterial species, are maintained via metabolic cooperation between bacteria and fungi, making them less sensitive to abiotic stresses than monocultures (Deveau et al., 2018). PGPR has been shown to create aminocyclopropane 1-carboxylate deaminase (ACC), which provides significant resistance to drought and heat conditions. It is well understood that ACC deaminase plays a role in plant tolerance processes (Ngumbi and Kloepper, 2016).

5.7 Conclusion and future prospective

Microbial infections of cultivated crops continue to pose a threat to food security in the twenty-first century, and the use of innovative alternatives to conventional fungicides in disease control offers a long-term solution for disease prevention and management. Biofilm-associated bacteria are important for the conversion of harmful pollutants to nontoxic chemicals, plant protection against phytopathogens, plant growth promotion, and the removal of surplus nutrients from wastewater. Furthermore, surface modification and QS signals can be used to induce the production of beneficial biofilms in a variety of industrial and environmental conditions. In the current scenario, any investigation should be focused on identifying rhizospheric biofilm-forming bacteria and their antagonistic mechanism against

pathogens. In addition, novel methodologies will be used to evaluate the behavior of the microorganisms' transit and collaborate in a range of surface microenvironments during biofilm formation. Also, in-depth research of the regulatory systems that control the production and dispersion of bacterial biofilms, with a focus on beneficial biofilms, is needed.

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CHAPTER 6

Health benefits of bacteriocins produced by probiotic lactic acid bacteria

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6.1 Introduction

There are different types of microorganisms present in the environment, including in the human body. Few are beneficial and others, harmful. The harmful microbes affect the organoleptic quality of food and cause many diseases to afflict humans through various methods (Choyam et al., 2019). Pathogenic bacteria are involved in many diseases like obesity, diabetes, inflammatory bowel disease (IBD), cancer, etc., in humans. These diseases can affect anyone anywhere, and their effects are widely spread all over the world, so there is felt a great need to control them (Huang et al., 2021). Many antibiotics and drugs are used to kill harmful bacteria, but their side-effects and resistance to drugs make them unsafe for therapeutic applications. Probiotics can be a safe option and alternative against the use of such antibiotics. They produce different antimicrobial compounds like hydrogen peroxide, acetoin, organic acids, and bacteriocins. These antimicrobials show a broad spectrum of inhibitory activity against pathogens and can also resolve the problem of microbial resistance and side-effects of antibiotics or other synthetic compounds (Pisoschi et al., 2018). Probiotics also improve the homeostasis of human gut microbiota (Kim et al., 2019). Gut microbiota is involved in the regulation of many physiological and immune responses. The dysbiosis in gut microbiota causes various types of diseases like diabetes, obesity, diarrhea, IBD, etc. (Tang and Lu, 2019).

Probiotics are classified as QPS (Qualitative presumption of safety) and Generally Regarded as Safe due to their nonpathogenic nature, tolerance to bile salt, and acceptability for industrial and technological processes (O'Connor et al., 2020; Reuben et al., 2020). They are resistant to bacteriophages, lactose, citrate fermentation, proteolytic activity, polysaccharides production, lyophilizing and freezing conditions, and have the capacity for colonization and adhesion of digestive mucosa. A few important examples of probiotics are *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, *Enterococcus, Cornobacterium*, etc. They have low guanine + cytosine (G + C) content and show

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antimicrobial activity through the production of organic acids, acetoin, antibiotics, diacetyl, hydrogen peroxide, and bacteriocins (O'sullivan et al., 2002). There are two types of antimicrobials produced by lactic acid bacteria (LAB): ribosomally synthesized, such as bacteriocins, and nonribosomally synthesized, such as έ-poly-L-lysine (Chikindas et al., 2018). Bacteriocins from probiotics are antimicrobials and are also beneficial for human health, given their control of various diseases like cancer, IBD, diarrhea, diabetes, etc. (Islam et al., 2012).

6.2 Bacteriocins

These are ribosomally-synthesized antimicrobial peptides, cationic, thermostable, and have a low molecular weight (Singh, 2018). They show bacteriolytic or bacteriostatic activity against a narrow or broad range of bacteria but protect themselves by the production of specific immunity proteins (Yang et al., 2014). Due to low molecular weight and positive charge, the bacteriocins can easily diffuse in the biofilms formed by pathogens and inhibit their growth, for example, the role nisin plays in the inhibition of biofilm formation has been documented (Choyam et al., 2019; Mathur et al., 2018). Bacteriocins can be used for human health because of their function as modulators for human gut microbiota, antimicrobial activity, lesser health risks, and specific activity against pathogens (O'Connor et al., 2020; O'sullivan et al., 2002). On the basis of structure, molecular weight, and mode of action, bacteriocins have been divided into three classes: Class I, Lantibiotics; Class II, Non-lantibiotics; and Class III, large bacteriocins. An overview of the classification of bacteriocins is mentioned in Fig. 6.1.

Class I: These are known as lantibiotics which are modified, heat-stable, and globular or linear peptides with molecular weight <5 kDa (Ghodhbane et al., 2015; Kumariya et al., 2019; Yang et al., 2014). The lantibiotics have lanthionine and methyl-lanthionine and dehydrated amino acids generated by post-translation modifications (Bali et al., 2016; Yang et al., 2014). Class I is further subdivided into six subclasses. (1) Class Ia: Lanthipeptides. In this, nisin is the best-studied example. (2) Class Ib: Head-to-tail cyclized peptides formed by linkage of N- and C- terminal by a peptide bond. These bacteriocins consist of only α-helices, for example, enterocin AS-48. (3) Class Ic: Sactibiotics. These peptides contain sulfur at alpha carbon, for example, subtilosin A. (4) Class Id: it contains linear azol(in) e peptide and possess cysteine, threonine, and serine residues derived from heterocyclic rings of (methyl)oxazole and thiazole, for example, streptolysin S. (5) Class Ie: this class contains glycosylated residues, for example, glycocin F having two α-helices linked with disulfide bonds. They have N-acetylhexosamine linked with C-terminal cysteine. (6) Class If: These bacteriocins are lasso peptides in which the first amino acid is linked with an amide bond and a ring formed at $+7$ to $+9$ positions (Alvarez-Sieiro et al., 2016).

Class II: These are non-lantibiotics, unmodified, small (30–60 amino acids), and thermostable, positively charged, having molecular weight <10 kDa (Bali et al., 2016; Yang et al., 2014).

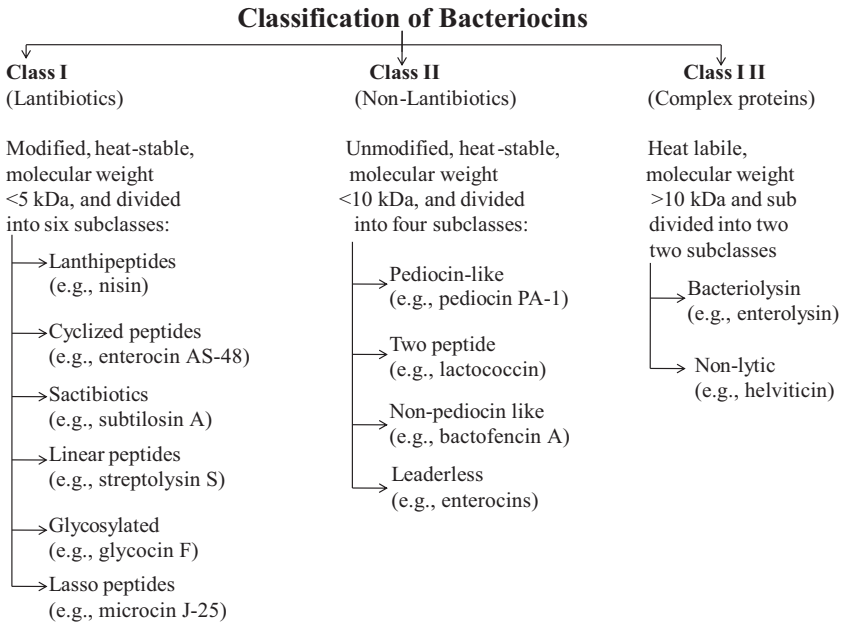

Figure 6.1
Classification of bacteriocins of lactic acid bacteria.

This class has four subclasses: (1) Class IIa: Pediocin-like bacteriocins. In this subclass, bacteriocins have two cysteine residues linked by disulfide bonds and motifs consisting of conserved sequence YGNGVXC at the N-terminal. The C-terminal is generally less conserved, for example, pediocin PA-1, leucocin A, and sakacin. (2) Class IIb: Two-peptide bacteriocins. These bacteriocins have a two-component system to form an active complex of pore formation, for example, lactococcin and plantaricin. (3) Class IIc: Leaderless bacteriocins. These bacteriocins lack a leader peptide at N-terminal, for example, enterocins. (4) Class IId: Non-pediocin-like bacteriocins, for example, bactofencin A (Kumariya et al., 2019).

Class III: These bacteriocins are heat-labile with larger molecular weight (>30 kDa) (Bali et al., 2016). This class has been divided into two groups: (1) bacteriolytic bacteriocins that lyse the cell wall of sensitive strains, for example, enterolysin; and (2) nonlytic bacteriocins that have bactericidal activity without causing cell lysis, for example, helviticin (Alvarez-Sieiro et al., 2016; Yang et al., 2014).

6.3 Mode of action of bacteriocins

The action mechanism of bacteriocins against target bacteria consists of interaction with the cell wall and destabilization of the structure of cytoplasmic membrane. The cell wall of Gram-positive bacteria is made up of peptidoglycan, which consists of anionic lipids. The

electrostatic interaction between positively charged nisin and negatively charged lipid leads to adsorption of nisin on the cell surface, which causes pore formation. The binding of lipid II molecule and nisin induces conformational changes in membrane that lead to pore formation and release of intracellular ions, amino acids, and ATPs leading to disruption of proton motive force. Such changes lead to cell lysis and, ultimately, bacterial cell death (Dobson et al., 2012; Moll et al., 1999; Islam et al., 2012). Pediocin interacts with mannose phosphotransferase system (Man-PTS), which acts as a docking molecule and brings pediocin closer to the cell membrane. It allows the insertion of bacteriocin in the membrane and oligomerization to form a channel (Colombo et al., 2018). Man-PTS is a complex of proteins responsible for phosphorylation and transport of molecules. Enterocins show activity through pore formation in cell membrane, causing depletion of membrane potential and pH gradient and loss of intracellular ions or other molecules. Enterolysin A degrades the cell wall, which has one catalytic domain at N-terminal and one recognition domain at C-terminal.

Bacteriocins act as competitive and colonizing agents when interacting with human cells in the gut. They act as a signaling molecule for the activation of immune cells, i.e., B-cells and T-cells, which produce antibodies and secondary molecules like defensins to kill pathogenic bacteria (Dobson et al., 2012), as shown in Fig. 6.2.

6.4 Modulation of gut microbiota, immune modulation, and anti-inflammation activity

Human organs are composed of complex and diverse microbiota. Dysbiosis in the microbiota can cause major health issues like AAD, obesity, IBD, urinary tract infection (UTI), etc. Dysbiosis depends on the composition of microorganisms and antimicrobial resistance within the gut microbiota. Disturbance in gut microbiota leads to overgrowth of pathogens causing bad effects. But bacteriocins and probiotics can exert a significant effect on pathogens without disturbing the commensal gut microbiota and is also nontoxic for human. Probiotics inhibit the growth of pathogenic bacteria and also improve immunity against food antigens and any other infection by activation of T-cells, T_{regs} or Th17 cells, and TLR signaling (Gopalakrishnan et al., 2018; Umu et al., 2017). An increase in expression of IL-8, $CD4^+$ + $CD8^+$ + double positive cells was measured by administration of salivaricin P in porcine ileum (Walsh et al., 2008). Nisin F exerts a stabilizing effect on the bacterial population in the gut of mice. Nisin F with lactoferrin and lysozyme was injected in a mice model, and it was observed that one injection of nisin F (640 AU) was sufficient to kill the bacterial population (e.g., *S. aureus* and Listeria) in the gut (Van Staden et al., 2011). Bacteriocin, salivaricins from *Streptococcus salivarius*, and ruminococcin A from *Ruminococcus gnavus* and *Clostridium nexile* inhibited the growth of closely related pathogens. Cytolysin, a two-component lantibiotic from *Enterococcus faecalis*, showed antibacterial activity against common human commensals and established colonization (Donia and Fischbach, 2015). Nisin

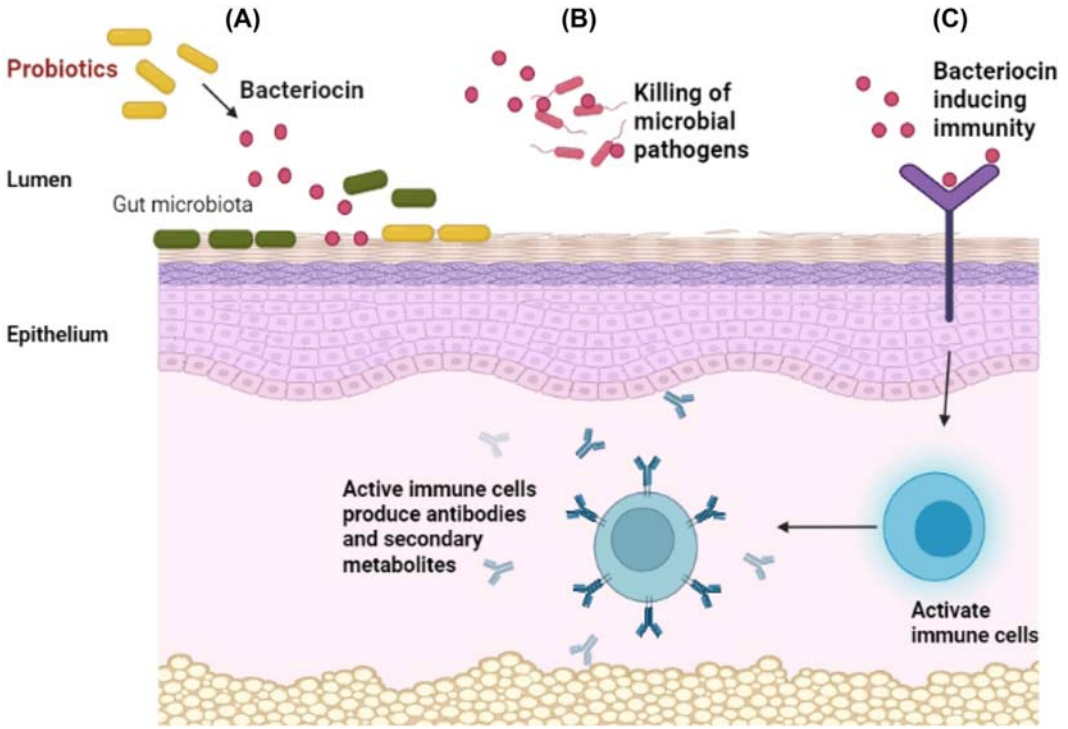

Figure 6.2
Mechanism of action of bacteriocins on human epithelial cells. (A) Competitive binding of bacteriocin-producer probiotics against other gut microbiota and pathogens. (B) Direct attack on pathogens to inhibit their growth. (C) Activate immune cells and help in the secretion of antibodies and secondary metabolites.

prevents infection caused by *Staphylococcus aureus;* whereas pediocin PA-1, lactocin AL-705, and enterocin CRL 35 inhibit the growth of *Listeria monocytogenes* and also their passage through the intestinal barrier. Pyocin was able to control *Pseudomonas* infection in patients with cystic fibrosis (Huang et al., 2021).

6.5 Antioxidant activity

Oxidative stress is a condition in which the level of intracellular oxygen radicals is increased, leading to abnormal DNA hydroxylation, denaturation of proteins, lipid peroxidation, and apoptosis. The highly active oxygen free radicals are superoxide anion radicals, hydroxyl, and hydrogen peroxide called Reactive Oxygen Species (ROS). ROS serves as a secondary messenger and activates NFκB and some other transcription factors, kinases such as hypoxia-inducible factors (HIF) and phosphatidylinositol (PI3K). The activation of these factors causes various diseases like arthritis, diabetes, neurodegenerative

disease, atherosclerosis, and cardiovascular disease. Probiotics show antioxidant activity by chelating ions like Fe^{2+} and Cu^{2+}, antioxidant enzymes like superoxide dismutase and catalase, and antioxidant metabolites like glutathione, butyrate, and folate. For example, *Lactococcus lactis*, and *L. rhamnosus* improve the immune system and oxidative status. *Bifidobacterium animalis* scavenges hydroxyl radicals and superoxide anions in mice. *L. plantarum* P-8 showed antioxidant activity and protected liver function (Wang et al., 2017). Plantaricin-producer, *L. plantarum* FLPL05 increased the activity of serum glutathione peroxidase (GSH-PX) and decreased malondialdehyde (MDA). MDA is the end product of membrane lipid oxidation and cause the formation of free radical in the body cells. A decrease in the level of MDA in serum of D-Gal aging mice was detected by *L. plantarum*. GSH-PX is intracellular antioxidant enzymes that may provide protection against oxidative stress (Yu et al., 2020). The plantaricin E and F recombinant also decreased MDA levels in the mice model (Hanny et al., 2019).

6.6 Antibiofilm activity

Biofilm is made up of bacterial cells enclosed in polymeric matrices. Many bacterial species are involved in the formation of biofilm, such as *E. coli*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas fluorescens*, and *Yersinia pestis* (Miquel et al., 2016). These bacteria form biofilm through quorum sensing (QS) and/or cyclic di-GMP (cGMP) signaling. QS is an intracellular mechanism that regulates the expression of genes in response to environmental small diffusible signaling molecules like acyl homoserine lactones, peptides, and autoinducer-2. Similarly, cGMP signaling provokes biosynthesis of extracellular polymeric substances and reduces the motility of bacteria leading to the formation of biofilm (Galie et al., 2018). It has been reported that exopolysaccharides from LAB generally lower the formation of biofilms. Thus autoaggregation of pathogenic bacteria is inhibited either by a weakening of cell surface modification or by lowering interaction between the cells (Angelin and Kavitha, 2020). Probiotics such as streptococci and lactobacilli showed competitive binding of the inhibitor to QS receptors and inhibited cell communication, enzyme degradation, and posttranscriptional control of QS signals (Kareb and Aïder, 2020; Galie et al., 2018). Subtilosin A purified from *Bacillus subtilis* inhibited *Listeria monocytogenes* and *Gardnerella vaginalis* by inhibiting the production of QS signals like autoinducer-2 and by forming a biofilm on the cell surface (Algburi et al., 2017).

6.7 Application of bacteriocins in a few important human diseases

6.7.1 Antibiotic-associated diarrhea

Antibiotic-associated diarrhea (AAD) results in the passing of liquid stools and causes a change in natural human gut microbiota. In addition, the use of antibiotics like

aminopenicillins, cephalosporins, and clindamycin disturbs the natural microbiota of the human gut by disturbing the colonization of bacteria and increasing the number of harmful bacteria causing AAD. *Clostridium difficile* is a major pathogen associated with AAD. The symptoms of AAD are frequent bowel movements and abdominal pain. A severe condition in AAD may lead to electrolyte disturbances, dehydration, toxicity in gut, and rarely death. Incidence of AAD ranges from 5%–62% and, in children, it ranges from 11% to 40% (Guo et al., 2019). Gassericin A-controlled diarrhea is reported as binding keratin 19, which enhances fluid absorption and decreases the loss of liquid (Hu et al., 2018). *Streptococcus thermophillus* DMST-H2 consists of 12 genes related to bacteriocin production, i.e., for example, lantibiotic biosynthesis protein, lantibiotic transporter, lantibiotic biosynthesis response regulator, etc., that help in antimicrobial activity of *S. thermophillus* DMST-H2 strain. Strain DMST-H2 also showed a reduction in proinflammatory cytokines (TNF-α) and enhancement in interferon-γ (IFN-γ) and antiinflammatory cytokines (IL-10), which reduce inflammation and intestinal injury due to AAD (Hu et al., 2020).

6.8 Inflammatory bowel disease

IBD is a major health issue caused by changes in human gut microbiota. Different spp. of *Lactobacillus* and *Bifidobacteria* showed antibacterial response and competition with pathogenic bacteria by producing bacteriocins. They interact with epithelial cells, reduce water secretion, and enhance the production of mucus or B-defensins, lactoferrin, phospholipase, or lysozyme by epithelial cells (Veerappan et al., 2012). Plantaricin B, plantaricin D, and plantaricin G purified from *L. casei*, *L. plantarum*, and *L. rhamnosus* strains isolated from breast milk competed with pathogens, lowered the level of total serum cholesterol level in mice after 14 days of treatment, and lowered loss in body weight after 8 days of treatment (Abdi et al., 2021). Adherent-invasive *E. coli* (AIEC) adhered to intestinal epithelial cells (IEC) and formed a biofilm. Also, they survived in macrophage cells for a long time and reduced the production of TNF-α and other inflammatory cytokines. Colicins E1 and E9 effectively killed cell-adherent and intracellular AIEC by entering the biofilm through endocytosis (Palmela et al., 2018).

6.9 Cancer

Cancer is one of the major causes of death globally, and the incidence of cancer is increasing very fast in developing countries. Some most commonly occurring cancers are colorectal, prostate, lung, stomach, liver, and breast cancers. Cancer is a disease that is caused by uncontrolled cell division. Tumor cells show many physiological alterations, self-dividing capability, and insensitivity to growth inhibitors, apoptosis resistance, unlimited cell proliferation capacity and are known to support angiogenesis. The use of standard drugs is doubtful because they not only kill cancer cells but also disturb healthy cells.

These drugs also result in drug resistance, many side-effects, and malignancy. Since no proper cure is available for cancer till now, scientists are looking for a treatment with minimal side-effects and high efficacy (Molska and Reguła, 2019).

Probiotic *L. casei* showed an inhibitory effect on colorectal cancer by decreasing the activity of fecal enzymes and provided protection against food mutagens. *L. fermentum* NCIMB 5221 modulated hyperinsulinemia, hypertriglyceridemia, and showed antiproliferative effect. *Weisella cibaria* JW15 increased the activity of natural killer cells (NK cells). *Saccharomyces cerevisiae* var. *boulardii* showed antiinflammatory and antibacterial activity by increasing secretion of IgA and maintaining epithelial barrier (Molska and Reguła, 2019). *L. plantarum* LS/07 reduced tumor necrosis factor (TNF)-α and increased CD4 (+) T-cells (Jahanshahi et al., 2020). *L. rhamnosus* GG showed an antiproliferative effect in gastric and colonic cancer (Yu and Li, 2016). *L. casei* SR1, *L. casei* SR2, and *L. plantarum* treatment in cancer cells increased the number of S-phase cells and decreased the number of G2/M phase cells, and encoded E6 and E7 genes, which further encoded for p53 and pRB tumor suppressor proteins. Nisin, a bacteriocin from *Lactococcus lactis*, has been used for induction of apoptosis, the arrest of the cell cycle, and the reduction of cell proliferation in cancer cells. It was able to form pores in the cells that caused a release of ions, amino acids, and ATP in the cancer cells (Veerappan et al., 2012). Colicin A, colicin D, and colicin E2 and E3 suppressed malignancy in the cells. Nisin controlled the head and neck squamous cells (HNSCC) and oral cancer by increasing apoptosis regulated by cation transport regulator homolog 1 (CHAC 1), a proapoptotic cation transport regulator, and a CHAC 1-independent influx of calcium ions (Shin et al., 2016; Lopetuso et al., 2019). Increased concentration of nisin induced DNA fragmentation or apoptosis after 24 h of treatment in HNSCC through the increasing influx of calcium ions, arresting cell cycle in the G2 phase by decreasing phosphorylation of Cdc2 and enhancing expression of CHAC 1 (Joo et al., 2012). Plantaricin A from *L. plantarum*, pediocin from *P. acidilactici*, and duramycin from *Streptoverticillium griseoverticillatum* showed an inhibitory effect against Jurkat, HeLa, and MCA-RH 7777 cell lines, respectively. Microcin E49 from *Klebsiella pneumoniae* triggered cancer cells through the formation of ion channels which resulted in shrinkage of cells, fragmentation of DNA, exposure of phosphatidylserine, and activation of caspases (Huang et al., 2021).

6.10 Obesity

Obesity is also one of the main health problems in both developed and developing countries. It occurs due to the presence of abnormal white adipose tissue. Probiotic bacteria such as *Prevotellaceae*, *Blautia coccoides*, *Eubacteria rectale*, *Lactobacillus*, and *Bifidobacterium* are known to reduce the obesity (Sáez-Lara et al., 2016). Bifidobacteria managed lipid metabolism and reduced obesity. *B. longum* with *Streptococcus thermophilus*

and *L. delbrueckii* subsp. *bulgaricus* lowered serum cholesterol in rats and humans. *L. curvatus* HY7601 alone or, in combination with *L. plantarum* KY1032, reduced body weight and adipose tissue in mice fed with a high-fat, high cholesterol diet for 9 weeks (Islam et al., 2012). Bacteriocin PJ4 purified from *L. helveticus* PJ4 isolated from rat feces showed an antiobesity effect in rats. The treatment of high fat-fed rats with PJ4 resulted lowered weight of adipose tissue and controlled the production of IL-6, TNF-α. The treatment showed a satisfactory effect in reducing obesity (Bai et al., 2020). Gassericin A purified from *L. gasseri* LA39 controlled the genes involved in adipogenesis. Stearoyl-CoA desaturase-1 (SCD-1 or Δ-9-desaturase) is an enzyme produced by monounsaturated fatty acids, which synthesized triglycerides, cholesterol esters, and very-low-density lipoprotein. Gassericin A decreased expression of SCD-1 gene in preadipocytes which reduced obesity-related complications. Also, Gassericin A enhanced production of Glucose transporter type 4 (GLUT4) in mice, which is associated with adiposity and increased serum free fatty acid levels (Taghizad et al., 2021). Plantaricin EFI from *L. plantarum* NCIMB8826-R was effective in reducing not only weight gain but also serum glucose levels in sham-fed high fat diet mice (Heeney et al., 2019).

6.11 Diabetes

Diabetes causes change in the intestinal microbiota from environmental or genetic factors because altered microbiota leads to an increase in intestinal permeability and mucosal immune response. When *L. johnsonii* was administered in diabetes-prone rats, it reduced the incidence of diabetes and increased the expression of claudin-1 gene, decreased oxidative stress, and modulated the response of TH17. *Dahi*, a fermented food consisting of *L. acidophilus*, *L. casei*, and *L. lactis* reduced glycemia and HbA1c. *L. plantarum* DSM15313, also reduced glucose level and insulin resistance. *Bifidobacterium longum*, *B. infantis*, and *B. breve* reduced inflammation of adipose tissue and modulated insulin signaling (Gomes et al., 2014). Nisin with paxiganan showed an inhibitory effect against *Staphylococcus aureus* with evidence of antibiofilm activity and pore formation in the bacterial membrane. *S. aureus* and *Pseudomonas aeruginosa* cause diabetic foot ulcers, which are major complications *of Diabetes* mellitus (Gomes et al., 2020).

6.12 Urinary tract infection

UTI are mainly related to kidneys, ureters, urethra, or bladder due to colonization of pathogenic bacteria. UTI can lead to strictures, abscess, fistula, bacteremia, sepsis, pyelonephritis, and kidney dysfunction (Schwenger et al., 2015), which can be treated with antibiotics, but the use of more antibiotics raises drug resistance and fails to restore natural barrier of the urinary tract from infection. Drugs can also have side-effects consisting of disturbances in natural microbiota leading to frequent UTI. Therefore probiotics and their

bacteriocins can be a better option for the treatment of UTI. *L. salivarius* subsp. *salivarius* produced a bacteriocin that inhibited the growth of *Enterococcus faecalis*, *E. faecium*, and *Neisseria gonorrhoeae*. *Lactobacillus* spp. isolated from vagina show bacteriocin activity against *Gardnerella vaginalis*. *L. fermentum* strain L23 showed a wide range of inhibition spectrum against both Gram-positive and Gram-negative pathogenic bacteria and *Candida* spp. (Borges et al., 2014) Salivaricin LHM inhibited the growth of multi-drug resistant *Pediococcus aeruginosa* by showing antibiofilm and antimicrobial effects, leading to rupture of the cell membrane and leakage of intracellular ions. Salivaricin LHM also stimulated immunity by overproduction of proinflammatory cytokines, IL-10, and IL-4, which relaxed inflammation during UTI (Mahdi et al., 2019). The role of bacteriocins in probiotic LAB has been mentioned in Table 6.1.

Table 6.1: Bacteriocins produced by different probiotic lactic acid bacteria and their role in the control of some human diseases.

S. no.	Diseases	Bacteriocins	Role of bacteriocins in diseases	References
1	**Antibiotic-associated diarrhea (AAD)**	Gassericin A	Enhance fluid absorption and decrease the loss of liquid in the intestine by binding with keratin 19	Galie et al. (2018)
2	**Cancer**	Nisin Plantaricins Microcins Colicins	Nisin controls the head and neck squamous cell carcinoma and oral cancer by increasing apoptosis and induction of DNA fragmentation. Plantaricin inhibited Jurkat, HeLa, A549, and MCA-RH 7777 cell lines. Microcins trigger formation of ion channels and result in shrinkage of cells, fragmentation of DNA, and activation of caspases. Colicins suppress malignancy in the cells	Abdi et al. (2021), Palmela et al. (2018), Hanny et al. (2019), Molska and Reguła (2019)
3	**Obesity**	Gassericin A Plantaricins	Bacteriocins PJ4 lower the weight of adipose tissue and control the production of interleukin-6, Tumor necrosis factor-α, and reduce obesity. Plantaricins control the genes involved in adipogenesis, reduce the level of triglycerides and cholesterol, thus reducing obesity	Yu and Li (2016), Shin et al. (2016), Lopetuso et al. (2019)

(*Continued*)

Table 6.1: (Continued)

S. no.	Diseases	Bacteriocins	Role of bacteriocins in diseases	References
4	**Diabetes**	Nisin	Nisin inhibits *Staphylococcus aureus and Pseudomonas aeruginosa*, which cause DFUs, which are major complications of Diabetes mellitus	Sáez-Lara et al. (2016)
5	**Inflammatory bowel disease**	Plantaricin Colicins	Plantaricin B, D, and G lower the level of total serum cholesterol and body weight. Colicins E1 and E9 will effectively kill cell-adherent and intracellular adherent-invasive *E. coli* by entering into biofilm endocytosis	Algburi et al. (2017), Guo et al. (2019)
6	**Urinary tract infections**	Salivaricin	Inhibits *Enterococcus faecalis*, *E. faecium, Neisseria gonorrhoeae*, and multi-drug resistant *Pseudomonas aeruginosa*, also stimulating immunity by overproduction of proinflammatory cytokines, interleukin-10, and interleukin-4	Taghizad et al. (2021), Heeney et al. (2019)

6.13 Summary

The human body is the host of billions of microorganisms that are present in a constant number of types known as microbiota. The change in the composition of microbiota causes dysbiosis and several diseases like cancer, IBD, diabetes, obesity, and diarrhea which drastically affect human health. Many antibiotics and drugs are used to cure these diseases, but due to their side-effects and drug resistance, there is a need for the development of safe alternatives. Probiotics and their antimicrobial compounds, especially bacteriocins, can be a better alternative due to their safe origin. Probiotics consist of various types of microorganisms like *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, etc. They produce different bacteriocins like nisin, pediocin, sakacin, enterocin, plantaricins, etc. Bacteriocins show antimicrobial activity, which either kills pathogens by forming pores or inhibits their growth. They also act as gut modulators and boost immunity by acting as signaling molecules. They show antibiofilm, antiinflammatory, antiobesity, and anticarcinogenic activity and, thus, are helpful for the treatment of these diseases and catalytic to the improvement of human health. Further, they show lesser health risks and no side-effects and, therefore, can be exploited for several therapeutic applications.

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CHAPTER 7

Actinomycetes, cyanobacteria, and fungi: a rich source of bioactive molecules

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7.1 Introduction

Actinomycetes are limitless resources of unique compounds with a variety of different therapeutic usage, and they occupy a significant place in microbial community. Actinomycetes, basidiomycetes, and fungi are being used in a variety of metabolites as a resource of unique bioactive compounds because of the diversification and demonstrated capability to create novel and unique bioactive chemicals. Actinomycetes are active members of marine microbial communities (Jensen et al., 2015) and form stable, long-term populations in a variety of marine environments. Microbial secondary metabolites have received a lot of interest because of their important biological functions, notably in terms of human health benefits. The biosynthetic pathway of these secondary metabolites via metabolic processes and biotechnology-related engineering has a number of advantages over traditional extraction techniques. They form a part of the marine group of microorganisms that produce new and distinctive secondary metabolites and have fascinating biological activity. Actinomycetes, like other species of marine bacteria, play a significant role in the healthcare and biopharmaceutical industries because of their capacity to manufacture secondary metabolites with a broad range of chemical structures and biologically active compounds (Fig. 7.1). Thousands of bioactive substances have been extracted and described, with many of them being turned into medications for a variety of ailments in the human, veterinary, and agricultural domains (Castillo et al., 2002; El-Shatoury et al., 2009; Singh et al., 2003).

The revelation of many new marine actinomycete taxa with distinct energy metabolism in their natural environments (Fenical and Jensen, 2006) has been accomplished, and also their potential to form stable populations in a range of ecosystems and generate novel bioactive

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Figure 7.1
Actinomycetes, cyanobacteria, and fungi as bioactive compounds.

compounds with an extended range of biological activities has been investigated. As a result, actinomycetes are considered the effective source of these secondary metabolites, antibiotics, and bioactive substances. Each actinomycete strain has a natural capability to

create 10–20 secondary metabolites, according to research. Actinomycetes are notable as research materials, according to a significant body of research, producing 75% of all known antibiotics being discovered. Discovering a novel actinomycete that digested the important component in a natural product like medicine has become increasingly fascinating and important. Antioxidants have a key function in blocking and scavenging free radicals, protecting humans from a variety of illnesses and, also, from degenerative disorders (Sosio et al., 2000). Oxidative stress can be caused by an increase in free radicals or a reduction in antioxidants, signaling the discovery of natural antioxidative agents. Several naturally occurring antioxidant properties have been shown to defend human cells from induced oxidative damage. Modern studies and research show that natural antioxidants derived from plants and microorganisms are increasingly being investigated as safe treatments (Suriyavathana and Nandhini, 2010) for humans.

Cyanobacteria, sometimes called blue-green algae, are a morphologically diverse genus of oxygenic phototrophic bacteria which make up one of the biggest groups of bacteria (Encarnação et al., 2015). Many useful molecules, including key bioactive and biotechnologically relevant substances, can be found in microalgae and cyanobacteria. Microalgae cultivations are a viable source for numerous innovative and new chemically and physiologically active compounds of great commercial uses and value, like lipids, proteins, and dyes, due to their tremendous biodiversity and resulting heterogeneity in biochemical composition. Modifying the cultivation media and conditions can change the type of chemical products. Algae are incredibly adaptable to a variety of cell culture settings, making them extremely versatile. They don't need arable land to grow, can be grown in saline water and effluent, and use a fraction of the water that plants do. They have an extraordinarily fast development rate, making them ideal for biofuel production. Some algae species may produce up to 100 times the amount of oil that oil seeds can. Furthermore, microalgae and cyanobacteria can accumulate a variety of biotoxins and, by producing biomass through carbon dioxide fixation, can help to reduce greenhouse gas emissions (Encarnação et al., 2015).

While fungi represent an enormous category of 3.8 million species, a few studies of antioxidant chemicals generated by these species are known. Khatua et al. (2013) investigated mushroom reactive potential compounds that are antioxidants in nature, whereas Gupta et al. (2020) examined fungal endophytic antioxidant compounds, and lately, Vitale et al. (2020) evaluated marine fungal antioxidant molecules with an emphasis on the production techniques and prospects.

Whenever fungal metabolites are discussed, the first discovery to be highlighted is "penicillin." The molding juice from *Penicillium notatum* was uncovered by Alexander Fleming in 1929 and was called "Penicillin" (Photolo et al., 2020) as a biologically active substance. In recent years, researchers are looking for a rapidly growing fungus strain that they think may create bioactive metabolism in submerged crops. Later, for the production

of penicillium on a bigger scale, the choice of *Penicillium chrysogenum* was made (Ayyanna et al., 2018). The most varied group of fungi show antioxidant activities produced from a wide variety of primary and secondary metabolites, including steroids, xanthones, phenols, flavonoids, and alkaloids, and are highly regarded and utilized for their medicinal purposes in the pharmacies and medicinal industries. Maximum medicinal and pharmacological studies have been conducted on Ascomycota and Basidiomycota species (Hameed et al., 2017) which comprise endophytic fungi and have greater potential for antioxidants. A variety of natural compounds has been described from endophytic fungus, including antiinsecticidal, antioxidants, antimycobacterial, anticancerous, antiviral, antimalarial, and immune-suppressant (Son et al., 2018). Antibiotics and bioactive chemicals are traditionally obtained from chemoheterotrophic microorganisms such as bacteria and fungi. Microalgae have been used as a source of physiologically active chemicals since the late twentieth-century era (Metting and Pyne, 1986).

7.2 Actinomycetes: *biology and bioactive compounds*

Actinomycetes are microbes that belong to the Actinomycetales order, having the phylum Actinobacteria. It is one of the largest taxonomic groups in the domain of bacteria. The genome of the obligate pathogen Tropherymawhipplei is an exception, with a G + C concentration of less than 50% (Ventura et al., 2007). The phylum has evolved into a variety of habitats, including soil, water (including salty water), and air. Nonetheless, the majority of these species prefer to live in alkaline soil with a high organic matter content (Barka et al., 2016). The production of secondary metabolites is controlled by a number of physical and chemical variables, including nutrition availability, oxygenation, temperature, and pH. In order to improve metabolite synthesis, these parameters have historically been managed and improved in industrial fermentations. In addition, the pharmaceutical sector has employed classic mutagenesis procedures to increase strain and manufacturing output (Olano et al., 2008).

Antibiotics are commonly thought of as compounds that kill harmful bacteria, and the roles performed by these antibiotics are more complex than we previously assumed. The oversimplification, which comes through a laboratory “distortion” in which this production of antibiotics was viewed as a separate method, is a cause of misunderstanding of the role of these antibiotics. Furthermore, in a composite environment where diverse microbe populations interact, these chemicals could play a variety of roles, including as signaling agents, biofilm promoters, and, more broadly, as the *prima donna* in regulating the expression of genes of the producer microbe or its neighbors (Aminov, 2009; Linares et al., 2006; Mlot, 2009). Surprisingly, a substantial number of antibiotics generated from actinomycetes have been identified from microbes that have completed their lifecycles in the soils, particularly the soils that are biologically damaged. This is furthermore evidence that microbial production of antibiotic chemicals can be an evolutionary benefit, allowing

microbial producers to compete more effectively for land and resources. Another item to consider is the specific conditions that exist in a deep-sea environment. Due to utmost variations in extreme ecological pressure, such as competition for habitat, predation, macro- and micro-nutrients, oxygen concentration, pressure, and light, it is predicted that marine actinomycetes may have peculiar nature of properties with unique and arranged structural elements that have not previously been discovered in the terrestrial actinomycetes (Casertano et al., 2020; Stincone and Brandelli, 2020).

7.3 Mechanism of production of bioactive compounds by actinomycetes

In certain ecosystems, actinomycetes remain the major source of genetic variety. Cell biology and genetics have provided us with new equipment that allows us to find new specific microbial molecular mechanisms and bioactive chemicals with greater sensitivity and accuracy. Screening procedures have evolved to include the use of antisense RNA, novel adjuvant compounds, novel antibiotic-resistant bacteria, different cultivation methods, and the stimulation of varying silent operons reacting to secondary metabolism (Genilloud, 2017). At this stage, extract from a fermentative broth or a compound might be introduced, and the antibiotic-related toxicity could be evaluated. In the final stage, the target can be studied, and the mode of action deduced. Naturally, antibiotic-resistant bacteria aid the drug development of humans as we begin with an assumption that naturally occurring resistant microbes like bacteria could manufacture the same antibiotic that is resistant (first filter). On the basis of open reading frames (ORFs), a second isolate is then used, and which—it is said—will boost our microbial library with potential novel drug makers (Thaker et al., 2013). Our culture processes are insufficient to allow a large fraction of the prokaryotic community to flourish (Rappé and Giovannoni, 2003).

Enhancing bacterial cultivation technologies and understanding their molecular biology could open up a slew of new avenues for studying biodiversity and enzymatic tools in the environment (Cardenas and Tiedje, 2008; van Wezel and McDowall, 2011). Strong selection factors, such as increased salinity, varied temperatures, and alkaline or acid growth conditions, can be used to achieve this (collections of novel or poorly represented strains). Furthermore, the microenvironment can be recreated in vitro to recognize the “cross-talk molecules” involving the connection between microbial populations. An important consideration is the development of novel synthetic antibiotics. Actinomycetes are makers of bioactive chemicals that belong to one of the most common antibiotic groups. Currently, these APIs are made using well-established, high-volume fermentation techniques. As a result, the pharmaceutical sector can benefit from these useful building blocks while developing new APIs. Antibiotic-resistant bacteria make the development of new antibiotics inevitable to produce, and chemical modification of existing medicines remains the most effective method of doing so.

To date, semisynthetic derivatives of substances produced from actinomycetes strain fermentation make up the majority of antibiotics used in clinical treatment. As a result, knowing the processes of antibiotic resistance can aid in the development of chemical modifications to an API to produce a different and new molecule with increased biological and metabolic activity. Chemical research will supply the synthetic and industrial capabilities needed for the commercial manufacturing of novel antibiotics (De Simeis and Serra, 2021).

7.4 Cyanobacteria: *biology and bioactive compounds*

Cyanobacteria are an oxygenic photosynthetic prokaryote that grows and multiplies on the basis of light, H_2O, CO, and inorganic nutrients (Issa et al., 2014). Because of their capacity to grow in a wide range of ecological conditions, including light, temperature, salinity, alkalinity, and pollution, they serve as model photosynthetic prokaryotes for the study of numerous metabolites generated by these organisms in such conditions (Alwathnani and Johansen, 2011; Comte et al., 2007; Papke et al., 2003).

Microcystis, Anabaena, Nostoc, and Oscillatoria are cyanobacteria that produce a variety of biochemically active chemicals (Sivonen and Börner, 2008). Toxic compounds are also produced by some cyanobacterial species (Carmichael, 1992). Cyanobacteria's secondary metabolites are rich sources of novel bioactive molecules that can be used to make medications and agriculturally essential chemicals. In the natural environment, several extracellular compounds released by cyanobacteria serve as poisons or allelochemicals (Pflugmacher, 2002). Lipopeptides from cyanobacteria have been found as biochemically active molecules. Toxic compounds are also produced by some cyanobacterial species (Carmichael, 1992). Cyanobacteria's secondary metabolites are rich sources of novel bioactive molecules that can be used to make medications and agriculturally essential chemicals. In the natural environment, several extracellular compounds released by cyanobacteria serve as poisons or allelochemicals (Pflugmacher, 2002). Lipopeptides from cyanobacteria have been found as biochemically active molecules. About 85% of them are bioactive, with cytotoxic (41%), anticancer (13%), antibiotic (12%), enzyme inhibitor (8%), antiviral (4%), and antifungal (4%) properties (Burja et al., 2001). Tumor-promoting, herbicide, antimycotic, antimitotic, antimalarial, antimicroalgal, cell-differentiation boosting, cardiac activity, and UV absorption activity that can be used as sunscreens account for the remaining activity (18%) (Burja et al., 2001).

Antifungal activity, cytotoxicity, sodium channel regulation, and protease inhibition are just some of the biological effects of cyanobacterial alkaloids isolated from a range of species (Harvey, 2008; Tan, 2007). The filamentous genera Lyngbya, Oscillatoria, and Symploca are the main producers of these alkaloids (Grindberg et al., 2008; Williams et al., 2002). Furthermore, cyanobacteria are known to produce toxins that are linked to water blooms.

There are approximately 40 genera of cyanobacterial species responsible for the synthesis of freshwater and marine cyanobacterial toxins (Grindberg et al., 2008). These cyanotoxins are classified as hepatotoxins, neurotoxins, irritants, dermatotoxins, and general cytotoxins based on their toxicity in vertebrates (Sivonen and Jones, 1999). Cyclic peptides, alkaloids, and lipopolysaccharides are the three types of chemical structures (Chorus and Welker, 2021; Stewart et al., 2006). Microcystins and nodularins, which are powerful inhibitors of protein phosphatases, are the most well-known cyanotoxins. Hundreds of cyanobacterial secondary metabolites have been described in the last few decades, in addition to those poisons (Carmichael, 1992; Harada, 2004; Newman and Cragg, 2012; Patterson et al., 1994).

Fatty acids, phenolics, terpenoids, N-glycosides, lipopeptides, linear and cyclic peptides, and alkaloids are among the metabolites that have diverse chemical structures. They also have a wide range of biological actions, including antibacterial, antifungal, antiviral, anticancer, cytotoxic, and enzyme inhibitory properties. The majority of putative bioactive chemicals are made with nonribosomal peptide synthetase machinery or in combination with polyketide synthase (Welker and Von Döhren, 2006). Some bioactive chemicals, such as microviridins and cyanobactins, used ribosomal routes in addition to the above biosynthetic pathway (Arnison et al., 2013). As a result, cyanobacteria are thought to be a source of potential pharmaceuticals, such as antifungal chemicals.

7.5 Mechanism of production of bioactive compounds by cyanobacteria

Ancient, typical, and modern technologies are used to extract biologically active compounds, like fatty acids, polysaccharides, etc., and pigments, such as carotenoids and polyphenols. Traditional solid−liquid extraction and other liquid−liquid extraction processes involve a lot of energy, organic solvents, money, and time. To control these limitations, the latest sustainable extraction technology, such as green technologies, demonstrated various advantages as compared to traditional methods that include decreased solvent usage, much short extraction times, and high-geared performance at lower temperatures. These approaches provide higher selective characters for isolating desirable chemicals, as well as the ability to minimize undesired interactions during extraction and large-scale recovery (Cheng et al., 2010). Because bioactive substances have distinct physical and chemical properties, an extraction that is size dependent is also used. Finding an appropriate method for extracting the desired bioactive chemical and, also, optimizing the extraction procedure are critical. Ultrasonification and other regular procedures, homogenization, freezing, maceration (in the presence of liquid nitrogen), and thawing were used to extract phycobilin proteins from Rhodophyta (Mittal et al., 2017). Modern methods such as ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), subcritical water extraction (SWE), and microwave-assisted extraction (MAE) have already been used for the extraction of bioactive compounds

because they have advantages over traditional methods. Microalgae and associated bioactive compounds (-3 fatty acids) are extracted utilizing revolutionary procedures, which are based on the principle of extraction with supercritical fluids at supercritical temperatures and pressure like SFE (without the use of enzymes). Carbon dioxide (CO_2) is the most often utilized SFE solvent to improve and increase the efficiency of extraction and isolation (Delattre et al., 2016) because it is safe, has low cost, and is less toxic. In microscale supercritical extraction equipment, carotenoids and chlorophylls were extracted from microalgae utilizing CO_2-modified ethanol for elevated extraction (del Pilar Sánchez-Saavedra et al., 2010).

The UAE approach was used to extract the bioactive component taurine from Porphyrayezoensis, which resulted in a greater yield. The SFE method, which is used to extract green, is primarily utilized to extract high-value bioactive chemicals, including fatty acids and algal pigments. When compared to other extraction processes, SFE has a quick processing time, a low degradability of product, and always requires minimum solvents because it uses supercritical fluids (Sosa-Hernández et al., 2018). Depending on the extracted chemical, this method uses both low and high-frequency ultrasound to enhance separation. The extraction temperature in the UAE method can be maintained using heat-exchange devices, which is very useful when extracting thermally labile chemicals, such as carotenoids. The procedures, on the other hand, require a considerable volume of solvents, are quite expensive, and are not environmentally friendly. Proteins and carotenoids are typically extracted by using a solvent-extraction process that employs a variety of solvents. During superheated water extraction and pressurized hot-water extraction in PLE, water is used as a polar solvent for the extraction of oxygen and light-sensitive carotenoids (Poojary et al., 2016). Microwave radiation is used in the MAE method to provide heat to the medium of extraction and aid in the dissolution, and improve the mass transfer of analytes. The efficiency of MAE is affected by the extraction condition, algal cell shapes, and microwave treatment (Poojary et al., 2016).

7.6 Fungi: *biology and bioactive compounds*

Fungi-derived biologically active chemicals are new scaffolds that may be exploited as bioresources with anticancerous, antiviral, antifungal, and antibacterial properties. Fungi are the decomposers that secrete extracellular enzymes, can be easily stored in the laboratory for culture processes, and grow rapidly. As a result, the fungus can produce metal nanoparticles and nanostructures by using intracellular or extracellular reducing enzymes (Srivastava, 2019). Plant endophytic fungi are a new and important source of natural bioactive chemicals with uses in the food sector, agriculture, and medicine. Endophytic fungi have yielded several important bioactive chemicals with anticancer, antimicrobial, cytotoxic, and insecticidal properties in the last two decades (Zhao et al., 2011).

7.7 Mechanism of production of bioactive compounds by fungi

Plant tissue samples are cultured on a specified medium to isolate a fungal endophyte linked with a medicinal plant. Fungal endophytes develop on a media plate after a specific amount of time has passed. After subculturing fungal endophytes, individual fungal strains may be identified using microscopic and molecular methods. Isolation of genomic DNA from fungal endophytes is followed by PCR for molecular identification of the internal transcribed spacer (ITS) region. The fungal strain is identified using BLAST analysis of raw sequences acquired from PCR product sequencing. Strains are cultivated in liquid culture for large-scale manufacturing of the fungal strain. Bioactive substances generated from fungal endophytes are separated using an ethanol or methanol extract. Biological tests are used to evaluate the efficacy of bioactive substances that have been extracted. For molecular identification and quantification, isolated bioactive chemicals are submitted to mass spectrometry investigations and nuclear magnetic resonance. After preclinical and clinical studies, a genome-editing technique may be used to improve the synthesis of the most effective bioactive chemicals, which could then be evaluated for drug discovery and development (Rai et al., 2021).

7.8 Applications of bioactive compounds of actinomycetes, cyanobacteria, and fungi

7.8.1 Production of antibiotics

Actinomycetes produce 75% of antibiotics, predominantly antibacterial antibiotics. Streptomycetes often reveal novel antibiotics, such as mediomycins A, B, and clethramycin from Streptomyces mediocidicus ATCC23936 and Streptomyces malaysiensis DSM4137 (Hussain et al., 2002). Because of their medicinal applications, polyketides are particularly important natural compounds. Nystatin (antifungal), avermectin, and Erythromycin (antibacterial) are examples of such polyketides (antiparasitic). *Streptomyces* sp., which is regarded as the primary manufacturer of antibiotics, created all of the prior antibiotics (Jalal and Hasan, 2021).

Chrysophytes, dinoflagellates, haptophytes, diatoms, chlorophytes, and cyanobacteria are all sources of antibiotics made by microalgae. Tannins, alcohols, bromophenols, peptides, alkaloids, fatty acids, halogenated compounds, amides, polysaccharides, lipopeptides, and a variety of other peculiar chemicals are among the active components (Hannon et al., 2010). In terms of the use of antibiotics by fungi, beta-lactams are the most common antibiotic class. They account for a significant portion of the antibiotic market. Clavulanic acid, penicillin, carbapenems, and cephalosporins are all included. Fungi are responsible for the synthesis of cephalosporins and penicillin, among other antibiotics (Masurekar, 2008).

7.8.2 Antiviral agents

Complestatins, also known as peptides, were generated by *Streptomyces lavendulae*. They did not demonstrate inhibitory efficacy against HIV enzymes, where these peptides engage with the target cells' cell surface molecules and prevent HIV-1 from adsorbing to the cells (Chiu et al., 2001). Protease inhibitor (PISC-2002) was generated by *Streptomyces chromofuscus* from culture supernatants. In the influenza virus, PISC-2002 performs a significant role as an antiviral agent (Angelova et al., 2006). Pimprinine is an extracellular alkaloid generated by *Streptomyces* sp. Pimprinine has antiviral efficacy against Enterovirus 71 (EV71) and has remarkable physicochemical qualities, antibacterial capabilities, and anticonvulsant activity (Wei et al., 2014).

Cyanobacteria are a never-ending supply of chemicals with antiviral potential, although just a few have been discovered thus far. Cyanovirin-N (CV-N) (Nisbet and Sleep, 2001), a very strong virucidal 11-kDa protein recovered from extracts of the farmed cyanobacterium Nostoc ellipsosporum, is one of the products identified. This renders a wide range of HIV strains inactive indefinitely. CV-N binds to the viral envelop glycoprotein gp120 rich mannose glycans with high affinity, preventing viral engagements with target cell receptors that are required for viral entry and cell-to-cell fusion (Jeong et al., 2006). Microvirin (MVN) (Payne et al., 2011), a newly discovered cyanobacterial lectin derived from *Microcystis aeruginosa* (Huskens et al., 2010), suppresses HIV-1 infection in a wide range of HIV-1 strains. MVN, like CV-N, attaches to a number of glycans on the HIV envelope and, thereby, prevents the virus from entering the body. MVN, on the other hand, does not promote viral replication in pretreatment peripheral blood mononuclear cells and is 50 times less cytotoxic than CV-N. Oscillatoria agardhii agglutinin (Macias et al., 2007) is a new cyanobacterial lectin that has been shown to be effective against HIV. Its structure hasn't been fully elucidated yet. Its basic structure and carbohydrate binding data are the only information available (Koharudin et al., 2011).

Fungi are a diverse group of organisms that produce a wide range of physiologically active substances. Thousands of compounds with various biological properties have been identified and are still being studied during the last several decades. Fungal compounds having antiviral properties have been investigated less thoroughly, but the number of these studies is growing (Linnakoski et al., 2018).

7.8.3 Antitumor compounds

Streptomycetes are unique families of bacteria that can produce cytotoxic chemicals with anticancer properties (Sahu et al., 2008; Soria-Mercado et al., 2005). Mitomycin is a natural substance that contains aminobenzoquinone and the aziridine ring systems, among other

functional groups. *Streptomyces lavendulae* produces mitomycin C (MC), which has been used clinically for anticancer therapy (Feng et al., 1999; Mao et al., 1999).

Structures with high cytotoxicity have been identified in recent years. The majority of these structures have been discovered in the *Leptolyngbya* cyanobacteria genus. The discovery of a class of secondary metabolites from cyanobacterium, the lipopeptides, was made possible by the pharmaceutical industry's efforts to produce new and more effective cancer medicines, as well as the screening of compounds from marine species. These compounds are utilized to trigger apoptosis by displaying their biological actions on tumor cells. Apratoxin A (DeMan, 1999) was also isolated from Lyngbya majuscule and has shown significant cytotoxicity against cancer cell types in vitro. But, in vivo action against a colon tumor was diminished, and it was inefficient against a breast tumor (Luesch et al., 2001). Dolastatins are antiproliferative drugs that stop cancer cells from growing and cause them to die (programmed cell death). Dolastatin binds to tubulin, a microtubule subunit protein that is essential for cell structure and kinetics, disrupting the cell cycle and halting mitotic cell division (Zheng et al., 2011). Bisebromoamide, a cytotoxic peptide, derived from the cyanobacterium *Lyngbya* sp., showed cytotoxicity and strong protein kinase inhibition in HeLa cells (Teruya et al., 2009). Given the study's findings of low camptothecin concentrations in tree roots and poor output from chemical synthesis, fungal fermentation appears to be a potential method for industrial camptothecin production. It is used to treat colon cancer and has remarkable anticancer efficacy against cancer in the uterus, lungs, and ovaries (Amna et al., 2006).

7.8.4 Production of pigments

Actinomycetes are distinguished by their pigment synthesis on natural and manufactured media. Blue, violet, red, rose, yellow, green, brown, and black are common hues. The pigments could either permeate into the media or remain in the mycelium (Thompson et al., 2002). Actinomycetes have been reported to produce a variety of antibiotics, and these antibiotics also contain a variety of colors (Wawrik et al., 2007). Melanins are widely used in medicine and cosmetics. In peptone-yeast extract-iron, *Streptomyces virginiae* produced the most pigment, followed by tyrosine liquid medium. Antibiotic and anticancer properties are found in microbial pigments, which are also safe to use in humans. A few of them have also been shown to be food-grade pigments. They are simple to make and cost little (Amal et al., 2011; Sathi et al., 2002). *Streptomycetes* are a big, industrially significant class of bacteria that can manufacture a variety of antibiotics and colors. The capability of these organisms to create colors is affected by various conditions regarding nutrition and culture of microorganisms, and it can be considerably augmented or completely lost. As a result, it's critical to develop the correct mix of culture-related conditions to promote growth and pigment production. The full range of pigment applications is yet to be discovered (Morens et al., 2004).

Chlorophyll (Falkowski et al., 2004) is found in the reaction centers of pigment–protein complexes in all oxygenic photoautotrophs, or organisms that do oxygenic photosynthesis. The pigment chlorophyll absorbs light and performs photochemistry, while the remaining pigments absorb light at wavelengths other than chlorophyll's and may do light harvesting as well as other tasks. Because of their densely conjugated network of single and double bonds, carotenoids absorb light (polyenes). Carotenoids are a class of organic chromophores that have an absorption band of 400–550 nm. Their main structure consists of two rings connected by conjugated double bonds to an 18-carbon chain. They are generally hydrocarbons (carotene and carotene (Singh et al., 2005)) or oxygenated hydrocarbons (lutein, violaxanthin (Connors et al., 2014), zeaxanthin (Arnold et al., 2003), etc.). Plants and microalgae have a number of defense mechanisms against external aggressors, such as too much light. Many of these mechanisms are utilized to improve photosynthesis as well.

Fungi generate carotenoids, which are tetra-terpenoid pigments that are powerful antioxidants. They're utilized as pharmaceuticals, food coloring agents and additives, animal feeds, and in the manufacture of cosmetics. They are made up of not only hydrocarbons, i.e., lycopene and carotenes, but also oxygenated derivatives such as xanthophylls and are used to prevent heart diseases, muscular degeneration, cancer, and aging (Roukas, 2016).

7.8.5 Biopesticide agents

To naturally control insects, microorganisms such as those that are averse to insects are utilized. Insecticidal active substances produced by actinomycetes are utilized in the biological control of the house fly *Musca domestica* (Sundarapandian et al., 2002). After employing actinomycetes pesticide, the mortality of larval and pupal stages was substantial, reaching up to 90% (Bloemberg and Lugtenberg, 2001). The mosquito *Culex quinquefasciatus* was successfully controlled by actinomycetes (Hasani et al., 2014; Sharma et al., 2014).

Nitrogen is a critical macronutrient for agricultural production and global food security. This macronutrient is necessary for biomolecules like DNA, proteins, and amino acids to develop. Despite the abundance of nitrogen in the atmosphere, water, and soil, most plants and microalgae are unable to digest dinitrogen, the most readily available form of nitrogen (N2). Diazotrophic heterocystous cyanobacteria are capable of ingesting N2 via the nitrogen-fixation process and have long been utilized as a rice biofertilizer. Anabaena azollae is a nitrogen-fixing cyanobacterium that lives in a symbiotic relationship with the water fern Azolla and can fix atmospheric nitrogen. Aside from biological nitrogen-fixation, another fascinating application of cyanobacteria in agriculture is the breakdown of pesticides and the production of plant pathogen inhibitors (Shi and Hall, 1988). Biopesticides, particularly fungi-based biopesticides, are a promising new option with high

specificity, low resistance, integrated pest control techniques, high biodegradability, and almost no known health concerns (de la Cruz Quiroz et al., 2019).

7.8.6 Antiinflammatory compounds

Marine actinomycetes generate saphenic acid and lipomycin, both of which have antiinflammatory properties (Manivasagan et al., 2014). *Micromonospora* sp. was also found to produce bioactive substances with antiinflammatory as well as antibacterial properties (Jayaprakashvel, 2012). *Streptomyces arenicola* also produces the antiinflammatory metabolites cyclomarin A and C, with cyclomarin A found to have antituberculosis and antimalaria properties (Barbie and Kazmaier, 2016). When cyclomarin A was used topically or intraperitoneally, it was found to reduce edema (Imada, 1995). Numerous biosurfactants were generated by *Streptomyces griseoflavus* and *Nocardiopsis* A17 (Chakraborty et al., 2015).

Organic extracts of solvents and -carotene, chlorophyll a, and chlorophyll b which are the pigments from *Chlorococcum humicola*, may inhibit the growth of *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, and other fungi. Antifungal activity of Nostofungicidine from Nostoc commune against *Aspergillus candidus* (Kim et al., 2014). Marine fungus has the capacity to create a wide range of antiinflammatory chemical compounds. Fungi found in or on sediments, water, corals, and algae have produced new secondary metabolites with significant antiinflammatory properties. Marine fungus compounds have gotten a lot of interest lately because of their unusual mode of action, and they have become a hotspot for antiinflammatory medication research (Xu et al., 2019).

7.8.7 Biosurfactant

A surface-active chemical generated mostly by microorganisms is known as a biosurfactant. The phrase refers to substances that have some effect on interfaces. Surface tension measurements are used to determine the effectiveness of biosurfactants. The terms surfactant and emulsifier are commonly used interchangeably in the literature (Campbell and Mougeot, 1999; Feller et al., 1998). Because of their selectivity, biodegradability, and low toxicity, biosurfactants have various advantages. Also, the ability of these biosurfactants to perform under high temperature, pH, and salinity conditions is well-known. In the generation of bio emulsifiers, actinomycetes play a critical role. Certain actinomycetes, such as *Nocardia* sp., produce trehalose dimycolates (Thampayak et al., 2008). Numerous biosurfactants were also generated by *Streptomyces griseoflavus* and *Nocardiopsis* A17 (Chakraborty et al., 2015).

Cyanobacteria's biosurfactant properties are stable under harsh settings where they exist without losing their emulsification capacity; therefore they can be used for bioremediation

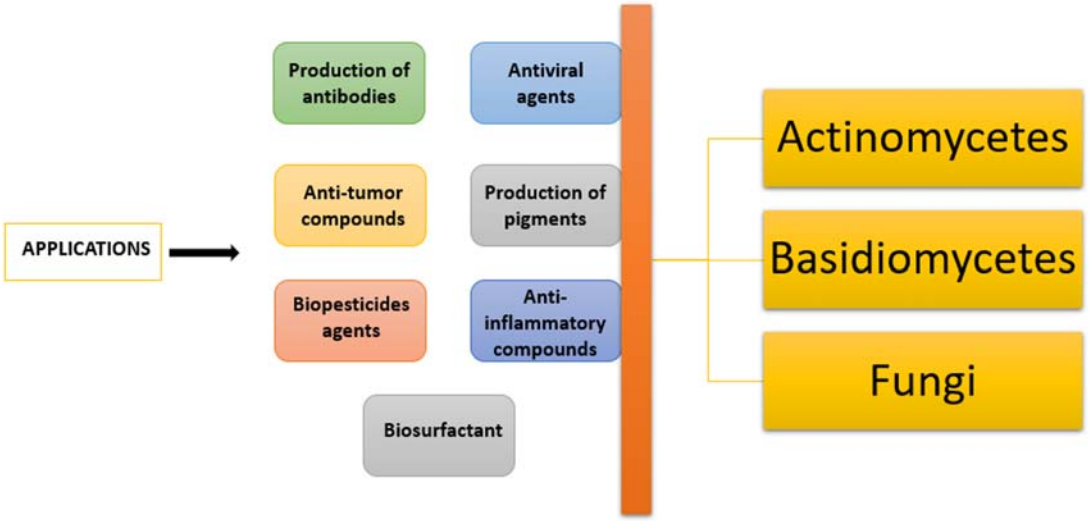

Figure 7.2
Applications of bioactive compounds produced by actinomycetes, cyanobacteria, and fungi.

(Tripathi et al., 2020). Biosurfactants are mostly generated by bacteria and yeast, although research in recent years have revealed that filamentous fungus may also create them (Castiglioni et al., 2009; Velioğlu and Ürek, 2015). These bacteria have the ability to generate a variety of biotechnologically important compounds, such as pigment enzymes and antibiotics, and their primary isolation sources are plants and soil (Adrio and Demain, 2003). Fig. 7.2 represents the applications of bioactive compounds produced by actinomycetes, cyanobacteria, and fungi.

7.8.8 Some specified applications

The real-life worth of commercial enzymes has increased significantly as a result of their various applications in the pharmaceutical, food, and detergent industries. Actinomycetes contain a unique active enzyme that can catalyze a variety of metabolic reactions with the help of additional enzymes (Kumar et al., 2014). Important enzymes generated by *Streptomyces* species include amylase, protease, and cellulose, which have economic applications in a variety of industries (Kundu et al., 2006). In natural water and sediments, L-glutaminase, L-asparaginase, and galactosidase play an important role in carbon and nitrogen biocycling. L-glutaminase and L-asparaginase, both generated by marine streptomycetes, have anticancer properties (Sahu et al., 2007; Vignardet et al., 1999). As a result of different mergers and acquisitions, the worldwide industrial enzyme market has grown steadily. In recent decades, food and beverage enzymes have received a lot of attention. On a straight scale, there are

additional patent number increments in the decades (Prakash et al., 2013). Actinomycetes from the sea are a promising source of enzyme inhibitors (Bode and Huber, 1993; Sun et al., 2015).

Marine algae seem to be the most important bioactive chemical with an anticoagulant action among all marine sources. Phlorotannins and SPs produced from marine algae have shown tremendous promise in biomedical research as anticoagulant medicines (Kim and Wijesekara, 2011). The prolongation of activated partial thromboplastin time, thrombin time, and prothrombin time determines these drugs' anticoagulant action. These substances have antiplatelet, anticoagulant proteins with fibrinolytic enzymes, which can regulate endothelial cell activities and activate the fibrinolysis system (Matsubara, 2004).

7.9 Conclusion

A promising natural resource is microalgae and cyanobacteria. The commercial possibilities of goods produced from them are limitless. However, there are currently very few goods available on the market. Commercialization is hampered by the high cost of acquiring some microalgae products and the difficulty of scaling up others. Furthermore, consumers are not yet used to this option, with the exception of a few isolated examples. For the separation of bioactive chemicals, the screening of fungal endophytes and their relationships with host plants is necessary. However, the rising scarcity of resources, along with improved extraction and manufacturing processes, is making microalgae, cyanobacteria, and fungus a viable renewable supply of bioactive compounds and fine chemicals.

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CHAPTER 8

Bioactive compounds from endophytic microorganisms

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8.1 Introduction

Anton de Bary, in 1886, introduced the term "Endophytes" for the group of organisms that resides within the plant tissues throughout their life without causing any disease symptoms. They are distinct from Epiphytes, which reside on plant surfaces, and mycorrhizal endophytes, which produce external structures from the host plant. Endophytes are reported to enhance host resistance against pathogens, which has mediated through secondary metabolites production, mainly, via their role in nutrient uptake, and improving host tolerance to environmental conditions like heat and salinity (Mishra et al., 2014). Whereas, "microbiome" refers to the different communities of microbes that grow collectively inside humans and help them to stay healthy (Wang et al., 2017). Endophytic fungi are considered highly diverse fungi, mostly belonging to ascomycetes, anamorphic fungi, basidiomycetes groups, etc., and categorized as obligate and facultative ones. Obligate endophytes reside inside the plant tissue throughout their lifetime, and the facultative ones are known to survive in the soil, on the plant surface, inside the plants, as well as on artificial nutrients. There are cultivable and uncultivable endophytes too (Christina et al., 2013). Endophytes are distributed widely in marine algae, mosses, ferns, grasses, lichens, and palms to large trees of coniferous and deciduous varieties and are known to be more diverse in the tropical forest, thus revealing major fungal biodiversity. The age of this fungal biodiversity is estimated at 2.27 million years on earth (Kumar et al., 2014). Many of the roots associated with mycorrhizal fungi are reported to have a role in host growth and nutrient uptake. They are capable of nitrogen (N) fixation, phosphate solubilization, its uptake, siderophores, 1-aminocyclopropane-1-carboxylate deaminase (ACCD) production, and production of plant hormones such as auxin, abscisins, ethylene, gibberellins, and indole acetic acid (IAA), which are important for plant growth and development regulation. The mechanisms of enhancement of nutrient uptake by plants colonized by endophytes have remained elusive but might be due to their extra metrical mycelium extending from the host roots that may increase the surface area and therefore increase host access to soil nutrients (Selim et al., 2012).

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Endophytes induce hyperparasitism by protecting the host plant from pathogens. It parasitizes around the hyphae of pathogens, penetrates them, and secretes enzymes to decompose their cell wall. Fungal endophyte-mediated plant resistance to pathogens has been well studied and explored as a biocontrol agent for crop diseases. A mass of bioactive compounds has been reported, which are alkaloids, peptides, steroids, terpenoids, quinones, flavonoids, aliphatic, and aromatic compounds produced during the amino acid, polyketide, shikimate, and mevalonate pathways (Gao et al., 2010). Apart from plants and animals as sources of drugs, endophytes are prolific producers of secondary metabolites and possess unique structural characteristics.
The discovery and importance of endophytes originated with the isolation of Paclitaxel (Taxol), a 1-million-dollar anticancer drug from the fungus *Taxomyces andreanae* derived from *Taxus brevifolia* in the early 1990s. Due to its unavailability and high price, other endophytic sources, namely, *Pestalotiopsis microspore, Pestalotiopsis guepini, Periconia* sp*., Fusarium solani, Podocarpus* sp*., Aspergillus* sp., *Alternaria* sp., *Taxus baccata*, and *Paraconiothyrium variabile* have been reported as producing strains (Somjaipeng et al., 2015; Malik et al., 2011).
A plethora of potentially useful plant entophytes have been explored for antimicrobial, antitubercular, antiinsecticidal, antiparasitic immunosuppressant, anticancer, antiviral, antioxidant, antiinflammatory, and enzyme-inhibitory compounds, thereby having potential application in the pharmaceutical and agricultural industry (Kaul et al., 2012; Joseph and Priya, 2011). Therefore the endophytes bear importance in drug discovery for treating dreadful human diseases.

8.2 Isolation, enrichment, purification, and characterization of endophytes for bioactive compounds

Endophytes are mainly isolated and reported from medicinal plants (35%), crops (29%), and plants grown in special environments (18%) like mangrove, arid and semiarid regions, and from other plants (18%). For the selection of a host plant, some strategies like plants from great biodiversity areas, plants growing in special habitats, infected plants without any promising symptoms, plants from traditional medicine systems, and plants from barely or desolated land are considered (Yu et al., 2010). Endophytes are mostly prevalent in the leaves due to their large surface area (67%), followed by the stem, the roots, the seeds, and finally, the least prevalence is noted in flowers due to wilting in a few days. Each time, healthy and mature samples of the host plant are selected, stored in separate zip lock plastic bags or containers and processed within 4−24 h after the collection. Furthermore, the fragile plant samples can be surface disinfected alone or in combination with distilled water, acidic electrolyzed water, sodium hypochlorites, ethanol, formaldehyde, and hydrogen peroxide, to remove any dirt and contaminants, followed by inoculation in specific culture media. This allows true endophytes to grow in culture media. Surface sterlization can also minimize the occurrence of the changes in color and texture of the

plant samples. Moreover, endophyte isolation depends on plant type, selection of plant tissue, sampling season, sterilization treatment and time, media type, and culture conditions (Gouda et al., 2016). Choice of growth medium is crucial for endophyte isolation as it directly affects the number and the type of endophytic bacteria associated with plant tissues. Different media like Potato dextrose agar (PDA), Starch casein agar (SCA), Czapek yeast extract agar, Yeast extract malt extract agar (YEMA), Yeast extract sucrose agar, corn meal agar, Actinomycetes isolation agar, chitin-vitamin B, Humic acid vitamin B agar, etc. are currently used for the isolation of endophytic actinomycetes. Most of the time, the media are supplemented with one of these: cycloheximide 50 μg/mL, or nystatin 50 μg/mL, or chloramphenicol (50 μg/mL), or penicillin G (100 μg/mL), or streptomycin sulfate (250 μg/mL) to suppress bacterial and fungal contaminant load. Sometimes powdered plant materials were added to the isolation media to enhance the growth of the endophytic fungi as well as to minimize fungal contamination. Endophytes are cultivated at 25°C−30°C temperature for 1−3 weeks in darkness. Secondary metabolites could be induced by various nonbiological stress factors such as UV light, heavy metal ions, or salt stress. 0.001%−1% of the endophytes present in plant tissues are cultivable. Selected promising endophytes can be identified by morphological methods and by the 18S rDNA analysis method (Waheeda and Shyam, 2017). Crude extracts from the fermentation broth of each endophyte should, firstly, be tested through the paper disk diffusion method, broth dilution assay, well diffusion assay, dual culture method, and mycelial radial growth test. The efficacy of the antimicrobial activity is measured by minimal inhibitory concentration (MIC) or 50% inhibitory concentrations value (IC_{50}). Fermentation broth or mycelial crude extract containing bioactive compounds is concentrated by solvent extraction using ethanol, butanol, ethyl acetate, followed by lyophilization. Separation and characterization of bioactive compounds from crude extract can be done by thin-layer chromatography (TLC), column chromatography, GC-Ms, and FTIR (Selim et al., 2012) (Fig. 8.1).

8.3 Antibacterial and antifungal compounds from endophytes

With the alarming rate of increase in the human population, a reality to be dealt with emphatically, another cause of worry nonetheless is a variety of new types of health-related issues popping up widely that need dealing with strongly too. As per the released report in January 2018 by WHO in support of the Global Antimicrobial Surveillance System (GLASS, May 2015) on global antibiotic resistance crisis owed to medicines in use for the treatment of hospital and community-acquired infections due to *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella* spp., *Neisseria gonorrhoeae, Shigella* spp. and *Acinetobacter baumanii* across the 52 countries, there are indications that *N. gonorrhoeae* is evolving as a superbug after *Salmonella* spp. and *S. aureus* and is followed by *K. pneumoniae* as high priority areas for research and development of new and effective

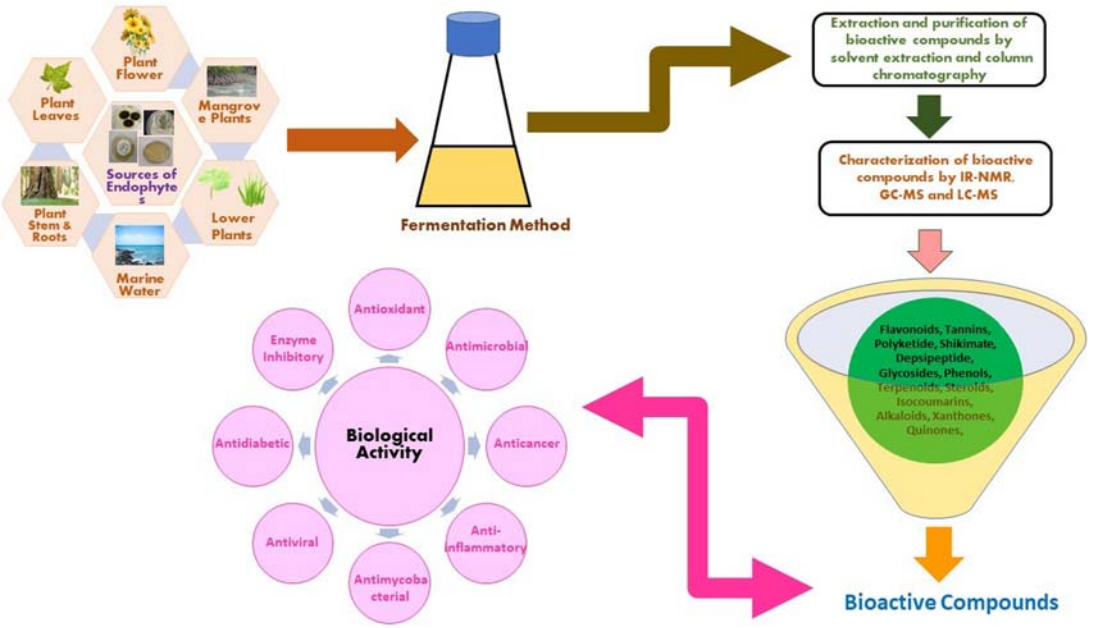

Figure 8.1
Isolation, enrichment, purification, and characterization of endophytes for bioactive compounds.

antibiotic treatment against such unprecedented incidences of incremental drug resistance (Hug et al., 2018). The development of bacteria and fungi resistance among pathogens due to widespread and uncontrolled use of antimicrobial compounds is pivotal for novel drug discovery. It involves the search for new sources or derivative synthesis from the existing drugs using combinatory chemistry. Due to high investment, low profit, and faster resistance to existing drugs, the strategies for novel bioactive compounds to be discovered from different sources are being explored, and endophytes are a priority area of action (Berdy, 2012). Different solvent extracts (ethyl acetate and ethyl ethanoate) of *Physalospora* sp., *Crataegus monogyna*, and *Plectophomella* sp., respectively, were screened for antimicrobial activity. The bioactive compound, namely, (-)-Mycorrhizin A (*Plectophomella* sp.), Cytochalasins E and K (*Physalospora sp.*), and Radicinin (*C. monogyna*) revealed promising inhibitory activity against *Chlorella fusca, Ustilago violacea, Eurotium repens, Fusarium oxysporum, Mycotyphamicrospora, Escherichia coli, and Bacillus megaterium* (Hussain et al., 2017). Entophyte *Penicillium sclerotiorum* PSUA13 was isolated from *Garcinia atroviridis*, and it revealed antimicrobial activity against *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans* as well as inhibitory effect on human immune deficiency virus (HIV)-1 integrase and protease enzymes. Broth analysis confirmed novel compounds as Penicilazaphilones A (1), Penicilazaphilones B (2), Penicilisorin (3) along with 2,4-dihydroxy-6-(5,7*S*-dimethyl-2-oxo-*trans*-3-*trans*-5-nonadienyl)-3-methyl benzaldehyde (6), (5 *S*, 6 *R*)

-5,6-dihydro-3,5,6-trimethylpyran-2-one(7) dechloroisochromophilone (4) and (+)-sclerotiorin (5) (Arunpanichlert et al., 2010). *Bacopa monnieri (L.) Pennell (Scrophulariaceae)*, a medicinal plant distributed widely in Asia, Australia, and America, is used effectively in ayurvedic formulations for treating gastrointestinal and neurologic disorders. Katoch et al. (2014) isolated twenty-six different fungi, namely, *Fusarium* sp., *Trichoderma* sp., *Flavodon* sp., *Fomitopsis* sp., and *Pleosporales* sp., from this plant which were screened for antimicrobial and cytotoxic activity. Solvent extracts of *Fusarium oxysporum* and *Fomitopsis* sp. were found to possess potent cytotoxicity (IC_{50} of <20 μg/mL) against HCT-116, followed by MCF-7, PC-3, A-549 cell lines. Isolate *Fomitopsis* sp. revealed promising antibacterial activity against *E. coli, S. typhimurium, P. aeruginosa, S. aureus, K. pneumonia*, and *C. albicans* (MIC 10−100 μg/mL) followed by *Fusarium* sp. and *Pleosporales* sp. (*E. coli, P. aeruginosa*, and *S. aureus*, MIC 10 μg/mL). The plant contains the active compounds Bacosides. *Phomopsis* sp., an endophyte isolated from *Cistus monspeliensis*, revealed two new chromones, Phomochromone A and B, and one new natural cyclopentenone derivative, Phomotenone, together with six known compounds, phomosines A−D, (1 *S*,2 *S*,4 *S*)-trihydroxy-*p*-menthane, and 5-methylmellein from crude ethyl acetate extract. Compounds Phomochromone A/B and Phomotenone showed good antifungal (*Microbotryum violaceum*), antibacterial (*Escherichia coli, Bacillus megaterium*), and antialgal (*Chlorella fusca*) activities. Again, Compound (1 *S*,2 *S*,4 *S*)-trihydroxy-*p*-menthane showed good antialgal and antibacterial activity (Ahmed et al., 2011). In another study on endophytic fungus, *Muscodor tigerii* was isolated from *Cinnamomum camphora* of the Northeastern Himalayas. Chemical analysis revealed 22 volatile organic compounds (VOCs) grouped as steroids (Campesterol and Stigmasterol), terpenoids (Caranol, Phytol, and Squalene), and aliphatic and aromatic compounds (4-Octadecylmorpholine and 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester). Strongest antifungal activity was observed by VOC against *Alternaria alternate* = *Cercospora beticola* (100% Inhibition)> *Rhizoctonia solani* (72% Inhibition)> *Penicillium marneffei* (42% Inhibition)> *Aspergillus flavus* (40.2% Inhibition). The antibacterial activity revealed for *Candida* sp. (54%−63% Inhibition) > *Staphylococcus aureus* and *Pseudomonas aeruginosa* (81% and 51% inhibition, respectively) (Saxena et al., 2015). 320 different endophytes from a Brazilian medicinal plant, *Stryphnodendron adstringens*, were screened against bacteria, fungi, cancer cell lines, and amastigote forms of *Leishmania amazonensis*. The extract of phylotype *Nigrospora cf. oryzae* exhibited a selective antifungal activity and inhibited the growth of *Candida albicans* (IC_{50} 104 μg/mL) and *Cladosporium sphaerospermum* (IC_{50} 213 μg/mL). The extracts of phylotypes *Diaporthe cf. phaseolorum* (IC_{50} 20 μg/mL) and *Xylaria* sp. (IC_{50} 20 μg/mL) displayed anticancer activity against MCF-7 (mammary) and TK10 (kidney) tumor cells. *Sesquiterpene* and *Phaseolinone* were reported to have cytotoxic activity against the CHO (Chinese hamster ovary) cell line from an endophytic *Xylaria* sp. Associated with *Piper aduncum* and (−)-epicytoskyrin from *Camelia sinensis* associated *Diaporthe* sp., no promising antibacterial and leishmanicidal activity were observed (Carvalho et al., 2012). Isolate *Muscodor crispans* from *Ananas ananassoides* produces organic compounds propanoic acid, 2-methyl-, methyl ester; propanoic acid, 2-methyl-; 1-butanol,

3- methyl-;1-butanol, 3-methyl-, acetate; propanoic acid, 2-methyl-, 2-methylbutyl ester; and ethanol. The mixture of all the compounds (VOCs) is effective against a wide range of plant pathogens (*Pythium ultimum, Phytophthora cinnamomi, Sclerotinia sclerotiorum, Mycosphaerella fijiensis*, and *Xanthomonas axonopodis pv. Citri* with 100% inhibition) and human pathogens (*Yersinia pestis, Mycobacterium tuberculosis*, and *S. aureus*) (Mitchell et al., 2010). Another species, *Muscodor yucatanensis*, was isolated from the leaves of *Bursera simaruba*. The endophyte VOCs were evaluated for allelochemical effects and found lethal to *Guignardia mangifera, Colletotrichum* sp., *Phomopsis* sp., *Alternaria solani, Rhizoctonia* sp., *Phytophthora capsici*, and *P. parasitica* but did not have any effect on *Fusarium oxysporum* and *Xylaria* sp. It inhibited the root elongation in amaranth, tomato, and barnyard grass. GC/Ms analysis revealed 23 compounds, including benzene derivatives, phenolic compounds, cyclopentadienes, esters, lactones, alkanes, aldehydes, and carboxylic acids. Allelochemicals are compounds produced by one organism that influence the germination, growth, survival, and reproduction of other organisms (Macías-Rubalcava et al., 2010). *Allamanda cathartica*, a medicinal plant, was used in the treatment of jaundice, malarial complications, enlarged spleen, and as laxative harbor endophyte *Phomopsis* sp. Antibacterial activity of ethyl acetate fraction of the endophytes revealed inhibitory activity against *Pseudomonas* sp (16 ± 0.32 mm), *Escherichia coli* (15 ± 0.30 mm), *Staphylococcus aureus* (25 ± 0.50 mm), *Salmonella typhi* (18 ± 0.36 mm), *Klebsiella* sp (16 ± 0.32 mm) and *Bacillus subtilis* (25 ± 0.50 mm) due to terpene compound which underwent an antibacterial activity (Nithya and Muthumary, 2011). *Plumeria acuminata* and *Plumeria obtusifolia* are medicinal plants from India known for their use as a purgative, a remedy for diarrhea, a cure for the itch, and in the treatment of inflammation and rheumatism. Entophytic fungi *Colletotrichum gloeosporioides* and *Fusarium oxysporum* effectively inhibit the growth of *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi* (MIC 20 μg/mL-100μg/mL). The active metabolites analysis of ethyl acetate extracts of *C. gloeosporioides* showed alkaloids and steroids, whereas *F. oxysporum* extracts revealed the flavonoids, phenol, and phenolic compounds (Ramesha and Srinivas, 2014). A new α-tetralone derivative, (3 S)-3,6,7-trihydroxy-α-tetralone, together with cercosporamide, β-sitosterol, and trichodermin, was reported from *Arisaema erubescens* associated *Phoma* sp. ZJWCF006 by Wang et al., 2012. Compound (3S)-3,6,7-trihydroxy-α-tetralone revealed promising antimicrobial activity against *F. oxysporium* (EC_{50} 413.22 μg/mL) and *R. solani* (EC_{50} 48.5 μg/mL). Compound 2 exhibited cytotoxic activity against the six tumor cell lines HT-29, SMMC-772, MCF-7, HL-60, MGC80−3, and P388 with IC_{50} values of 9.3 ± 2.8, 27.87 ± 1.78, 48.79 ± 2.56, 37.57 ± 1.65, 27.83 ± 0.48, and 30.37 ± 0.28 μM, respectively. *Aspergillus fumigatus* LN-4, an endophytic fungus isolated from the stem bark of *Melia azedarach*, revealed two novel alkaloids, 12β-hydroxy-13α-methoxyverruculogen TR-2 (6) and 3-hydroxyfumiquinazoline A (16), from the fermentation broth. Among 1-39 reported compounds, 16 compounds revealed potent antifungal activities against phytopathogenic fungi viz. *Botrytis cinerea, Alternaria solani, Alternaria alternata, Colletotrichum gloeosporioides, Fusarium solani, Fusarium oxysporum f.* sp. *niveum, Fusarium oxysporum f.* sp. *vasinfectum*,

and *Gibberella saubinettii*. Four compounds named 12β-hydroxy-13α-methoxyverruculogen TR-2 (6), Fumitremorgin B (7), Verruculogen (8), and Helvolic acid (39) exhibited antifungal activities at MIC values of 6.25 − 50 μg/mL (Li et al., 2012). *Gossypium hirsutum*, commonly called cotton plant, was screened for endophytes isolation from root, stem, and leaf tissues. The nonvolatile substances produced by CEF-818 (*Penicillium simplicissimum* 79.9 ± 2.7% growth inhibition), CEF-325 (*Fusarium solani*, 78 ± 3.1% growth inhibition), CEF-714 (*Leptosphaeria* sp. 88.1 ± 0.2% growth inhibition), and CEF-642 (*Talaromyces flavus* 83.8 ± 0.3% growth inhibition) completely inhibited highly virulent phytopathogenic *Verticillium dahlia* (Li et al., 2014). *Rafflesia cantleyi* is a parasitic flowering plant distributed in Borneo, Indonesia, Malaysia, and the Philippines and known for its largest-sized flower in the world. Endophytes from flower and buds revealed taxa *Colletotrichum*, *Cytospora*, and *Gliocladiopsis* and showed promising growth inhibition of *Candida albicans* (IC_{50} of 3.5−8.2 μg/mL). *Colletotrichum and Cystospora* are reported to produce active metabolites, namely, Colletotric acid, Colutellin A, 6-isoprenylindole-3-carboxylic acid, Cytoskyrin A, Cytosporon D, Cytosporon E, 3, 5-dimethyl-8-hydroxy-7-methoxy-3,4-dihydroisocoumarin,3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin, respectively, against bacteria and fungi (Refaei et al., 2011). *Casuarina junghuhniana* associated endophyte *Aspergillus* sp. showed maximum zone of inhibition against bacteria, namely, *Pseudomonas aeruginosa* (24 ± 0.1 mm) followed by *B. subtilis* (21 ± 0.3 mm) and fungi, namely, *Fusarium oxysporum* (34 ± 0.2 mm), followed by *R. solani* (27 ± 0.4 mm), *M. phaseolina* (26 ± 0.5 mm), *F. solani* (25 ± 0.4 mm), *C. lunata* (12 ± 0.3 mm), and *A. alternata* (10 ± 0.3 mm) at 100 μg/mL concentration. HPLC study revealed the presence of gallic acid and salicylic acid, and GC-MS analysis indicates the presence of different secondary metabolites, namely, Phytol, 4H-1-Benzopyran 4-one, 2-(3,4-dimethoxy phenyl-7-hydroxy), Eicosanoic acid, methyl ester, 2-Cyclohexene 3,6, diol-1, one,2-tetradecenoyl , (E)-9-Octadecenoic acid ethyl ester, 4,5,7-Trihydroxy isoflavone and Estra-1,3,5(10)-trien 17 a-ol (Bose and Gowrie, 2017). Phenylpyridone derivatives, Citridones E, and curvularins were isolated from *Garcinia multiflora* associated endophytic fungus *Penicillium sumatrense* GZWMJZ-313. The compound revealed antifungal and antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium perfringens, E. coli*, and *Candida albicans* with MIC values ranging from 8 μg/mL to 64 μg/mL (Xu et al., 2019). Endophytic fungus isolated from *Taxus baccata* bark displayed considerable antimicrobial activity against *Staph. aureus, Staph. epidermidis, B. subtilis, K. pneumoniae, E. coli, Shg. Flexneri, C. albcans, C. topicalis* with zone of inhibition 10 to 27 mm at 1 mg concentration. The active fractions confirmed the metabolites, namely,1-tetradecene, 8-octadecanone, 8-pentadecanone, octylcyclohexane, and 10-nonadecanone (Tayung et al., 2011).

8.4 Antiviral compounds from endophytes

Viruses are the obligate intracellular parasites responsible for various life-threating diseases to humans and animals. In plants, it affects overall growth and crop yield. Current

medications, including antiviral drugs and vaccines, have limitations due to the rate of viral mutations and the reduced efficacy of medications (Manganyi and Ateba, 2020). Antiinfluenza A viral (H1N1) activity of two isoindolones derivatives emerimidine A and B from *Aegiceras corniculatum* associated endophytic fungal strain *Emericella* sp. R was revealed by Zhang et al., 2011. Further, Hydroanthraquinone and Azaphilones derivatives, namely, 6-O-dimethyl-4-dehydroxyaltersolanol A, 4-dehydroxy altersolanol A, Altersolanol B, and Chermesinone B were isolated from the culture broth of *Nigrospora* sp. YE3033, an endophytic fungus obtained from *Aconitum carmichaeli*. All these four compounds exhibited the antiviral activity against the H1N1 influenza virus (Stain A/Puerto Rico/8/34) with the IC_{50} values of 2.59, 8.35, 7.82, and 0.80 μg/mL, respectively. The compound Chermesinone B being low cytotoxic would be a future potential drug candidate in drug discovery against influenza A virus (Zhang et al., 2016). In another study, carried out on the endolichenic fungal strain *Nigrospora sphaerica*, revealed two new heptaketides, (+)-(2*S*,3 *S*,4a*S*)-altenuene and (−)-(2*S*,3*S*,4a*R*)-isoaltenuene, together with six known compounds, (−)-(2*R*,3*R*,4a*R*)-altenuene, (+)-(2*R*,3*R*,4a*S*)-isoaltenuene, 5′-methoxy-6-methyl-biphenyl-3,4,3′-triol, alternariol, alternariol-9-methyl ether, and 4-hydroxyalternariol-9-methylether. The similar compounds were also reported from two endolichenic fungal strains *Alternaria alternata* and *Phialophora* sp. The compounds alternariol and alternariol-9-methyl ether showed in vitro antiviral activity against herpes simplex virus (HSV) with IC_{50} values of 13.5 and 21.3 μM respectively (He et al., 2012). Two Sesquiterpenoid, compounds were extracted from the fermentation broth of endophytic fungus *Phoma* sp. isolated from *Aconitum vilmorinianum* plant roots. The compounds phomanolide and (−)-6-methoxymellein showed antiinfluenza A virus activity (H1N1) with IC_{50} values of 2.96 ± 0.64 and 20.98 ± 2.66 μg/mL, respectively (Liu et al., 2019). In another study, similar compounds were extracted from *Ephedra aphylla*, associated endophytic fungi *Pleospora tarda* for antiherpes simplex (HSV-2) and vesicular stomatitis viruses with 40.7% and 15.2 μM (Selim et al., 2018). Antidengue inhibitory activity of Brefeldin A compound extracted from *Angelica keiskei* associated endophytes *Penicillium* sp. FKI-7127 was reported by Raekiansyah et al., 2017. Endophytic fungi like *Fusarium equiseti, Scopulariopsis fusca*, and *Geotrichum candidum*, were isolated from brown alga *Padina pavonica*, and screened for antiviral activity. The fungi *F. equiseti* extract exhibited the highest inhibitory against the hepatitis virus (HCV) NS3-NS4A protease enzyme, with IC_{50} 23 ± 2.5 μg/mL. Four active principles were identified as diketopiperazine (IC_{50} 8.20 ± 1.7 μg/mL) and ω-hydroxyemodin (IC_{50} 10.7 ± 2.3 μg/mL), Ara-A (IC_{50} 21.3 ± 4.12 μg/mL), and Cordycepin (IC_{50} 25.4 ± 2.3 μg/mL) that exhibits a potent inhibitory effect on HCV NS3-NS4A protease (Hawas and Al-Farawati, 2017). Several studies demonstrated endophytic fungi for antiretroviral activities against human immunodeficiency virus type 1 (Wellensiek et al., 2013). *Quercus emoryi* plant-associated endophyte *Alternaria tenuissima* QUE_1Se revealed antiviral activity against the HIV-1 virus. The extract revealed five altertoxins derivatives which inhibit the HIV-1 completely

up to 1.5 μM concentration (Bashyal et al., 2014). In another study, antiviral activity against HIV-1 was evaluated using *Achyranthes bidentata* associated *Phomopsis* sp. CGMCC No. 5416, fungal extract. The active principle chromanones showed inhibitory activities against HIV-1 with IC_{50} values of 20.4 and 32.5 μg/mL, respectively (Yang et al., 2020). Indolosesquiterpene compound (xiamycin) was first reported time from endophytes Streptomyces sp. GT2002/1503, isolated from mangrove plant Bruguiera gymnorrhiza for anti-HIV-1 activity (Ding et al., 2010). *Cytonaema* sp. F32027 was isolated from *Quercus* sp. and evaluated for cytomehalovirus protease inhibitory activity. The extract revealed two compounds, cytonic acid A and B, as having the highest enzyme-inhibitory activity at IC_{50} 43 μmol and IC_{50} 11 μmol, respectively (Guo et al., 2000).

8.5 Antiinflammatory compounds from endophytes

Inflammation is a cytological response induced by trauma, infections, or postischemic, toxic, or autoimmune injury. The studied inflammatory biomarkers are interleukins (IL-1β, IL-6; IL-8); tumor necrosis factor (TNF-α); nuclear factor-ĸB (NF-ĸB), intercellular adhesion molecule-1, inducible type cyclooxygenase-(COX-) 2, prostaglandin E2 (PGE2); 5-lipooxygenase (5-LOX); and inducible nitric oxide synthase (iNOS). Overproduction of these inflammatory mediators leads to different kinds of cell damage. In addition, prolonged inflammation causes many inflammatory diseases such as inflammatory bowel disease, juvenile idiopathic arthritis, multiple sclerosis, gastritis, rheumatoid arthritis, bronchitis, and atherosclerosis. A plethora of studies is reported to tackle the endophytic sourced bioactive compounds as antiinflammatory agents from associated medicinal and mangrove plants. (Abdel-Azeem et al., 2019). New diterpenoid, sesquiterpenoids, and α-pyrone derivatives were isolated from *K. candel* associated mangrove endophytic fungus *Diaporthe* sp. QYM12. Compounds diaporpenoid A (IC_{50} 21.5 μM) and diaporpyrones A (IC_{50} 12.5 μM) showed potent antiinflammatory activities by inhibiting the production of nitric oxide (NO) in lipopolysaccharide (LPS)-induced RAW 264.7 cells (Chen et al., 2021). *Fusarium* sp. was isolated from rotten roots of *Scutellaria baicalensis* and investigated for metabolite extractions. Compounds fusarubin, 8(Z)-lucilactaene,4(Z)-lucilactaene, solaniol, javanicin, lucilactaene, 9-desmethyl herbarine, NG391 and NG393 revealed to inhibit NO production and suppress proinflammatory cytokines (IL-6 and TNF α) expression in LPS-stimulated macrophage cells (Maharjan et al., 2020). The endophytic fungus *Aspergillus fumigatus* isolated from Stem of *Erythrophloeum fordii Oliv* produces diketopiperazine alkaloid, i.e., Pseurotin A demonstrated antiinflammatory activity by inhibiting proinflammatory factors with IC_{50} 5.20 μM in BV2 microglial cells (Shi et al., 2015). Endophytic fungus *Fusarium* sp. isolated from *Mentha longifolia* L. (Labiatae) roots revealed three new ergosterol derivatives that were assessed for 5-lipoxygenase (5-LOX) inhibitory activity. Compounds fusaristerols B (IC_{50} 3.61 μM) and (22*E*, 24 *R*)-5β,8β-epidioxyergosta-22-en-3β-yldecanoate (IC_{50} 2.45 μM), showed 5-LOX inhibitory activity as compared to indomethacin (IC_{50} 1.17 μM) (Khayat et al., 2019). An ergoflavin, from *Mimosops elengi*

associated endophytic fungus, revealed inhibition of human TNF-α (IC_{50} 1.9 μM) and IL-6 (IC_{50} 12.3 μM) as compared to dexamethasone (IC_{50} 0.06 μM and 0.01 μM, respectively) in (Deshmukh et al., 2009). Periconianone A and periconianone B that were isolated from the endophytic fungus *Periconia* sp. showed promising inhibition of LPS-induced NO production with IC_{50} 0.15 and 0.23 μM in mice microglia BV2 cells as compared to standard curcumin (IC_{50} 3.9 μM) (Zhang et al., 2014). Lasiodiplactone A from the mangrove endophytic fungus *Lasiodiplodia theobromae* ZJ-HQ1 showed α-glucosidase inhibitory (IC_{50} 29.4 μM) and antiinflammatory activity (IC_{50} 23.5 μM) by inhibiting nitric oxide (NO) production in lipopolysaccharide activated in RAW264.7 cells (Chen et al., 2017).

8.6 Enzyme-inhibitory activity of compounds from endophytes

Enzymes are biocatalysts, running biochemical reactions in cells. The majority of the marketed drugs are either inhibitors/inactivators or activators used to alter these reactions to treat various pathological conditions and diseases. In the coming couple of years, the industrial market for enzyme-based drugs is expected to increase at an annual growth rate of 6.8%. Enzymes-substrate interactions are alternated by the inhibitors to prevent the product formation either competitively, noncompetitively, or uncompetitively. For example, hyperuricemic disease management includes xanthine oxidase (XO) inhibitors, tyrosine kinases for oncology, Angiotensin-converting enzymes (ACE) for hypertension, 3-hydroxy-3-glutaryl CoA reductase for hypercholesterolemia, Cytochrome P450 (CYP) for stroke and fatty acid metabolism, Cytochrome P450 (CYP), DNa gyrases for urinary tract infections, cyclooxygenase for inflammation, Acetylcholinesterase for Alzheimer's and Parkinson's diseases, monoamine oxidase for depression, etc. Therefore understanding the role of enzymes in disease is a key focus for drug discovery, and the implementation of strategies to modulate their activities for therapeutic benefit remains so in years to come. Various entophytic metabolites are screened as enzyme inhibitors and have potential in medicines (Holdgate, et al., 2018; De la Fuente et al., 2021). The fungal endophyte *Bipolaris sorokiniana* LK12, has been isolated from the leaf parts of *Rhazya stricta*. The obtained compound sorokiniol (EC_{50} 3.402 ± 0.08 μg/mL) exhibited significantly higher AChE enzyme inhibition (Ali et al., 2016). In another similar study on *B. sorokiniana* LK12, the compound bipolarisenol was revealed as a urease and acetyl cholinesterase (AChE) inhibitor. Bipolarisenol significantly inhibited the AChE (IC_{50} 67.23 ± 5.12 μg/mL) and urease (IC_{50} 81.62 ± 4.61 μg/mL) activity in a dose-dependent manner. It has antilipid peroxidation potential. (IC_{50} = 168.91 ± 4.23 μg/mL) (Khan et al., 2015). Endophyte *Aspergillus niger* IFB-E003, residing on Cyndon dactylon revealed aurasperone A as promising XO (IC_{50} 10.9 μM) comparable with standard allopurinol (IC_{50} 9.8 μM) (Song et al., 2004). Fusaruside, a cerebroside derivative from the extract of *Fusarium* sp. IFB-121, residing on *Quercus variabilis* revealed promising XO inhibitors (IC_{50} 43.8 ± 3.6 μM) as compared to standard cerebroside (IC_{50} 55.5 ± 1.8 μM) (Shu et al., 2004). In another study,

penisimplicissin from endophyte *Penicillium simplicissimum*, conjugated with C60 fullerene, was studied for promising XO inhibitory activity. The endophyte was isolated from *Loranthus micranthus* (Govindappa et al., 2021). Ali et al., 2017 isolated endophytic fungi *Penicillium citrinum* from *Boswellia sacra* for active metabolites as inhibitors for urease and α-glucosidase enzyme. The compound, 11-oxoursonic acid benzyl ester (IC_{50} 273.87 ± 1.59 μg/mL), has the potential to inhibit the α-glucosidase enzyme.

8.7 Antimycobacterial compounds from endophyte

Mycobacterium, an infectious bacterial genus, causes infectious tuberculosis and leprosy diseases in humans. These diseases are difficult to treat due to emerging drug-resistant strains. *Mycobacterium tuberculosis* is the causative agent of the deadly tuberculosis. Further, there is a rise in multidrug-resistant-TB and HIV coinfection, in patients making it difficult to control. Piperine (5-(3,4-methylenedioxyphenyl)-1-piperidinopent-2,4-dien-1-one) compound from the endophytic fungus *Periconia* sp., residing on *Piper longum* exhibited strong activity against *M. tuberculosis* (MIC 6.09 μM) and *M. smegmetis* (MIC 9.18 μM) (Verma et al., 2011). *Chaetomium globosum* (HG423571), an endophytic fungal strain, was isolated from Avena sativa (Gramineae). Bioactive compound chetomin displayed significant antitubercular activity (MIC 0.78 μM) (Marmouzi et al., 2017). Two new azaphilone derivatives, namely, biscogniazaphilones A and B revealed promising antimycobacterial activity against *M. tuberculosis* H37Rv strain with IC_{50} value 5.12 and 2.52 μg/mL, respectively. The compounds were reported from the bark of the *Cinnamomum* species associated endophytic fungus *Biscogniauxia formosana* BCRC 33718 (Cheng et al., 2012). Endophyte *Phomopsis* sp. PSU-D15, from *Garcinia dulcis*, produced three new metabolites named phomoenamide, phomonitroester, and deacetylphomoxanthone B. Phomoenamide revealed antimycobacterial activity against *M. tuberculosis* H37Ra (MIC 6.25 μg/mL (Rukachaisirikul et al., 2008). In a similar study, *Garcinia nigrolineata* was used to isolate endophytic fungi PSU-N24. The fungal extract forms a Hydronaphthalenone compound with antimycobacterial activity at a MIC value of 12.50 μg/mL against *M. tuberculosis* (Sommart et al., 2008). An endophyte *Penicillium* sp., of *Garcinia nobilis*, produces six metabolites with antimycobacterial activity against *M. smegmatis*: penialidin A, B, and C, citromycetin, p-Hydroxy-phenyl-glyoxalaldoxime, and Brefeldin A. All the metabolites exhibited MIC values between 193.93 and 891.71 μM (Jouda et al., 2016). A report on fusaric acid from endophyte *Fusarium* sp. DZ-27, and its metal complex, revealed enhanced antimycobacterial activity against *M. bovis* BCG (MIC 4 μg/mL for Cd (II) complex) and *M. tuberculosis* H37Rv strain (MIC 10 μg/mL for Cu (II) complex) as compared to fusaric acid alone (MIC 20 μg/mL and MIC 40 μg/mL, respectively). *Fusarium* sp. DZ-27 was isolated from the bark of the mangrove growing plant *Kandelia cande* (Pan et al., 2011). In another study, an anthraquinone compound 4-Deoxybostrycin and Nigrosporin were isolated from the endophytic fungus *Nigrospora* sp. residing on the

mangrove plant *Kandelia candel*. Both the compounds showed inhibitory activity against *M. bovis* (MIC 39 and 15 μg/mL) and *M. tuberculosis* (MIC 15 and 20 μg/mL, respectively) (Wang et al., 2013). *Fusarium solani* endophytes associated with *Glycyrrhiza glabra* demonstrated antimycobacteial activity. Fusarubin showed good activity against *M. tuberculosis* strain H37Rv (MIC 8 μg/mL), whereas compounds 3,6,9-trihydroxy-7-methoxy-4,4-dimethyl-3,4-dihydro-1H-benzo[g] isochromene-5,10-dione, 3-*O*-methylfusarubin, and javanicin exhibited moderate activity with MIC 256, 64, 32 μg/mL, respectively (Shah et al., 2017).

8.8 Antidiabetic compounds from endophytes

Diabetes mellitus is an endocrine system disease that afflicts a large number of people worldwide and may expect to reach 366 million by 2030. Endophyte *Aspergillus awamori* isolated from *Acacia nilotica* was investigated for alpha-amylase and glucosidase inhibitory activity. A peptide of 22 kDa exhibited mixed-type inhibition against α-amylase (IC_{50} $3 \cdot 75$ μg/mL) and α-glucosidase (IC_{50} $5 \cdot 625$ μg/mL) (Singh and Kaur, 2016).Three biosynthetic compounds, (*S*)-(+)-2-*cis*-4-*trans*-abscisic acid, 7'-hydroxy-abscisic acid, and 4-des-hydroxyl altersolanol A from the endophytic fungus, *Nigrospora oryzae*, isolated from *Combretum dolichopetalum* leaf was studied for their antidiabetic potential in alloxan-induced diabetic mice (Uzor et al., 2017). *Boswellia sacra*—associated endophytic fungi *Aureobasidium pullulan* BSS6 was reported for antidiabetic activity. The compound methyl-5-docosenoate exhibited potent α-glucosidase inhibitory activity with IC_{50} 23.3 mM (Al-Hosni et al., 2020). Endophytic fungi *Epicoccum nigrum* SCNU-F0002 from *Acanthus ilicifolius* L. produces five new isobenzofuranone derivatives. Compounds (±)-epicoccone C, epicoccone E, epicocconigrone A, and epicoccolide B revealed potent α-glucosidase inhibitory activity within the range of IC_{50} 32.3 to 63.3 μM. Moreover, the compounds (±)-epicoccone C, flavimycins A, and epicocconigrone A showed promising antioxidant activity in the range of IC_{50} values ranging from 10.2 to 15.3 μM by DPPH assay (Yan et al., 2020). Vermistatin derivatives, namely, 6-demethylpenisimplicissin and 2″-epihydroxydihydrovermistatin were isolated from *Cerbera manghas* associated endophytic fungus *Penicillium* sp. HN29−3B1. Compounds 6-demethylpenisimplicissin and 2″-epihydroxydihydrovermistatin exhibited α-glucosidase inhibitory activity with IC_{50} values 9.5 ± 1.2 and 8.0 ± 1.5 μM, respectively (Liu et al., 2014). Endophytic fungi *Paecilomyces formosus* LHL10 isolated from root *Cucumis sativus* plant revealed α-glucosidase enzyme-inhibitory activity due to extracted sester-terpenoid YW3548 (IC_{50} 61.80 μg/mL) and a cyclic peptide paecilodepsipeptide A compounds. (IC_{50} 75.68 μg/mL) (Bilal et al., 2018). Xanthone compounds, namely, 1,2,3, pentahydroxy-8-methylxanthone, 1,3,5,6-tetra hydroxy-8-methylxanthone, and 1,6-dihydroxy-3-methoxy-8-methylxanthone were purified from *Juniperus polycarpos* associated endophytic culture *Penicillium canescens*. All three compounds exhibited α-glucosidase inhibitory activities with IC_{50} values of 38.80, 32.32, and

75.20 μM, respectively. Compounds revealed mixed-mode inhibitor, competitive inhibitor, and noncompetitive inhibitor, respectively (Malik et al., 2020). Endophytic fungus strain MEXU 27905 that was isolated from healthy leaves of *Hintonia latiflora* was studied for the production of tridepsides thielavins A, J, and K. The compounds inhibit the α-glucosidase enzyme activity noncompetitively with IC_{50} 23.8, 15.8 and 22.1 μM, respectively. Thielavins K at 10 mg/kg induces hypoglycemic activity in diabetic mice (Rivera-Chávez et al., 2013). Indrianingsih and Tachibana, 2017 reported an endophytic fungi *Xylariaceae* sp. QGS 01 from the stem of *Quercus gilva* Blume. The fungi produce an 8-hydroxy-6,7-dimethoxy-3-methylisocoumari compound with promising α-glucosidase inhibitory activity (IC_{50} 41.75 μg/mL). Naphthaquinone, herbarin from endophytic fungi *Dendryphiom nanum* (Nees) S. Hughes residing on leaves of *Ficus religiosa* enhances the cellular glucose uptake in skeletal muscles in the presence of insulin (ED_{50} 0.8 ± 0.09 μM). The results were comparable with rosiglitazone (ED_{50} of 0.3 ± 0.04 μM), a standard glucose uptake activator (Mishra et al., 2013). Lectin (N-acetylgalactosamine) was extracted from endophytic fungal isolate *Alternaria* species from the *Viscum album*. The antidiabetic activity was screened using inhibition of α-amylase (IC_{50} 85.26 ± 1.25), α-glucosidase (IC_{50} 93.41 ± 1.27), and sucrase (IC_{50} 81.61 ± 1.05) (Govindappa et al., 2015). Protein tyrosine phosphatases are potential drug targets for the treatment of type II diabetes and are responsible for the negative regulation of insulin signaling. Moreover, silent information regulator T1 (SIRT1) enzymes are also important. Thus, in drug discovery designing, PTP1B inhibitors have applications for the treatment of type II diabetes. Neglectine A, a Polyketide-derived metabolite, was purified from *Kandelia candel* associated endophytic fungus *Pestalotiopsis neglecta*. The compound showed PTP1B enzyme-inhibitory activity with an IC_{50} value of 6.7 μg/mL (Gao et al., 2019). In another study, novel guignardins (spirodioxynaphthalenes) were isolated from cultures of the endophytic fungus *Guignardia* sp. KcF8, from the similar mangrove *K. candel*, revealed significant anti-PTP1B (IC_{50} 25.7 μM) and anti-SIRT1 (IC_{50} 43.9 μM) enzyme-inhibitory activity (Ai et al., 2014). PTP1B inhibitory activity of four endophytic fungi isolated from fresh roots of the medicinal plant *Vernonia anthelmintica* was reported for antidiabetic activity. The crude extracts of *Aspergillus* sp. XJA6 (IC_{50} 5.662 ± 1.099 μg/mL) and *Talaromyces* sp. XJA4 (IC_{50} 4.789 ± 1.222 μg/mL) exhibited significant inhibition of PTP1B, respectively (Rustamova et al., 2020). The fungal endophytic strain *Scedosporium apiospermum* F41−1 was isolated from the inner tissue of *Lobophytum crissum* and studied for triglyceride-promoting activity using 3T3-L1 adipocytes. The compound Scequinadoline D, a Fumiquinazoline alkaloid, was confirmed to have potent PPARγ pathway triggering activity (EC_{50} value of 0.27 ± 0.03 μM).Thus scequinadoline D is a potent insulin sensitizer that targets adipocytes and may be useful for the treatment of type 2 diabetes mellitus (Li et al., 2020). Asperpyridone A, a pyridone alkaloid produced from *Hypericum perforatum* leaves-associated endophytic fungi *Aspergillus* sp. TJ23, revealed antidiabetic activity by regulating the PPAR signaling pathway, insulin signaling pathway, and insulin resistance signaling pathway under both normal and insulin-resistant conditions in liver HepG2 cells.

Moreover, protein interaction analysis confirmed FGF21 as a potential target for Asperpyridone A, which is involved in the modulation of cellular glucose homeostasis (Qiao et al., 2019).

8.9 Anticancer compounds from endophytes

Cancer is a major cause of death worldwide. Various plant-derived metabolites, namely, vinblastine, vincristine, irinotecan, topotecan, etoposide, camptothecin, and paclitaxel (Taxol), are clinically used for cancer treatment. Some of the new promising metabolites, namely, combretastin A-4- Phosphate and flavopiridol are under clinical trials. However, the requirement of high plant samples, lower yield, limitations in extraction and purification, compound stability, etc., demand alternative sources for the same entity, namely, plant tissue culture and microorganisms. Taxol, the first line of FDA-approved anticancer drugs, was discovered in 1962 from the bark of *Taxus brevifolia* plant by researchers of the US Department of Agriculture, National Cancer Institute. Soon after, in 1993, a fungal endophyte, *Taxomyces andreanae*, was isolated from the phloem (inner bark) of the *T. brevifolia* for the production of Taxol and Taxanes. Soon after, various endophytic fungal cultures, namely, Aspergillus, Cladosporium, Alternaria, Botrytis, Botryodiplodia, Ectostroma, Metarhizium, Fusarium, Periconia, Mucor, Monochaetia, Ozonium, Xylaria, Penicillium, Pithomyces, Phyllosticta, and Pestalotiopsis has been reported for the production of paclitaxel, and for analogs of paclitaxel. It has been the recommended drug for the treatment of cancers of the breast, ovaries, lungs, colon, and Kaposi's sarcoma (Hao et al., 2013; Uzma et al., 2018). *Aspergillus fumigatus* isolated from *Juniperus communis* L. Horstmann plant is reported for deoxypodophyllotoxin and podophyllotoxin production. Moreover, deoxypodophyllotoxin demonstrated the anticancer activity against several tumor cell lines, further including A-549, SK-OV-3, SK-MEL-2, HCT15, B16F10, and K562 (Ejaz et al., 2020). Camptothecin (CPT) is the second-most highly demanded anticancer drug derived commercially from *Camptotheca acuminata* and *Nothapodytes nimmoniana* plants. It prevents DNA replication by inhibiting the DNA topoisomerase I complex. Endophytic fungi *Entrophospora infrequens*, *Alternaria burnsii*, and *Fusarium solani* S-019 were isolated from *N. nimmoniana* and *C. acuminata*, respectively. Irinotecan and topotecan, the two analogs of CPT, have been revealed as a promising anticancer agent and safer than the CPT parent compound. The activity was determined using Caco-2 (human colorectal adenocarcinoma cells), MCF-7 (breast cancer cells), and SK-OV-3 (human ovarian cancer cells) for up to 72 h. The maximum compound was received from the petiole, followed by the leaves, the stem, and the bark of the above plants. Other reported endophytes include Fusarium, Xylaria, Trichoderma, Botryosphaeria, Entrophospora, and Colletotrichum. The derivatives 9-methoxycamptothecin and 10-hydoxycamptothecin have been isolated from the *Fusarium* sp. of the *C. acuminata* plant (Kaur et al., 2020; Mohinudeen et al., 2021, Ruan et al., 2021). A significant amount of Chaetocochins A-C for anticancer activity was revealed from

Chaetomium cochliodes isolated form *Sapium ellipticum* on cell lines Bre-04 (MDA-MB-231), Lu-04 (NCIH460), and N-04 (SF-268). In another study, the co-culturing of *Chaetomium* sp. with *B. subtilis* using a rice medium further increased the production by threefold with the accumulation of other compounds, namely, acremonisol A, 3-and 4-hydroxybenzoic acid methyl esters, isosulochrin, protocatechuic acid methyl ester, mainly (Li et al., 2006; Akone et al., 2016). Chaetominine alkaloids from endophytic fungi *Chaetomium* sp. IFB-E015 isolated from *Adenophora axilliflora* leaves revealed anticancer activity against the human leukemia K562 and colon cancer SW1116 cell lines (Jiao et al., 2006). Cytochalsians like multirostratin A, 20-oxo-deoxaphomin, other analog deoxaphomin, cytochalasin A, cytochalasin B, cytochalasin Z_2, and cytochalasin F have been isolated from the endophytic fungus *Phoma multirostrata* EA-12 collected from *Ageratina adenophora*. Compounds multirostratin A and 20-oxo-deoxaphomin revealed moderate cytotoxicity against five tumor cell lines: SK-BR-3 (breast cancer cell line), SMMC-7721 (hepatocellular carcinoma cell line), HL-60 (human myeloid leukemia cell line), A- 549 (lung cancer cell line), and PANC-1 (pancreatic cancer cell line). The cytotoxicity was achieved in between IC_{50} 7.8−15.8 and 7.7−14.2 μM by compounds 1 and 2, respectively, against the selected cell lines. Other genera of fungi, such as *Aspergillus*, *Spicaria*, *Xylaria*, and *Periconia*, are also reported. (Chen et al., 2015). Vincristine and Vinblastine bind with spindle proteins and microtubules in S-phase, thereby inhibiting the cancerous cell multiplication at metaphase. The semisynthetic vinorelbine and vindesine are used for the treatment of leukemia, lymphomas, breast cancer, and lung cancer. Historically the compounds were first extracted from *Catharanthus roseus* plant leaves. Endophytes, *Botryosphaeria laricina*, *Alternaria sesami*, *Streptomyces antibioticus, Fusarium oxysporum*, *Talaromyces radicus*, *Eutypella* spp CrP 14, *Nigrospora sphaerica, Curvularia verruculosa*, and *Chaetomium globosum* Cr 59, were reported for the their productions (Parthasarathy et al., 2020, Andriambeloson et al., 2021, Birat et al., 2021, Bandara et al., 2021). *Cylindrocarpon* sp., an endophytic fungus isolated from the tropical plant *Sapium ellipticum*, produces alkaloids cylindrocarpones A − C, cylindrocarpyridones A − B, and pyrone cylindropyrone in solid rice media at 20°C under static conditions. Pyrrocidine A revealed cytotoxicity against human ovarian cancer cell line A2780 with an IC_{50} value of 1.7 μM (Kamdem et al., 2018a). *Bionectria* sp. endophytes obtained from *Raphia taedigera* seeds produce 1,2-dihydrophenopyrrozin, Agathic acid, and Penicolinate A. Further co-culturing of fungus either with *Streptomyces lividans* and *B. subtilis* forms new compounds via induction, namely, o-aminobenzoic acid derivatives, and bionectriamines A and B. Penicolinate A exhibited potent cytotoxic activity against the human ovarian cancer cell line A2780 with an IC_{50} value of 4.1 μM (Kamdem et al., 2018b). *Anvillea garcinii* (Burm.f.) DC. leaves-associated endophyte *Fusarium chlamydosporium* produces aminobenzamide, Fusarithioamide B having promising anticancer activity against BT-549 (IC_{50} 0.09 μM), MCF-7 (IC_{50} 0.21 μM), SKOV-3 (IC_{50} 1.23 μM), and HCT-116 (IC_{50} 0.59 μM) cell lines (Ibrahim et al., 2018). Endophytic fungus *Diaporthe* sp. isolated from *Datura inoxia* significantly produces xylarolide A-B and diportharine A compounds.

Xylarolide A and xylarolide revealed significant cytotoxicity against pancreatic cancer MIAPaCa-2 (IC_{50} of 20 and 32 μM) and prostate cancer cell lines PC-3 (IC_{50} 14 and 18 μM), respectively. The compound's induction was initiated in the presence of valproic acid at 100 μM (Sharma et al., 2018). Bostrycin and deoxybostrycin were isolated from the marine endophytic fungus *Nigrospora* sp. from *Kandelia candel* (L.) Druce showed moderate antitumor activity (Xia et al., 2011).

Overall, until now, more than 200 anticancer compounds have been reported from 100 different fungal species that were revealed as having antiproliferative and/or cytotoxic properties against more than 60 different cell lines. Moreover, in the past three decades, the bioactive compounds belonging to alkaloids, terpenes, quinone, polyketides, chromones, depsidone, ergochromes, lactones, spirobisnaphthalenes, and xanthone are reported and still it is ongoing (Hridoy et al., 2022).

8.10 Antioxidant compounds from endophytes

Metabolic reactions-induced reactive oxygen species (ROS) are involved in pathological conditions like aging, cancer, coronary heart disease, and Alzheimer's disease. A plethora of studies that revealed the importance of antioxidants is thought beneficial in the management of this ROS-mediated tissue injury. *Cephalosporium* sp. IFB-E001, an endophytic fungus harbored in the roots of *Trachelospermum jasminoides*, produces Graphislactone A. The compound revealed promising antioxidant activity (IC_{50} 2.9 and 2.2 μg/mL) as compared to BHT (IC_{50} 3.2 and 3.4 μg/mL) by DPPH assay and lipid peroxidation assay, respectively (Song et al., 2005). Pestacin, a 1,3-dihydro isobenzofuran from endophytic fungi *Pestalotiopsis microsporea* has greater antioxidant activity than trolox (Harper et al., 2003). Several endophytic fungal isolates revealed exopolysaccharide mediated promising antioxidant activities, namely, *Fusarium oxysporum* from *Dioscorea zingiberensis* rhizome and *Otoba gracilipes* leaves (Li et al., 2011; Caicedo et al., 2019). Cui et al., 2015 studied Alpine Plants (Rhodiola crenulata, R. angusta, and R. sachalinensis) associated with 347 endophytic fungi for antioxidant activities, namely, Phialophora sp., Penicillium sp., Aspergillus sp., Lachnum sp., and Dothideomycetes sp. revealed promising antioxidant activity using ethanol extract by DPPH assay at different concentrations (1 mg /mL to 10 mg/mL). This might be due to phenolics and flavonoids compounds, like salidrosides, p-tyrosol, and rosavins (Cui et al., 2015). In another study, flavonoids and phenolic compounds-mediated antioxidant activity was examined from *Viola odorata* associated endophytic fungi *Aspergillus* sp.(IC50 17.4 μg/mL)(Katoch et al., 2017). *Elaeocarpus sylvestris* associated endophytic fungi *Pseudocercospora* sp. ESL 02 showed the highest antioxidant activity due to two compounds terreic acid (0.22 ± 0.02 mmol/L) and 6-methylsalicylic acid (3.87 ± 0.27 mmol/L) by DPPH radical scavenging assay

(Prihantini and Tachibana, 2017). Chaetomium globosum CDW7 endophytes from the *Ginkgo biloba* plant produce flavipin with promising antioxidant activity (IC_{50} 6.33 μg/mL) by DPPH assay (Ye et al., 2013). Forty-nine fungal endophytes were isolated from the bulbs of *Fritillaria unibracteata var. wabuensis* (FUW) and evaluated for antioxidant activity. *Fusarium* sp. dominantly showed rutin, gallic acid, phlorizin, 2,4-di-tert-butyl-phenol, and 2,6-di-tert-butyl hydroquinone mediated antioxidant activity by DPPH assay (Pan et al., 2017). Thirty-three fungal endophytes from the genus *Pestalotiopsis*, *Colletotrichum*, *Russula*, *Fusarium*, *Diaporthe*, *Arthrinium*, and *Cladosporium* were isolated from *Aquilaria subintegra*. The endophyte *Diaporthe* sp. revealed promising antioxidant activity due to bioactive compounds, namely, Phenyl butanone, agarospirol, and oxoagarospirol (Monggoot et al., 2017). Among 13 endophytes isolated from *Mangifera casturi, Aspergillus minisclerotigens* AKF exhibited the strongest antioxidant activity IC_{50} 142.96 μg/mL and IC_{50} 145.01 μg/mL due to dihydropyran and 4H-Pyran-4-one,5-hydroxy-2-hydroxymethyl-(CAS) Kojic acid, respectively (Nuraini, et al., 2019). *Nerium oleander* L. associated endophytic fungal strain *Chaetomium* sp. showed the strongest antioxidant capacity due to the highest level of phenolics (Huang et al., 2007). In another study, the *Chaetomium* sp., *Aspergillus peyronelii*, and *Aspergillus niger* strain from *Eugenia jambolana* Lam showed the highest antioxidant activity due to phenolic content (Yadav et al., 2014). Endophytes *Trichoderma longibrachiatum* LMA 1684, *Chaetomium globosum* LMA 1793, and *Aspergillus nidulans var. dentatus* LMA 1705 associated with *Passifora incarnate* plant revealed promising antioxidant activity by phenolic compounds, methoxymethylphenol, orcinol (Da Silva et al., 2020). Eugenol from fungal endophyte *Neopestalotiopsis* sp. (IC_{50} 22.92 ± 0.67 μg/mL) and *Diaporthe* sp.(IC_{50} 37.61 ± 0.49 μg/mL) from *Cinnamomum loureiroi* leaves revealed promising antioxidant activity (Tanapichatsakul et al., 2019).

8.11 Conclusion

In conclusion, discovering new chemical entities/compounds from natural sources is very important for formulating a new drug. Endophytes have proven to be a rich source of novel natural compounds with a wide spectrum of biological activities and, thus, have promising potential in human health concerns and in drug discovery. Many novel and valuable bioactive compounds with antimicrobial, antimycobacteial, antioxidant, antiinflammatory, antidiabetic, antiviral, and anticancer activities have been reported from endophytes, and yet they are indeed still a hidden treasure. Modern biotechnology methods such as genetic engineering, metabolomics, and microbial fermentation process would further make the commercialization of bioactive compounds possible.

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Conflict of interest

No conflict of interest

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CHAPTER 9

Microbial metabolites in plant disease management

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9.1 Introduction

Agriculture has been associated with mankind since civilization's dawn. Even today, agriculture plays a substantial role in feeding the world population and generating income, especially in third-world countries. It contributes 20% of the total GDP and provides jobs to 52% population, thus boosting India's economy (Arjun, 2013).

Despite the advancement in agriculture technology, the damage caused by plant pathogens is still significant and relevant in reducing plant productivity. The pathogens can cause a reduction in the quality and quantity of agriculture products resulting in the loss of billions of dollars per year (Afonso et al., 2021).

Many conventional approaches have been utilized for controlling phytopathogens for long which includes the use of microbial pesticides, traditional breeding approaches, and biocontrol agents (El-Baky and Amara, 2021). The microbial pesticides focus on the direct killing of pathogens, whereas the breeding and biocontrol agents induce disease resistance in plant variety. The evolving resistance towards pesticides, emerging human and environmental concerns, disturbing biodiversity, and climate change (Kumari et al., 2021) have urgently asked for an economical and eco-friendly solution for controlling the pesticide-resistant phytopathogens. Microbial secondary metabolites being naturally produced from diverse microbes can play a key role in sustainable plant disease management.

Secondary metabolites are organic compounds produced by plants or microbes in the late phase of their life cycle, which help them to cope with biotic and abiotic stress while helping in their growth and catering to their defense mechanism (Demain and Fang, 2000). They do not have a primary role in the metabolism and development of organisms. Microbes are capable of producing a myriad of secondary metabolites, including phenolics,

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flavonoids, alkaloids, sterols, terpenoids, depsides, and enzymes. Many of the isolated secondary metabolites have shown antimicrobial, antioxidative, and cytotoxic properties (Kamat et al., 2020), which can further be employed in plant disease management. The diversity and synergism between microbes provide them an added advantage of producing novel and potential antimicrobials (Boustie and Grube, 2005; Stierle and Stierle, 2014). Many bacteria, actinomycetes, and fungi are prolific producers of bioactive secondary metabolites, which will be described in detail in further sections.

Microbe-derived secondary metabolites are potent antimicrobials and also possess the added advantage of low toxicity on nontargeted cells and induction of disease resistance in plants. The multiple modes of action shown by the microbial metabolites also make it difficult for phytopathogens to acquire drug resistance by fighting against them. *Bacillus* sp. are the well-known producers of iturin and surfactin, which can suppress the growth of phytopathogenic fungi *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, and *Phytophthora infestans* in greenhouse conditions by inducing oxidative stress, dysfunction of the energy cycle, and disruption of cell structure (Kim et al., 2020; Wang et al., 2020). Similarly, siderophores produced by many microbial metabolites deprive phytopathogens from iron and elicit plant defense responses (Gu et al., 2020).

This chapter presents the microbial metabolites produced by different fungi, bacteria, and actinomycetes, along with a state-of-art of their mechanistic aspect against phytopathogens. The new potential sources which can provide novel and potent antipathogenic compounds have also been discussed, along with the challenges which are yet to be overcome for the journey of microbial metabolites from lab to land in their fight against phytopathogens.

9.2 Secondary metabolites

Biotic factors such as plant pathogens/parasites cause adverse effects on the growth of plants and the productivity of crop plants. The alternative for the management of plant health problems/diseases is a much-needed system for sustained crop production. To search for alternatives to plant health problems/diseases, the interest in plants and their chemo-biodiversity as a source of bioactive secondary metabolites has amplified. Many studies reported the potential of secondary metabolites for plant health in different strategies such as integrated or ecological pest management acting directly on the target pest or inducing resistance. Biocontrol agents owned one or more of the several mechanisms of disease suppression, such as siderophores production, auxin production, etc. Production of antimicrobial secondary metabolites with inhibiting effects against pathogens is another direct mode of action. Various microorganisms including fungi, nematode, bacteria, and actinobacteria, produce a diverse array of small bioactive molecules with significant potential to be used in both medicine and agriculture (Van Bergeijk et al., 2020). Microorganisms inhabiting in rhizospheric systems produce bioactive compounds, including

secondary metabolites (SMs); these SM induce plant defense reactions leading to a systemic resistance to pathogen infection. These microorganism-produced SMs are low-molecular-weight type diverse compounds having a wide range of biological activities that are involved immensely in combating pathogens, particularly by developing a disease-suppressive soil and acting as plant growth promoters (Buddhika and Abeysinghe, 2021). SMs such as antibiotics, toxins, terpenes, phenolics, nitrogen (N) and sulfur (S) containing compounds, ribosomal peptides (RPs), low-molecular-weight volatile organic compounds (VOCs), polyketides (PKs), alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, phlobatannins, polyphenols, steroids, non-ribosomal peptides (NRPs), and hybrids between PKs and NRPs, where all have shown a diverse performance in antagonistic activity against numerous plant pathogens (Breen et al., 2015). Biological control of plant diseases through microbial metabolites is an eco-friendly and effective means of reducing or mitigating crop losses. Such plant protective metabolites are produced by many microorganisms such as actinomycetes like *Streptomyces*, *Actinoplanes*, *Actinomadura*, *Micromonospora*, *Streptosporangium*, *Streptoverticillium*, and *Spirillospora*, bacteria belonging to the genera *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacte*r, *Pseudomonas*, *Serratia*, and a few fungal genera such as *Ampelomyces*, *Aspergillus*, *Gliocladium*, *Laetisaria*, *Penicillium*, *Phlebiopsis*, *Sporodesmia*, *Trichoderma, Trichothecium*, and nonpathogenic *Fusarium* are prolific producers of secondary metabolites. Production of antimicrobial metabolites is commonly observed during microbial interaction of antagonistic microorganisms and pathogens (Kohl et al., 2019). Various microbial secondary metabolites have been used in plant disease control, such as Phenazine-1-carboxylic acid, pyocyanin, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, hydrogen cyanide, siderophores, zwittermicin a 14, kurstakin, azole compound, ammonia, wuyiencin, viridin, trichodermin, 6-pentyl-2h-pyran-2-one, gliovirin, gliotoxin, harzianopyridone, harzianolide, massoilactone and d-decanolactone, viridepyronone, koningins, t22 azaphilone, t39 butenolide, volatile compounds, and trichothecin (Fig. 9.1). These metabolites can be used as a substitute for chemical fungicides and pave the way for their use in sustainable agriculture as biopesticides. SM of endophytic bacteria proved to be a new source of potential biopesticides that act as elicitors of plant defenses and act as an alternative for synthetic pesticides. The influence of different biotic factors, including bacteria, fungi, actinomycetes, etc., on secondary metabolites in plants is of great importance. In this chapter, we focused on the current state of the use of beneficial microbial metabolites to improve disease suppression in plants and in the management of soil-borne diseases.

9.2.1 Bacterial secondary metabolites

Plants are exposed to biotic stresses in their habitats and exploited by phytopathogens at various stages of their development. These phytopathogens often result in several diseases and a decline in crop production of economically important crops. The effects of pathogenic

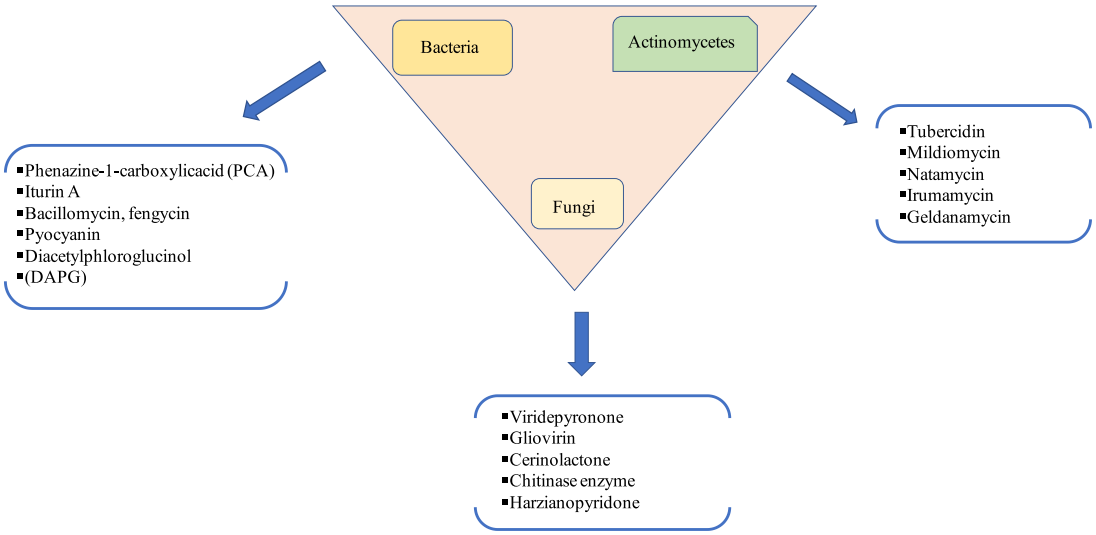

Figure 9.1
Bioactive compounds produced by bacteria, fungi, and actinomycetes.

microorganisms on plants can be decreased by the presence of bacteria that reduce the impact of the pathogen on the plant. Many beneficial bacteria produce SMs that interfere with the pathogen's disease potential by either direct inhibition of pathogen growth, induction of plant defense, or enhancement of plant growth (Keswani et al., 2020) (Table 9.1). The SMs play an important role in plant growth and development and confer protection against environmental stresses. They play a crucial role in the adaptation of plants to the environment, in overcoming stress conditions, and in sustainable pest management (Jan et al., 2021). Major microbial secondary metabolites include antibiotics, pigments, toxins, alkaloids, etc., that proved effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immune-modulating agents, receptor antagonists and agonists, pesticides, antitumor agents, and growth promoters (Jayaprakashvel and Mathivanan, 2011). Beneficial microbes interact with plants by inducing resistance or priming plants without any direct interaction with the targeted pathogen (Kohl et al., 2019). Major biological control protects crops from damage by inhibiting pathogens via different modes of action such as the production of antimicrobial secondary metabolites production and competition for nutrients and space, siderophores production, microbial cyanide, and lytic enzymes (Keswani et al., 2020). Antimicrobial metabolites produced by biological control agents are an alternative to chemically synthesized pesticides and are unique sources for pharmaceuticals, food additives, and flavors, as well as used in plant disease control (Jayaprakashvel and Mathivanan, 2011). In recent years, the use of secondary metabolites is an alternative to chemical control as these SMs are biologically synthesized and highly target-oriented and, hence, eco-friendly for beneficial organisms (Verma et al., 2020).

Table 9.1: List of secondary metabolites from bacteria and their antagonistic activity.

S. no.	Secondary metabolite(s)	Target pathogens/ resistance against	Microbes/source	Reference
1	Phenazine-1-carboxylic Acid (PCA)	*Gaeumannomyces gramini var. tritici*	*Pseudomonas fluorescens P. aureofaciens P. aeruginosa*	Chin-A-Woeng et al. (2001)
2	Pyocyanin (PCN)	*P. aeruginosa*	*Septoria tritici*	Yu et al. (2018b)
3	2,4-Diacetylphloroglucinol (DAPG)	*Pseudomonas fluorescens*	*Gaeumannomyces gramini var. tritici*	Kwak et al. (2012)
4	Hydrogen cyanide (HCN)	*P. fluorescens, P. aeruginosa*	*Thielaviopsis basicola Gaeumannomyces gramini var. tritici Rhizoctonia solani Meloidogyne javanica*	Verma et al. (2020)
5	Bacillomycin, fengycin	*Bacillus amyloliquefaciens FZB42*	*Fusarium oxysporum*	Zhao et al. (2014)
6	Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*R. solani and Pyricularia oryzae*	Verma et al. (2020)
7	Chitinase enzyme	*Serratia marcescens*	*Sclerotium rolfsii*	Reyes-Ramirez et al. (2004)
8	Iturin A	*Bacillus subtilis*	*Colletotrichum gloeosporioides, B. cinerea and R. solani*	Kim et al. (2010)
9	Xanthobaccin A	*Lysobacter sp. strain SB-K88*	*Aphanomyces cochlioides*	Islam et al. (2005)
10	PRN	*P. chlororaphis O6*	*F. graminearum and R. solani*	Park et al. (2011)
11	Polyketides 1,2-bezenedicarboxyl acid, Methyl ester, Decanodioic acid, bis(2-ehtylhexyl) ester	*Bacillus atrophaeus, Bacillus mojavensis*	-	Fadiji, and Babalola (2020)
12	1,3-glucanase and chitinase	*B. amyloliquefaciens strain TB2*	*Peronophthora litchi*	Jiang et al. (2018)

Since these secondary metabolites are of biological origin, these metabolites are inherently biodegradable and often do not persist in nature and are safe for the environment (Adeyemi and Mohammed, 2014).

The antagonistic *Fluorescent pseudomonads* are used as effective biocontrol agents (BCA) against an array of phytopathogens. Prominent secondary metabolites involved are known for antifungal, antibacterial, antiviral, antitumor, and antinematicidal properties and include phenazines (PHZ), 2, 4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylicacid (PCA), pyoluteorin (PLT), oomycin A, pyrrolnitrin (PRN), cyclic lipopeptides (CLPs), and volatile organic compounds (VOCs) such as hydrogen cyanide (HCN) (Velusamy and Gnanamanickam, 2008). DAPG has recently been recognized as an important feature in the biological control of plant diseases by antagonistic bacteria such as the root rot of wheat caused by *Fusarium oxysporum* f. sp. *graminis*, black root rot of tobacco caused by *Thielaviopsis basicola*, damping-off of sugarbeet caused by *Pythium ultimum* and *Rhizoctonia solani*, and the "take-all" of wheat caused by *Gaeumannomyces graminis tritici* (Sindhu et al., 2016). The production of many saprophytic microorganisms colonizing plant

roots has the ability to protect plants from damage caused by parasitic nematodes, bacterial and fungal pathogens, and several strains of them have been used as BCA (Xu et al., 2011). Biocontrol bacteria belonging to the genera *Agrobacterium, Bacillus, Burkholderia, Enterobacter, Erwinia, Lysobacter, Pseudomonas*, and *Serratia* were successfully used as BCAs against many plant diseases.

9.2.2 Fungal secondary metabolites

Biotic stress caused by phytopathogenic fungi contributes substantially to the overall loss in yield among crop plants. In plants, microbes produced SMs that showed potential against phytopathogens during fungal attacks. Secondary metabolites possess a wide range of biological activities, such as in medical, pharmaceutical, or agricultural areas, and also play a pivotal role in plant disease management that leads to the development of disease resistance. These low-molecular-weight type secondary metabolites often act in potent physiological ways and induce systemic defense responses in the plant. In addition, SMs are considered to aid the producing organism in survival and basic functions, such as competition, symbiosis, metal transport, differentiation, etc. SMs are widely used for medical, pharmaceutical, or agricultural purposes that include antibiotics and play a pivotal role in plant disease management.

Many fungal species are known producers of SMs with antimicrobial activity. Several fungal biocontrol genera such as *Penicillium, Aspergillus, Laetisaria, Phlebiopsis, Ampelomyces, Sporodesmia, Talaromyces, Gliocladium, Tilletiopsis, Trichoderma, and Trichothecium* are prolific producers of SM. *Trichoderma* is one of the most popular genera of fungi available as plant growth promoting fungus (PGPF) and biological control agent (BCA) that are successfully used as biopesticides worldwide also (Verma et al., 2019). SMs play a pivotal role in the suppression of plant pathogens; for example, penicillin, cephalosporin, ergotrate, and the statins are well-known fungal secondary metabolites. SM interacts not only with other microbes but especially with plants and soil components, enhances plant growth, and activates plant defense mechanisms. Numerous antimicrobial agents have been used for many years to ensure the safety and quality of stored products (Table 9.2). In the last decades, the demand for novel natural fungicides has been known to increase. For example, *Trichoderma* has attracted global interest due to its prominent antagonistic activities against a wide range of soil-borne phytopathogens. *Trichoderma* produced SM include volatile and nonvolatile antifungal substances, such as 6-n-pentyl-6H-pyran-2-one (6PP), gliotoxin, viridin, harzianopyridone, harziandione, and peptaibols (Heflish et al., 2021). *Trichoderma* spp. secrete a variety of lytic enzymes and a chemically diverse range of secondary metabolites, of which a broad spectrum of antimicrobial properties such as 6-pentyl-2H-pyran-2-one is active against *Rhizoctonia solani* and *Fusarium oxysporum f. sp. lycopersici* (Keswani et al., 2014). Many studies reported the

Table 9.2: List of secondary metabolites of fungi and their target pathogen/s.

S. no.	SM	Target pathogens/ resistance against/disease	Microbes/source	Reference
1	5-hydroxyvertinolide	*Mycena citricolor*	*T. longibrachiatum*	Khan et al. (2020)
2	Gliotoxin	T. viride, T. hamatum	Rhizoctonia solani	Ahmed and El-Fiki (2017)
3	Chitinase enzyme	*Serratia marcescens*	*Sclerotium rolfsii*	Zeki and Muslim (2010)
4	Viridepyronone	*T. viride*	*Sclerotium rolfsii*	Contreras-Cornejo et al. (2016)
5	Chitinases and cellulases	*Trichoderma harzianum* and *Trichoderma viride*	*Sclerotium rolfsii*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*	Kotasthane et al. (2015)
6	Gliovirin	*Glioclaudium virens* (*Trichoderma virens*)	*Pythium ultimum*	Howell (2006)
7	Pyrone 6-pentyl-2H-pyran-2-one	*T. viride, T. harzianum, T. koningii*	*Rhizoctonia solani and Fusarium oxysporum* f. sp. *lycopersici*	Woo et al. (2014)
8	Koningin D	*T. koningii*	*Rhizoctonia solani, Phytophthora cinnamomi, Pythium middletonii, Fusarium oxysporum*, and *Bipolaris sorokiniana*	Verma et al. (2020)
9	Harzianopyridone	*T. harzianum*	*Botrytis cinerea, Rhizoctonia solani Gaeumannomyces graminis* var. *tritici*, and *Pythium ultimum*	Marra et al. (2006)
10	Pyrones [6-pentyl-2H-pyran-2-one (6-pentyl-α-pyrone) (**1**), cytosporone S (**2**)]	*T. viride*, *T. atroviride*, *T. harzianum*, *T. koningii*	*Fusarium moniliforme*	Vinale et al. (2014)
11	2-Phenylethanol, tyrosol (**30**), sorbicillin	*T. harzianum*, *T. longibrachiatum*	*Enterobacter dissolvens*, *Paecilomyces variotii*, *Armillaria mellea*	Tarus et al. (2003)
12	Cerinolactone (Cerinolactone)	*T. cerinum*	*P. ultimum*, *R. solani*, and *B. cinerea*	Arjona-Girona et al. (2014)

role of a variety of novel genes and gene products, including ABC transporters, enzymes, and other proteins that produce or act as novel elicitors of induced resistance, proteins responsible for a gene-for-gene avirulent interaction between *Trichoderma* spp. and plants, mycoparasitism-related inducers, plant proteins specifically induced by Trichoderma, etc. (Keswani et al., 2014) *Colletotrichum lini, Fusarium caeruleum, Botrytis allii*, and *Viridio fungins* have shown microbicidal activity against *Candida, Aspergillus*, and *Cryptococcus* spp. (Keswani et al., 2014) Thus, the increased demands for agrochemicals have promoted the sustainable and healthy production of foods, provided alternative control strategies, and encouraged the use of plants as a source of secondary metabolites with antifungal properties. Several beneficial microbes are a rich source of bioactive secondary metabolites of a wide variety, such as tannins, terpenoids, saponins, alkaloids, flavonoids, and other compounds, reported to have antimicrobial properties. SM with antifungal activity reduce the heavy reliance on synthetic pesticides used to control them. Synthetic fungicides have a

negative impact on the environment and are more restricted due to the appearance of highly resistant isolates that derive from the indiscriminate use of products. The use of microbial products provides an alternative to synthetic fungicides to control phytopathogenic fungi.

9.2.3 Secondary metabolites from actinomycetes

Phytopathogens are the cause of many plant diseases and much loss of crop yields, especially in subtropical and tropical regions. SMs produced from *Actinomycetes* have antimicrobial potential and other properties. These SM are widely used to combat phytopathogens. SM produced by several species of actinomycetes belongs to genera *Streptomyces, Spirillospora, Micromonospora, Actinomadura, Streptosporangium, and Actinoplanes*, which show potential against plant pathogens (Verma et al., 2019).

Streptomyces are profile producers of previously known secondary metabolites. Actinomycetes are a group of bacteria with high guanine−cytosine content in their genomes with various metabolic possibilities. They are widely distributed in the agroenvironment, particularly in the plant rhizosphere, and influence plant growth in a significant manner. They are widely spread in different habitats, including soil environments, where they are involved in dead organic matter decomposition, nitrogen fixation (around 15% of nitrogen fixation is done by actinomycetes), and phosphate solubilization. Several actinomycetes genera are potential prolific producers of valuable bioactive secondary metabolites as antibacterial, antifungal, antibiotic, antitumor, antiparasitic, insecticide, and herbicide (Table 9.3). The use of synthetic pesticides has led to negative effects on human health and the development of pesticide-resistant pathogens and insect pests, thereby causing ecological imbalance. The use of SM-producing microbes is a good alternative for the management of insect pests and diseases that enhance the sustainability of agricultural production due to their eco-friendly behavior, low production cost, and reduced use of nonrenewable resources. Actinomycetes showed biocontrol potential against Wilt disease of banana caused by *Fusarium oxysporum* f.sp. *cubense* (FOC). Actinomycetes are well-known for the production of primary and secondary metabolites having antibiotic activities against a variety of pathogens; for example, it inhibited the growth of *Pythium ultimum* and *Erwinia carotovora* fungal pathogens that cause damping-off and post-harvest rot (Keswani et al., 2020). Among the actinomycetes, *Streptomyces* are effective in controlling plant pathogens and mobilizing and acquiring the nutrients, which are aided by several metabolites and hydrolytic enzymes such as cellulase, amylase, lipase, xylanases, peptidase, collagenase, protease, chitinase, and ligase (Aggarwal et al., 2016). The use of actinomycetes having antimicrobial properties has become one of the most attractive options for enhancing the sustainability of agricultural production due to their eco-friendliness, low production cost, and reduced use of nonrenewable resources. These metabolites are known to possess antibacterial, antifungal, neuritogenic, anticancer,

Table 9.3: List of antagonistic actinomycetes and their disease-suppressing activity against plant pathogens.

S. no.	SM	Target pathogens/ resistance against/disease	Microbes/source	Reference
1	Mildiomycin	*Rhodotorula rubra*	*Streptoverticillium rimofaciens B-98891*	Solanki et al. (2016)
2	Geldanamycin	*R. solani*	*S. hygroscopicus var. geldanus, S. griseus*	Shih et al. (2003)
3	Tubercidin	*P. capsici, Magnaporthe grisea, and Colletotrichum gloeosporioides*	*Streptomyces violaceusniger*	Solanki et al. (2016)
4	Daunomycin	*Actinomadura roseola Ao108*	*P. capsici and R. solani, Phytophthora*	Solanki et al. (2016)
5	5-Hydroxyl-5-methyl-2-hexenoic Acid	*Actinoplanes sp. HBDN08*	*B. cinerea, C. cucumerinum, and Corynespora cassiicola*	Ding et al. (2019)
6	1H-Pyrrole-2-carboxylic acid (PCA)	*Streptomyces griseus H7602*	*P. capsici*	Nguyen,et al. (2015)
7	Blasticidin S	*Streptomyces griseochromogenes*	*Rice blast*	Law et al. (2017b)
8	Validamycin A	*Streptomyces hygroscopicus var. limoneus*	*Rice sheath blight*	Yu et al. (2005)
9	Xanthobaccin A	*Lysobacter sp. strain SB-K88*	*Lysobacter sp. strain SB-K88*	Islam et al. (2005)
10	Bafilomycins B1 and C1	*S. halstedii K122*	*Aspergillus fumigatus, Mucor hiemalis, Penicillium roqueforti, and Paecilomyces Variotii*	Solanki et al. (2016)
11	Natamycin	*Streptomyces lydicus strain A01*	*F. oxysporum, B. cinerea, Monilinia laxa*	

antialgal, antimalarial, and antiinflammatory activities. *Streptomyces* genus is well-known for their potential to produce a large number of inhibitory metabolites used in industry and pharmacy; for example, SM produced from *S. plicatus* inhibited *Phytophthora infestans* and *Sclerotium rolfsii* pathogenic fungi. Actinomycetes are reported to produce SM such as Siderophores, hydrocyanic acid (HCN), Indole acetic acid (IAA), etc., while some of them play a role in plant growth promotion, induce various physiological and defense pathways, act as BCA, and improve nutritional values. Many studies reported the biocontrol potential of actinomycetes against a range of plant pathogens such as *Alternaria, Fusarium, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Verticillium*, and *Streptomyces* spp. (Vurukonda et al., 2018). Antibiotics including aminoglycoside, anthracyclines, chloramphenicol, β-lactams, macrolides, and tetracyclines have been discovered from members of this genus (Solecka et al., 2012). Many studies reported various biological applications of Actinomycetes SM along with the antimicrobial potential for the protection of soil fertility and control of phytopathogen. Therefore, actinomycetes are a good alternative for the management of insect pests and diseases, and there are many reports well documented with its potential explored extensively.

9.3 Endophytes

Biotic stresses are the major constraint to the important crop production and inflict significant losses to global agriculture. Microbes produced SM induces plant defense reactions leading to a systemic resistance to pathogen infection. Endophytes are beneficial microbes that inhabit the interior of plant tissue without causing apparent disease in the host plant, are also a source of SMs, and promote host plant growth, increase plant nutrient uptake, prevent plant pathogen development, may act as elicitors of plant defenses, reduce disease severity, and increase tolerance to environmental stress (Lacava and Azevedo, 2013). In the last years, the use of endophytic microorganisms has produced bioactive compounds that have been found to play an important role in the biocontrol of plant disease and plant growth promotion. Many studies reported SMs such as antibiotics, non-ribosomal peptides (NRPs), toxins, polyketides (PKs), ribosomal peptides (RPs), low-molecular-weight volatile organic compounds (VOCs), and hybrids between PKs and NRPs that have showed antagonistic activity against plant pathogens (Buddhika and Abeysinghe, 2021). These secondary metabolite compounds are immensely involved in combating pathogens, particularly by developing a disease-suppressive soil. Several endophytic microorganisms are well-known producers of SM; for example, the *Trichoderma* genus showed activity against phytopathogens and compounds that substantially affected the metabolism of the plant (Vinale et al., 2014). *Aspergillus* spp. and *Penicillium* spp. increased plant growth and showed biocontrol potential against soil-borne pathogenic fungi (Boughalleb-M'Hamdi et al., 2018). In addition, antimicrobials against plant pathogens and biocontrol-related metabolites may also increase disease resistance by triggering systemic plant defense activity and/or by enhancing root and shoot growth. Endophytes represent an eco-friendly and a powerful tool for the implementation of IPM strategies through the production of different secondary metabolites (Fig. 9.2). The endophytes communicate by various signaling molecules in order to modulate

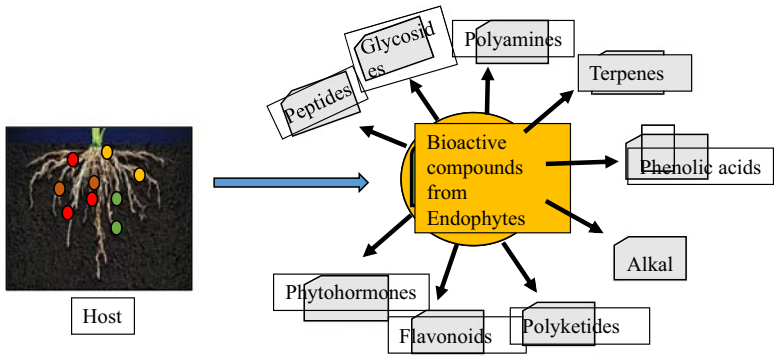

Figure 9.2
Secondary metabolites produced by endophytes.

their functional mechanism by controlled release of antibiotic and toxins and the regulation of gene expression (Netzker et al., 2015). The microbes that are capable of SM production include various bacteria, yeast, fungi, and symbionts which are not only antagonistic to phytopathogens but also help in the enhancement of plant growth and development by acting as a sink for nutrients that function as a site of colonization of plant parts.

9.4 A new source of microbial metabolites for plant disease management

Many bacteria, fungi, and actinomycetes have been unleashed for the presence of potential secondary metabolites, though there are many untapped sources. One of them is extremophiles which comprise halophiles, thermophiles, psychrophiles, archea, anaerobes, and microbes living in extreme conditions (Hui et al., 2021). The unique environment the microbes face for their survival and growth in extreme conditions can induce the synthesis of novel secondary metabolites, which are difficult to produce as far as production by other microbial counterparts is concerned. Table 9.4 illustrates the microbial metabolites isolated from extremophiles for their potent antimicrobial activities.

Lichens are the prolific producers of many novel compounds, such as usnic acid, atranorin, and physodic acid, which are highly potent in combating plant pathogen attacks (Schinkovitz et al., 2016). Many isolated secondary metabolites of lichens have shown significant antibacterial and antifungal activities against plant pathogens, but their large-scale application is still in infancy.

Recently, endophytes have also emerged as potent sources of bioactive compounds (Taritla et al., 2021), which can be used against plant disease management. They have emerged as prolific producers of novel antimicrobial compounds as well as substitutes for secondary metabolites producing plants. Table 9.5 summarizes some of the metabolites produced from lichens and endophytes, which can potentially be utilized against plant pathogens.

With the dawn of the genomic era, many omics-based strategies have pioneered the discovery of potent microbial metabolites for plant disease management. With high throughput sequencing (454, Illumina and ion torrent) and newly developed fourth-generation sequencing (Oxford Nanopore Sequencing), a large amount of data has been generated, which can allow the genome mining in more depth and accuracy (Paterson et al., 2017). Genome mining is the exhaustive search inside the available genomic, proteomic, and metabolomics sequences for the discovery of gene clusters, proteins, and metabolites of interest with the help of bioinformatics tools. Mullins et al. (2019) identified cepacin as a plant protective metabolite from biopesticidal bacterium *Burkholderia ambifaria* by genome mining. Genome mining and metabolomics analysis revealed the presence of many known and unknown biosynthetic gene clusters in a panel of 64 *Bacillus ambifaria* strains. Genome mining of plant endophyte *Streptomyces scabrisporus* NF3 revealed a number of genes associated with secondary metabolite production, xenobiotic degradation, and genes present

Table 9.4: Microbial metabolites isolated from extremophiles.

S. no.	Secondary metabolites	Producer	Characteristic features	Antipathogenic activity	Reference
1.	Perfluorotributylamine, Cyclopentane	*Halomonas salifodinae* MPM-TC	Isolated from Solar salt works, India	Antibacterial and antiviral	Velmurugan et al. (2013)
2.	Sulfolobicins	*Sulfolobus islandicus*	Grow at a low pH range of 2–4 and high temperatures between 65°C and 85°C	Antibacterial	Charlesworth and Burns (2015)
3.	Diketopiperazines	*Haloterrigena hispanica*	haloarchaeon	Antibacterial and antiviral	Charlesworth and Burns (2015)
4.	Volatile emission	*Nitrosocosmicus oleophilus* MY3	Ammonia oxidizing	Antibacterial activity against Pectobacterium carotovorum and Pseudomonas syringae in Arabidopsis thaliana	Song et al. (2019)
5.	4-oxo-1,4-dihydroquinoline-3-carboxamide	Nocardiopsis terrae YIM 90022	novel halophilic actinomycete	Antimicrobial activity against plant pathogens	Tian et al. (2014)
6.	streptoclorine, nigericin and piericidin A1	*Streptomyces fildesensis*	Isolated from the Antarctic region	Antibacterial activity	Núñez-Montero et al. (2019)
7.	Bacteriocins	*Neocallimastix californiae*	Anaerobic fungus of the gut microbiome of herbivores	Antibacterial activity	Swift et al. (2021)
8.	halocin H4	*Haloarchaeon mediterranei* R-4	Resides in hypersaline environment	Antibacterial activity	Kumar et al. (2021)
9.	Ethyl acetate extract of Al-Dhabi-1	*Streptomyces* sp. Al-Dhabi-1	Isolated from Tharban hot spring, Saudi Arabia	Antibacterial activity	Al-Dhabi et al. (2016)
10.	n-butanol extract	*Bacillus licheniformis* strains VK2 and VK21	Isolated from thermal springs of the Kamchatka Peninsula	Antibacterial activity	Esikova et al. (2002)
11.	N-propionylanthranilic acid	*Thermophilic Laceyella sacchari*	Isolated from a green alga of the genus Spirogyra	Antibacterial, herbicidal activity	Akiyama et al. (2014)
12.	Austinol,	*Penicillium sp.*	Isolated from Ghamiqa Hot Spring in Saudi Arabia	Antibacterial activity	Orfali and Perveen (2019)
13.	Cycloaspeptide D	Psychrophilic fungus Penicillium reibeum		Antibacterial activity	Wickneswary et al. (2016)
14.	Psychrophilin D	Psycrophilic fungus Penicillium rivulum	Isolated from Greenland	Antibacterial activity	Wickneswary et al. (2016)

(*Continued*)

Table 9.4: (Continued)

S. no.	Secondary metabolites	Producer	Characteristic features	Antipathogenic activity	Reference
15.	Chetracins B and D	Psychrophilic fungus Oidiodendron truncatum	Collected from the soil under lichens in Antarctica	Antibacterial activity	Wickneswary et al. (2016)
16.	Anthraquinones	*Alternaria* sp.	Halotolerant	Antifungal activity	Baranova et al. (2020)
17.	botryorhodine I	*Lasiodiplodia theobromae* M4.2—2	Isolated from mangrove soil	Antifungal activity	Baranova et al. (2020)

Table 9.5: Microbial metabolites isolated from endophytes and lichens.

S. no.	Secondary metabolites	Producer	Host/mechanism	Reference
1.	Usnic acid	*Usnea, Evernia, Lecanora, Cladonia, and Parmelia*	Inhibition of DNA and RNA synthesis	Maciąg-Dorszyńska et al. (2014)
2.	Atranorin	*Parmotrema rampoddense, Eernia*	Antibiofilm activity	Pompilio et al. (2013)
3.	Physodic acid	Evernia prunastri and Pseudoevernia furfuraceae, Hypogymnia physodes	DNA damage and antienzymatic activity	Studzińska-Sroka and Zarabska-Bożjewicz (2019)
4.	Vulpinic acid	Letharia vulpina	DNA damage	Ranković and Kosanić (2021)
5.	Diaporone A	Fungus *Diaporthe* sp.	Plant endophyte showing antibacterial activity	Guo et al. (2020)
6.	Cephalosol	Endophytic Cephalosporium acremonium IFB-E007	Isolated from the root of *Trachelospermum jasminoides* showing strong antimicrobial potential	Zhang et al. (2008)
7.	eupenicinicols A and B	Fungus, *Eupenicillium* sp. LG41	Isolated from the roots of *Xanthium sibiricum* with antimicrobial activity	Li et al. (2014)
8.	Ethyl acetate extract	Endophytic actinomycetes *Nocardiopsis* sp. GRG1	Isolated from brown algaeActive against MDR pathogens	Rajivgandhi et al. (2016)
9.	lateropyrone	fungal endophyte *Fusarium tricinctum*	Coculturing the fungus with bacterium *Bacillus subtilis*, antibacterial activity against pathogens	Ola et al. (2013)
10.	Ethyl acetate extract	*Pseudomonas aeruginosa* CP043328.1	Isolated from the leaves of *Anredera cordifolia* CIX with good antimicrobial activity	Nxumalo et al. (2020)

for symbiotic association (Ceapă et al., 2018). Similarly, genome mining of seven bacterial strains isolated from plant rhizosphere revealed the presence of novel biosynthetic gene clusters, including two NRPSs, four NRPS-PKS hybrids, and five bacteriocins (Li et al., 2020). The increasing number of microbes screened for biosynthetic gene clusters by genome mining has been increasing, which can help in uncovering novel strains, novel gene clusters, and novel microbial metabolites for revolutionizing plant disease management.

9.5 Challenges and future perspectives

Microbial metabolites have shown promising *in vitro* and *in vivo* results against plant diseases, though very few metabolites have been made for commercial production till now. AAL toxin isolated from *Alternaria alternate*, bialaphos, and herboxidiene isolated from *Streptomyces* sp. are some of the microbial metabolites which have been commercialized in the USA and Japan under different generic names (Saxena and Pandey, 2001). For the journey of microbial metabolites to the farmers to complete, the many factors standing between their production by microorganisms and their use by farmers must be researched and are described as follows.

9.5.1 Increase in yield of microbial metabolites

Any bioactive compounds isolated from the microbial source have faced the problem of low yield. For microbial metabolites to combat the plant pathogen attacks, a higher quantity of product is required in comparison to that required in biomedical applications. Several approaches have been researched for increasing the yield of microbial metabolites. One of the most successful approaches is the chemical synthesis or semisynthesis of natural products; however, due to the structural complexity and chirality of the natural product, their chemical synthesis has never been easy (Pham et al., 2019). The second approach employed is media optimization by optimizing physicochemical parameters, stress induction, and the use of elicitors. The yield of iturin A was increased by optimizing culture conditions of inulin, L-sodium glutamate, and $MgSO_4$ by response surface methodology to 99.73 mg/L from 37.35 mg/L (Dang et al., 2019). Genetic engineering of the strain is another approach used to increase the yield of secondary metabolites from microbes. Employing mutation, genetic engineering, and protoplast fusion, the synthesis of many secondary metabolites has been enhanced (Adrio and Demain, 2006). Dang et al. (2019) inserted a strong constitutive promoter C2up upstream of the *itu* operon for increased production of iturin A.

9.5.2 Sustained release and low cost

For effective functioning of microbial metabolites in plant disease, it is necessary that they should be released in a sustained manner and should be absorbed by the plant cells. Several mechanisms such as microencapsulation, nanoencapsulation, and nanoemulsions have been

researched for the sustained delivery of microbial metabolites. Starch–alginate beads were used for the controlled release of BCA for controlling aflatoxin contamination (Feng et al., 2019). Similarly, Pandey et al. (2021) described the synthesis of eucalyptus oil (ENE) and peppermint oil (PNE) nanoemulsions for the effective control of plant pathogens. However, during agriculture application, costing becomes a major controlling factor. Nanoencapsulations and nanotechnology applications can drastically reduce the application cost by increasing the efficacy of microbial metabolites (Kumari et al., 2017); however, during nanoencapsulation, the choice of polymers should be kept in mind. Polymers such as chitosan and alginate not only show up as cost-effective but can also protect plants from biotic and abiotic stresses.

9.5.3 Environmental safety and toxicological assessment

Before the commercialization of any microbial product, the safety should always be assessed for its potential impact on the environment, food chain, and native microflora. The final concentration of natural products should be optimized for efficient plant disease management without causing phytotoxicity or cytotoxicity. Many guidelines from government bodies of India, Europe, and the United States have been proposed, which should strictly be adhered to for the industrialization of microbe-derived products against plant pathogens.

9.5.4 Academics and industrial collaborations

Any transition of research from lab to land requires a fruitful collaboration between academics and industries. To converge the success stories of microbial metabolites as plant pathogen antagonists in the laboratory into the great saga of success in the field, the enhanced production, costing policies, timely delivery, and promotional activities are required. A collaboration between academic research will not only guarantee the large-scale production of microbial metabolites but will also overcome the challenges standing in between.

9.6 Conclusions

Microbial metabolites are a unique pandora's box of nature that has a remedy for every kind of illness of mankind and the plant system. The antimicrobial metabolites of microbial origin can be a sustainable and cost-effective substitute to the traditional chemical pesticides owing to their targeted toxicity to the pathogens. Their additional role in enhancing plant immunity provides them a superior edge over conventional antimicrobials. A number of bacteria, fungi, and actinomycetes are known to produce antimicrobials that can effectively be used against plant pathogens. The multiple mechanisms shown by them

include iron chelation, ROS generation, and membrane and DNA damage. With the emergence of next-generation sequencing, genome mining and searching for new hosts from different niche areas guarantee the discovery of novel and potential antimicrobials in the future; however, certain limitations are yet to be overcome to make the metabolites successful on a large scale.

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CHAPTER 10

Secondary metabolites from marine fungi: current status and application

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10.1 Introduction

Natural fungal products have a glorious history in alleviating human illness. Harold Raistrick's group began the systemic study of fungal secondary metabolites that eventually resulted in the characterization of 200 fungal metabolites (Raistrick, 1950). However, with the discovery of the blockbuster metabolite penicillin, pharmaceutical companies were instigated into putting place a thorough screening of microbial metabolites. The transformative capacities of the fungal kingdom are rendered by the peculiar and unusual biosynthetic pathways. These have resulted in the production of several toxins, poisons, anticancer, and antibiotic compounds. Today, low-molecular-weight natural products have garnered widespread attention (Keller et al., 2005). The contemporary technologies are, in most cases, unable to produce commercial quantities of most bioactive compounds. Hence, the natural sources of these compounds, bacteria and fungi, are employed as biocatalysts for large-scale production (Panagiotou et al., 2009). The discovery of bioactive compounds can be achieved using two strategies: screening samples from various ecological spaces or identifying specific gene clusters in their genomic sequences. With the advances and ease of whole-genome sequencing, the genome mining approach has revealed an incredible number of secondary metabolite gene clusters. This signifies the potential of fungi as a treasure mine for the discovery of novel bioactive compounds (Vassaux et al., 2019).

Fungi surviving in several environments are constantly challenged by ranges of biotic and abiotic stress. These may include pH, temperature changes, and limited nutrients. The production of secondary metabolites is a physiological response to these conditions. These metabolites are a heterogenous group of small molecules representing immense structural diversity.

Fungal secondary metabolites can be classified into the following.

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10.1.1 Polyketides

This is the most abundant class of fungal secondary metabolites. Fusarin C, lovastatin, aflatoxin, naphthopyrone, griseofulvin, and mycophenolic acid are some of the best characterized fungal polyketides. The polyketide backbone is built by two iterative polyketide synthases: polyketide synthases (PKSs), a highly reducing PKS, and a nonreducing PKS (Agarwal and Moore, 2014). Several fungal genome sequences deposited in public databases have revealed the biosynthetic gene clusters bearing the PKS genes using SMURF and AntiSMASH tools (Minami et al., 2020). Benzenediol lactones were generated using a bioengineering approach by Xu et al. (2014). They coexpressed the reducing and nonreducing PKSs from different biosynthetic pathways to produce a wide chemical space of benzenediol lactones. Such studies prove that metabolic engineering can assist in the heterologous biosynthesis of any desirable compound.

10.1.2 Nonribosomal peptides

This class of compounds is derived from proteinogenic and nonproteinogenic amino acids by nonribosomal peptide synthetases (NRPSs), a class of multimodal and multidomain enzymes. Several variations in the peptides (linear/cyclic) are introduced by the modules in NRPSs that introduce adenylation, pantothenylation, condensation, peptide bond formation, and thioesterase activity (Keller et al., 2005). *Trichoderma virens* contains peptaibol synthetase, an NRPS, that produces an 18- amino acid linear peptide bearing aminoisobutyric acid (Wiest et al., 2002). The cyclic undecapeptide cyclosporin in *Tolypocladium niveum* is also produced by an NRP lacking the thioesterase domain (Weber et al., 1994). Although most functions of NRPs remain obscure, the best-studied NRPs function as siderophores involved in the transport of iron and its homeostasis and host defense (Bills et al., 2014). Novel peptides and depsipeptides continue to be discovered from the fermentation extracts of fungi, with varied biological applications.

10.1.3 Terpenes

This diverse class of secondary metabolites has simple to complex structures derived from five-carbon isoprene units. Their diversity and pharmacological properties have aroused interest from synthetic chemists. The classical subclasses of terpenes are monoterpens, sesquiterpens, diterpenes, triterpens, sesterterpenes, steroids, and carotenoids or tetraterpenes (Ebel, 2010). Monoterpenes are rarely reported from the fungi. A chloro-monoterpene was reported from a mangrove fungus *Tryblidiopycnis* sp. from Hong Kong (Huang et al., 2006). Terpenes are generated by the defining enzyme terpene cyclase, which has been observed in fungi: trichodiene synthase from *Fusarium sporotrichioides*, a bifunctional terpene cyclase from *Gibberella fujikuroi*, etc. (Keller et al., 2005). The

biosynthesis of carotenoids has been studied extensively in *Neurospora crassa* (Schmidhauser et al., 1990). Some popular examples of bioactive terpenes include curcumin, geraniol, eugenol, and myrcene.

10.1.4 Indole alkaloids

They are usually biosynthesized from dimethylallyl pyrophosphate and tryptophan. Indole alkaloids are nitrogen-containing metabolites. The ergotamine synthesis pathway in *Claviceps purpurea* is the best-understood indole alkaloid pathway (Keller et al., 2005). This class includes the several hallucinogenic and psychedelic drugs used for recreation and medical purpose. A recent investigation of the secondary metabolites produced by the plant *Psychotria nemorosa* revealed the presence of 10 new alkaloids: nemorosines A and B, nemorosinosides A−F, 10-hydroxyisodolichantoside, two β-carboline derivatives, and nemorosinoside G, among others (Klein-Júnior et al., 2020). Novel indole alkaloids Cristatumins A−D with striking antibacterial properties were reported from *Eurotium cristatum* EN-220 (Du et al., 2012).

10.1.5 Phenylpropanoids and flavonoids

These secondary metabolites are valued in the medicinal and agricultural industries. The pathway begins with phenylalanine and further involves the action of key enzymes phenylalanine ammonia lyase (PAL), coumarate hydroxylase and ligase, chalcone synthase, reductase, and isomerase. In early 2000, the genes encoding these core enzymes for phenylpropanoid and flavonoid biosynthesis were discovered in *Aspergillus* sp., an industrially important fungus. This eventually led to identifying novel flavonoids from fungus (Juvvadi et al., 2005; Seshime et al., 2005). These genes are now targeted for metabolic engineering to produce a higher yield and other metabolites. Chrysin, a dihydroxyflavone, commonly found in passionflower and honey, was recently reported from a marine fungus *Chaetomium globosum* (Kamat et al., 2020a,b).

10.2 What makes marine fungi unique?

The search for bioactive secondary metabolites has moved from terrestrial sources to marine sources. In the marine world, the vertebrates and invertebrates, algae, etc., continue to be explored extensively; and it was learned that they have unique adaptations to changes in their environment that produce a diverse natural productome. The marine environment is amazingly unique, challenging, and extreme where the organisms have to survive by adapting to low or no light, low oxygen, high pressure, intense competition, species richness, pollution, acidification, etc. As a way to adapt to living in these stressful conditions, the organisms have evolved with biochemical and physiological adaptations that often have no counterparts in the terrestrial habitats. Thus, marine secondary metabolites

are more potent, more diverse, and of higher significance in drug discovery (Saide et al., 2021) than terrestrial ones. Around 70% of structures identified as marine natural products are only found in marine organisms (Spainhour, 2005).

Marine fungal communities have attracted attention for the search for bioactive secondary metabolites, for they also display the adapted chemical space. Marine fungi have been obtained from every possible marine habitat: soil, sand, marine wreckage, artificial substrates, marine algae, sea grass, mangroves, sponges, corals, bivalves, holothurians, crustaceans, fish, and sea sediment. They are widely distributed in deep sea and polar ice covers. Endophytic fungi cohabiting with higher forms of marine life are a rich candidate for a sustainable drug discovery process. From the ecological perspective, marine fungi can be obligate (grow and sporulate only in the sea) or facultative (can be from terrestrial or freshwater originally but have adapted physiologically to survive, grow, and sporulate in the marine environment) (Kohlmeyer and Kohlmeyer, 1979). The unique nature of the marine environment transcends into the survival of marine fungi by activating secondary metabolite genes (Raghukumar, 2008). It is observed that mangroves harbor the maximum diversity of endophytic fungal life forms. However, it is also observed that maximum reports on endophytic fungi and their compounds have been from those associated with algae. Marine sponges also demonstrate a rich source of endophytic fungal diversity. Most of the reported marine fungi belong to *Ascomycota* (Rateb and Ebel, 2011; Pang et al., 2016). Several investigations have demonstrated a wealth of fungi often represented by *Aspergillus, Acremonium, Fusarium, Penicillium, Phoma, Trichoderma, Talaromyces* sp., etc. (Imhoff, 2016). Out of the 20 endophytic fungi isolated from the marine algae found in the Konkan coast, India, a majority of 18 belonged to *Ascomycota*. The endophytes demonstrated extraordinary cytotoxic activities on cancer cells, with moderate antioxidant and antibacterial activity (Kamat et al., 2020a; Sajna et al., 2020).

Although marine natural products have been reported from an exhaustive list of sources, such as algae, invertebrates and vertebrates, the ecological concerns relating to biodiversity losses cannot be ignored. Marine fungi provide several answers to these concerns because they provide options for sustainable drug discovery. Yield enhancement of the desired metabolite just by means of media manipulation, biotic and abiotic elicitors, incubation interval, genetic engineering approaches, etc., can be achieved (Kumari et al., 2018; Taritla et al., 2021).

The area of nanotechnology has also started using biodegradable materials for the synthesis, reduction, and stabilization of nanoparticles, given the need for a green, environmentally friendly and sustainable process to balance the benefits of NPs vis-à-vis costs of production (Mahanty et al., 2019). The biologically active scaffolds from fungi fit perfectly for this purpose. Fungi secrete extracellular enzymes and are fast and easy to grow in laboratory settings. The ease of a fungal scale-up is also an advantage here (Castro-Longoria et al., 2012). The economic advantages and opportunities to use biomass are of enormous merit

for a green approach mediated by fungi to synthesize green nanoparticles. The biosynthesis of nanoparticles could be triggered by terpenoids, phenolics, flavonones, amines, pigments, alkaloids, and other reducing agents in the extract generally containing a carbonyl group (Asmathunisha and Kathiresan, 2013; Boroumand et al., 2015). *Penicillium fellutanum* from coastal mangrove sediment was reported to extracellularly produce silver nanoparticles when exposed to silver nitrate (Kathiresan et al., 2009).

In comparison with bacteria, fungi are economical, relatively easy to handle, and can withstand agitation in bioreactors due to their filamentous nature. To add to this is the unique nature of marine fungi to produce a diverse natural productome with high potency. Hence, exploring the potentiality of marine fungi for drug discovery presents a novel avenue of biomedical research.

10.3 Secondary metabolites from marine fungi and their application

The 56% of the total marine bioactive compounds, reported between 1985−2012 and surveyed by Hu et al. (2015), show anticancer activity. This was followed by antibacterial activity (13%), and the remaining 31% included other bioactivities. The statistics remained fairly the same even in 2021. Several seminal discoveries in marine natural products can be categorized according to their chemical structures into classes: alkaloids, terpenes, polyketides, polysaccharides, anthraquinones, among others (Ruiz-Torres et al., 2017).

The pipeline for discovery of bioactive marine natural products from fungi to proceed to drug development includes (Jaspars et al., 2016; Ghareeb et al., 2020):

1. collection of a marine organism or marine sediment;
2. isolation and identification of its fungi based on ITS region;
3. growing the fungi in an appropriate growth medium;
4. preparation and evaluation of fungal extracts for a desirable bioactivity—*in vitro*; anticancer, antibacterial, antioxidative, antiinflammatory, etc.
5. purification of the potent compound/s using HPLC, TLC;
6. structural elucidation using integrative spectroscopic techniques NMR, FTIR, CHONS analyses;
7. understanding the mechanism of the bioactive property;
8. enhancing the yield, changing the compound chemistry, nanoencapsulation of the compound;
9. understanding the compound bioactivity in 3D culture models/ pharmacokinetics *in vivo* using animal models;
10. clinical trials in primates, human subjects;
11. file for FDA approval.

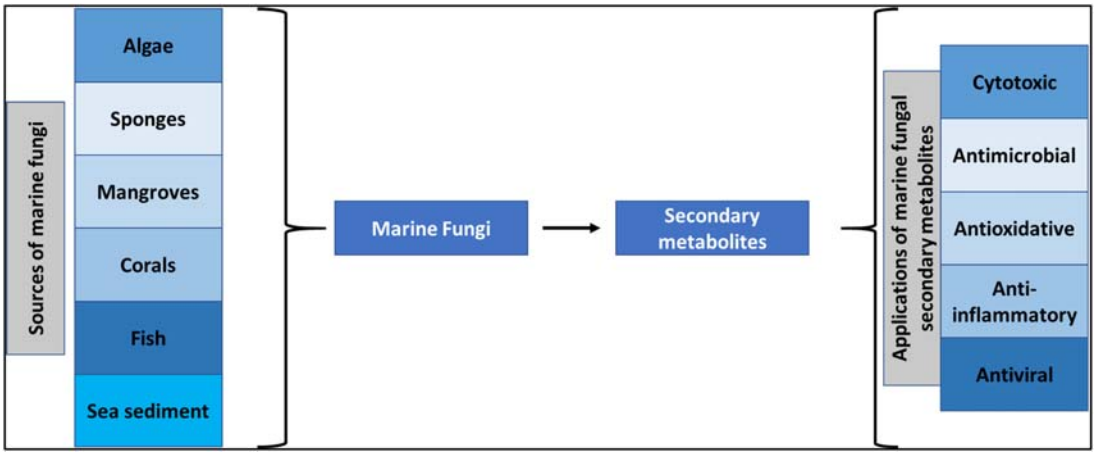

Figure 10.1
The versatility of marine fungi in biomedical application.

The applications of marine natural products from fungi can be grouped according to their bioactivities into cytotoxic, antimicrobial, antiviral, antioxidative, among others (Fig. 10.1).

10.3.1 Cytotoxic secondary metabolites from marine fungi

The drug worth mentioning in this category is Plinabulin, an artificial analog of the natural product Halimide (diketopiperazine) from a marine fungus *Aspergillus* sp. The drug acts against nonsmall cell lung cancer (NSCLC) by preventing tubulin polymerization and is currently in phase 1 of clinal trials sponsored by Nereus Pharmaceuticals, Inc (https://clinicaltrials.gov/ct2/show/NCT00322608). Another study in the pipeline conducted by BeyondSpring Pharmaceuticals is to develop the drug for the prevention of chemotherapy-induced neutropenia in the treatment of late NSCLC and other solid tumors (https://beyondspringpharma.com/pipeline/plinabulin/). The US FDA has asked for additional studies before approving the drug for clinical use. Jiangsu Hengrui Pharma, China, is also in the process of regulatory revie to develop the drug in China (https://www.reuters.com/business/healthcare-pharmaceuticals/beyondspring-says-us-fda-declined-approve-neutropenia-prevention-drug-2021-12-01/). The drug is reported to trigger antiangiogenic action in multiple myeloma cells by inducing JNK-mediated apoptosis (Singh et al., 2011).

While there are several cytotoxic drugs—either FDA approved or in clinal trials—isolated from marine invertebrates and vertebrates, algae, those of fungal origin still have to catch up. Current work on marine fungi isolated cytotoxic secondary metabolites can be summarized based on the source of fungus and its mechanism of action (Table 10.1). Cytotoxic compounds can induce cell death through apoptosis, necroptosis, ferroptosis, autophagy via DNA damage, regulating kinases, by changing the biophysical features of a

Table 10.1: Examples of cytotoxic secondary metabolites from marine fungal sources.

Secondary metabolite	Fungal source	Source of the fungus	Mechanism of cytotoxicity	Targeted cancer cells	References
Cytotoxic compounds from algae-associated fungi					
Chrysin	*Chaetomium globosum*	*Chaetomorpha media*	Apoptosis, HDAC-8 inhibitor	MCF-7, MDA-MB-231	Sun et al. (2012), Kamat et al. (2020b)
Varioloid A and B	*Paecilomyces variotii EN-291*	*Grateloupia turuturu*	–	A549, HCT116, HepG2	Zhang et al. (2016)
Cinnamolide derivative, insulicolide A	*Aspergillus ochraceus* Jcma1F17	–	–	H1975, U937, K562, – 823, Molt-4, – 7, A549, HeLa, HL60, Huh-7	Fang et al. (2014)
Physcion, Neoechinulin A	*Microsporum* sp. (MFS-YL)	*Lomentaria catenata*	Apoptosis	HeLa	Wijesekara et al. (2014)
Asperolides A–B, wentilactones A	*Aspergillus wentii* EN-48	–	–	HeLa, HepG2, MCF-7, MDA-MB-231, NCI-H460, SMMC-7721, SW1990	Sun et al. (2012)
Pentanorlanostane, dendryphiellide A	*Paradendryphiella salina* PC 362 H	*Pelvetia caniculata*	Altered expression of HSP60, HSP70, PRAS40, increased phosphorylation of p53	MCF7, MCF7-Sh-WISP2	Dezaire et al. (2020)
Conidiogenones C,H,I	*Penicillium chrysogenum* QEN-24S	*Laurencia* genus red alga	–	HL-60	Gao et al. (2011)
Cytotoxic compounds from sea sediment fungi					
Scopararane I	*Eutypella* sp. FS46	–	–	MCF-7, NCI-H460, SF-268	Liu et al. (2017)
Simplicilliumtides A-H	*Simplicillium obclavatum* EIODSF 020	–	–	HL-60, K562	Xiao et al. (2016)
Penicitrinine A	*Penicillium citrinum*	sediment samples	Apoptosis, downregulates the expression of MMP-9 by upregulating its specific inhibitor TIMP-1	A-375, SPC-A1, HGC-27	Liu et al. (2015)
falconensins O and P	*Aspergillus falconensis*	Red Sea sediment	NF-κB inhibitory activity	MDA-MB-231	El-Kashef et al. (2020)

(*Continued*)

Table 10.1: (Continued)

Secondary metabolite	Fungal source	Source of the fungus	Mechanism of cytotoxicity	Targeted cancer cells	References
Cytotoxic compounds from mangrove endophytic fungi					
Secalonic acid D	Endophytic fungus No. ZSU44	–	Apoptosis, activation of GSK-3β	HL60, K562	Zhang et al. (2009a,b)
spirobrocazines C, brocazine G	*Penicillium brocae* MA-231	–	–	A2780, A2780 CisR	Meng et al. (2016)
2,4-Dihydroxy-6-nonylbenzoate	*Lasiodiplodia* sp. 318	*Excoecaria agallocha*	–	MMQ, GH3	Huang et al. (2017)
5-methyl-8-(3-methylbut-2-enyl) furanocoumarin	*Penicillium* sp. ZH16	–	–	KB, KBV200	Huang et al. (2012)
3-O-(6-O-α-L-arabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone	*Phomopsis* sp.	*Excoecaria agallocha*	–	HEp-2, HepG2	Huang et al. (2013)
chloropreussomerins A and B, spreussomerin K, preussomerin H,G,F	*Lasiodiplodia theobromae* ZJ-HQ1	*A. ilicifolius*	–	A549, HepG2, MCF-7	Chen et al. (2016)
7-O-methylnigrosporolide, pestalotioprolides D-F	*Pestalotiopsis microspore*	*Drepanocarpus lunatus* (Fabaceae)	–	A2780	Liu et al. (2016)
Campyridones D, ilicicolin H	*Campylocarpon sp. HDN13–307*	*Sonneratia caseolaris*	–	HeLa	Zhu et al. (2016)
Dihydroaltersolanol C, alterporriol E, altersolanols A, B, N	*Stemphylium globuliferum*	*Avicennia marina*	Altersolanol A induces apoptosis, anti-invasive, inhibits NF-κB	L5178Y, 34 human cancer cell lines (altersolanol A)	Debbab et al. (2009), Teiten et al. (2013), Mishra et al. (2015)
Beauvericin	*Fusarium* sp. (No. DZ27)	–	Apoptosis	KB and KBv200	Tao et al. (2015)
Waol A, pestalotiopene A, cytosporone E	*Acremonium strictum*	*Rhizophora apiculata*	–	human cisplatin-sensitive and resistant A2780 cell lines	Hammerschmidt et al. (2014)

Cytotoxic compounds from sponge and coral associated fungi					
Cytochalasin K	*Arthrinium arundinis* ZSDS1-F3	*Phakellia fusca*	Disrupts cytoskeletal filaments	K562, A549, Huh-7, H1975, HL60, HeLa, MOLT-4	Schliwa (1982), Wang et al. (2015)
Aszonapyrone A, 13-oxofumitremorgin B, sartorypyrone A,B	*Neosartorya laciniosa* (KUFC 7896)	–	–	MCF-7, NCI-H460, A375-C5	Eamvijarn et al. (2013)
disydonols A and C	*Aspergillus* sp.	*Xestospongia testudinaria*	–	HepG-2, Caski	Sun et al. (2012)
Marilines A1 and A2	*Stachylidium* genus	*Callyspongia* cf. *C. flammea*	Inhibited the human leukocyte elastase (HLE)	–	Almeida et al. (2012)
Cytotoxic compounds from other marine fungi					
Quinolinone derivative	*Aspergillus versicolor* Y31–2	Indian Ocean sea water	–	MCF-7 and SMMC-7721	Li et al. (2016)
Penicilazaphilones B,C	*Penicillium sclerotiorum* M-22	rotten leaf collected on the west coast of Haikou, China	–	B-16, SGC-7901	Zhou et al. (2016)
Furan derivative	*Penicillium chrysogenum* HGQ6	Lianyungang sea mud sample	–	BGC823	Guo et al. (2016)
Pimarane diterpenoids	*Epicoccum* sp. HS-1	*Apostichopus japonicas* (sea cucumber)	–	KB, KBv200	Xia et al. (2012)
Trichodermamides (modified dipeptides)	*Trichoderma virens*	*Didemnum mole* (ascidian), green alga *Halimeda* sp	S-phase accumulation, DNA double strand breaks	HCT-116, HeLa	Jans et al. (2017)
Cephalimysin A	*Aspergillus fumigatus OPUST106B- 5*	*fish* Mugil cephalus	–	P-388, HL- 60	Hasan et al. (2015)

cancer cell, etc. It is necessary to not just report the structure of the compound but also study the ways in which it induces cell death.

10.3.2 The need of antimicrobial secondary metabolites from marine fungi

The oceans—which constitute a large part of the earth's surface—have remarkable chemical and biological diversity. Marine ecosystems are made up of groups of organisms that rely upon each other and their environment. Marine ecosystems are biologically more distinct than their corresponding terrestrial habitats. In the early stages, the most commonly isolated marine natural products (MNPs) were from terrestrial fungi, but steadily, with analysis of the diversity of microbes in the marine environment, it became practical to isolate marine fungi to generate a huge variety of natural products. In a recent chemoinformatics study, it was found that approximately 71% of the natural products derived from marine organisms are not expressed in terrestrial organisms (Montaser and Luesch, 2011). Initially, the identification of MNPs was concentrated mostly on macroorganisms like algae, sponges, and corals (Almeida et al., 2011; Leal et al., 2012), but modern studies have fixed their attention on identifying bioactive compounds in microorganisms like bacteria and fungi which make up a substantial part of marine biomass (Gerwick and Moore, 2012). Natural compounds obtained from endophytic fungi have shown promise as potent sources for drug discovery. Fungi obtained from the marine origin are known to have yield in a variety of unique biologically active secondary metabolites, which have become a vital resource of distinct pharmacologically active metabolites. Endophytes are a rich source of many compounds having distinct properties, which may help in acquiring significance in the agricultural and pharmaceutical fields due to their anticancerous, antibacterial, immunosuppressive, antifungal, and antioxidative properties (Zhang et al., 2006; Mishra et al., 2017).

The development of multidrug-resistance (MDR) among various microorganisms in the last two decades has become a major concern in the field of modern medicine. MDR is a phenomenon that is revealed as acquired in a species of microorganism due to many factors such as gene mutations encoding resistance towards a definite agent, mechanism of efflux pumps, the buildup of transposons, and resistance plasmids. MDR microorganisms are generally nonsusceptible to at least one drug in three or more antimicrobial categories. Most commonly known developing global concerns are MDR-TB and XDR- TB, i.e. (Extensively drug-resistant TB), which is brought about by species resistant to drugs like isoniazid, rifampin, and any fluoroquinolone (Gillespie, 2002; LoBue, 2009).

There has been a large increase in Methicillin-resistant *S. aureus* (MRSA), which is not just resistant to methicillin but shows nonsusceptibility towards macrolides, chloramphenicols, aminoglycosides, tetracycline, and disinfectants too. Penicillin resistant *S. pneumoniae* (PRSP), Vancomycin-resistant *Enterococcus faecium* (VRE), and other types of MDR

microorganisms have become a matter of concern (Menichetti, 2005). The recently developed drugs such as dalfopristin and linezoid have already developed resistance (Mutnick et al., 2003; Skiest, 2006). Reports have shown that *Staphylococcus aureus*, *Enterococcus faecium*, *P. aeruginosa*, *Klebsiella pneumoniae*, *A. baumannii*, and *Enterobacter* species are the most common species responsible for the majority of the infections in the US hospitals (Rice, 2008).

Recent efforts and studies have been exclusively focused on the search for natural compounds showing a broad spectrum of antimicrobial activities from endophytic fungi. They produce a variety of groups of secondary metabolites like polyketides, nonribosomal peptides, alkaloids, isoprenoids, proteins, lipids, terpenes, etc. (Mayer et al., 2011). These endophytes are present throughout the world in extremely cold Arctics, temperate regions, and tropical climates as well. They can be cultivated on a small scale as well as industrial-scale fermenters to produce adequate quantities of biologically active agents; hence, they are a promising candidate for commercial exploitation.

10.3.2.1 Secondary metabolites extracted from marine endophytic fungi showing antibacterial activity

Table 10.2 presents bioactive products obtained in endophytic fungi in a marine environment which showed antibacterial activity against several bacterial species.

10.3.2.1.1 Inhibitory mechanism of antibacterial compounds

It is hypothesized that the antibacterial mechanism of the marine compounds is mainly dependent upon the chemical properties and functional groups present in the compound. It is speculated that the cells of bacterium interacting with the compounds that cause inhibition of respiratory enzymes encourage the generation of ROS (reactive oxygen species), including peroxides, superoxide, hydroxyl radical, singlet oxygen, etc., and thus, lead to cell damage.

Studies focused on understanding the inhibitory mechanism of marine compounds against bacterial species revealed that upon treatment with the metabolite, the organism loses its ability to carry out DNA replication. It also causes the inactivation of key enzymes necessary in energy production, that is, ATP, and the microorganism sometimes loses its ability to express cellular proteins, ribosomal subunit proteins, etc. Reports show the antibacterial activity of a secondary metabolite, Trichoderin A, derived from *Trichoderma sp*., is attributed to its ATP production inhibition mechanism (Pruksakorn et al., 2011).

Ascochytatin, a naphthalene derivative extracted from marine *Ascochyta sp*., showed the most potent activity against *B. subtilis*, where the organism's growth regulator system is attacked by targeting the TCS gene, that is, two-component regulatory system gene, so as to

Table 10.2: Antibacterial compounds produced from marine endophytic fungi.

Crude extract/ metabolite	Fungal sp.	Marine source	Susceptible microorganisms	Reference
Malettinin E	*Cladosporium*	Wadden Sea	*Xanthomonas campestris*, *Trichophyton rubrum*	Silber et al. (2014)
Penicillosides A and B	*Penicillium*	Red Sea	*Candida albicans*, *Staphylococcus aureus*, *E. coli*	Murshid et al. (2016)
Dinemasones A and B	*Dinemasporium strigosum*	Baltic Sea	*Bacillus megaterium*	Krohn et al. (2008)
Pestalone	*Rosenvingea*	Bahamas Islands	*Bacillus subtilis*, MRSA, VRE	Augner et al. (2013)
Nigrosporin	*Nigrospora*	South China Sea	*M. tuberculosis*	Wang et al. (2013)
Brevianamide M	*Sargassum thunbergii*	Red Sea	*S. aureus*, *E. coli*	Miao et al. (2012)
Siderin	*Halimeda opuntia*	Red Sea	*Bacillus cereus*, *B. subtilis*, *S. aureus*	Hawas et al. (2012)
Anhydrofusarubin	*Cladosporium*	South China Sea	*Staphylococcus aureus*	Shao et al. (2008)
HF13	*H. fascigera*	South Coast of West Sumatra	*S. aureus*, *E. coli*, *C. albicans*	Handayani et al. (2015)
Phomoxanthone	*Phomopsis longicolla*	–	*Plasmodium falciparum*, *M. tuberculosis*	Choi et al. (2013)
Trypethelone	*Coniothyrium cereale*	–	*Mycobacterium phlei*, *S. aureus*, *E. coli*	Elsebai et al. (2011)
Xanalteric acid	*Alternaria*	Hainan Island, China	MRSA	Kjer et al. (2009)
Aspergillusol A	*Aspergillus aculeatus*	Ton Sai Bay, Phi Islands	*Bacillus stearothermophilus*	Ingavat et al. (2009)
Yicathin A-C	*A. wentii*	–	*Escherichia coli*, *Staphylococcus aureus*, *Colletotrichum lagenarium*	Sarasan et al. (2017)

alter its function (Kanoh et al., 2008). *Aspergillus versicolor* produced amide compounds called Brevianamides, which were selectively active against BCG (Bacillus Calmette Guerin), suggest a novel inhibition mechanism that could illuminate the development of upcoming antitubecular drugs (Song et al., 2012).

The inhibitory mechanism of the compound Psammaplin A extracted from the sponge was determined by analyzing its DNA gyrase inhibition against MRSA (Kim et al., 1999).

Phlorofucofuroeckol-A (PFF) is a compound from a brown algal sp. which showed antibacterial activity by exploiting the function of PBP2a, that is, Penicillin-binding protein 2a. PFF also showed potent inhibition towards MRSA by supressing expression of its regulatory genes mecI, mecR1, and mecA (Eom et al., 2014).

Jeon and his associates studied the mechanism of action of the marine *Sceptrella sp.* isolated compound, discorhabdin Z. It was determined that proteins present on bacterial cell walls act as an attachment site for the bacterial transpeptidase, Sortase A, which binds covalently to cell wall proteins. This enzyme has likely become a new target for antibacterial drugs (Jeon et al., 2010).

Table 10.3: Antiviral compounds produced from marine endophytic fungi.

Crude extract/metabolite	Country	Inhibitory activity	Reference
Neoechinulin B	China, Germany	H3N2 and H1N1 A influenza virus	Chen et al. (2015a,b)
Chartarutine B	China, Germany	HIV-1	Li et al. (2014)
Truncateol M	China, Germany	H1N1 A influenza virus	Zhao et al. (2015)
Thaixylomolin I	Thailand, Germany, China	H1N1 A influenza virus	Li et al. (2015)
Aflaquinolone B derivative	China	Respiratory syncytial virus	Chen et al. (2014)
(+)-pestaloxazine A	China	Enterovirus 71	Jia et al. (2015)
Trichobotrysin A	China	Herpes simplex virus (HSV-1)	Sun et al. (2015)
Territrem D and Arisugacin A	China	HSV-1	Nong et al. (2014)

10.3.2.2 Secondary metabolites extracted from marine endophytic fungi showing antiviral activity

Table 10.3 denotes the information taken from reports published between 2014–15, specifically about bioactive products obtained from endophytic fungi in the marine environment showing antiviral properties.

10.3.2.2.1 Inhibitory mechanism of antiviral compounds

Unlike other drugs, antiviral drugs do not target to kill the infectious microorganism but try to inhibit their growth. It is quite challenging to produce a drug that does not interfere with or harm the host cells, which are a source for viral replication. A viral infection takes place in five main steps, viz. attachment, penetration/invasion, uncoating, replication, and assembly and release. Based on the approach of viral inhibition, antiviral drugs may be classified into two types: drugs that target cellular function that is essential to the virus or drugs targeting the viral function itself. Drugs that directly affect viral function mainly target the key enzymes like proteases, integrases, neuraminidases, and nucleic acid polymerases (DNA dependant DNA polymerase, RNA dependant RNA polymerase, DNA dependant RNA polymerase) (Kausar et al., 2021).

Truncateol M isolated from fungal sp. *Truncatella angustata* was studied to analyze its antiviral inhibitory mechanism, which was found to target the assembly and release step of viral replication, which may serve as a novel lead for antiviral drugs. Marine fungus *Eurotium rubrum* produced secondary metabolite neoechinulin B. The possible mechanism of neochinulin B involved in antiviral activity displayed against the influenza virus was explained by Chen and his associates. The alkaloid was found to bind to the viral envelope hemagglutin, thereby succeeding in blocking its association with the host cell's sialic acid receptors. Chen and coworkers found a dihydroquinolone (Aflaquinolone B) derivative, produced on *Aspergillus* sp. mycelia, that showed inhibition against H1N1 virus at a high

therapeutic ratio (Chen et al., 2015a,b). Bioactive products isolated from marine fungi can emerge as potential therapeutics in the management of SARS-CoV-2. *In silico* studies showed that marine fungi-derived tirandamycins targeted SARS-CoV-2 methyltransferase nsp16/10, while alteramide A resulted in inhibition of RdRp (Zahran et al., 2020). Further in vitro and in vivo investigations are required for the development of marine-based drugs against SARS-CoV-2.

10.3.3 Antioxidant compounds derived from marine fungi

Antioxidants are a vast group of molecules that serve as the first line of defense against free radical damage, making them necessary for good health and acting as protective agents capable of deactivating or stabilizing free radicals before they destroy cells (Kalam et al., 2012). An antioxidant is essentially a molecule (or an ion or a relatively stable radical) that can delay or even inhibit other molecules from oxidizing. The rising trend of the antioxidant market reflects the prospect of curing a wide range of diseases possibly caused or impacted by oxidative stress (Halliwell and Gutteridge, 2007). Oxidative stress is usually associated with high levels of reactive oxygen and nitrogen species, which is defined as an imbalance between pro-oxidative and antioxidative components (ROS and RNS, respectively). It is also indicated by high levels of oxidation products, such as DNA fragments and hydroperoxides (Sies, 1986). These species have the ability to be both harmful and useful. The balance between their two opposite effects is clearly an important aspect of life. ROS and RNS have favorable effects on cellular responses and immunological function at low to moderate levels. They cause oxidative stress at high doses, which is a harmful process that can affect all cell structures. It is to be noted that oxidative stress has been linked to the pathogenesis of a variety of diseases, including diabetes, atherosclerosis, various cancers, and viral infections, including AIDS (Halliwell and Gutteridge, 2007; Niki, 2010). Antioxidants can prevent the rise of degenerative, cardiovascular, and neurological diseases by inactivating reactive oxygen and nitrogen species. (Vitale et al., 2020).

Microorganisms offer a great way to get a steady supply of bioactive chemicals like antioxidants, which can be used to meet the needs of industries that need massive amounts of biomass for clinical trials and industrial production. Despite the fact that microorganisms play an important role in ecosystem functioning and global biogeochemical cycles of the primary elements (Stocker, 2012), we still know little about the diversity, the ecological role, and the metabolic capabilities of marine microorganisms. Marine microbial eukaryotes, including fungi, are abundant and ecologically significant constituents of the marine microbiota, and several studies have shown their before unthinkable diversity, even in deep sea hydrothermal habitats, using molecular and metagenomics methodologies (Heidelberg et al., 2010). Marine fungi produce a wide range of well-known bioactive

compounds, among which are anticancer, antioxidant, antibiotic, antiviral compounds, and molecules having antiproliferative activity (Blunt et al., 2018). Interestingly, a large number of antioxidants molecules isolated from marine fungi are reported which, due to their unique properties, can be used in a variety of fields, including food, cosmeceuticals, and pharmaceuticals (Vitale et al., 2020). Recent findings on the use of various marine microorganism-derived products, primarily in cosmetics and feed, have opened the door to using marine fungal metabolites in this as well as other domains (Martins et al., 2014). In terms of production costs and process sustainability, the utilization of marine fungi-derived products would have significant advantages over synthetic antioxidants and those derived from sources such as plants and algae.

The following Table 10.4 gives a list of antioxidative molecules extracted from marine fungi, along with the source of isolation of the respective marine fungal strains.

10.4 Commercial application and clinical investigations of marine fungi-derived secondary metabolites

Though marine fungi-derived secondary metabolites are able to show myriad applications in pharmaceutical, food, agriculture, textile, leather industries, and bioremediation, still commercial development is in its infancy. Table 10.1 describes the industrial applications of some of the secondary metabolites isolated from marine fungi. Many of the natural products isolated from different marine flora and fauna are under different phases of clinical trials. As of January 2019, Aplidin, PM00104, pseudopterosin A, kahalalide F, hemiasterlin, and Plinabulin (NPI-2358) are some of the examples of biologically active marine products which are in different stages of clinical trials (Ghareeb et al., 2020). Till December 2018, six marine drugs have the status of FDA approved, including cytarabine Eribulin mesylate, Brentuximab vedotin 63, Keyhole Limpet Hemocyanin for viral treatment, Vidarabine for pain, Ziconotide and omega-3-acid ethyl esters for hypertriglyceridemia (Ghareeb et al., 2020), but the contribution of marine fungi-derived products has been scarce (Table 10.5).

10.5 Challenges ahead and opportunities

The rapid growth in isolation and characterization of novel bioactive compounds and novel sources in marine fungi has not been utilized to its fullest potential. Novel and potential cytotoxic, antibacterial, antiviral, and antioxidative compounds have been produced by this untapped source (Kumari et al., 2018; Kamat et al., 2020a,b), but their journey is still limited to the laboratory, which needs to be further upscaled for the commercial production of the natural products to take a step forward. Though the marine fungi derived bear the huge potential for industrial upscaling, many challenges are yet to overcome, which are listed below:

Table 10.4: Summary of metabolites with antioxidant activity produced by marine fungi.

Fungi strain	Antioxidative metabolites/crude extracts	Ocean/sea	Reference
Acremonium sp. (Isolated from brown alga *Cladostephus spongius*)	Hydroquinone	Moraira, Mediterranean Sea	Abdel-Lateff et al. (2002)
Aspergillus chevalieri TM2-S6 (isolated from the sponge *Axinella*)	Crude Extracts	Israeli Mediterranean coast	Letsiou et al. (2020)
Epicoccum nigrum JJY-40	ENP2 (Extracellular Polysaccharide)	South China Sea Hainan, China	Sun et al. (2011)
Myrothecium sp. BZO-L062	2-benzoyl tetrahydrofuran	Sea bottom near Yongxing Island	Lu et al. (2020)
Aspergillus versicolor SH0105	Violaceol-I	Mariana Trench	Yang et al. (2020)
Fusarium oxysporum (Mangrove Associated)	Extracellular Polysaccharide Fw-1	South China Sea, China	Chen et al. (2015a,b)
Trichoderma EMFCAS8	Crude Extracts	Andaman and Nicobar Islands	Kandasamy and Kandasamy (2014)
Talaromyces islandicus EN-501 (isolated the marine red alga *Laurencia okamurai*)	Hydroanthraquinones	Yellow Sea	Li et al. (2016)
Aspergillus sp.	Golmaenone, neochinulin A	Golmae village, Ulsan city, Seoul	Li et al. (2004)
Fusarium equiseti ANP2	MF-1-EPS	Krishna estuarine mangrove	Prathyusha et al. (2018)
Pha ffia rhodozyma	Astaxanthin	Marine environment not specified	Ni et al. (2008)
Rhodosporidium babjevae (Isolated from *Calanus finmarchicus*)	Torularhodin	Grøtsundet	Sperstad et al. (2006)
Aspergillus parasiticus	Parasitenone	Ulsan city, Seoul	Son et al. (2002)
Chrysosporium synchronum	1-O-(a-D-mannopyranosyl)chlorogentisyl	Yokji Island Korea	Yun et al. (2011)
Penicillium brevicompactum	Sinapic acid, Syringic acid	Red Sea Coast	El-Hawary et al. (2018)
Epicoccum sp. (Isolated from brown algae *Fucus vesuculosus*)	4,5,6-trihydroxy-7-methylphtalide, (-)-(3 R)-5-hydroxymellein	North Sea Coast	Abdel-Lateff et al. (2003)
Chaetomium globosum	Chaetopyramin	Marine environment (not specified)	Wang et al. (2006)
Fusarium sp. strain T-1	Glycosyl Ester	Tanegashima Island	Sakaki et al. (2002)
Microsporum sp.	Flavoglaucin and isodihydroauroglaucin	Marine environment (not specified)	Wang et al. (2006)
Aspergillus versicolor (A-21—2—7)	versicolorin B, UCT1072M1, dihydrosterigmatocystine, averythrin, averufine, nidurufin, averufanin, oxisterigmatocystin D, oxisterigmatocystin C, sterigmatocystine, and methylaverantin	South China Sea	
Aspergillus europaeus WZXY-SX-4—1	euroxanthone A, wentiquinone C, yicathin A, dermolutein, methylemodin, 1-O-demethylvariecolorquinone A, variecolorquinone A, 1-methoxy-14-dehydroxywentiquinone C and calyxanthone	Weizhou Island (Gulf of Tonkin)	Du et al. (2012)

(*Continued*)

Table 10.4: (Continued)

Fungi strain	Antioxidative metabolites/crude extracts	Ocean/sea	Reference
Gymnascella dankaliensis	Crude extracts	Mediterranean	Abdel-Monem et al. (2013)
Nigrospora oryzae	Crude extracts	Mediterranean	Abdel-Monem et al. (2013)
Chaetomium globosum	Crude extracts	Mediterranean	Abdel-Monem et al. (2013)
Engyodontium album	Crude extracts	Mediterranean	Abdel-Monem et al. (2013)

Table 10.5: Commercial applications of marine fungi-derived natural products.

Secondary metabolite	Source	Application	Industry	References
Lipase	Many marine fungal strains, *Aspergillus awamori* from the Kerala coast	Used as a digestive enzyme	Pharmaceuticals, cosmetics, Oil effluent treatment	Basheer et al. (2011), Murray et al. (2013)
Protease	*Many marine fungal strains*, Aspergillus ustus (NIOCC 20) isolated from a coral lagoon of the Lakshadweep Islands in the Arabian Sea	Digestive and antiinflammatory drugs	Pharmaceuticals and leather industry	Bonugli-Santos et al. (2015), Damare et al. (2006)
Ligninase	*Marasmiellus* sp. CBMAI 1062, *Tinctoporellus* sp. CBMAI 1061, and *Peniophora* sp. CBMAI 1063	Bioremediation of recalcitrant pollutants	Food and agriculture industry, textile industry	Bonugli-Santos et al. (2015)
Tannase	Many marine ascomycetes, Aspergillus awamori BTMFW032	clarification of beer and fruit juices, production of flavored drinks	Food industry	Bonugli-Santos et al. (2015), Beena et al. (2011)
Chitinase	Many marine ascomycetes, Penicillium oxalicum k10, Acremonium sp. YS2−2	Control of pathogens, Industrial biocatalyst	Pharmaceuicals and Agriculture industry	Chung et al. (2019), Xie et al. (2021)
Carotenoids	Many marine fungi, *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp.	Pro-vitamin A activity, can prevent many human disorders, and potential antioxidant	Pharmaceutical and cosmetic industry	Vitale et al. (2020)
Anthraquinones and xanthones	*Aspergillus* spp., *Eurotium* spp., *Fusarium* spp., *Dreschlera* spp., *Penicillium* spp., *Emericella purpurea*, *Culvularia lunata*, *Mycosphaerella rubella*, *Microsporum* sp.	Potential antioxidant, a cytotoxic and antibacterial agent	Pharmaceutical and cosmetic industry	Vitale et al. (2020)
Plinabulin (NPI-2358)	*Aspergillus* sp.	Inhibition of tubulin polymerization (cytotoxic agent)	Under phase III clinical trial by BeyondSpring Pharmaceuticals	Jaspars et al. (2016)

10.5.1 Low yield of the marine fungi-derived natural products

Though marine products are very potent bioactive compounds, their low yield remains their bottleneck and hampers their upscale production.

Media and physicochemical parameter optimization is generally used to enhance the production of desired secondary metabolites (Kumari et al., 2018). Taritla et al.(2021) optimized different media and found that maximum cytotoxic activity was observed in potato dextrose broth in comparison to nine other media. Salinity also plays a crucial role in optimizing media for the production of the desired secondary metabolite from marine fungi. Wang et al. (2011) observed that marine fungi *Spicaria elegans* produced metabolites "(2E,2'Z)-3,3'-(6,6'-dihydroxybiphenyl-3,3'-diyl)diacrylic acid (1), aspulvinone E (2), aspochalasin E (3) and trichodermamide B (6)" only in the presence of 10% salt. Different elicitors and stress have also been used to enhance the secondary metabolite production from marine fungi.

Coculture is another technique employed for improved production of marine fungi-derived secondary metabolites. The co-culturing of marine-derived fungi *Aspergillus sulphureus* KMM 4640 and Isaria felina KMM 4639 has induced production of five novel prenylated indole alkaloids (Chen et al., 2020).

Genetic engineering for strain improvement and chemical engineering for derivatization or semisynthesis of the natural products have also been employed to overcome the low yield of a secondary metabolite of interest from marine fungi. Heteroexpression of *Aspergillus nidulans laeA* in marine-derived fungus *Aspergillus nidulans* increased the expression of biosynthetic gene clusters for increased production of quinolactacin A (Khan et al., 2020). Marine-derived *Exserohilum* sp. fungus resulted in the synthesis of eight natural isocoumarins. Coronado et al. (2021) semisynthesized 22 new derivatives by substituting the aromatic ring and the aliphatic side chains, which showed excellent antiplasmodial activities.

10.5.2 Marine environment ex situ and the uncultured microbiome

The marine environment is completely different from the terrestrial environment in terms of pH, salinity, humidity, flora and fauna. Mimicking marine microenvironment ex situ in lab conditions is quite difficult, which can suppress the production of secondary metabolites which were produced in situ. Salt and osmotic stress are one of the strategies employed for optimizing the growth of marine fungi ex situ (Wang et al., 2011).

The diverse and complex niche of marine fungi often makes it difficult to replicate the same growth conditions in the lab, resulting in many uncultured marine fungi. Genome and

resource mining can be employed to discover the untapped potential of uncultured marine fungi (Jung et al., 2021).

10.5.3 Relatively unexplored marine fungi

The ocean and seas are vast resources of life on earth that have not been explored completely. About 95% of marine fungi residing in the deep sea have not been explored. Marine-derived endophytic fungi, the hidden gems of marine wealth, have also been explored partially. Recently, Kamat et al. (2020a,b) documented the diversity of algal-derived marine fungi of Konkan coasts, India. Previously, Hassett et al. (2019) documented the distribution of marine fungi in an extremophilic environment of the Arctic region and found the involvement of fungal genes in the degradation of biomass and the assimilation of nitrate. The successful expeditions to remote locations of ocean and sea can bring out novel marine fungi and novel secondary metabolites.

10.5.4 Solubility at physiological pH

The major drawback with any natural product is their hydrophobicity resulting in interference with their solubility and reduction in functionality at physiological pH. Many physical and chemical methods such as derivatization, salt formation, cocrystallization, solid dispersion, and use of surfactant have been employed to increase the solubility of natural products (Savjani et al., 2012). Though these techniques have been used extensively for the terrestrial compounds, the efforts for the derivatization of marine fungi-derived natural products are limited.

Further, nanotechnology can be employed to increase the solubility and sustained release of products by forming liposomes, micelles, and dendrites or by encapsulating them in polymers (Pateiro et al., 2021), though safety regulations should always be adhered to Bazana et al. (2019). The application of these new technologies can help in the further growth of natural product chemistry derived from marine fungi.

10.5.5 Regulatory, government policies, and risk assessment

For the growth and commercialization of any product or industry, the support of regulatory and government policies is always required. Costing policies are another important factor that play a key role in the successful commercialization of a product. Till now, most governments have no clear policies on the exploitation of marine-derived natural products.

Further, risk assessment of the product on humans and the environment is equally necessary. Risk assessment parameters should be strictly followed in every step of their efficacy evaluation (Malve, 2016).

10.5.6 High throughput screening and artificial intelligence

The major drawback of isolation of the potential natural product from marine fungi is the prolonged time and effort needed during screening. High throughput screening (HTS) is a method to isolate bioactive compounds rapidly with specific targets with the help of a library of compounds screened with high-throughput techniques. However, for the rapid screening, the continuous addition of compounds in the existing library is a must. Bugni et al. (2008) created a MNPs library for phenotype-selective and high-throughput screening for the compounds that inhibited the growth of BRCA2-deficient cells. Itaconate derivatives from marine *Penicillium antarcticum* were screened by HTS, which inhibited mesenchymal stem cell differentiation (Marchese et al., 2020). Deep learning and artificial intelligence approaches with a mechanism-driven neural network-based method can provide a better understanding of mechanistic aspects of drug action screened through HTS (David et al., 2019; Pham et al., 2021).

10.5.7 Omics and genetic engineering approaches for increasing the production of secondary metabolites

Integrated natural product chemistry, bioinformatics, and genetic engineering approach can result in an enhanced yield of secondary metabolites of interest and the discovery of novel biosynthetic pathways (Mohana et al., 2018). Multi-omics technology can provide an insight into the biosynthetic pathways, the temporal dynamics of transcription, epigenetic changes, and product assembly (Machado et al., 2017). Omics-based approaches can help in the prioritization and targeted isolation of secondary metabolites by establishing the molecular network and structural link between the natural products (Wolfender et al., 2019). Though nascent in marine-derived natural products, many plants and terrestrial fungal-derived secondary metabolites synthesis pathways have been engineered genetically. Sah et al. (2020) synthesized baccatin III enzymatically and investigated its cytotoxic potential.

10.6 Conclusions

Marine fungi have demonstrated immense potential as prolific producers of cytotoxic, antibacterial, antiviral, and antioxidant natural products. Though they remain largely unexplored, the emergence of new diseases such as COVID-19 and the increasing rate of resistance against conventional antimicrobial and cytotoxic agents have asked for in-depth studies of diverse compounds and their mechanisms produced by marine fungi. The last decade has seen a tremendous increase in the research with marine fungal-derived compounds, constrained by certain limitations such as high-throughput screening, increased

yield, bioavailability, and regulatory policies that are yet to be optimized for increased commercialization and success of marine fungi-derived products.

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CHAPTER 11

An eco-friendly quick-fix biosurfactant approach with wide range of roles and potential

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11.1 Introduction

11.1.1 Transforming the world from chemical surfactant to "biosurfactant"

The scientific era discoveries highlighting "the arrival of microorganisms" have always justified our existing relationship to nature, our planet Earth or our so-called mother "Earth." The historical lineage has always upgraded our knowledge regarding the beneficial aspects of microorganisms and their utilization in various aspects that ranges from fighting alarming conditions of pandemics, ensuring food safety and its security, assisting the geochemical cycles, securing cleaner energies, and last but not least, purifying water sources and cleaning the environment (Liu et al., 2015).

The ever-increasing harmful activities professed by humans as part of their livelihoods have brought an adverse threat to the environment that has caused some serious fluctuations and alterations in physiology, biochemical, geochemical, and community characteristics of the environment. Furthermore, the disparity in nature is noticed due to high pollution upsurging at an alarming rate that has led to the drastic transformations in pollutants and their accumulation over the decades (Sobrinho et al., 2013). The environmental degradation and contamination issues caused by the organic pollutants are widespread and costlier to manage by society at the present time. The categories of organic pollutants that pose a great threat to the environment comprise a wide range of aqueous wastes like chemical fertilizers and pesticides, oils (ranging from hydraulic oils and fuels to lubricating oils), liquids (solvents), and organic solids (sludge; ranging from tars to painting operations) (Deleu and Paquot, 2004). Similarly, the risks faced

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by the agricultural section, mostly in terms of soil degradation, are the result of accidental poison-like spills, which is an operational activity due to the processes like cleaning of equipment, use of outdated chemicals and residues, excessive and indiscriminate use of pesticides, interference by the landfill mixture (toluene, chlorinated hydrocarbons. and pesticidal residues) and presence of some inorganic sources (heavy metals) (Abbasi et al., 2013). In addition, the other contaminant sources include chemical contaminants from automobile services, improper landfills, household waste, industrial outlets, etc.

11.1.2 Strategies for remediation of toxic contaminants: tagging bioremediation with bioaugmentation and biostimulation processes

The wide use of various detection strategies has been already incorporated for decades to solve the challenges of augmentation in contaminants and pollutants in the environment and ecology (Jagtap et al., 2010).

The progress in detection strategies has resulted in better resolution to detect even more the minute range (parts per billion) of toxicants prominent in soil and water sources. The premature techniques used for the remediation were the physical and chemical strategies which (Bhardwaj et al., 2013) embrace processes like the correct processes of disposal of toxic landfills and incineration of the left-out waste, etc. But the zeal to move towards some of the eco-friendly and cost-effective processes introduced (Morita et al., 2007) an alternative remediation technology known as "Bioremediation." Depending on the advanced detoxification and degradation approach of toxic pollutants, bioremediation uses two successful routes to achieve it: (1) initiating accumulation intracellularly; and (2) active transformation from a more toxic contaminant to a lesser toxic or non-toxic contaminant (Stancu, 2015). Bioremediation is a two-step tagged process wherein the first initial step highlights "Biostimulation," which refers to the incorporation of specific nutrients to the site identified as the "contaminated target site" processed by the indigenous communities of microbial flora, present in abundance and that carry out the process of degradation efficiently (Muthusamy et al., 2008). The second step focuses on the "Bioaugmentation" process, which involves the addition of upgraded and modified microorganisms to a contaminated site (Shekhar et al., 2015).

The investigations are in the pavement to uncover factors like the capability of the processes, feasibility, and verification of microorganisms to study the basic route of degradation, transformation, and/or chelation of the toxic chemicals (Marques and Silva, 2013). The process of bioremediation needs complete profiling of microbial communities as they get down to play a key role in transforming and mineralizing the toxic pollutants, rendering them harmless by reducing their toxicity to zero levels so that they don't pose threats to the habitat (Dave and Joshi, 2017). The process further uses both "natural" as well "recombinant" traits of microbes to degrade the hazardous contaminants by anaerobic

or aerobic processes (Bratovcic et al., 2018). The most crucial application of this remediation process is its applicability in "on-site" and "off-site" targets. Furthermore, it includes the incorporation of pure microbial cultures, mixed consortiums, and also microbes associated with plant sources (phytoremediation) (Sil et al., 2017). Several processes like biostimulation, biosorption, bioaugmentation, bioleaching are being mentioned for increasing the efficacy, specificity, and selectivity of the remediation process (Akbari et al., 2018). The efficiency of these remediation processes depends upon three important prerequisites: the first one is the source and availability of microbial flora, the second one is the accessibility of the toxic contaminant, and the third one is the maintenance of the conducive environment (Rhein, 2002). The degradation efficiency relates to the ability of microbes to degrade complex mixtures of toxic contaminants.

Alwadani and Fatehi (2018) related kinetics, intrinsic and extrinsic factors like temperature, pH levels, soil composition, concentration, the bioavailability of toxicants, physical and chemical attributes of the targeted contaminants (Lukic et al., 2016). To attain a successful phase of the remediation processes, it should be efficient in the removal of toxicants and should attribute a better degradation ability than ordinary deterioration processes (Bucci et al., 2018).

11.1.3 Bridging two streams: bioremediation and biosurfactants

The low bioavailability, high persistent nature, and deleterious effects of xenobiotic compounds on human health have heightened the standards of bioremediation processes (Yuewen et al., 2017). These xenobiotic compounds have a high hydrophobicity ratio, rapid availability, and capacity to interact with nonaqueous phases and organic matter (Al-wazni, 2016). These properties generally make them unavailable to microbial degradation in a targeted way because bacterial communities initiate degradation only when they get to interact with the water dissolved chemicals (Pasiar et al., 2016). The conversion of these chemicals or toxicants during bioremediation depends on the intrinsic process of cell and the mass transfer (Rodrigues et al., 2006).

The bioavailability of toxic chemicals is controlled by processes like dissolution, diffusion, sorption, etc. (Gharaei-Fathabad, 2011). To increase the chances of bioavailability, microbes have devised strategies to enhance the availability of hydrophobic pollutants such as biofilm formation and "biosurfactant production," which has opened doors for the microbes to identify and act upon the various targeted contaminants (Kitamoto et al., 2002). Although, the fact remains that much more efforts are required in the direction of understanding bioremediation, for example, to identify the routes related to standardization and optimization of the microbial communities, to enhance the inherent microbial activities, and to enhance the bioavailability of the toxicants for easy targets and deterioration processes (Sekhon Randhawa and Rahman, 2014a,b).

11.1.4 The amphiphilic moiety: surfactants

Surfactants are a well-recognized chemical-amphiphilic (hydrophilic and hydrophobic) moiety derived (Invally et al., 2019) synthetically or biologically. They have a great advantage of distributing themselves between two immiscible surfaces via reducing the interfacial surface tension and increasing the solubility of polar entities into nonpolar ones (Araújo et al., 2019). The structural domain of surfactants usually consists of two domains, the hydrophobic (hydrocarbon) domain and the hydrophilic (positive, negative, or can be non-ionic in nature) domain, which together can thus reduce the tensions at interfaces (Liu et al., 2018). Usually, the hydrophobic domain is a hydrocarbon, whereas the hydrophilic domain can be non-ionic, positively or negatively charged, or amphoteric (Singh et al., 2018). The variations in surfactant head domain categorize them into different groups such as zwitterion complex, ionic complex, and non-ionic complex (Biniarz et al., 2017). At lower concentrations, these surfactants act as monomers that accumulate at the interfaces and, finally, on the increase of the aqueous concentrations, they converge into larger groups called "micelles," which at the initial stages are known as "the critical micelle concentration." Above these concentrations, their solubilizing capacities are increased in terms of activity and specificity (Yazid et al., 2017). The potential examples of non-ionic surfactants include propylene, sorbitan esters, ethoxylates, etc., whereas fatty acids, sulfates, and quaternary ammonium salts are being categorized (Shah et al., 2019) as the potential surfactants of ionic categories. The advantages are increased solubility, wetting abilities, good adhesion power, flocculation, excellent detergency power, demulsifying nature, and lower surface tension that contribute to the properties as excellent agents to be utilized in industries for numerous applications (Khan et al., 2014).

Surfactants are commonly used in various industries, including food, cosmetics, petroleum, and pharmaceuticals (Global oil, 2019). These synthetic surfactants have some disadvantages in terms of results and hazardous products that, in turn, pollute the environment (Luna et al., 2012). So, a research drive has been initiated in search of eco-friendly surfactants that would provide easy solutions to the present condition in the degradation of the environment from toxic contaminants (de Almeida et al., 2016). Synthetic surfactants possess two types of toxic pollutants that can be both hazardous to the environment as well as their receptors. The two broad categories are the 'toxic by-products' and the 'remains of the pollutants'. These pollutants are very resistant and persistent in the environment as they do not get degraded easily and also consume more energy compared to the processes mediated by the biosurfactants. With these associated disadvantages, it becomes a foremost need to go for alternative strategies which are target-specific, pure, and green in approach (Mazaheri-Assadi and Tabatabaee, 2010).

11.1.5 Biosurfactants: an eco-friendly strategy of microbial consortium

Unconventional strategies are needed to deal with the issues related to environmental safety by devising some eco-friendly, safer methods to ensure parameters of security and human health.

Biosurfactants are a different category of surfactants derived naturally from biological entities/microbial flora (yeasts, algae, and bacteria). There have been various genera identified as potential candidates for the production of a wide variety of biosurfactants, such as *Pseudomonas*, *Acinetobacteria*, and *Bacillus*, whereas the species related to the bacterial genus which are active invaders are *Bascillus subtilis, Acinetobacter radioresistens, Lactic acid bacterium*, etc. (Satpute et al., 2016; Alagorni et al., 2015; Sun et al., 2017; Crecente et al., 2005). However, *Pseudozyma rugulosare* and *Candida bombicola* have been recognized as potential yeast and fungi active biosurfactant producers.

Further, biosurfactants are widely grouped into low molecular weight (LMW) and high molecular weight (HMW) biosurfactants based on their nature, given the biochemical structure (Camara et al., 2019; Negin et al., 2017; Yu and Huang, 2011; Geetha et al., 2018). The former category serves as an excellent emulsion stabilizing agent, whereas the latter category lowers the interface tied tensions. Furthermore based on chemical composition, they are further categorized into lipopeptides, glycolipids, fatty acid surfactants, and particulate surfactants (Al-Bahry et al., 2013; Park et al., 2017).

The application of biosurfactants has been proved to be a cost-effective strategy that is environmentally sound, biodegradable, biocompatible, has low toxicity, chemically diverse and specific, and their most important characteristic, i.e., their nonfluctuating nature against the unpredictable changes in the environment (Sarafzadeh et al., 2014). Some of the loopholes in the pathway are the heavy cost associated with the isolation and purification, as well as lower output in global markets. This can be sorted out by incorporating some alternative inputs like agricultural wastes, for e.g., various carbon sources, growth optimization, and modification through computational gene modeling will help to meet the challenges and overcome the flaws related to biosurfactant processes. The current driving force of research is towards advanced biosurfactants that will help in promoting the health and the sustainability criteria for the environment in increasing its competitive phase against the cheap and dangerous chemical surfactants (She et al., 2019; Maudgalya et al., 2007; Khire, 2010a,b; Golabi et al., 2012; Weidong et al., 2014).

11.2 Potential strengths of biosurfactants

The specific advantageous properties make biosurfactants an important tool for replacing synthetic surfactants. These properties include salient features related to their surface/interface activities, pH tolerance factors, ionic strength, low toxicity,

biodegradability, emulsifying and demulsifying properties, and antimicrobial characteristics (Basafa and Hawboldt, 2019).

The major distinctive features of each property of biosurfactant are discussed below.

11.2.1 Surface and interface activity

The property of reducing the exerted interfacial surface tensions is being performed at an excellent pace by the biosurfactant produced via microbial communities. For example, bacterial species like *Bacillus subtilis* produce a biosurfactant known as "Surfactin," which reduces the surface tension of water to 20−25 mN mG1 and further reduces interfacial surface tension to less than 1 mN/m (Astuti et al., 2018).

Similarly, the rhamnolipids produced by another bacterial species *Pseudomonas aeruginosa* decreases the surface tension considerably to about 20−26 mN mG1 and interfacial tension to 1 mN mG1 (El-Sheshtawy et al., 2015). Furthermore, they are more efficient and specific as their critical micelle concentration is lower than the synthetic surfactants', which adds up to the advantages of maximum decrease in interfacial tension junction and requirement of lesser surfactant (Hong et al., 2019).

11.2.2 Temperature and pH tolerance

The biosurfactant displays an important feature related to its stability against fluctuating behavior of intrinsic and extrinsic factors like temperature and pH. The extraction from extremophiles has caught the interest of researchers and collaborating industries for the commercialization process. The production of extremophiles has gained attention in the last decades for their considered commercialization (Zhang et al., 2016). Researchers reported that lichenysin from the bacterial species *Bacillus licheniformis* was found to persist in a temperature hovering in a range of 40°C−50°C with a pH between 4 and 9.0, and concentrations of sodium chloride or calcium in ranges 50−55 and 20−25 g LG1, respectively. A similar biosurfactant produced by *Arthrobacter protophormiae* was reported to be a thermostable biosurfactant tolerating a temperature range from 25°C to 100°C and a pH range from 2 to 14. These resistant advantages make them suitable to work in an unstable environment as they can tolerate fluctuation in extrinsic and intrinsic factors (Youssef et al., 2007).

11.2.3 Biodegradability

Since the synthetic chemical surfactants impose environs' menace, hence we need some safer microbial-derived compounds that can be easily degraded (Udoh and Vinogradov, 2019; Mukherjee, 2007; Bezza and Chirwa, 2017a,b; Abass, 2017) and are reported safe for

applications in actual site with actions such as bioremediation/ bioaugmentation/ biosorption. The effective application of biodegradation can be easily witnessed in research related to the control of increasing algal blooms. In addition, the utilization of biosurfactant "Cochlodinium" has the algal bloom removal efficacy of 80%−90% in 30 min treatments, respectively (Sai-Chaitanya et al., 2016).

11.2.4 Low toxicity

Biosurfactants are low toxic or non-toxic categories that are finding their potential applications in cosmetics, food, and pharmaceuticals. Researchers also reported higher toxicity of "Corexit," a chemical-derived surfactant displaying 10 times lower activity than rhamnolipids and LC50 against *Photobacterium phosphoreum* (Syafiq et al., 2017). Similar research conducted depicted the mutagenic profile and related toxigenicity of biosurfactants from *Pseudomonas aeruginosa* and of chemically (El-Din et al., 2013) derived surfactants, and found that the microbial biosurfactants were non-toxic and nonmutagenic when compared to the toxic chemical surfactants. A similar example is "sophorolipids from *Candida bombicola*," which finds its potential application in food industries due to their non-toxic nature (Hegde et al., 2016).

11.2.5 Emulsion forming and breaking process

Biosurfactants may act as emulsifiers and de-emulsifiers. Emulsions are categorized into broad categories: one, in oil-in-water (o/w); while another, in water-in-oil (w/o) emulsions classification. The biosurfactants generally stabilize the additives, which can be maintained for longer periods. For example, liposan is a water-soluble emulsifying agent isolated from Candida lipolytica (yeast), which possesses the quality of emulsifying edible oils by providing a coating of oil droplets and stabilizing them (Leng et al., 2015).

11.2.6 Antiadhesive agents

Biosurfactants are used for altering and modifying the hydrophobicity of the surfaces, which increases the interaction of microbes over the targeted surfaces. Thus, a biofilm can be defined as a group of bacteria/other organic matter that has accumulated on any particular surface (Pekdemir et al., 2005). For example, a surfactant from *Streptococcus thermophilus* reduces the accumulation and fouling process initiated by other thermophilic strains of Streptococcus over the steel surface. Similarly, a biosurfactant reduces the chances of attachment of *Listeria monocytogenes* on steel surfaces produced by *Pseudomonas fluorescens* (Perfumo et al., 2010).

11.3 Production of microbial surfactants

These microbial surfactants are produced by bacteria, yeast, and fungi as a fragment of the cell membrane or while occurring outside the cell body (Fig. 11.1). For example, rhamnolipids produced by *Pseudomonas aeruginosa* utilize substrate, including succinate, fructose, glucose, mannitol, olive oil, and citrate (Copper and Paddock, 1984). High yield production of a microbial surfactant such as sophorolipids is made possible from vegetable oils, sugar, and waste (Coscolin et al., 2018) by a few yeasts, which include *Candida bombicola*. Production of lipopeptide called surfactin by *Bacillus subtilis* is another example. Hydrocarbon is a major substrate used as the source for the production of microbial surfactants (Daniel et al., 1999). The production of microbial surfactants can be growth-associated. There are atypically two possibilities: first, substrate emulsification (extracellular); and second, ease the movement of the substrate through the membrane (cell membrane-associated). However, microbial surfactants are produced during the late exponential phase and stationary growth phase, usually as secondary metabolites. These secondary metabolites can be classified into higher and lower molecular weight biosurfactants (De Roubin et al., 1989). The renewable substrates can be used to produce biosurfactants as they possess low toxicity and also prevent their accumulation in the environment (Desphande and Daniel, 1995).

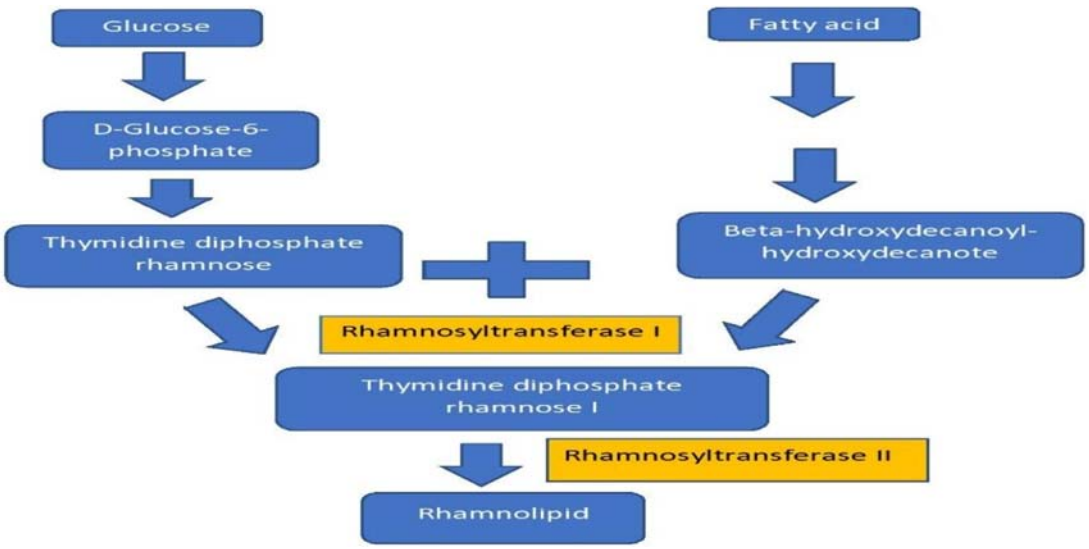

Figure 11.1
Biosynthesis of rhamnolipid by *P. aeruginosa*.

11.3.1 Rhamnolipids

11.3.1.1 Production and properties

Rhamnolipids are microbial surfactants that have been studied in *Pseudomonas aeruginosa* (Fox and Bala, 2000). These compounds are classified based on surface tension. Either these rhamnolipids contain identical fatty acids connected to one rhamnose or two molecules of rhamnolipids are attached to alpha-hydroxy decanoic acid. Production of rhamnolipid can be significantly increased by the use of nitrogen as an energy source, for example, ammonium (Hitsatsuka et al., 1971). Fermentation in the presence of ammonium sulfate and trace metal leads to increased production of rhamnolipids (Ishigami et al., 1995). The use of phosphorus as an energy source also leads to the production of rhamnolipids (Jackson et al., 2015). The hydrocarbon present on polluted sites is used as a substrate by these microbes for the generation of microbial biosurfactants (Johann et al., 2016). The rhamnolipids-derived biosurfactant serves a biodegradable property making them less toxic in (Fig. 11.1) the environment and, simultaneously, they can be a good substitute for the chemical detergent used for cleaning (Kakinuma et al., 1969).

11.3.2 Surfactin

Surfactins are the biosurfactant produced by *Bacillus subtilis*, which is composed of seven amino acids linked to the hydroxyl and carbon groups of a fourteen-carbon acid (Fig. 11.2) (Lin, 1996). These surfactins are folded into a beta-sheet structure (Mata-Sandoval et al., 2001). Even a small concentration of surfactin, as low as 0.005%, can reduce the surface tension up to 27 mN/m, which makes them one of the most effective and powerful surfactins. Using glucose for the production of surfactin results in low surfactin production (0.7 g/L), while the addition of iron or manganese could result in increased production of surfactant up to 3.5 g/L (Mulligan and Gibbs, 1989). Soybean can be used as a substrate for the production of surfactin by solid-state fermentation (Nishizaki et al., 2007). Using potato waste as a substrate can lead to a decrease in the cost of production (Nunez et al., 2001). The hydrolysis of peat with 0.5% sulfuric acid for one hour at 120°C results in a higher yield of surfactant (Ohno et al., 1995). The addition of citric acid to glucose has also shown an increase in the production of surfactin (Robert et al., 1989). There are various factors that determine the production of a surfactant such as carbohydrate metabolism, oxygen concentration, and lipid forming regulating factors (Sekhon Randhawa and Rahman, 2014a,b). The *Bacillus subtilis* serves as a good host for the generation of a recombinant type of biosurfactant (Sheppard and Mulligan, 1987). Alanine and arginine decrease the production, while the addition of glycine and leucine shows no varied effect. By mining, the design of the

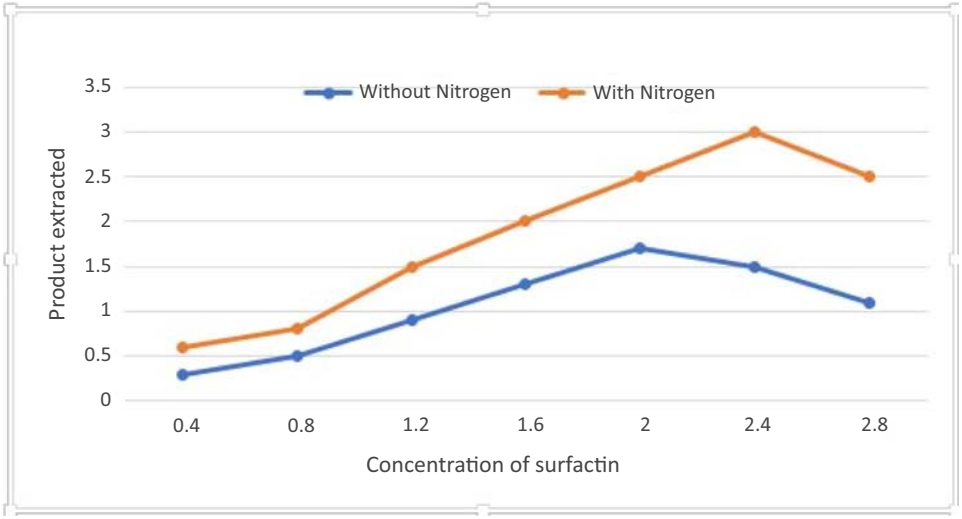

Figure 11.2
Production of surfactin in the presence of nitrogen.

final product molecule quality can be optimized (Soberon and Maier, 2011). The production of surfactant can be determined by blood agar plate screening.

11.3.3 Sophorolipids

There are only a few yeasts that can produce biosurfactants, such as *Candida bombicola* (Syldatk et al., 1987). The high yield of sophorolipids can be obtained using substrates like glucose or soyabean oil, as the concentration of the product can be obtained at around 150 g/L (Wie and Chu, 1998). Oils, too, help in regulating intrinsic circuit induction for the biosynthesis of biosurfactants (Wittgens and Rosenau, 2018). Alternative carbon sources such as lactose, canola oil, and animal fat (Zhou and Kodaric, 1995) are also used as substrates. Researchers (Sachdev and Cameotra, 2013 Feb) have developed a two-step process to utilize lactose using deproteinized whey where the process involves, firstly, concentrating by growing *Cryptococcus curvatus* and, then, using nitrogen limiting environment to extract crude cell extract by *Candida bombicola* for growth. Using an initial concentration of whey around 24 g/L can nearly produce a 12 g/L concentration of sophorolipids. The properties of biosurfactants can be modified by inserting palmitic, linoleic acids, and glycerol. These modifications can be determined by using the process of liquid chromatography-atmospheric pressure mass detection.

11.4 Applications of biosurfactants

Biosurfactants have the potential for the replacement of synthetic chemical surfactants in many different ways and processes (Paulino et al., 2016). They can be used in various food,

cosmetic, pharmaceutical, softening, lubricating, dye fixing, and decontamination processes. Major reasons for the attraction toward biosurfactants are the increasing hazardous issues on the environment and the need of climate-friendly methods for the waste management system. Today, the waste sludge treatment (Olasanmi et al., 2018), organic or inorganic chemical hazards, oil, and synthetic chemicals are causing soil and water toxicity. These toxic elements are indeed very difficult to remove. Another major issue that is also a key concern is "heavy metals management and its removal." Heavy metals, like copper, lead, chromium, cadmium, iron (Banat et al., 2010), etc., are causing deprivation of soil and aquatic life as these metals leach out to the soil and aquatic bodies and even to the groundwater (Tiso et al., 2018) creating a great imbalance and toxicity. Biosurfactants can help in these issues as they are biologically synthesized by several microorganisms. A class of biosurfactants, the glycolipids contain (Díaz De Rienzo et al., 2016) mono and disaccharide linked to long acids that work well in bioremediations, food industry, pharma and cosmetic industries, in the removal of chelating agents, reduction of oil concentration in contaminated soil and water treatment processes, and are indicative of promising antimicrobial activities (Joshi-Navare and Prabhune, 2013).

11.4.1 Biosurfactants in remediation of heavy metals

Heavy metals (HM) are becoming a significant risk to various organisms of the ecosystem. They are becoming a major reason for varied severe diseases in humans. These HM are highly toxic even at a very low concentration and can affect the natural properties of soil drastically. Several methods have been developed for the removal of heavy metals from soil (Hage-Hülsmann et al., 2018) which can be nonbiological or biological. Biological methods include plants and microorganisms for the clean-up process. The methods which need plant involvement are known as phytoremediation, and the one which needs the association of microorganisms is called bioremediation (Balan et al., 2016). As heavy metals cannot be fully degraded, they are just transformed from one form to another, which is less toxic. Metal mobility is also changed by microorganisms for remediation purposes, in which they use the redox or alkylation processes for the transformation of these heavy metals. Their accumulation can be passive, i.e., metabolism independent or active metabolism dependent on intracellular uptake. Heavy metals are transformed by changing pH or releasing substances that change the mobility of metals. Soil washing and soil flushing are the two methods for soil remediation and removal of heavy metals from soil (Hamza et al., 2017).

Biosurfactants work very efficiently by forming complexes with the metals that are then removed from soil by creating interfacial tension. Then, the cationic biosurfactant helps in the removal of charged metal (Ronco and Klein, 2014) ions by forming biosurfactant micelles, in which procedure their hydrophobic fragments attach to metal ions and leaches out with water (Fig. 11.3).

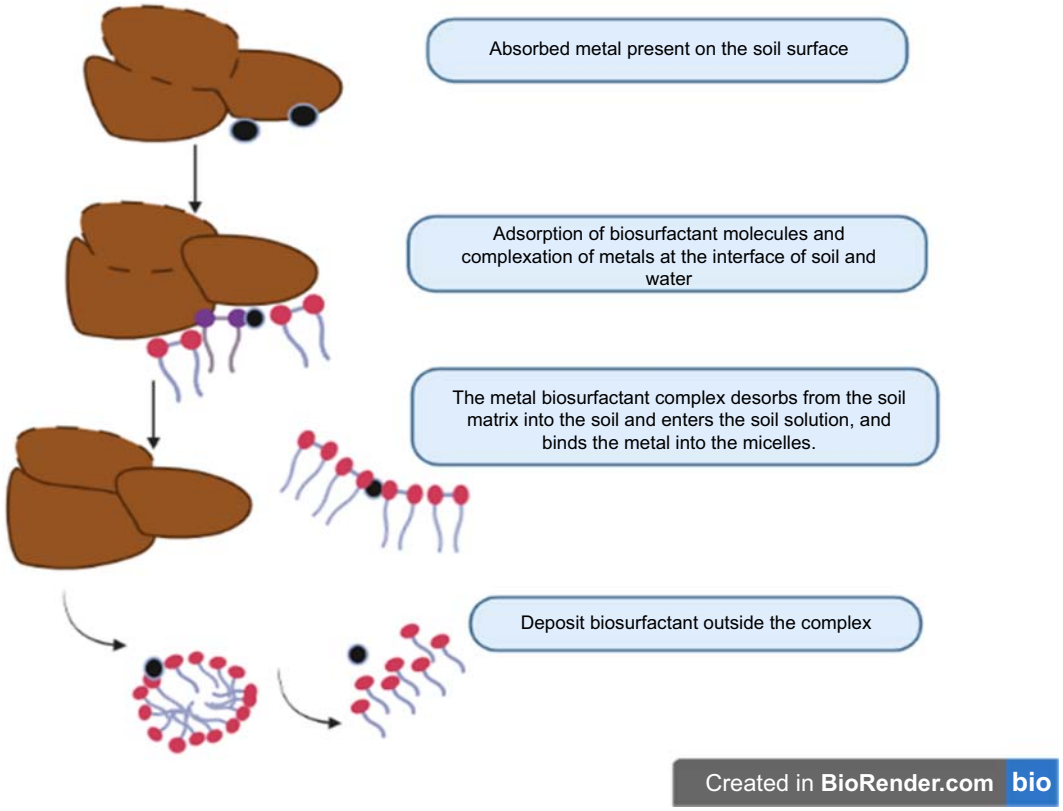

Figure 11.3
Micelles formation: mechanism of biosurfactant activity in metal contaminated soil. *Created by Biorender.com*.

Di-rhamnolipid is a biosurfactant produced by *Pseudomonas aeruginosa* that shows potent activity for the removal of multi-metal contaminants from the soil (Pacwa-Płociniczak et al., 2011). An alternative method that shows effectiveness for heavy metal remediation is biosurfactant foam technology. Studies have shown that this method also gives eminent results for the remediation of heavy metals. Contaminated soil is also treated with the method of phytoremediation by several plants that are introduced by biosurfactant-producing bacterial strains. *Bacillus* sp. promotes the plant growth and uptake of heavy metals in plants like maize, rapeseed, tomato, etc., in contaminated soil (Das et al., 2016).

11.4.2 Biosurfactants as biocides

Overuse of chemicals-based products vitiates the environment. It is not the living form but also our abiotic environment which shows up as the losers. There are thousands of limitations of chemical biocides and its urgent need for time to develop more organic

and less harmful products for safer use (Callaghan et al., 2016). We have limitless bioactive compounds produced by various wonder microorganisms (Jiang et al., 2016). These bioactive compounds are generally secondary metabolites that are produced by several specified bacteria, fungi, or yeast. Some of the dominating species that are well known for this purpose include *Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans*, and *Acinetobacter calcoaceticus* (Stipcevic et al., 2006). These biologically synthesized surfactants have several advantages over chemically synthesized products (Vatsa et al., 2010). As they are produced by microorganisms, they are less toxic, highly selective, have the ability to work in a wide range of pH levels, and temperatures while exhibiting broad antimicrobial properties. They are used in various agricultural, oil, food, pharmaceuticals, and environmental remediation processes (Renfro et al., 2014).

Biocides are classified into five subclasses on the basis of their nature and composition. The major groups are glycolipids, lipolipids, phospholipids, polymeric and neutral lipids. These compounds have great variability in size ranging from 500 Da to 1000 KDa (Kiran et al., 2017). They have hydrophobic and hydrophilic fractions (Luepongpattana et al., 2017). They also involve a variety of biomolecules like fatty acids, dicarboxylic acids, lipoproteins, glycolipids, alkyl glycosides, lactones, and other sugar molecules (De Araujo et al., 2014). Once these compounds are produced, extracted, and purified, the next step is—depending on their nature—to spread them extra-cellularly or to be attached to the membrane of specified cells. Some specialized processes like gene uptake (Bezza and Chirwa, 2017a,b) and nutrient uptake are also designed for intracellular biosurfactants so that they can reduce the toxicity level and promote carbon and energy storage. Mobility of an organism is also enabled so that it can easily move from one phase to another phase (Souza et al., 2014).

Biocides also have the properties of disrupting the membrane of microbial cells, so it becomes difficult for any cell to develop resistance against biocides (Tiso et al., 2017). The mechanism behind this process is pore formation on the walls of the target cell and creating ionic imbalance. They also involve the lytic effect against some of the target cells; hence, they work from a very broad spectrum of microorganisms (Vecino et al., 2015; Shah et al., 2016; Nicolopoulou-Stamati et al., 2016). The lipid compound of a biocide is well known to destroy many bacteria, viruses, and even fungi. When used in combination with several selected organic acids and sodium dodecyl sulfate (SDS), the biosurfactant helps in the disruption of biofilms. Studies also showed that sophorolipids are effective in destructing biofilms on glass cover slips produced by a variety of bacterial species like *E.coli, Staphylococcus aureus*, *Bacillus subtilis*, etc., where the properties of biocides like hydrophobicity, hydrophilicity, emulsifications, growth enhancement, antimicrobial properties, detergency, etc. make them an attractive approach towards environmental remediation (Karlapudi et al., 2018).

11.4.3 Role of biosurfactants in sustainable agriculture

To meet the agricultural commodity demand of an expanding population worldwide is a matter of concern. The role of green products as an eco-friendly, waste-minimizing alternative to traditional wasteful goods offers a solution to moving towards sustainable agriculture while alleviating the consequences of the present conditions (Fenibo et al., 2019). The current review highlights the pitfalls of synthetic surfactants on agricultural soil and agrochemical industries and the role of biosurfactants produced by bacteria, fungi, and yeast which can serve as green surfactants (Das and Mukherjee, 2007), considered to be less noxious and nonpolluting. The biosurfactants synthesized by environmental isolates also have a promising role in the agricultural industry (Olasanmi and Thring, 2018). Many rhizosphere and plant-associated microbes produce biosurfactants that have a vital role in biofilm formation (Kubicki et al., 2019), signaling, and motility, and that also govern the plant-microbe interaction. In agriculture, biosurfactants can be used to increase the bioavailability of nutrients for beneficial plant-associated microbes and plant pathogen elimination (De Almeida et al., 2016). Biosurfactants can generally be applied to improve the agricultural soil quality, which helps in soil remediation. Thus, exploring biosurfactants from natural isolates to explore their potential role in plant growth promotions is important (Rincon-Fontan et al., 2019). Some methods are followed for screening the microbial population for the production of biosurfactants. However, molecular methods are fewer in reaching out to biosurfactants from a diverse microbial population, and there is a need to (Rodriguez-Lopez et al., 2019) explore novel biosurfactants from uncultured microbes in the soil biosphere by using advanced methodologies like functional metagenomics. The use of chemical fertilizers and their application methods ultimately become the main cause of soil pollution, and it is also a major challenge that we must deal with to reaffirm a healthy and pollution-free environment (Vecino et al., 2017). Biosurfactants, such as rhamnolipids, lipopeptides, and sophorolipids, not only aid in constructing a hygienic and nutrient-rich environment but also improve the texture/ fertility of agricultural soil while showing potential as a nutrient (Van Renterghem et al., 2018). The implementation of biosurfactants as a regular practice will help to create resistance from the infection-causing pathogens and pests. Reports have shown that biosurfactants have successfully removed the Para-naphthalene or green oil (Vecino Bello et al., 2012), phenanthracene (Patel et al., 2015), polycyclic aromatic hydrocarbon, and different heavy metals (Khire, 2010a,b). These biosurfactants excel in the bioaccumulation rate of hazardous waste from the soil ecosystem and dreg it to microbial cells (Fig. 11.4) (Chong et al., 2016). Due to the decrease in interfacial and surface tension by the biomolecules, the rate of dispersion increases, which has the upshot that an elevated emulsification of toxic compounds and effortless removal of these compounds from the soil (Arora and Singh Cameotra, 2014) are completed.

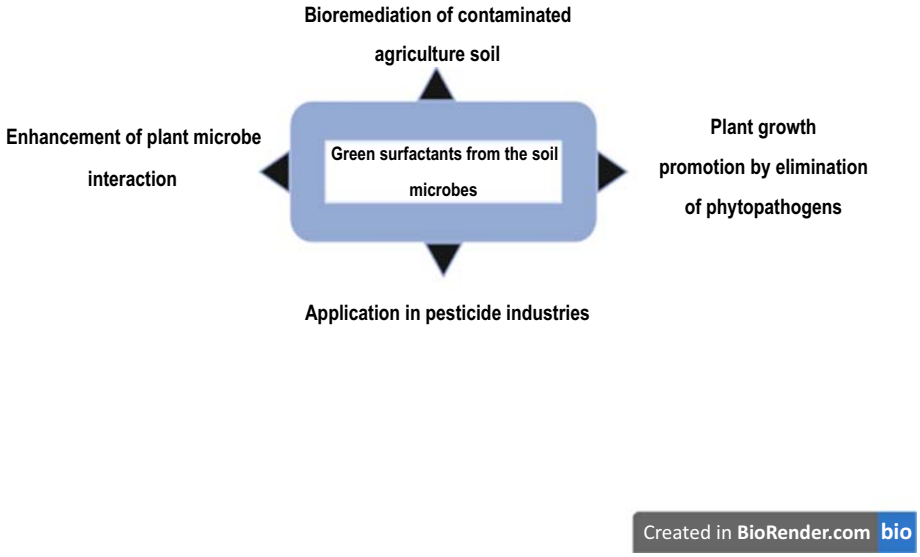

Figure 11.4
Multifunctional prospective of biosurfactants in agriculture. *Created by Biorender.com.*

11.4.3.1 Mechanism of MEOR: microbial enhanced oil recovery

Biosurfactants can also be used in MEOR. These methods are used to recover oil remaining in reservoirs after the primary (mechanical) and secondary (physical) recovery procedures (Araújo et al., 2019) have taken place. They are too used in important tertiary processes where microorganisms or their metabolites, including biosurfactants, biopolymers, gases, solvents, biomass, acid, and enzymes, are used to increase the recovery of oil from depleted reservoirs (Kim et al., 2019). The application of biosurfactants in enhanced oil recovery is one of the most advanced and appropriate methods to recover a significant proportion of the residual oil (Mehetre et al., 2019). The remaining oil is often located in regions of the reservoir that are difficult to access (Fig. 11.4), and the oil is trapped in the pores by capillary pressure. Biosurfactants reduce the interfacial tension between oil or water and oil or rock. This reduces the capillary forces averting the oil from moving through rock pores (Ravindran et al., 2020). Biosurfactants can also bind tightly to the oil–water interface and form micelles, which stabilize the desorbed oil in water and allow the removal of oil along with the injecting water (Fig. 11.5).

11.4.4 Biosurfactants in commercial laundry detergents

Almost all surfactants are an important component of and regularly used in modern-day commercial laundry detergents but are chemically synthesized and pernicious (Sarubbo et al., 2015) to freshwater living organisms. This has begun the search for a safer and

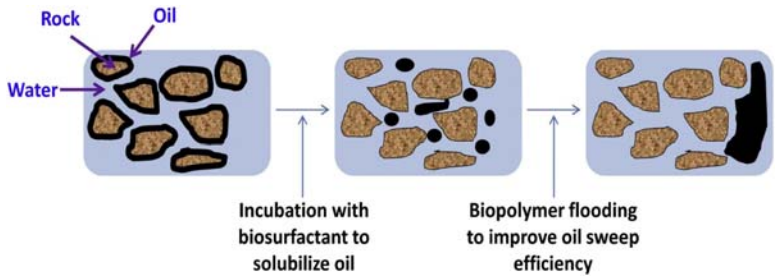

Figure 11.5
Biosurfactants' role in the oil recovery process. *Created by Biorender.com.*

organic substitute for their use as laundry detergents in place of chemical surfactants. Biosurfactants such as Cyclic Lipopeptide (CLP) maintain the pH range (approx. 7.0−12.0), and heating them at high temperatures does not result in any loss of their surface-active property (Gudina et al., 2015). They showed good alloy formation capability with vegetable oils and demonstrated excellent stability with commercial laundry detergents favoring their inclusion in laundry detergents formulation (Janek et al., 2018).

11.4.5 Biosurfactants in food processing industries

There is already a wide use of biosurfactants in various food processing applications, but a significant role is well documented as a food formulation ingredient and as an antiadhesive agent. As a food formulation ingredient (Garg and Priyanka, 2018), it promotes the stabilization of emulsion due to its ability to decrease interfacial tension. It is also used to control the agglomeration of fat globules, stabilize aerated systems, improve the texture, and improve consistency and texture of fat-based products, increase the shelf-life of starch-containing products, modify rheological properties of wheat dough, and improve food consistency. For food production, the utilization of biodegradable and non-toxic components is very important. The current scenario (Heidary et al., 2018) shows that for the continuous increase in growth of the human population, it's important to simultaneously increase the agricultural productivity, and for that, biosurfactants can aid by providing (1) improvement in microbes-plant symbiont relationship or interaction (Ye et al., 2019), (2) resistance in plant (Kalyani et al., 2016), (3) enhancement in soil nutrients (Gomaa, 2013), (4) improving fertilizer uptake by plants (Pacwa-Płociniczak et al., 2011), etc. Moreover, biosurfactants can help in sanitizing equipment used for production by utilizing their antimicrobial and antibiofilm effect to cure food spoilage in the food industry (Paulino et al., 2016). In addition to this, compounds such as massoia lactone, which have been produced by marine fungi, have been known to display pleasant odor and flavor (Olasanmi et al., 2018). These natural compounds can be used as a substituent to the present predominating production route using artificially or synthetically produced derivates of

chemistry. At last, the savage or the waste product generated by the food industry can be used as a source or feedstock to produce microbial biosurfactants at the beginning (Banat et al., 2010).

11.4.6 Application of biosurfactants in the textile industry

The textile industry is considered one of the world's most polluted industrial effluents releasing sources with a high concentration of organic compounds and heavy metals. Some essential metals necessary for plant metabolism (Tiso et al., 2018) act as enzyme activators, e.g., Fe, Ni, Zn, Mn, and Cu, which are also found in textile effluents. Textile waste streams containing heavy metals and minerals also contain organic dyes, which are characterized by bright colors, total solids (TS), severe pH fluctuations, chemical oxygen demand (COD), biological oxygen demand (BOD), and total dissolved solids content (TDS); and biological surfaces containing sulfur, ammonia, and nitrogen increase the availability of nutrients when using textile wastewater. Further, the aim of this study is to appraise the correlation between soil enzymatic activity with the physiochemical and microbial activity of soil, as well as to assess the intra- and inter-specific variation in nutrient uptake capacity under biosurfactants augmentation with textile effluent fertilization (Díaz De Rienzo et al., 2016; Joshi-Navare and Prabhune, 2013; Hage-Hülsmann et al., 2018).

The biological and physical features of microbial biosurfactants make them suitable in various fields such as emulsifiers (Balan et al., 2016), separators, detergents, wetting, and foaming agents (Hamza et al., 2017). Further, the bio-based compounds have been increasingly recognized by the chemical industries drawing their attention to bio-based economy (Ronco and Klein, 2014). The importance and utilization of these chemical compounds have been indicated by an increase in the number of patents, highlighting their show of industrial interest (Das et al., 2016).

11.4.7 Application of biosurfactants in the clinical field and medicine

Many microbial biosurfactants have surface-active lipids which exhibit bioactivity properties, e.g., lipopeptides. There are many investigations that have shown the role of microbial surfactants as antimicrobial agents. For example, the rhamnolipids which are present naturally demonstrate the bacterio-static effect, such as in mono rhamnolipids. In addition to this, di-rhamnolipids are found to be bacteriocidal, which act on *P. aeruginosa* (Callaghan et al., 2016). Exceptionally, there are many biosurfactants that are present as mixtures that display variable mechanisms of action. In several cases, microbial biosurfactants have displayed a striving for reinforcing or interactive or synergistic effect (Jiang et al., 2016), with antibiotics, such as, the penetration efficiency of antibiotics has increased into the cell by the microbial biosurfactant (Stipcevic et al., 2006). Many pathogenic bacteria can produce biofilms, which make them resistant to a wide range of

immune cells and antibiotics, even as microbial biosurfactants manifest effectiveness in disruption of biofilm (Vatsa et al., 2010). The disruption of biofilm enhances the application of antibiotics (Renfro et al., 2014). Many lipopeptides are used to absorb the liposaccharide endotoxins to prevent sepsis reactions and alleviate or soften inflammation (Kiran et al., 2017). Moreover, there are a few biosurfactants that have been attracting attention because of their anti-tumor cell activity (Luepongpattana et al., 2017). Marine compound didemnin B, a surface-active natural product, has victoriously set foot in real-life for clinical trial as an anticancer agent by enhancing metastasis by liberating cells from the primary tumor (De Araujo et al., 2014). The bioactivities of microbial biosurfactant properties can be excelled or changed by using various modifying processes such as muta-synthesis, molecular engineering, or semisynthesis (Bezza and Chirwa, 2017a,b). Burn wounds have been successfully treated with the help of microbial biosurfactants by easing wound healing and preventing scar formation by decreasing collagen contents (Souza et al., 2014). The pathogenic strains such as *S. aureus*, *Streptococcus agalactiae*, and *P. aeruginosa* show growth that is successfully inhibited by the biosurfactant produced by *Lactobacillus agilis*, which shows antimicrobial activity (Tiso et al., 2017). Pathogens such as *Proteus vulgaris* and *Vibrio harveyi* are drug-resistant, so they can be successfully killed by the trehalose lipid produced by *Rhodococcus fascians* which shows antimicrobial activity against them (Vecino et al., 2015). Research conducted on *Candida parapsilosis* reported that biosurfactants produced by these bacteria have a substantial antimicrobial activity which is very effective against pathogens such as *S. aureus* and *Escherichia coli* (Shah et al., 2016). Saleable antibiotic status has been accomplished by some lipopeptides, for example, daptomycin (Nicolopoulou-Stamati et al., 2016). This is more relevant in the context that *Streptomyces roseosporus* also produces daptomycin, which is used to cure or treat microbial infection caused by most gram-positive infectious bacteria (Karlapudi et al., 2018). The antiadhesive feature shown by biosurfactants resists the attachment of infectious bacteria to the host cell, preventing various numbers of diseases (Fenibo et al., 2019); for example, biosurfactants from *Lactobacillus* spp. showed antiadhesive characteristics against a wide range of yeast and pathogenic microbes (Das and Mukherjee, 2007). The progression of the cancerous cells by apoptosis, growth of cells, and arrest of the cell cycle were successfully halted by surfactin which is produced by different *Bacillus* strains (Olasanmi and Thring, 2018).

11.4.8 Bioremediation: a process initiated by microbial biosurfactants with excellent biodegradability and low ecotoxicity

The main properties of biosurfactants are biodegradability and low ecotoxicity, which make them an ideal fit for the treatment of waste that has been released into the environment. The pollutants such as hydrocarbons (Olasanmi and Thring, 2018), pesticides, heavy metals, and other pollutants are addressed as a bigger global worry for terrestrial and marine ecosystems

(Kubicki et al., 2019). The bioremediation concept can be applied by using bio-based compounds at the site of pollution used for the remediation process (De Almeida et al., 2016). The microbial biosurfactant also facilitates the removal of hydrophobic pollutants by increasing or promoting their water solubility (Rincon-Fontan et al., 2019). Petroleum hydrocarbon is the major cause and concern of soil contamination because of the hydrophobic properties of hydrocarbon that are difficult to destroy and eliminate from the environment (Rodriguez-Lopez et al., 2019). These hydrocarbons are responsible for degrading the nutrient quality and fertility of the soil and, hence, make them unsuitable for the growth of plants (Vecino et al., 2017). Biosurfactant has a great and effective potential for degradation of these hydrocarbons from the environment (Van Renterghem et al., 2018). In addition to this, biosurfactants have been successfully used in the processing of petroleum products, refining, in transportation, and petrochemical production (Vecino Bello et al., 2012). The extensive use of pesticides such as nematicides, fungicides, herbicides, and insecticides results in extensive hazardous impacts, mainly on the soil ecosystem (Vecino Bello et al., 2012). Microbial biosurfactants such as rhamnolipids, sophorolipids, etc., have a prominent capacity to remove hydrocarbon pollutants from the marine and soil ecosystems (Patel et al., 2015). Biosurfactants not only increase reservoir life but also provide better recovery of oil from an endangered reservoir (Khire, 2010a,b). Therefore, these microbial biosurfactants can be used as prominent substituents for typical surfactants used in remediation. The use of biosurfactants in combination with living organisms and biochemical supplements can also help pace out the processing rate of bioremediation. The role of biosurfactants played in heavy metal removal is explained in Fig. 11.6.

11.4.9 Consumer products

The biosurfactant application allied to cosmetics majorly relies on their wetting, emulsifying, solubilizing, and foaming properties, which not only solubilize the hydrophobic constituents in the by-product but also assist in movement through different skin barricades (Chong et al., 2016). Due to their natural origin, biosurfactants exhibit low irritancy and inflated skin togetherness, which serves them with a major advantage over synthetic or chemically produced, or non-natural analog (Arora and Singh Cameotra, 2014). Recent reviews showed that the antimicrobial properties of biosurfactants could play a promising role in cosmetics products (Araújo et al., 2019). The surface-active feature and intrinsic emulsifying characteristics of biosurfactants make them suitable for the replacement of chemically manufactured derivates of detergents used for cleaning purposes (Kim et al., 2019). The environmental release of biosurfactants makes them less toxic to the ecosystem because of their biodegradability property. In addition to this, the biosurfactant is more tolerant to unfavorable conditions and can work over a wide range of temperature, pH, and salinity levels (Mehetre et al., 2019).

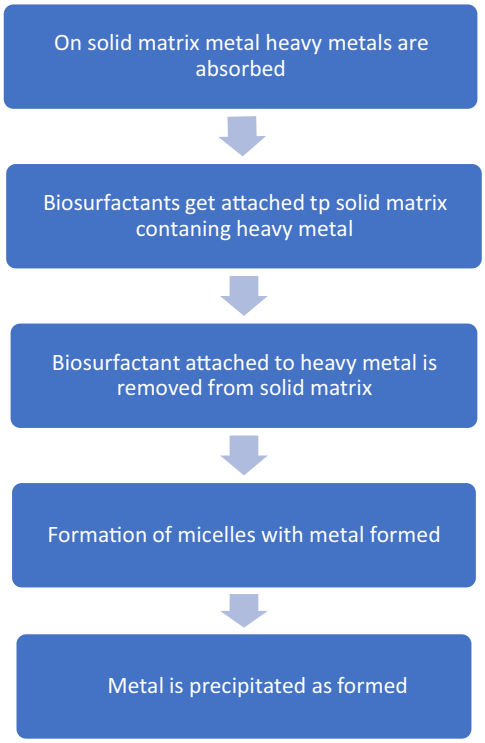

Figure 11.6
Role of biosurfactant in heavy metal removal.

11.4.10 Fossil fuel enhanced recovery

Being aware of variable disadvantages, in particular the problem of environmental pollution caused by petroleum based-products and crude oils, biosurfactants play a very noteworthy role in today's world. But due to the lower efficiency of the traditional oil recovery process, which is only 10%−40%, the need for new recovery methods is required (Ravindran et al., 2020). The concept of MEOR utilizes the natural potential of microbes to emulsify or solubilize oil (Sarubbo et al., 2015). The formation of ice with gas molecules such as methane is trapped, known as gas hydrates. They display as energy and carbon source, predominantly present below the seafloor. These depositories are considered a future energy source (Gudina et al., 2015; Janek et al., 2018; Heidary et al., 2018; Ye et al., 2019; Kalyani et al., 2016; Gomaa, 2013; Zin et al., 2018; Monnier et al., 2020; El-Sheshtawy et al., 2016; Zarinviarsagh et al., 2017; Ribeiro et al., 2020; Raza et al., 2014; Lee et al., 2012; Kulkarni and Choudhary, 2011; Thakur and Kumari, 2022; Thakur, 2018a,b; Thakur, 2017a,b,c; Thakur, 2018a,b; Thakur, 2017a,b,c; Kashyap and Thakur, 2017; Sharma et al., 2020; Thakur, 2021). Therefore, the present study is going on to evolve technologies for

efficient and timid storage of gas hydrates. Various studies reported that the microbial biosurfactant has a prospective advantage in advancing the reformation of methane hydrate and results in improved storage (Heidary et al., 2018; Ye et al., 2019).

A snapshot of microbes producing biosurfactants with potential application in respective fields.

11.5 Conclusions and future challenges

The driving forces responsible for the biodegradation processes of various organic contaminants in the environment can be attributed to the utilization of microbial communities. The urge for the processes like enhanced bioremediation technologies such as biostimulation and bioaugmentation is increasing day by day, which is helping to open new outlooks for the molecular microbial ecology. Keeping in view the need for an eco-friendly and clean approach to be utilized in the industrial systems, the use of microorganisms seems the way out. The use of this microbial mechanism and metabolism has proven climate-friendly and is an economically viable means of eliminating the toxic compounds in the ecological surroundings both under lab and field conditions. However, the excessive accumulation of some of the major toxic compounds which are persistent in the environment poses a big challenge to the microbial process. Future research holds a promising role in screening out the potentiality of microbes in terms of their metabolic potential, degradation availability, the bioavailability of the substrates, diffusion and transport of substrates into the cell, the degradation potential, and the site of degradation. A budge towards the advent of modern technologies like quantitative Polymerase Chain reaction and Microarrays will permit deeper insight into the loopholes and will improve the existing technologies when integrated. Furthermore, the approaches like metabolic engineering or the utilization of synthetic biology for designing the process of microbes have led to increased efficiency of the clean-up process of contaminants by the microbes. However, a lot of work is still needed to reveal the correct adsorption patterns of microbial surfactants, micellar solubilization of organic hydrocarbons, degradation and partitioning behavior of surfactants onto the soil, liquid organic phase, and process optimization at the biological and engineering levels, respectively.

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CHAPTER 12

Microbial enzymes as a robust process to mitigate pollutants of environmental concern

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12.1 Introduction

Aside from the unprecedented scale of the industrial revolution, modern industrialization methods have exacerbated environmental degradation by emitting a slew of dangerous pollutants. For instance, antibiotics, active pharmaceutical residues, poisonous heavy metals, corrosive phenols, and other hazardous substances have a negative impact on the complete biological ecosystem due to their free movement in water matrices (Bilal et al., 2019). As a result, there is a pressing need to successfully prevent, reduce, or degrade new environmental toxins to maintain a healthier and greener environment (Liu et al., 2019).

Several remediation techniques have been devised and used against a variety of environmental-related hazardous pollutants (Aleya et al., 2019). However, none of them has been able to totally eliminate the contaminants. High computational costs, the use of nasty chemicals in surplus, poor cost-effective ratios, and the creation of hazardous metabolic end-/by-products or secondary environmental pollutants are all major challenges that impede the practical deployment of in-practice remediation systems or approaches (Bilal and Iqbal, 2020, Dasgupta et al., 2015).

A huge variety of enzymes are a product of microorganisms and certain plants and are crucial in the treatment of organic contaminants via bioremediation. Bioremediation, an ecologically sound technology that is both cost-effective and environmentally friendly, is generated by enzymes produced by microbes. This field's research might be beneficial in the development of improved bioprocess technology to lower pollutant toxicity while also obtaining innovative valuable compounds. Enzymes active for bioremediation, such as oxidoreductases and hydrolases, have had their processes well explored.

This chapter gives a detailed description of the enzymes produced by diverse microorganisms that are engaged in the disintegration of a range of pollutant types. The bioremediation of contaminants is carried out by a variety of enzymes, including

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oxygenases, peroxidases, hydrolases, lipases, phosphotriesterases, and others. Different mechanical and agricultural sources discharge extremely dangerous and cancer-causing toxic substances into the environment, like esters, dyes pesticides, heavy metals, oil-based things, synthetic nitrogen-containing materials, etc.

Toxic waste disposal and expulsion have been major concerns for environmental researchers in recent years. Bioremediation potential has been spotted in a variety of archaea, tiny organisms, plants, and parasites. Bioremediation is the action of using microorganisms and their metabolites to disintegrate and alter contaminants into a less poisonous structure. Microbes and their enzymes are an effective, safe, and cost-effective approach for removing contaminants. Several enzymes are involved in bioremediation processes, including oxidoreductases, laccases, hydrolases, and peroxidases. Microorganism growth is influenced by environmental conditions such as light, moisture levels, and pH. The tuning and modification of these variables can drastically accelerate the occurrence of degradation.

A tremendous amount of untreated sewage and other trash is disposed of as a consequence of scientific, industrial, and technological advancement, posing a serious threat to mankind's survival on Earth. Previously, trash was traditionally disposed of by landfilling. Due to a shortage of new locations and cleanliness concerns, this method of trash disposal proved difficult to sustain. Later, systems evolved that used high-temperature burning and chemical degradation. These procedures not only offer the advantage of efficient contamination but also have certain disadvantages in terms of cost, complexity, and social acceptability (Karigar and Rao, 2011).

Bioremediation is the modification or breakdown of pollutants by microorganisms into less dangerous or nonhazardous substances. Bioremediation commonly employs microorganisms such as bacteria and fungi. Bioremediation, which depends on the catalytic activity of enzymes generated by organisms rather than a specific microorganism's growth under hazardous settings, is based on a pure and substantially purified catalyst. A pure protein can be used to accomplish bioremediation in nutrient-poor soil. When enzymatic biotransformation is implemented, poisonous by-products of microbial biotransformation are not provided, making it safe for varied surroundings (Leung, 2004). Proteins, in contrary to organisms, are more substrate-specific and portable due to their smaller size. The current cases of biodegradation of a substance mediated by an enzyme of stubborn, persistent, and refractory contamination are described here in the chapter (Dave and Das, 2021) (Fig. 12.1).

12.1.1 Controlling the fate of hazardous contaminants through microbial degradation

In broad terms, microbial degradation is the activity of using microorganisms and their associated distinct enzyme systems to break down, degrade, detoxify, or convert environmental contaminants. Effective contaminant degradation is not expected every now

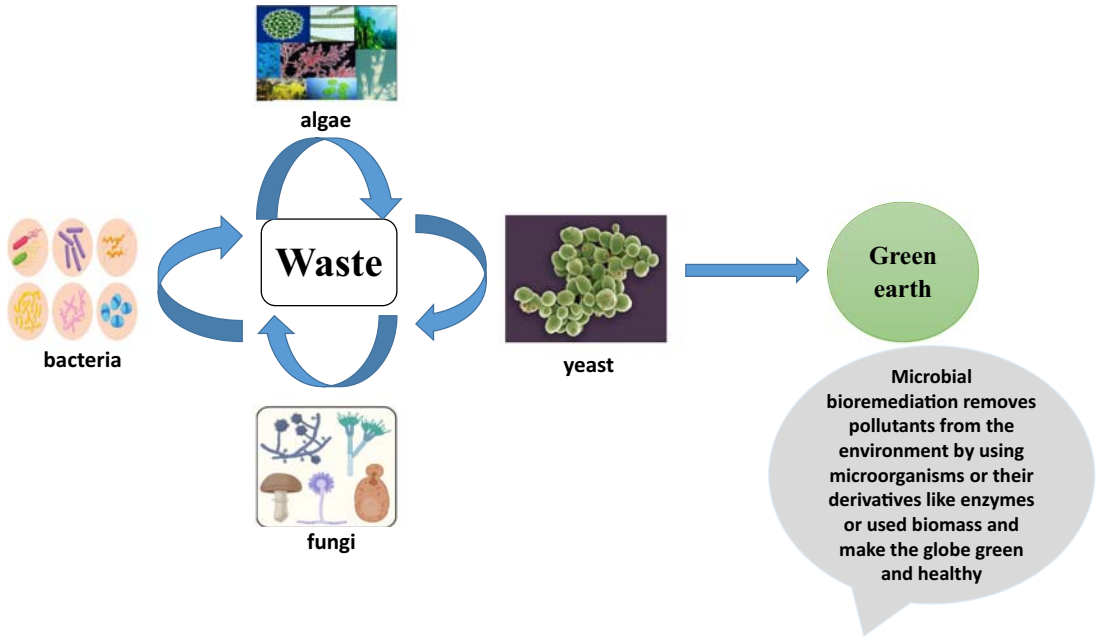

Figure 12.1
Microbial bioremediation of pollutants of environmental concern.

and then under constrained growing conditions for bacteria and even microbes with degradation capabilities exist in the environment (Liu et al., 2019).

As a result, designing and developing varied bioremediation arrangements based on available contaminants in the environment through the cultivation of microorganisms having catalytic potentialities that have been created is crucial.

Aromatic hydrocarbons are a synthetic category of hydrocarbons, designated as polychlorinated biphenyls (PCBs) are frequently employed all over the world. However, because of their reluctance and accumulating potential, pervasive contamination, and suspected impacts to cause cancer, broad-spectrum PCB use poses long-term and detrimental hazards to the ecosystem and human health (Aldhafiri et al., 2018). Genetically modified microbial cultures have the potential to greatly boost PCB elimination and mitigation. The gene bphA encodes a protein called biphenyl 2,3-dioxygenase (BDO), which is involved in the reduction of PCBs (Shumkova et al., 2015).

Anthropogenic sources are mostly to blame for the dumping of petro-based hydrocarbons into aqueous systems such as the beach, coastline waters, and landscape, which, in return, have severe harmful impacts on environmental patterns all over the world. Petro-based hydrocarbons leaked, can be harmful for a long time, based upon the total volume and duration of the exposure (Ndimele, 2017).

All countries across the world should ensure efficient offshore oil pollution deterioration properly. Given that the spill oil contains a variety of hydrocarbons (along with 103 aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and *n*-alkanes), the advancement of genetically mutated microbes with current promising applications to reduce or degrade various petro-hydrocarbons is a move in the right direction for reducing offshore oil pollution (Gallo et al., 2012).

To evaluate their biodegradation capacities, biodegradation aptitude, and translocations, the genomes of 26 Sphingomonas and Sphingobium strains were analyzed. A crucial phase in the disintegration of aromatic hydrocarbons has been identified as catechol. While horizontal gene transfer-related elements are common, this suggests that genome sculpting and evolution are ongoing. Furthermore, genomic analysis of the test strains revealed homogentisate pathways and the ketoadipate were the predominant biodegradation routes seem to be the most predominant (Zhao et al., 2017, Bilal and Iqbal, 2020).

12.1.2 General reaction of bioremediation

In situ and ex situ types of bioremediation are the two major types of bioremediation based on microorganisms. The treatment of pollution in its natural environment is an in situ bioremediation. This decentralized process is both efficient and cost-effective (Leung, 2004). It is advantageous because the soil is not exposed. This is accomplished through the use of nonpathogenic microorganisms. Because this process requires aerobic conditions, oxygen is delivered to the soil via bioventing.

In bioventing, air at a low flow rate is employed to provide adequate oxygen concentration to maintain microbial activity in the unsaturated area going, and there are nutrients like nitrogen, phosphorous, and sulfur that are provided to assist organisms to flourish. The decrease in the rate of breakdown and the viability of microorganisms on waste material in the soil makes in situ type of bioremediation difficult. Ex situ bioremediation, on the flip side, is the treatment of pollutants in contaminated water or soil after they have been evacuated from their natural setting.

Bioremediation in the slurry phase and bioremediation in the solid phase are key techniques for doing so. In the first method, a bioreactor is used to grow microorganisms, and reagents and water are added to it. Nutrients and oxygen are used to maintain aerobic conditions. The water is removed, and the soil is restored at the end of the treatment period, but in solid-phase bioremediation, the contaminated soil is lifted and dispersed on a treatment surface with an air pump in solid-phase bioremediation.

To optimize bioremediation efficiency, minerals, air, humidity, pH, and temperature should all be regulated. Composting and land farming are two examples (Adams et al., 2015). Microorganisms that enzymatically attack contaminants and modify them to harmless

byproducts are used extensively in both ex situ and in situ bioremediation treatments. Bacteria and fungi both of them depend upon the engagement of several internal and extracellular enzymes to remediate refractory substances.

In the bioremediation of harmful and refractory compounds in the environment, many enzymes are involved. The enzyme's name is according to the function of the enzyme, that is, the type of reaction it catalysis (Sharma et al., 2018). A protein or glycoprotein with at least one polypeptide moiety is called an enzyme. The active sites of an enzyme are the parts of it that are actively involved in the catalytic activity. With such an enzyme, the protein or glycoprotein constituent is known as the apoenzyme, whereas a nonprotein moiety is known as the prosthetic group. One or maybe more groups that are crucial for catalytic activity and are coupled to the active site through covalent or noncovalent bonds can be found in an enzyme. To make the holoenzyme, the apoenzyme is coupled with the prosthetic group.

Each of the six groups contains all of the enzymes that have been discovered. The six major distributions are as follows: isomerases, hydrolases, ligases, transferases, oxidoreductases, and lyases.

Lyases were shown to facilitate the removal or addition reactions that break similar bonds resulting in the molecule becoming unsaturated or saturated.

Isomerases are crucial for structural or geometrical isomerization and also for rearrangements that transform one molecule to another, as their name implies.

Ligases catalyze the combining of two molecules to make a new one.

According to the study, oxidoreductases facilitate the transfer of an electron and protons from one which is termed as a donor to an acceptor.

A transferase is a protein that allows a functional group to be transferred from a donor to an acceptor.

Hydrolases assist in the cleaving of carbon−carbon, carbon−oxygen, carbon−nitrogen, and other bonds in water (Fig. 12.2).

12.1.3 Enzymes

12.1.3.1 The basics of enzymes

Enzymes are known catalysts that aid in the transfer of certain substrates in the products by lowering the reaction's activation energy. A protein or glycoprotein that has at least one polypeptide moiety is called an enzyme. The active sites of an enzyme are the parts of it that are actively involved in the catalytic activity. The apoenzyme is the protein or glycoprotein moiety of an enzyme, whereas the prosthetic group is the nonprotein

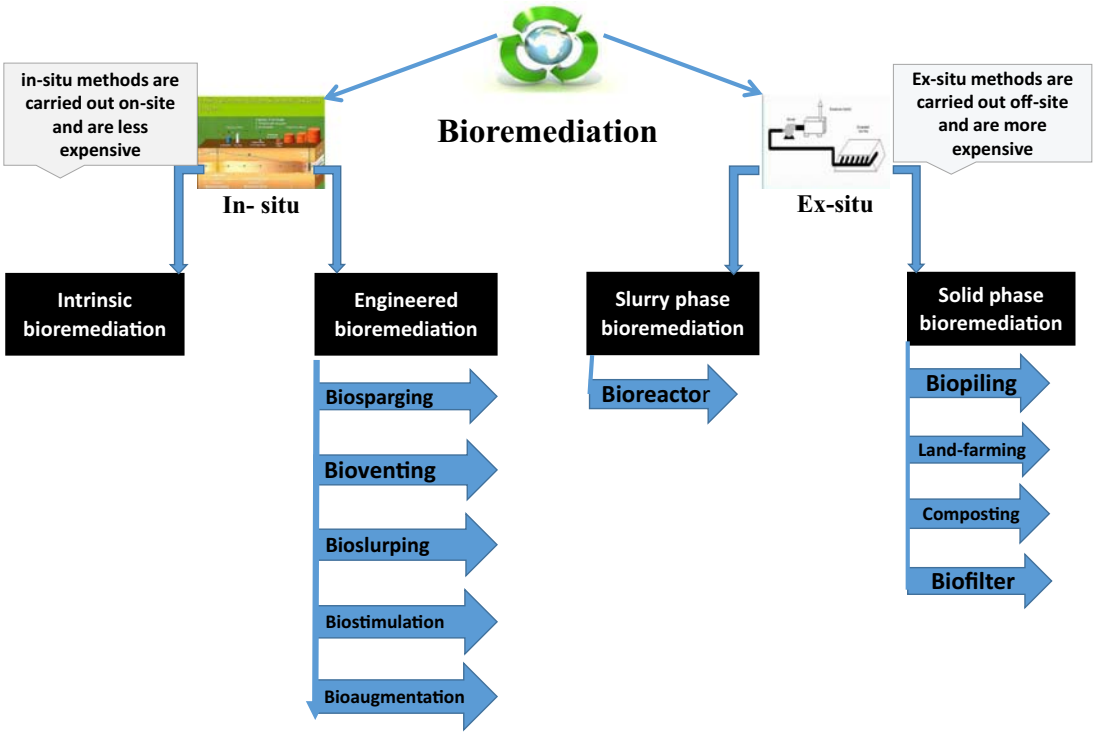

Figure 12.2
Bioremediation and its types.

component. One or even more than one groups are necessary for catalytic activity and are either covalently or noncovalently connected to the active site in an enzyme. The apoenzyme and the holoenzyme are combined to generate the holoenzyme. To make the holoenzyme, the apoenzyme is connected to the prosthetic group.

12.1.3.1.1 Enzyme classification

The enzyme commission (E.C.) number is used to determine a given enzyme's identity. E.C. numbers are assigned according to the International Union of Biochemistry's rules.

12.1.3.2 The importance of microbial enzymes

The most well-known employed enzyme classes in the remediation of polluted environment include hydrolase, transferases, and oxidoreductases. The principal producers are microorganisms, such as bacteria and fungi (especially the white fungus), plants, and microbe–plant interactions. Many of these enzymes have been tested primarily in the laboratory for their ability to transform various xenobiotic substances. Many of these enzymes have reagents and activity assay conditions available, and they are also defined in length to make identification and implementation easier (Karigar and Rao, 2011).

12.2 Decontaminating agents based on enzymes

The usage of enzymatic proteins could be a viable alternative to microbes for eliminating the majority of their downsides (Nannipieri and Bollag, 1991). Enzymes have a variety of beneficial qualities. They are the principal catalysts for all biota changes. They comprise catalysts with either narrow or wide specificity, allowing them to be used with a variety of chemicals in mixtures. They may cause severe structural and toxicological changes in pollutants, as well as their complete conversion to inorganic end products that are safe. They could be utilized to do processes for which there is no effective chemical modification. Enzymes do not inhibit by microbial metabolism inhibitors. They can be used in harsh environments to limit microbial activity. They are efficient at low pollution concentration and in the presence of microbial predators or antagonists. They seem to be more mobile than microbes and have a specialized substrate on which they work (Nannipieri and Bollag, 1991). All of these characteristics enable enzymes environmentally friendly catalyst. According to our societies' current ecological standards, these latter may be capable of remediating a large number of environmentally hazardous substances.

According to Alcalde et al. (2006), enzyme-catalyzed biocatalysis (also called white biotechnology) completely embraces the "green chemistry" movement that began in the 1990s by Sheldon and Rantwijk (2004). Its long-term impact on stability has now been established: intracellularly—inside their sourced cells; extracellularly—both in the existence and absence of originating cells; free—soluble in solution with homogeneous catalysis; or immobilized—linked to a solid matrix through various links with heterogeneous catalysis (Gianfreda and Rao, 2004). Polyelectrolyte multilayers, micropatterning, and self-assembled monolayers appear to fit the bill in the first case, whereas hollow polyelectrolyte shells or colloidal particles coated in polyelectrolytes (and phospholipids) can host proteins or other types of functional molecules and allow molecules to pass through the shell wall in the second case (Rao et al., 2010).

12.3 Significant microbial enzymes for bioremediation

Some of the most significant microbial enzymes for bioremediation are listed below.

12.3.1 Cytochrome P450

The heme enzyme superfamily Cytochrome P450 is found in all three biological domains: eukaryotes, bacteria, and archaea (Bak et al., 2011). It is in charge of a wide range of functions in living systems, along with the creation of complex natural products, the metabolism of medications, and the biotransformation of hazardous chemicals (Li et al., 2020). P450s have the ability to break down xenobiotics by nature (Anzenbacher & Anzenbacherova, 2001). Dehalogenation, dealkylations, epoxidations, aliphatic

hydroxylations and epoxidations, and various mechanism-based inactivations are all essential chemical transformations in bioremediation chemistry (Guengerich, 2018).

For catalytic function, ferredoxin and ferredoxin reductase are also employed as electron sources for bioremediation of organic pollutants and hydrocarbons (Kellner et al., 1997). *Bacillus megaterium* model P450, CYP102A1 (P450BM3), has been shown to be able to oxidize PAHs. For polyhalogenated aromatics, some microbial P450s showed bioremediation capability. Lamb et al. studied *Acinetobacter calcoaceticus* strain BD413 that had been genetically engineered to express the P450 from *Streptomyces griseus*, allowing microorganisms to grow on persistent pollutants, herbicides, and agrochemicals.

Modified CYP102A1, which showed an improvement in productivity against PAHs, PCBs, and linear alkanes, which are usually applied in toxic chemical bioremediation, gaseous alkane detoxification, and terpenes (Kumar et al., 2012). In bacteria, there are catabolic genes, plasmids, and genomes encoding P450s for the degradation and removal of POPs from the environment (Chakraborty, 2016). *Sphingobium* sp. strain YC-JY1 decomposed the plasticizer bisphenol A (BPA). This strain's sole carbon sources could be 4′-hydroxyacetophenone, 4-hydroxybenzaldehyde, and 4′-hydroxyacetophenone. The *Escherichia coli* strain YC-JY1bisdB was created to examine the influence of the P450 enzyme (Jia et al., 2020).

12.3.2 Microbial oxidoreductases

Biodegradation of a wide spectrum of natural as well as anthropogenic pollutants has been accomplished using oxidoreductases.

The removal of hazardous organics produced by oxidoreductases and released by numerous types of bacteria and parasites via oxidative coupling comprises electron transfer from reductants toward the oxidants. During the degradation process, heat is evolved which is then utilized by microbes in their metabolic operations. Contaminants are oxidized to nontoxic molecules during these oxidation−reduction reactions. Various phenolic anilinic complexes are humified, polymerized, and copolymerized with the help of oxidoreductases.

Biodegradation of a vast spectrum of natural and anthropogenic contaminants has been accomplished using oxidoreductases. *Bacillus safensis* CFA-06, that is a Gram-positive bacteria, generates oxidoreductase to biodegrade the oil mixtures. Various oxidoreductases enzyme catalysts, such as peroxidases and laccase, discharge and break down dyes produced in the textile industry (Novotný et al., 2004).

Maximum microscopic organisms transfer radioactive substances from an oxidative soluble structure to a reduced insoluble setup. Microbes discard electrons from natural mixtures and use radioactive substances as the final electron acceptor in this procedure. In the decolorization of azo dyes, microbial enzymes have been utilized (Bansal and Kanwar, 2013).

With the assistance of a mediator electron donor, several bacterial species decrease radioactive elements indirectly. Chlorinated phenolic combinations are the richest refractory waste encountered in effluents from the paper and pulp industries.

These blends arrive in the middle of the lignin hydrolysis process. The action of extracellular oxidoreductases which including manganese laccases, LiP, and peroxidase which are secreted by fungus mycelium in close enough proximity influences the behavior of organisms. Therefore, these organisms can reach contaminants more effectively than bacteria because they are filamentous (Rubilar et al., 2008).

12.3.3 Microbial oxygenases

Oxygenases add a few oxygen atoms to aromatic compounds to catalyze ring cleavage. The two subclasses of oxygenases are monooxygenases, which catalyze the addition of one oxygen molecule, and dioxygenases, which catalyze the addition of two oxygen molecules. They influence organic molecule metabolism by enhancing their reactivity or solubility in water, or by triggering aromatic ring cleavage. Oxygenases show a broad range and thus are effective against a vast range of compounds, include chlorinated aliphatics. When oxygenase introduces O_2 atoms into an organic molecule, the aromatic rings are often cleaved. Bacterial monooxygenase or dioxygenases were formerly the most characterized enzymes in bioremediation (Arora et al., 2009).

Halogenated organic compounds make up one of the major classes of environmental contaminants due to their extensive utilization as herbicides, insecticides, fungicide, hydraulic and heat transfer and also have a role of intermediates for chemical synthesis. These contaminants are degraded by certain oxygenases. (Fetzner and Lingens, 1994). In collaboration with multifunctional enzymes, oxygenases also mediate the dehalogenation processes of halogenated methanes, ethanes, and ethylenes.

12.3.4 Monooxygenase

In a variety of metabolic activities, these enzymes add one OH group to substrates. Two O_2 atoms are converted to one OH group and one H_2O molecule in this process. Monooxygenases are a diverse category of enzymes that catalyze oxidative reactions in a variety of substrates such as fatty acids, steroids, and alkanes. Because of their strong regioselectivity and stereoselectivity on a wide range of substrates, they are employed as biocatalysts in bioremediation and synthetic chemistry.

Other monooxygenases, such as tetracenomycin F1 monooxygenase isolated from the bacteria *Streptomyces glauscens* and quinol monooxygenase recovered from *E. coli*, do not require a cofactor (Shen and Hutchinson, 1993).

These enzymes use the substrate as a reducing agent and catalyze reactions with only molecular oxygen. Because of the existence of a cofactor, monooxygenases are further split into two categories:

1. NADP or NADPH is used as a coenzyme, and flavin is used as a prosthetic group, in flavin-dependent monooxygenases.
2. Both eukaryotic and prokaryotic organisms have P450 monooxygenases, which are heme-containing oxygenases.

Desulfurization, denitrification, ammonification, dehalogenation, hydroxylation, biotransformation, and microbial degradation of aromatic and aliphatic compounds are all catalyzed by monooxygenases. Methane monooxygenase is the most well-known enzyme, and it is associated with the breakdown of hydrocarbons such as substituted alkenes, methanes, haloalkenes, alkanes, cycloalkanes, ethers, aromatic and heterocyclic hydrocarbons, and aromatic and heterocyclic hydrocarbons, along with aromatic and heterocyclic hydrocarbons (Karigar and Rao, 2011).

Monooxygenase assists oxidative dehalogenation reactions when oxygen rates are elevated, but reductive dehalogenation happens when concentration is low. Monooxygenases are enzymes that degrade chlorine-containing pesticides like endosulfan (Sharma et al., 2018).

Methane monooxygenase cometabolizes aliphatic molecules that contain halide, aromatic chemicals, and heavy metals. Monooxygenase is a type of enzyme that produces oxygen. In many sectors, cytochrome P450 is used to oxidize the contaminant that has been emitted. The P450 oxidoreductase subfamily has approximately 200 members in both prokaryotes and eukaryotes. P450 monooxygenase, generated by *B. megaterium* BM3, may destroy a wide range of substrates, including fatty acids and aromatic compounds (Roccatano, 2015). P450 oxidoreductases all include an iron-containing porphyrin group and reprocess their redox center with a noncovalently bound cofactor like NADPH. An oxidoreductase is a type of cytochrome CYP1A1 found in the liver of mammals that catalyzes the breakdown of herbicides including atrazine, norflurazon, and chlortoluron (Kawahigashi et al., 2005, Didierjean et al., 2002). Using an ice-nucleation protein from *Pseudomonas syringae*, researchers identified that whole cell biocatalyst showing NADPH cytochrome P450 oxidoreductase on the surface of *E. coli* (Yim et al., 2006).

12.3.5 Microbial dioxygenases

Before their oxygenase constituents, all members of this group include one or two electron transport proteins. Dioxygenases are multiphase enzyme processes that break down their substrate into molecular oxygen. Rieske nonheme iron oxygenases include aromatic hydrocarbon dioxygenases. Enantiospecific oxygenation of a vast variety of substrates is catalyzed by dioxygenases. They are fundamental enzymes in bacteria's degradation of

aromatic hydrocarbons. To disintegrate aromatic pollutants, two molecules of oxygen are introduced into the ring. Because they oxidize aromatic compounds, these pathways can be exploited in environmental remediation.

This multicomponent enzyme can function as both monooxygenase and dioxygenase. It can act as dioxygenase for a variety of contaminants, comprising aromatic and aliphatic hydrocarbons. Toluene dioxygenase (TOD) can also act as monooxygenase for monocyclic aromatic compounds, aliphatic olefins, and other molecules. The biotransformation of aromatic precursors into aliphatic products is carried out by catechol dioxygenases found in soil bacteria (Sharma et al., 2018).

Quinaldine bioremediation is facilitated by ring-opening 2,4-dioxygenases and 1*H*-4-oxoquinoline catalyzes the dissociation of two carbon−carbon bonds, leading to the production of carbon monoxide (Khatoon et al., 2017). The pharmaceutical, dye, and chemical industries emit a huge proportion of aromatic chemicals into the atmosphere. Dioxygenase causes the destruction of the aromatic ring at the 1,2-position, incorporating two molecules of oxygen into the substrate. Naphthalene dioxygenase, for example, is involved in naphthalene breakdown and was isolated from *P. putida* (Sharma et al., 2018) (Table 12.1).

12.3.6 Microbial laccases

Several organisms, such as higher plants, bacteria, fungi, and insects, possess laccases (*p*-diphenol: dioxygen oxidoreductase). They catalyze the oxidation of a huge range of reduced phenol and aroma-containing substrates, as well as the conversion of oxygen molecules into water (Ahn et al., 2002, Mai et al., 2000).

Laccases are among the most well understand and oldest enzymes. Copper-containing 1,4-benzenediol:oxygen oxidoreductases are observed in plants and microbes with a carbohydrate concentration of 15%−30%. These enzymes are glycosylated polyphenol oxidases with four copper ions with respect to a single molecule that electron oxidizes phenolics and related substances by reducing oxygen to water (Leung, 2004). When a laccase oxidizes a substrate, it gives up one electron and produces a free radical, which can be further oxidized or undergo nonenzymatic processes including polymerization, disproportionation, and hydration (Dua et al., 2002).

Laccases target the methoxy groups of phenolic and methoxy-phenolic acids by oxidizing and decarboxylating. The enzymes are responsible for lignin depolymerization, which causes a diversity of phenols. These chemicals are also employed as microbe nutrition or are repolymerized into other materials (humic) by laccase. Laccases are an oxidoreductase enzyme family that has performed admirably in biotechnological and biodegradation settings. Laccase substrate specificity and affinity can vary as pH fluctuates. Laccase

Table 12.1: Distinct microbial enzymes from various microorganisms have varied characteristics and applications (Bhandari et al., 2021).

S. No.	Microorganism and enzymes involved	Substrate	Ideal temperature (°C)	Ideal pH	Molecular weight (kDa)	Applications	References
1	*Pseudomonas putida* F6 and laccase	Syringaldazine (SGZ)	30	7.0	59	Synthetic dye degradation	McMahon et al. (2007)
2	*Streptomyces cyaneus* and laccase	2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)	60	4.5	—	Micropollutants such as DFC, BPA, and MFA are then oxidized	Margot et al. (2013)
3	*Geobacillus thermocatenulatus* and Laccase	ABTS	60	4.5	42.5	It involves in decolorizing of dyes used in textile, particularly Congo red and bromophenol blue	Verma and Shirkot (2014)
4	*Bacillus safensis* and Laccase	—	37	6.2	—	Commercially available dyes are decolorized by this	Singh et al. (2014)
5	*A. gonensis* and Laccase	ABTS	60	5.0	160	It involves in wastewater bioremediation	Efe et al. (2016)
6	*Rhodococcus rhodochrous* and P450	Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	30	7.2	—	It involves in RDX degradation	Seth-Smith et al. (2002)
7	*S. rhizophila* and dehydrogenase	Polyvinyl alcohol and vinyl alcohol oligomer	30	7.2	—	It involves in degradation of polyvinyl alcohol	
8	*Mycobacterium* sp. and P450	—	30	6.6	—	It involves in biodegradation of morpholine	Besse-Hoggan et al. (1998)
9	*Bacillus* sp.; *Geobacillus* and purified amylase	Diethylethanolamine (DEAE)	90	8.0	—	It involves in liquefaction of starch	Nigam (2013)
10	*Bacillus subtilis* and lipase	Olive oil	45	7.0	—	It involves in wastewater bioremediation	Mazhar et al. (2017)

11	*Bacillus pumilus* and lipase	Palm oil	50	7.0	–	It involves in degradation of palm oil containing industrial wastewater	Paranji et al. (2019)
12	Chromobacterium viscosum and lipase	–	37	7.0	–	It involves in PBSA, PCL, and PBS degradation	Hoshino and Isono (2002)
13	*Bacillus subtilis* and lipase	Olive oil	37	8.0	–	It is responsible for removing tough oil and grease stains from detergent	Saraswat et al. (2017)
14	*Sphingobacterium* sp. strain S2 and lipase	Chromogenic PNPL	37	7.0	40	It involves in PLA degradation	Satti et al. (2018)
15	*Pseudomonas putida* and dehydrogenase	4-Hydroxybenzaldehyde and 4-hydroxy-3-methylbenzaldehyde	30	8	–	It involves in 2,4-xylenol catabolism	Chen et al. (2013)

function could be halted by a number of chemicals, including halides, azide, hydroxide, and cyanide. Different laccases tend to respond to halide inhibition in diverse ways, showing that halide availability is different. Laccase synthesis in fungus is influenced by nitrogen content. When nitrogen levels are high, laccase is often obtained in significant quantities. Laccase recombinant can be synthesized in either a homologous or heterologous manner. Laccase also decolorized azo dyes by oxidizing their bonds and converted them to less harmful compounds (Legerska et al., 2016).

Two laccase isozymes isolated from the fungus *Trametes hispida* were used to decolorize the samples. Laccase from *R. practicola* degrades and biotransforms phenolic substances (Strong and Claus, 2011). Laccase stability, its half-life, and protease enzyme resistance are all improved when laccase is immobilized on solid supports (Dodor et al., 2004).

Laccase was isolated and immobilized on porous glass beads from the fungus *Trametes versicolor*. Among other things, it can be bioremediate phenolic chemicals, aromatic heterocyclic compounds, and amine-containing aromatic compounds. Laccase removes electrons from the organic substrate to minimize toxic dioxygen molecules in water. In the paper and pulp business, synthetic laccase was employed to improve pulp and textile bleaching (Tuomela and Hatakka, 2011).

Trametes villosa produces laccase, which improves soil quality by digesting 2,4-DCP (2,4-dichlorophenol). In his experiment, Ahm used two types of soil: in soil 1, both free and immobilized laccase removed 100% of 2,4-DCP (regardless of moisture content), whereas in soil 2, immobilized laccase removed 95% of 2,4-DCP (regardless of moisture content) (55%, 75%, and 90%, at 30%, 55%, and 100% highest water holding capacity, respectively). *Cerrena unicolor* produces laccase in a low-nitrogen environment, reducing the lignin content of sugarcane bagasse by up to 36% in 24 hours at temperature of 30°C (Shraddha et al., 2011).

12.3.7 Microbial peroxidases

Hydrogen peroxide oxidoreductases are used as a source of hydrogen peroxide by peroxidases. They catalyze the oxidation of lignin and some other phenolic compounds in the absence of hydrogen peroxide and in the presence of a mediator (H_2O_2). In nature, proteins can be heme or nonheme. Auxin metabolism, lignin and suberin synthesis, cell wall cross-linking, pathogen defense, and cell elongation are all involved in the hormone regulation, immune system, and other biological mechanisms in mammals, and auxin metabolism, lignin and suberin synthesis, cross-linking of cell pathogen defense, and elongation of cell are all involved in plants (Koua et al., 2008, Lloret et al., 2010).

Hemeperoxidases are divided into two categories: those found in animals and those found in fungi, plants, and prokaryotes. When sequence comparison is done, the second group of

peroxidases has been divided into three categories. Intracellular enzymes, yeast cytochrome peroxidase, plant ascorbate peroxidase, and bacterial gene-duplicated catalase peroxidases are all examples of Class I enzymes. Class II peroxidases include secretory fungal peroxidases such as LiP and manganese peroxidase (MnP) from *Phanerochaete chrysosporium*, as well as *Coprinus cinereus* peroxidase and *Arthromyces ramosus* peroxidase.

The breakdown of lignin in wood is the most important activity of Class II peroxidases. Secretory plant peroxidases, such as those found in horseradish, soybean, and barley, are classified as Class III hemeperoxidases. These peroxidases appear to be biosynthetic enzymes involved in the lignification and development of plant cell walls. The five groups of nonheme peroxidases that have evolved separately include manganese catalase, thiol peroxidase, alkylhydroperoxidase, nonheme haloperoxidase, alkylhydroperoxidase, manganese catalase, and NADH peroxidase. Peroxy redoxins and glutathione peroxidases are two subfamilies of the thiol peroxidase class, which is the largest of the five (Koua et al., 2008). Horseradish peroxidase (HRP) peroxidase was identified to cause the oxidative paradechlorination of 2,4,6-trichlorophenol, a hazardous pollutant and cancer-causing. The degradation of thiazole compounds is also being studied using soybean peroxidase and chloroperoxidase (Sharma et al., 2018).

12.3.8 Categorization of peroxidase enzymes

Peroxidases are categorized according to their source and manner of action. LiP, manganese-dependent peroxidase (MnP), and versatile peroxidase have all gotten a lot of press because of their ability to break down hazardous chemicals found in nature (Abdel-Hamid et al., 2013).

12.3.8.1 Microbial lignin peroxidases

LiPs seem to be heme proteins that are generally released in the presence of cosubstrates such as H_2O_2 and mediators such as veratryl alcohol. In secondary metabolism, LiP destroys lignin and other phenol-containing compounds produced by the white-rot fungus. During the reaction, H_2O_2 is reduced to H_2O by obtaining one electron from LiP, which will then be oxidized. After acquiring one electron from veratryl alcohol, the oxidized LiP switches back to its reduced state, yielding veratryl aldehyde. By receiving an electron from the substrate, the generated veratryl aldehyde is reconverted to veratryl alcohol (Abdel-Hamid et al., 2013). LiPs have a wide range of applications, including wastewater treatment and bioremediation. In terms of specificity and thermostability, bacterial peroxidases disintegrate lignin more readily than fungal peroxidases (Behbahani et al., 2016).

This causes polycyclic aromatic compounds, halogenated phenolic compounds, and other aromatic hydrocarbons to be oxidized, and also a series of nonenzymatic reactions. LiP is required for the breakdown of lignin, a constituent of plant cell walls. LiP may oxidize aromatic compounds with redox potentials greater than 1.4 V owing to single-electron

abstraction (NHE). The specific redox mechanism underpinning biodegradation and the participation of microbial enzymes is still unknown (Piontek et al., 2001).

12.3.8.2 Microbial manganese peroxidases

MnPs, exogenous enzymes which are produced by heme-containing lignin-degrading fungi, are effective of oxidizing Mn21 to Mn31 in a multistep reaction. MnP is a manganese-binding enzyme with a heme group and several acidic amino acid remains. Furthermore, because Mn21 donates a single electron to MnP compound I, it is the best reducing agent. This chelator is thought to break down lignin and xenobiotic compounds in an indirect manner. These enzymes catalyze the degradation of dyes, phenols, and amine-containing aromatic compounds (Have & Teunissen, 2001).

MnP-Tra-48424 was isolated and purified using *Trametes* sp. 48424, a white-rot fungus. When combined with heavy metal ions and an organic solvent, this enzyme may decolorize a wide range of dyes, including anthraquinone, Indigo, triphenylmethane and azo, as well as pigments like indigo carmine and methyl green. Purified MnP-Tra-48424 kills a wide spectrum of PAHs (Sharma et al., 2018). MnP had also been immobilized using glutaraldehyde-activated chitosan beads, revealing that dye effluent from the textile sector has a lot of decolorization potential (Bilal et al., 2016).

12.3.8.3 Microbial versatile peroxidases

Mn21, methoxybenzenes, and phenolic aromatic substrates can be directly oxidized by versatile peroxidase enzymes, much like other peroxidases. Versatile peroxidase has a high substrate selectivity and a predisposition to oxidize substrates in the absence of manganese when compared to other peroxidases. It has also been proven that flexible peroxidase may oxidize phenolic or nonphenolic lignin model dimers. As a result, a multifunctional peroxidase overproduction system with high efficiency and versatility is a desirable component for biotechnological industrial applications and biological treatment of refractory contaminants.

12.3.9 Microbial hydrolases

The bonds of hazardous compounds are disrupted by hydrolytic enzymes, resulting in a decrease in their toxicity. Oil spills, as well as organophosphate and carbamate pesticides, benefit from this biodegradation pathway. Insecticides containing organochlorines, such as DDT and heptachlor, have been proven to be in well-aerated soils, it is persistent, and but in anaerobic environments, it quickly degrades. Condensation and alcoholysis are two related processes that hydrolases catalyze. The primary advantages of this enzyme type are its convenience of use, lack of cofactor stereoselectivity, and resistance to the introduction of water-miscible solvents.

Hydrolases, which belong to the group III enzyme system, are divided into three categories based on the nature of hydrolyzed bond. Lipases, xyllanases, amylases, and proteases are extracellular hydrolytic enzymes with a variety of applications in the food industry like chemical industries, feed additives, and medical fields, etc. Because of their use in biomass breakdown, hemicellulase, cellulase, and glycosidase are particularly essential (Singh, 2002).

12.3.10 Microbial lipases

Lipases are lipid-degrading enzymes found in a wide range of organisms such as bacteria, animals, and plants. Lipase has been linked to organic pollutants that are mainly found in soil, according to a recent study. Lipase action was discovered to be the cause of a significant reduction in total hydrocarbons in polluted soil. As soon as the triglycerides develop an emulsion, activity of the enzyme accelerates, and in this field, lipases having a protein loop covering the active site have been studied. The key element of natural oil or fat is triglyceride, which can be hydrolyzed into fatty acids, diacylglycerol, glycerol, and monoacylglycerol by a series of reactions. Glycerol and fatty acids are used as raw materials for a variety of applications, whereas monoacylglycerol is being used as an emulsifiers in a variety of food, pharmaceutical products, and cosmetics.

In a biphasic oil−water system, a study of triolein breakdown by *Candida rugosa* lipase was successful. Lipase adsorbs on the oil−water interface in the majority of the liquid medium. Lipase breaks the ester bonds in triolein, resulting in monoolein, diolein, and glycerol in that sequence.

Oleic acid is synthesized during every point of the catalytic reaction. As a result, the resultant glycerol is hydrophilic and dissolves in water. The best indicative metric for assessing hydrocarbon breakdown in soil was identified to be lipase activity. Lipase is expected to have a critical role in the manufacture of regiospecific chemicals operating in the pharmaceuticals. Lipase has various potential applications in the food, chemical, detergent, cosmetic, paper, and other industries, in contrast to its clinical use in bioremediation. Despite the value it delivers, however, its own manufacturing expenses have limited its industrial consumption (Karigar and Rao, 2011, Sharma et al., 2018).

12.3.11 Microbial cellulases

Cellulase technology has come a long way in recent years, and it has been the subject of a lot of research. It has given use the opportunity to investigate the idea of transforming waste cellulosic material into food products to feed the world's fast-rising population. Some species form cell connections, cell envelopes, and extracellular cellulases. Numerous bacteria and fungi have been reported to express hemicellulases, pectinases, and extracellular cellulases constitutively at really low concentrations.

Although the hydrolysis process usually requires three major types of cellulases, cellulases are generally a combination of several enzymes. The first type is endoglucanase break the cellulose molecule by removing cellobiose units from free chain ends; Exoglucanase or cellobiose hydrolase is the second type that degrades the cellulose molecule by removing cellobiose units from the free chain ends, and the third one glucosidase is a hydrolase that converts cellobiose to glucose. Aside from the most significant enzymes, supplementary enzymes are also present in this scenario.

12.3.12 Microbial proteases

Proteases are enzymes that could hydrolyze and synthesize peptide bonds in both aqueous and nonaqueous environments. Proteinaceous compounds that escape into the atmosphere are degraded by them as a result of appendage shedding and molting, animal death, and byproducts of businesses including poultry, fisheries, and leather. Food, leather, detergents, and pharmaceuticals are just a few of the businesses that use them (Sun and Cheng, 2002, Auriol et al., 2007).

There are two types of proteases: endopeptidases and exopeptidases. This categorization is dependent on peptide chain catalysis. Endopeptidases are classified as metallopeptidases, serine endopeptidase, aspartic endopeptidases, and cysteine peptidase, depending on the active site location. It is important to remember that exopeptidases mainly work near the chain's terminal amino or carboxylic position. Proteases that activate on free amino and carboxyl terminals are designated as aminopeptidase and carboxypeptidase, accordingly. The endopeptidase enzyme works on the peptide chain's central region. It has been discovered that the presence of free amino and carboxyl terminals reduces enzyme activity.

Proteases have historically been utilized in commercial operations such as cheese making and detergent synthesis. In the leather business, alkaline proteases have been used to remove hairs and other contaminants from animal skin. Dipeptide aspartame, a noncalorific artificial sweetener, was made with the help of proteases. To generate effective therapeutic treatments, the pharmaceutical industry employs a variety of proteases. Clostridial collagenase or subtilisin is used in concert with broad-spectrum antibiotics to heal burns and wounds.

12.3.13 Phosphotriesterases

Phosphotriesterases having the ability to break down industrial waste as well as insecticides like parathion is a pesticide that is used in agriculture fields (Sharma et al., 2018). Herbicides and insecticides include the organophosphate chemical parathion (Gao and Yan, 2016).

Phosphotriesterases, also known as aryldialkylphosphatase or organophosphorus hydrolase, are enzymes that break down the phosphoric acid ester organophosphate. *Thalassospira tepidiphila*,

Ruegeria mobiliz, and *Phaeobacter* sp. have all been found to decompose the phosphate triester especially in the coastal oceanic environments. In addition, a new enzyme comparable to phosphotriesterases was discovered in the bacteria *Geobacillus stearothermophilus*. Both lactone and organophosphate-containing compounds could be hydrolyzed by this enzyme. *G. stearothermophilus*' phosphotriesterase-like lactonase is extremely thermostable, remaining active at temperatures as high as 100°C (Sharma et al., 2018).

12.3.14 Haloalkane dehalogenases

Natural and man-made activities both result in the formation of halogenated chemicals. They can be dangerous, poisonous, mutagenic, or carcinogenic, and they are all over the place in soil. Haloalkane dehalogenase hydrolyzes halogen-containing contaminants, breaking down carbon−halogen bonds to generate alcohol and halides. Haloalkane dehalogenase's active site is sandwiched between two areas, the largest of which is an eight-stranded b-sheet surrounded by a-helices.

In the bacterium *Xanthobacter autotrophicus* GJ10, the first haloalkane dehalogenase capable of digesting 1,2-dichloroethane was discovered. Following that, various dehalogenases from Gram-positive and Gram-negative haloalkane-degrading bacteria were cloned and characterized. The activity and integrity of enzymes are increased when they are mobilized (Das et al., 2018). Affinity-tag binding, adsorption on glass, alginate beads, or a matrix can all be used to immobilize enzymes. They could be able to create covalent bonds with an insoluble support, such as silica gel (Sharma et al., 2018).

The method of immobilization utilized has a big influence on enzyme characteristics. However, it should have no effect on the structure or function of enzymes (Sharma et al., 2018). Microbial enzymes employed to immobilize biodegradation should be low of cost, have a large enough contact area, and avoid substrate and product diffusion limitations (Shin et al., 2015). Catalytic efficiency is improved by immobilizing enzymes on a solid support. The activity of an immobilized enzyme lasts longer than that of a free enzyme. The immobilized enzyme can be retrieved and reused several times. The usage of immobilized enzyme for xenobiotic chemical degradation may show to be cost effective due to their stability and capacity to be reused (Kulkarni et al., 1999). Laccase stability and protease resistance improve when laccase is immobilized on a solid substrate (Dodor et al., 2004).

Laccase was immobilized on glass beads extracted from *Trametes versicolor*, a basidiomycete. Despite being completely immobilized, glass beads retain 90% of their activity. Immobilized enzymes work at a wide variety of temperatures and pH levels. They also improve the enzyme's heat stability. In a cell-free environment, intracellular enzymes may not be as effective. In certain cases, immobilization can assist enhance enzyme stability. Some of the methods for immobilizing enzymes that have been established include

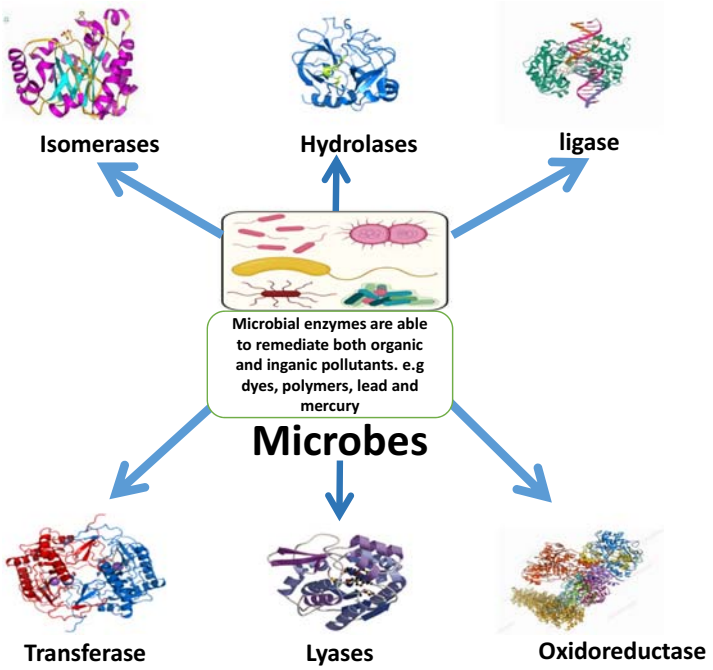

Figure 12.3
Enzymes from different microbes that have a role in bioremediation.

solid support adsorption methods, covalent adhesion to solid supports, cross-linking with biofunctional reagents, trapping in polymeric gels, and encapsulated within a solid support (Bilal et al., 2016) (Fig. 12.3).

12.3.15 Nanozymes

12.3.15.1 Biomimicry based on nanomaterials (nanobiomimicry)

Nanozymes are called as cutting-edge artificial proteins having properties matching to enzyme, are nanoparticle-based catalyst impersonators. In biological settings, they can catalyze substrate changes in the same manner as conventional proteins can (Gao and Yan, 2016). Because of their simplicity and high potential, nanoparticle-based nanozymes have piqued the interest of researchers. Nanoparticle-based nanozymes have grabbed researchers' curiosity due to their simplicity and tremendous potential (Zhang et al., 2016, Shaheen et al., 2017). They can be used to handle nucleic acids, proteins, carbohydrates, and other biomolecules.

Peroxidase, esterase, phosphatase, catalase, protease, and oxidase are just a few of the typical enzymes that have been discovered to imitate nanomaterials. Nanozymes are often

devoid of a functional site; they can only connect to a certain substrate and produce a synthetic reaction (Wang et al., 2015).

These nanozymes offer a wide range of bioremediation uses. They are employed to break down poisons like dyes and lignin-based chemical compounds, among other things.

The breakdown of a few natural poisons such as methylene blue, phenol, and rhodamine has been explored using magnetic nanomaterial Fe_3O_4-MnPs that imitate peroxidases (Wen et al., 2010). Peroxidase-like impetuses are simulated by carbon nanomaterials such as graphene oxide and graphene quantum dots (Dave and Das, 2021; Mai et al., 2000) (Table 12.2).

Table 12.2: Microbial enzymes involve in bioremediation and their function (Bhandari et al., 2021).

S. No.	Enzyme involved	Mechanism of action	Function in bioremediation	References
1	Cytochrome P450	Reduces or oxidizes heme iron to perform electron transfer reactions and catalysis. Pyridine nucleotides are used as electron donors, resulting in carbon substrates and oxidized products.	Steroids, fatty acids, and xenobiotics are oxidized within cells by the manufacture and metabolism of numerous compounds and substances.	Guengerich (2018)
2	Laccase	Reduction of the O_2 molecule using a wide range of aromatic chemicals, including one electron oxidation.	In aromatic compounds, ring cleavage reduces one oxygen molecule in water, culminating in free radicals.	Shraddha et al. (2011)
3	Dehalogenase	Three mechanisms were mostly responsible for this: 1. In the SN reaction, the halogen group is substituted by that of the hydroxyl group, and the molecule of water serves as a cofactor. 2. Oxygenlytic mechanism: mono/ dioxygenase catalyzes the addition of one/two atoms of oxygen molecules to the substrate. 3. It belongs to the carbamide group; in this case, in aerobic conditions, halogen is replaced by hydrogen with organohalides serving as terminal electron acceptors.	The halogens are eliminated when the carbon–halogen link is broken.	Jugder et al. (2015)

(*Continued*)

Table 12.2: (Continued)

S. No.	Enzyme involved	Mechanism of action	Function in bioremediation	References
4	Dehydrogenase	To catalyze the processes, use coenzymes like NAD + /NADP + or flavins like FAD and FMN as electron acceptors. It takes two hydrogen atoms from organic molecules and transfers them to electron acceptors.	Creating energy by oxidizing organic compounds	Dotaniya et al. (2019)
5	Hydrolase	The peptide bond of three-mole fatty acids (P) is broken down by hydrolyzing when one-mole of triglyceride (T) mixes with three moles of molecules of water (W) to generate one-mole glycerol (G).	Fat and protein degradation	Karigar and Rao (2011)
6	Protease	Dissolve protein peptide connections by catalyzing the reaction.	Protein degradation, comprising keratin and casein degradation, as well as treatment of wastewater	Razzaq et al. (2019)
7	Lipase	The carbonyl group of the substrate is targeted by a proton transfer between the aspartate, histidine, and serine residues of the lipase, followed by the hydroxyl residue of the serine. During the deacylation stage, nucleophile assaults the enzyme, renewing it and releasing the product.	This enzyme catalysis the hydrolysis glycerol and fatty acid. Additionally, catalyze the esterification and the process of transesterification.	Casas-Godoy et al. (2012)

12.4 Conclusion

As a result of urbanization, population increase, and technological improvement, toxin buildup has reached a critical juncture in recent years. The most environmentally friendly way to solve this issue is bioremediation, which can be accomplished in situ or ex situ. In bioremediation, the employment of a catalyst is a viable and economical strategy. The segregation of biodegradable catalysts has been researched using a variety of microorganisms from a diversity of typical sources. Various bioremediation-capable molecules have been studied, including oxidoreductase, laccases, and peroxidases. Initially, the protein provided by bacteria in certain conditions was not apparent in catalyst-based bioremediation. As recombinant DNA is generated and suitable circumstances are supplied for these microorganisms, the number of chemicals produced grows. In a wide range of

environmental situations, microorganisms show extraordinary adaptability. There are a plethora of complex and varied methods of communication between microbial networks that play key roles in organisms' response to toxins. These microbial systems transport and exhaust a few compounds and supplementary metabolites. To create a desirable future, it is crucial to examine the perplexing behavior and recognize the metabolites and underlying routes. Due to their distinctive features and the broad application potential in a range of disciplines, cost-effective technologies for creating nanoparticles and materials based on nanoparticle have recently caught the interest of researchers. These nanoparticles can bind to xenobiotic substances and totally breakdown or change them into less hazardous counterparts, assisting in environmental remediation. Nonetheless, the technologies outlined above are sufficient for effective bioremediation, and ideal mechanical intervening is required for developed, efficient, and environmentally pleasant ways.

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CHAPTER 13

Consequences of pharmaceutically active compounds and their removal strategies

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13.1 Introduction

Pharmaceutically active compounds (PhACs) are a prominent issue among Environmental Priority Strategies (EPs). Due to the constant entry of emerging contaminants (ECs) into aquatic systems, the effluents released from plants for the treatment of wastewater are the principal birthplace of PhACs. Antibiotics, tranquilizers, diuretics, and psychiatric medicines are among the common PhACs. The growth of technology and resources has changed the manufacturing process, resulting in a rise in the number of chemicals and certain compounds. Overuse and unchecked emission of PPCPs, on the other hand, pose serious hazards to the environment and living creatures (Yoon et al., 2009). All pharmaceuticals, including prescription and nonprescription medicines, along with substances (inert/active) used in personal care products, are included in PPCPs. Antibiotics, antiseptics, analgesics, plasticizers, hormones, sunscreens, antiepileptics, heavy metals, organic and some inorganic pollutants, and stimulants are being regularly detected in the effluents of wastewater treatment facilities (WWTPs) around the world, though in varying amounts. This frequent occurrence suggests that harmful pollutants are not effectively eliminated during wastewater treatment (Ryu et al., 2014).

PhACs are a family of man-made chemicals that target human and animal metabolic and molecular pathways. Antibiotics, analgesics, hormones, antiinflammatory medications, -blockers,

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agents for lipid regulation, and antiepileptic pharmaceuticals are all examples of PhACs (Liu & Wong, 2013). On a global scale, PhACs are employed for prevention and treatment of disease and in protection of public health. In the European Union, for example, roughly three thousand distinct PhACs have been licensed for the usage of humans (Ferreira-Silva et al., 2011). Antibiotic medication usage grew by 36% from fifty-four to seventy-three billion standard units between 2000 and 2010 (Boeckel et al., 2014), according to comprehensive database research. However, it is difficult to forecast the rates of synthesis, the use, and release of PhACs in the coming 10−50 years. PhAC excess in the environment is expected to enhance due to population growth, ageing, and the affordability of compounds or medicines. Residues of PhACs have been found in practically all environments and on every continent in the last three decades (Kumar, 2019). In the European Union, about 3000 medicinal compounds are utilized. Pharmaceuticals have been found in quantities ranging from ng to g per liter in groundwater, urban wastewater, and drinking water (Ahad, 2019). Pharmaceuticals are difficult to remove from water systems using traditional wastewater treatment procedures due to their ineffective biodegradability and greater hydrophilicity (Szabó et al., 2011). As a result, wastewater treatment plants are a key source of concern (Mansouri et al., 2014). Pharmaceuticals are, in fact, one of the most significant classes of environmental contaminants to be concerned about. The most commonly used medicinal products may vary from country to country. For instance, analgesics, antihistamines, and antidepressants, for example, are the most commonly prescribed medications in Spain (Petrovic et al., 2009); while antibiotics, ofloxacin, sulphamethoxazole, and ciprofloxacin, diuretics such as furosemide, hydrochlorothiazide, and NSAIDs such as ibuprofen are the most commonly identified medications in Italy.

Pharmaceutical production and consumption have both expanded significantly in recent years (Boeckel et al., 2014). The untreated discharge and leakage of these drugs into natural ecosystems pose a risk of proving toxic to aquatic species and have the potential to affect human health (Yang et al., 2017). Salicylic acid (SA) is commonly employed in chemical synthesis, while acetylsalicylic acid (ASA) is another the most commonly prescribed nonsteroidal antiinflammatory medicine. SA and ASA are released into the environment both directly through wastewater and indirectly as metabolites of other pollutants. SA and ASA concentrations in wastewater effluents have been detected up to the g mL1 level (Essandoh et al., 2015; Rakić et al., 2014). Consumption of SA and ASA can cause headaches and nausea, as well as interfere with the liver and kidneys' natural functions in humans. PhACs are found in large quantities in the plant for wastewater treatment influents, surface waters, and hospital effluents which have detrimental effects on many life forms due to their toxic effects. Different pharmaceuticals were detected in disturbingly high amounts in different regions of the world, and it has also been discovered that the quantity of PhACs which exist in water could be linked to the region's climate and socioeconomic conditions.

13.2 Classes, structures, and therapeutic application of most common pharmaceuticals

In the European Union, about 3000 medicinal compounds are utilized. Antibiotics are the most frequently used compounds in human medicine and the veterinary field. Over the recent decade, 3 of 35 consumptions had above 12,500 tonnes per year. Non-steroid antiinflammatory medicines (NSAIDs), which contain aspirin, diclofenac, and ibuprofen, inhibit some enzymes that are involved in prostaglandin formation. Ibuprofen, which is also known by the name 4-isobutylphenyl-2-propionic acid, is among one of the most extensively taken or used as NSAIDs in the world. Other kinds of medications, such as analgesics, with paracetamols as its most prominent example, act as antipyretics. It blocks cyclo-oxygenase present in the central nervous system without having any effect on the peripheral nervous system, which is why it has no antiinflammatory properties. Naproxen (NPX), an NSAID, is one of the most effective analgesics, and it has been found in both surface water and in wastewater in concenterate form (Fu et al., 2018). It's also found in wastewater treatment plants in amounts ranging from 250 to 1.5 g/L, with a clearance rate of roughly 71% (Méndez-Arriaga et al., 2008). Although NPX has a favorable cardiac profile, it has been observed that those who consume small quantities of the compound have an increased risk of heart attack or bladder cancer (Lubet et al., 2015). The type and accumulation of medicines in wastewater differ by country, according to their health services. Natural hormones and synthetic too, such as 17-ethinylestradiol (EE2) and 17-estradiol (E2), are found in sewage at very low levels in the Northern hemisphere including Germany, Hungary, Spain (Mansouri et al., 2021) (Table 13.1).

Table 13.1: Classes of PhACs (pharmaceutically active compounds).

Class	PhACs	pKA	Reference
Hormones	Estriol	10.54	Kim et al. (2015), Fernandes et al. (2021)
	Estrone	10.34	
Analgesic	Diclofenac	4.2	Simazaki et al. (2015), Kim et al. (2018), Behera et al. (2011), Tran et al. (2017), Behera et al. (2011)
	Codeine	10.6	
	NaproxenIbuprofen	4.2	
	Paracetamol	4.9	
	Salicylic acid	9.4	
		3.49	
Antibiotics	Ciprofloxacin	6.38	Papageorgiou et al. (2016), Batt & Aga (2005), Tran et al. (2017)
	Trimethoprim	6.8	
	Erythromycin	8.9	
	Azithromycin	8.74	
	Levofloxacin	6.24,	
	Ofloxacin	8.74	
		5.97,	
		9.28	
Stimulant	Caffeine	10.4	Batt & Aga (2005), Behera et al. (2011)
	Para xanthine	8.5	
Antiepileptic drugs	Carbamazepine	13.9	Santos et al. (2009)

13.3 Occurrence and sources of pharmaceutically active compounds

Pharmaceuticals and personal care products (PPCPs) are not biodegradable, and these are active (biological) compounds that are easily soluble in water. They penetrate water, and then move to freshwater resources, and then eventually accumulate inside aquatic life as a result of their disposal (Zhao et al., 2019). More than 80 different forms of PPCPs have been discovered in sewage effluents. PPCP levels in various wastewater/sewage effluents range from some ng/L to greater than certain g/L, according to numerous studies (Lim et al., 2017). Because of their frequent use, analgesics and various antibiotics are mostly found in aquatic species. It means that because of the excessive use of the antibiotics and analgesics, their residues remain in the auqatic environment hence found in the auqatic species (Küster & Adler, 2014). The two commonly used antibiotics—tetracycline and sulfonamide—are both very stable and environmentally permeable. Similarly, as an analgesic medicine, ibuprofen (IBP) is among the most commonly discovered pharmaceutical compounds in the environment (Jiang et al., 2013). Pollutants (EPs) generated through cosmeceuticals, pharmaceuticals, certain sunscreens, and synthetic musks, in addition to their metabolic products, were investigated by Daughton and Ternes (1999). PPCPs was the name they gave to this broad group of active chemicals. In addition, pharmaceutical compounds are categorized into seven classes on the basis of their uses: analgesics, X-ray contrast, antibiotics, anticonvulsants, cytostatic, -blockers, and some lipid regulators, with antiinflammatory (analgesics), -blockers, antibiotics, X-ray contrast being the most well-known. Personal care items, on the other hand, contain steroid hormones, synthetically produced hormones, perfumes, lipid regulators, shampoos, analgesics, and cosmetics. Waste Water Treatment Plant (WWTP) is also an important source of PPCPs, which enable paths for micropollutants to be transferred to the surface and sometimes even to tap water. It is due to the fact that traditional wastewater treatments are antiquated and ineffective that removal efficiency in eradicating PPCPs is less than 20% (Qarni et al., 2016).

13.4 Environmental impacts of pharmaceutically active compounds

PhACs are present in huge amounts in the environmental matrices, where they range between some ng/L to g/L. Nonetheless, PhACs may have a major cumulative influence on nontarget organisms' metabolism and ecosystems as a whole (Schulman et al., 2002). The estrogenicity seen owing to the releasing of disruptors of endocrine was the first indication of a negative effect of PhACs on ecosystems. For example, zebrafish exposed to 5 ng/L ethinyloestradiol causes retardation of development of the embryo, whereas rainbow trout exposed to 0.1 ng/L ethinyloestradiol induced vitellogenin. Endocrine disruptor concentrations in wastewater vary from 0.3 to 150 ng/L, and in surface water from 0.3 to 7.2 ng/L, according to the LOES experiment. In India and Pakistan, a high death rate that is

(5%–86%) of vultures was recorded in 2004 as a result of diclofenac, a commonly used antiinflammatory PhAC and antibiotic. A link between exposure to diclofenac leading to renal failure was seen in Oaks et al. investigations (Oaks et al., 2021). Following that, antibiotics became a source of public worry, as prolonged antibiotic exposure might cause the generation of antibiotic-resistant strains of bacteria (Wang et al., 2017). Antibiotic resistance genes (ARGs) are currently thought to be borne by certain bacteria which are naturally found in the environment, and they may serve as reservoirs of resistance to antibiotics that could be passed on to disease-causing bacteria and cause harm to human health in the next centuries (Berglund et al., 2014) (Table 13.2).

For decades, environmental issues around PPCPs have been known. The EPA had noticed the lack of cooperative controlling procedures for its PPCP geographic updates. As a result, concerns about health and the environment surrounding PPCPs are not new, and they are only getting worse as the use of prescription and nonprescription medications grows. Antibiotics, opioids, steroids, depressants, and stimulants are examples of EPs commonly present in the environment. Sulfonamides are the class of antibiotics that are used most frequently (Gao et al., 2012). It is also common in the environment because of its great water solubility and stability. Tetracycline, chlortetracycline, and oxytetracycline, for example, are widely used as animal medicines and produce byproducts that harm the environment. The most well-known opioids for pain relief include oxycodone, hydrocodone, and codeine. Antidepressant medications such as barbiturates and benzodiazepines are also regularly administered (Bagheri & TermehYousefi, 2017). NSAIDs are the most widely used antiinflammatory drugs in the world, and they are regularly discovered in water

Table 13.2: Impacts of PhACs (pharmaceutically active compounds).

PHACs	Impacts	References
Diclofenac	Lessens hematocrit value of fish Causes change in cells of kidney, liver, and gills of fishes Causes renal tumors	Santos et al. (2009), Tran et al. (2017)
Codeine	Increase in plasma conc. Organism became intoxicated	Boyer (2012)
Ibuprofen	Effect on reproduction system DyspepsiaGastric ulceration Effect on CNS Bowel inflammation Damage to mucosa Effects on kidney Cardiovascular issues	Ali et al. (2016), Santos et al. (2009)
Ketoprofen	Harmful for aquatic species	Illés et al. (2014), Tran et al. (2017)
Naproxen	Proves toxic for fishes	Santos et al. (2009)
Paracetamol	Toxic impact on liver	Li et al. (2016), Tran et al. (2017), Santos et al. (2009)

(Méndez-Arriaga et al., 2008). Carbamazepine (CBZ), found in WWTP effluents, has been shown to play a role in causing toxicity that stays for a long time (Khetan & Collins, 2007).

EPs have extremely low concentrations, but their prolonged exposure and their reactive nature endanger the ecosystem. The information provided about this is insufficient to determine their environmental impact. As a result, numerous concerns remain, like how many PPCPs are affecting human health now, both directly and indirectly, and what their long-term consequences for the ecosystem are. We don't know for sure, but the constant presence of traces in the environment attests to their long-term effects (Rasheed et al., 2020).

13.5 Adverse impacts of PhACs on the environment

Hundreds and even thousands of PhACs are in use around the world for various purposes. The direct or indirect discharge of PhACs in the environment in many ways poses hazards to many environmental compartments. However, the problems associated with their acute toxicity are minor in comparison to those associated with their toxicity that stays for a long time. The treatment plants developed for wastewater are ineffective in clearing out PPCPs, allowing them to spread and cause irreversible environmental damage. Some PPCPs are present constantly in nature, and their presence in drinking water can be used to assess their risk. Other non-persistent PPCPs, on the other hand, became pseudopersistent as a result of their continued use and release into the environment (Löffler et al., 2005). They have a greater negative impact due to their constant discharge into the environment by the source. PPCPs are categorized into three classes on the basis of their dissipation time (DT50):

1. extremely persistent (DT50 = 119−328 days) e.g., clofibric acid,
2. moderately persistent (DT50 = 15−54 days) e.g., ivermectin,
3. low persistent (DT = 3−7 days) e.g., paracetamol, ibu

PPCPs are introduced into soil by the use of manure as fertilizer or the application of sludge (Verlicchi et al., 2012). Soil pollutants leach into groundwater or get accumulated in plants as a result. It has been discovered that irrigation with wastewater causes greater PPCP buildup in soil than irrigation with freshwater. Because of their high leachability, PPCPs like naproxen, triclosan (TCS), and clofibric acid might contaminate groundwater. Microbial activities such as their enzymatic activity, nitrification, soil respiration, and soil biodiversity are also harmed by PPCPs. At a dosage of 150 g/kg, antibiotics like ciprofloxacin and sulfamethoxazole reduce soil respiration (Conkle & White, 2012). TCS, at concentrations of more than 1 mg/kg, can disrupt the soil nitrogen cycle and the nitrification process. Their prevalence in water, where they pose harm to aquatic life, is a major source of worry. Musk fragrance prevalent in perfumes and some detergents are longlasting, and their fat solubility allows them to build up in fish tissues. PCPs were observed in water samples at low amounts (50−200 ng/L) but at greater amounts

(50−400 ng/g) in algae (Anekwe et al., 2017). Diltiazem, caffeine, ibuprofen, and CBZ were found to have bioaccumulation factors of 2, 16, 28, 16, and 14 in aquatic life, respectively. In mussels from the River Ontario, 145 PPCPs were found. Similarly, 43 PPCPs with various bioaccumulation factors were discovered in mussel tissues. Even at smaller amounts than those found in the environment, long-term exposure to PPCPs in aquatic life can produce ecotoxicity and impair several physiological functions. Hormones' propensity to behave as endocrine disruptors is one of the primary issues for aquatic life, given their negative impact on the aquatic's endocrine system, resulting in a large decrease in the population (Rasheed et al., 2020).

13.6 Presence of PhACs in different water bodies

North America had the highest pharmaceutical sales, followed by Africa and Europe according to (Statista, 2018) accessed 11.8.18. Even though western countries' pharmaceutical consumption was much more than the Asian countries', still the reported quantities of various PhACs in various water bodies in Asia were at par with, or much more than, those in Europe and North America. This demonstrates that the presence of pharmaceuticals in aquatic ecosystems is influenced by a variety of elements such as the region's water and soil properties, climate, people's socioeconomic conditions, pharmacokinetics, available treatment strategies, and so on. These PhACs were identified in high concentrations in hospital effluent, WWTP influent, and surface water. The only significant distinction between the two sources is the concentration range in which they emerge. Because of their widespread use and availability in the population, analgesics are the most studied class of medications (Tran et al., 2017). The most typically found analgesics in influents are paracetamol, ibuprofen, and diclofenac. Paracetamol concentrations of more than 60 and 50 g/L were discovered in the influent of WWTPs in Canada (Guerra et al., 2013). In Canada, South Africa, and Singapore, the maximum detectable amount of ibuprofen, diclofenac, and naproxen were 55.975, 25, and 22.628 g/L, respectively (Guerra et al., 2013). In comparison to Africa (39 g/L) and Europe (43 g/L), the average concentration of analgesics in WWTP influent was lower in Asia (4 g/L). The consumption of analgesics was found to be highest in nations such as Sweden and France. In comparison to countries in Europe, the United States consumed about half as many analgesics per capita. The residues of analgesics in the wastewater of respective countries reflect this tendency. The quantity of analgesics found in hospital wastewater followed the same pattern as the influent from the WWTP (Majumder et al., 2019) (Fig. 13.1).

13.7 Impact on ecosystem

PhACs are durable in water habitats and may have a negative impact on microbes and other living creatures in the water when exposed to them (Bhatia et al., 2017). They invade

Figure 13.1
Sources of PhACs in water bodies. *PhACs*, pharmaceutically active compounds.

aquatic species and begin to move up the hierarchy, causing biomagnification of pollutants. When humans and animals consume previously tainted water, they can get a direct dose of these toxins. Extended exposure to these PhACs in nontarget animals can result in a variety of abnormalities (Zenker et al., 2014).

13.8 The threat to the aquatic environment

The rise in consumption of pharmaceutical substances in humans and also in animals has created an environmental crisis, and as a result of this, there is a growing interest in learning more about their existence in the environment and the negative consequences on human and ecological systems. Antibacterial, medicines, and hormones are examples of this class of molecules, which are frequently employed in agriculture, pharmacology, and biotechnology. PhACs molecules' prevalence and fate have become a significant source of

worry and studies in the twenty-first century. More than 80 chemicals have been discovered in the aquatic environment in different affluent countries, like the United States, Japan, Korean, Australia, and many countries in Europe. The usage of active chemicals is expected to be over 100,000 tonnes or more per year. The widespread use of medications in a variety of settings results in the constant dumping of pharmaceuticals and some metabolites into the surroundings, resulting in their "Pseudo permanence." Even in these small quantities, their existence in water has prompted questions among stakeholders, including drinking-water agencies, governmental ministries, and the general public, about the possible hazards to public health and aquatic life from contact to pharmaceutical residues. As the world's population grows and pharmaceutical use rises, the problem gets more serious. Excretions from human and animal systems, seeping from landfills, compost, or biosolids uses, and incorrect disposal are all ways for PhACs to enter the aquatic environment. Some PhACs have been detected at quantities that may have harmful impacts on aquatic established communities. One of the increasing challenges in environmental chemistry is the occurrence and fate of PhACs in the aquatic environment. Antibiotics, antiinflammatory medications, lipid regulators, X-ray contrast media, and beta-blockers are among the most common pharmaceuticals discovered in sewage, surface, and samples taken from groundwater. Surface waters, and treated water, have all been found to contain PhACs (drinking water). Streams, rivers, WWTP effluents, hospital sewage, and pharmaceutical manufacturing effluents have all been studied. Pharmaceutical concentrations in the environment, their temporal evolution, and probable synergistic and antagonistic effects are all affected by the level of pharmaceuticals released by WWTPs, as well as the affecting the geographical region and causing changes in climate. These compounds travel from domestic waste streams to municipal wastewater treatment plants, private septic systems, or, in rare situations, directly traveling to receiving water without processing. The persistence of these medications could also be influenced by photodeterioration in the aquatic environment. A broad range of levels of light deterioration of pharmaceuticals has been documented based on lab trials. The two major sinks for PhACs have been identified as photodegradation and microbial degradation. The physical and chemical features of PhACs determine their removal efficiency in wastewater and potable water.

To check pharmaceuticals in sewage and, as a result, check the number of pharmaceuticals released into the aquatic system, sensitive and accurate procedures are required. The advancements in analytical techniques and instruments are largely responsible for the increase in detection. Pharmaceuticals have been identified in water samples. The majority of the studies released provide up-to-date information on PhACs in aquatic environments in the more advanced countries in sense of development. Due to a shortage of effective sewage and unwanted pharmaceutical chemical treatment facilities in developing nations, the information may differ. In addition, the volume and types of medications utilized in

developing nations may paint a very different image on this subject. Due to the lack of studies exploring their identification, medicinal metabolites are yet to be discovered.

13.9 Adverse health issues

Pharmaceuticals, cosmetics industries, manufactured musks, and some other PPCPs are more likely to be exposed to humans in their daily routines via polluted water, air, and soil (Bilal & Iqbal, 2019). Prolonged exposure to these chemicals can lead to major health problems such as allergies, illnesses, and cancers (Mathew, 2014). Resistance against antibiotics is among the most serious health issues, given antibiotics can become inefficient against infection caused by antibiotic-resistant microorganisms. During an Australian assessment conducted on buying and usage of antibiotics and their left over which was found in environment, many antibiotics, such as tetracycline, sulfa drugs, and erythromycin, pharmaceuticals were discovered in wastewater, which meant resistance to antibiotics in humans, if exposed (Jamil, 2019). Bioaccumulation and entry into the food web via WWTPs, including the usage of animal wastes as fertilizer in the agricultural field, are two of the reasons for exposure. Despite its presence in minimal amounts ranging from ng/L to g/L, it has the potential to induce serious health effects with unclear chronic outcomes (Rajapaksha et al., 2015). 1,4-dioxane was discovered with an amount of 28% in twenty-seven thousand (27,000) PCPs, as per the EPA of the United States. Researchers also discovered 16 different dangerous compounds, including 2-benzene dicarboxylic salt and synthesized musks (Fig. 13.2).

13.10 Ecotoxicology and genotoxicity aspects

Underground, ocean systems, and surface water have all been contaminated with various kinds of PPCPs in diverse proportions as a result of industrialization and urbanization. Several clinical and preclinical tests have been carried out while developing drugs. Their negative consequences, if any, have only been observed in laboratory settings. The amount of some substances that may prove toxic to organisms, such as Daphnia magna (*D. magna*), has been assessed in identifying risks based on toxicity testing (Bound & Voulvoulis, 2006). A high-dose contact can result in death, which is uncommon in the case of a production line accident. Pharmaceuticals, on the other hand, are unclear as to how they pose a threat to wildlife. Even if the effects of pharmaceuticals on ocean organisms are not well-known, biologically active chemicals pose significant dangers (Christensen, 1999). Their concentrations of fewer than 1 g/L are not known to cause toxicity. Constant exposure, on the other hand, is likely to have long-term consequences. Likewise, even at low doses, certain substances such as organotins and growth hormones may have unexpected impacts on nontarget organisms (Larsson et al., 1999). Several substances, such as antibiotics related to veterinary science, have been discovered to have impacts. Several

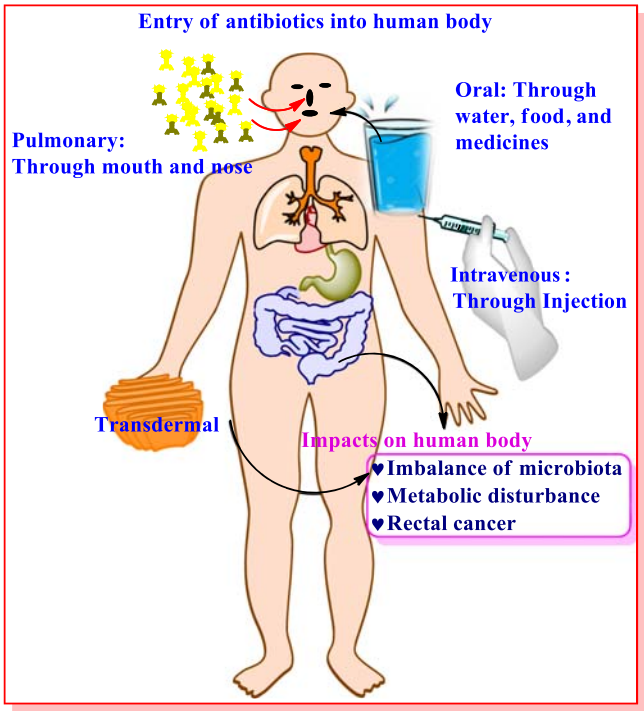

Figure 13.2
Residues of pharmaceuticals in environment and entry to the human body.

substances can potentially affect the species that live in the environment. It gets more problematic when some drugs cause harm to germs and animals, sometimes even at lower doses than the recommended dose. The creation and use of a broad range of drugs of diverse medicinal groups, which are constantly discovered in the aquatic system, is the primary cause of this problem (Yang et al., 2017). So far, the amounts are lower than the typical amounts used in clinical trials. Therefore, while the concentrations of PPCPs may be lower, the cumulative concentrations of all PPCPs might be significant enough to have an impact on aquatic species. This impact is amplified when many PPCPs have the same mechanism of action. Because antibiotics are frequently used at small exposure levels, their presence in the environment is creating serious concern. As a result, bacterial pathogens have become more resistant to a variety of chemicals, rendering them ineffective in controlling diseases. Further, antibiotics can potentially have an impact on the microbial ecology in sewage treatment plants. Suppression of bacteria in sewage water may have a significant impact on the breakdown of organic matter, as well as nitrification and the process of denitrification. However, a study found that the bacteria separated from digested sludge and processed sewage do not act as antibiotic-resistant as bacteria isolated from raw sewage (Luo et al., 2014). Antibiotics in sewage discharges, as well as their potential

impacts on microbes, are thus a major source of worry. Using a bacterial genotoxicity experiment in hospital effluents, researchers identified fluoroquinolone as a primary reason for genotoxicity. When compared to other antibiotics, Ciproxin and noroxin (fluoroquinolone antibiotics) had the highest induction probability and outperformed them by a scale factor. The outcomes were confirmed by testing a sample of urine from hospital patients. Using fluorescent and reverse-phase high-performance liquid chromatography, the researchers evaluated the concentration of ciprofloxacin in hospital wastewater which was deemed to be 3−87 g/L. A log-linear association was found for the assay-inducing factor of sixteen (16) samples collected from the hospital effluent for ciprofloxacin. The authors have suggested that fluoroquinolone drugs, notably ciprofloxacin, were mostly responsible for genotoxicity.

13.11 Control measures and removal fate

When discharged into the environment, the PPCPs' fate is determined by the environmental compartments making up the composition of active medicinal components (Gurr & Reinhard, 2006). Some pharmacological chemicals may build up in the animals' tissues. Bioaccumulation can occur for a variety of reasons, including utilization through a surrounding phase or through food. Because of the medications' potential impacts on different trophic levels, bioaccumulation is critical (Lai et al., 2002). Water fleas (Monia macrocopa) have been shown to be carriers of norfloxacin (an antibiotic) in fish. Several other medications, on the other hand, are easily digested and do not pass through the food chain. As a result, making broad statements about the future of PPCPs is problematic. As the PPCPs are discharged into the water habitats, sorption through solid-phase medium—such as sand and suspended particles—reduces their quantity. The majority of PPCPs are hydrophilic and have low volatility; hence, sorption is unlikely to reduce their concentration in the aqueous phase. The steroid estrogen in PPCPs has ability to bind effectively to organic compound containing materials because PPCPs have phenolic group. When iron oxide sorption and sediment sorption were compared, iron oxide sorption of 40% estrogen showed that carbon could not be a sorption precondition.

PPCPs with equivalent features are likely to behave similarly. There might be some aquaphobic elements present because of huge groups with several chemicals for the ability to bind to the deposits (Jones et al., 2003). These chemicals are significant in terms of bioavailability, transportation, and degradation.

Because PPCPs sorb at a higher amount in sediments when compared with water, benthic creatures would receive a higher dose than pelagic ones. The octanol-water partition coefficient indicates the likelihood of chemicals to partition to organic substances (Kow). The higher the Kow of compounds, the greater the tendency to partition towards organic stuff, which is only confirmed by modeling data for a few medications and lacks

scientific proof. The more repulsive a PPCP is, the more tightness it will collect on the solid, and the more friendly a PPCP is, the greater its quantity will be in the liquid state (Rasheed et al., 2020).

13.12 Treatment techniques

WWTPs are an important line of defense against PhAC discharge. Traditional WWTPs, on the other hand, are planned to remove components in a bulk amount, such as organic matter present in wastewater and nutrients, but not PhACs. The compounds that exist in the mixture at concentrations ranging from ng to g/l then were previously overlooked when improving WWTP. As a result, the release of WWTP outflow into surface water is now a primary conduit for the liberation of PhACs. PhACs were not adequately eliminated in WWTPs, according to a review, with 24 out of 50 PhACs eliminated at less than 50% (Verlicchi et al., 2012). To make up for the lack of removal, the WWTPs should take care that post-treatment processes are established or existing post-treatment procedures are optimized to reduce PhACs to a greater extent in WWTPs. Indeed, along with the detection and recognition of PhACs in the environment, a variety of approaches in post-treatment for PhAC removal have been utilized and studied, including physically, chemically, biologically, or hybrid methods (He, 2017).

13.12.1 Physical techniques

Nano-filtration (NF), which is a membrane-based method, is extensively used as physical techniques that may eliminate PhACs, although the pore diameters of ultra-filtration are significantly bigger than the particle mass of PhACs (Luo et al., 2014). Reverse osmosis (RO) has been shown to have a high potential for partially or completely removing PhACs (Yangali Quintanilla et al., 2011). NF and RO membranes, on the other hand, are permeable to some minor PhACs. Membrane-based approaches also need a lot of energy and have a lot of fouling difficulties (Zhou et al., 2017). Activated carbon, in the form of powder activated carbon (PAC) or granular activated carbon (GAC), is another sort of physical approach. Adsorption, on the other hand, can only pass PhACs from the liquid phase to the carbon phase without transforming them, and it works best for hydrophobic chemicals (Snyder et al., 2007). Furthermore, because of the competing attraction of the natural organic matter (NOM) and the blockage, the NOM present in sewage would impair the functionality of PAC and GAC in the sorption process of PhACs (Heijman et al., 2007).

13.12.2 Chemical techniques

PhACs have been found to be oxidized to readily biodegradable compounds using disinfection procedures such as chlorination, ferrate (FeO4 2)(Zhou et al., 2017), among

others. Ozonation has been shown to reduce PhACs considerably as an after-treatment technique at full-scale WWTP (Hollender et al., 2009). The reaction rate of ozonation was three orders of magnitude higher than that of chlorination. However, ozone synthesis is energy-intensive, and in a WWTP, this might raise energy consumption by 40%−50%. Furthermore, ozonation performance can be unstable due to interference from reducing agents. Ferrate is an effective disinfectant because it oxidizes pollutants with Fe6+ and coagulates them with Fe3+ (Jiang et al., 2013). At the ng/L level, ferrate has been shown to oxidize up to 90% of PhACs. Despite this, ferrate ions have poor liquid phase stability and are expensive to produce. The oxidation of organic pollutants by •OH generated by the interaction of hydrogen peroxide and Fe2+ (Liu et al., 2016) is well recognized using Fenton-based procedures. PhAC biodegradability in wastewater can be improved by Fenton oxidation. During Fenton oxidation, however, iron sludge is formed as a result of the precipitation of Fe(OH)3, which must be handled and disposed of correctly. Fenton method was upgraded to Photo-Fenton and Electro-Fenton by adding any source of electric current or electricity and light to decrease the use of catalytic iron to lessen the creation of iron sludge. The improved Fenton procedures can effectively oxidize PhACs, but their effectiveness is highly reliant on the pH of the aqueous medium and the dosage of H_2O_2 and Fe^{2+}, much as it is with conventional Fenton. Furthermore, when dealing with the treatment of hydrophobic organic pollutants with a high partition coefficient Kow(octanol water), Fenton-based approaches are less effective (Shemer et al., 2006). Photolysis is a method of decomposition in which these molecules are dissolved by consuming light, directly or indirectly. In the presence of manually applied photosensitizers such as H_2O_2, indirect photolysis occurs. To replicate photolysis, researchers have investigated many light sources. Yes in these days UV light is the most widely used wavelength of light As UV plays a great role in process of photolysis. Furthermore, due to the expensive nature of the artificial light application, sunlight-induced photolysis is gaining in popularity.

13.12.3 Treatment using biological procedures

The membrane bioreactor (MBR) is commonly regarded as cutting-edge technology for removing PhAC. MBRs have the ability to eliminate a broad spectrum of PhACs, which can be attributed, in part, to the extended sludge retention time—allowing the sediment to respond to the breakdown of micropollutants—and, in part, to physically intercepting PhACs. Activated sludge treatment and membrane filtration are thus combined in MBR procedures. High energy and operational expenses, and the expensive demand for membranes, however, are major barriers to MBR adoption (Chae et al., 2009). Attached growth approaches have been found to be promising for treating pollutants by culturing microbes on inert carriers that are either fixed or even mobilized in reactor suspensions. Attached growth techniques include trickling screens, soil washing, and biological activated carbon (BAC) sieving. The fixed bed film reactor and the sliding bed biofilm reactor are

two types of biofilm reactors. Biofilm reactors have recently been used to extend the attachment growth concept for PhAC elimination by Accinelli et al.. In addition, new cost-effective bio-plastic materials such as pellets and granules have been investigated to replace the regularly used Kaldnes-like carriers in the future. Other microorganisms, such as algae or fungus, can lessen PhACs in a cost-effective and long-term manner, in addition to microorganism-based biological therapy approaches. To biodegrade PhACs, a pond which is high in algal rate, for example, uses microorganisms. PhACs are destroyed in the pond rich in algal growth by heterotrophs that utilize oxygen which is produced by microalgal photosynthesis, requiring no aeration (Matamoros et al., 2015). Furthermore, the recovery of the biomass of algae into fertilizer and biofuel is gaining popularity for the microalgae-based technology (cytochrome P450) which is intracellular and external (e.g., laccases, peroxidases) these enzymes can be produced by white-rot fungi. By functioning as biocatalysts, enzymes with limited substance specificity can assault PhACs. Till now, only a small amount of research has been done on PhAC bioremediation employing microorganism-based approaches.

13.12.4 Phytoremediation technique

Plants (agricultural and wetland plant species) and related rhizosphere microbes are used in phytoremediation to extract, convert, and detoxify pollutants (Carvalho et al., 2014). These green procedures are generally described as low-energy, low-manpower techniques that have been shown to be effective in treating a variety of pollutants, including PhACs. However, pollutants accumulating in plant biomass may cause phytotoxicity (Gerhardt et al., 2009), which can reduce or prohibit plant development in the field, reducing the effectiveness of phytoremediation (He, 2017).

13.12.5 Removal of pharmaceuticals by biological method

Bacteria, the major organisms in the environment supporting the metabolic cycle, are essential to all life on planet Earth (Martínez-Espinosa, 2020) through their varied metabolic capabilities. Researchers are looking at the ability of diverse bacteria to decompose medicines and personal care products (PPCPs) into environmentally benign monomers that might be a new way to remove drug residues from the environment (Molina et al., 2020). Sludge-based methods (aerobic, activated, or granular) have proven to be particularly effective in the treatment of wastewaters (Nancharaiah et al., 2019). This is mostly due to the involvement of the microbial population. Some genus and varieties involved in pharmaceutical substance removal procedures, such as venlafaxine, fluoxetine, metoprolol, alprenolol, bisoprolol, propranolol, salbutamol, norfluoxetine, and 17-estradiol, had shown a removal efficiency of 90% or greater (Muter et al., 2017). Another study (Marchlewicz et al., 2016) found that utilizing a Gram-positive bacterial strain B1 (2015b)

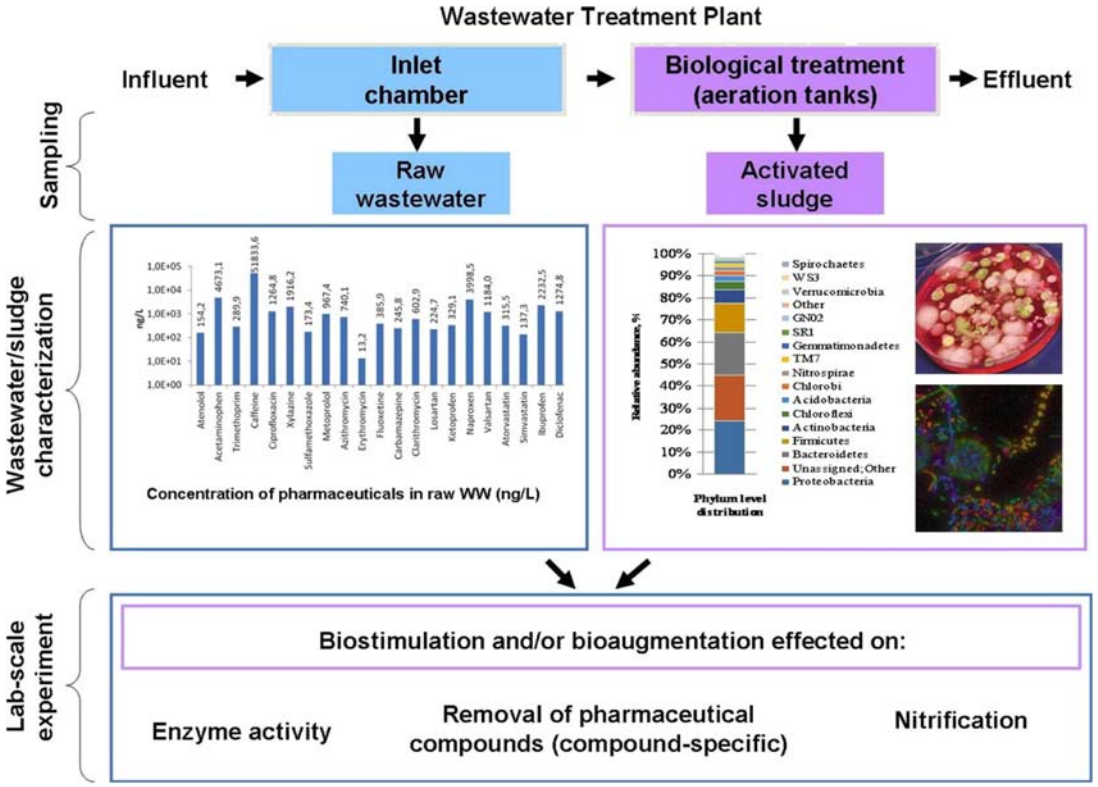

Figure 13.3
Laboratory-scale biosimulation and activated sludge treatment of pharmaceuticals removal from municipal wastewaters (Muter et al., 2017).

improved efficiency in the breakdown of ibuprofen and naproxen after six days for a 20 mg/L concentration and 35 days for a 6 mg/L concentration. Microbial degradation of PPCPs could be a viable option for removing this residue. Microorganisms degrade pharmaceutical debris into methane, CO2, biomass, and water, along with other inorganic chemicals, with the organism's enzymes playing a key role. The process of biodegradation is used to remove pharmaceuticals depending on the configuration required. Many researchers have explored sand columns, WWTPs, artificial wetlands, and membrane bioreactors, among other reactor configurations (Fig. 13.3).

13.12.6 Electrocoagulation method

Electrocoagulation is a therapy method in which there is a generation of cations in an electrical field by metal electrodes (McPhie, 1992). It makes coagulants with a dielectric material, which are more capable of interacting with pharmaceutical products, and it has a

number of advantages, including sludge reduction, automatic processing, and low and efficient running expenses (Dey et al., 2021). Ensano et al. (2017) evaluated the elimination efficiency of the three most commonly used pharmaceuticals: carbamazepine (70%), diclofenac (90%), and amoxicillin (77%). Husein et al. (2020) investigated the antibiotic oxytetracycline. For both anodes, the optimum density was seen at 20 mA/cm^2: removal efficiency for iron and aluminum was 93.2% and 87.75%, respectively. The impact of the initial concentration on extraction efficiency was also investigated: raising the initial amount of oxytetracycline hydrochloride to the extent of 200 mg/L had no discernible effect on its eradication. During the studies, the pH, and Eh of all specimens were examined: pH increased significantly with both anode−cathode combinations, whereas Eh and dissolved oxygen declined significantly. Furthermore, 100% ciprofloxacin elimination was achieved utilizing an electrocoagulation technique at a density of roundabout 15 mA/cm^2, pH was 7.5, and a starting CIP dosage of 60 mg. Within 20 minutes, the electrolyte dose was 0.07 M NaCl, and the inter-electrode distance was 1.58 cm (Mansouri et al., 2021).

13.12.7 Removal of pharmaceutical products by sorption method

The solid adsorbents being used in the adsorption process have the possibility to be one among the most effective for treating a large range of pharmaceutically contaminated fluids and wastewaters (Kumar et al., 2015). Indeed, there have been some outstanding trials of carbon materials being used to eliminate pharmaceutical contaminants, which is a cost-effective wastewater treatment option. Natural adsorbents and synthetic adsorbents are two types of adsorbents. The natural adsorbents include clay minerals, clays, sorbents, charcoal, and some ores. These organic resources are often cheap and readily available, and they contain a lot of potential for modifying and improving their capacities of adsorption. Synthetic adsorbents are those adsorbents created from agricultural waste and goods, household garbage, industrial pollutants, sewage sludge, and polymer adsorbents. Porosity, porous structure, and micropore surface type, all, are distinct to each adsorbent. Fruit waste, cash crops, date nuts, bark, used tires, and other tannin-rich substances, rice husk, saw dust, petroleum waste, fly ash, fertilizer waste, sugar mills waste, blast furnace slag, chitin and seafood disposal, algae, sandy clay, peat, foundry sand, zeolites, soil particles and soil, ores, and some other wastes are among the many waste products used. Activated carbon (AC), clays, and biochar are three types of advanced materials now being used to remove PPCPs from wastewater. Other more contemporary materials, like molecularly imprinted polymers (MIPs), high-temperature gel, and magnetic nanoparticles, have also been reported, albeit theirs are yet to be developed and supported by case studies.

13.13 Removal of different pharmaceuticals by advanced oxidation processes

Aqueous phase oxidation technologies are known as advanced oxidation processes (AOPs), according to Klavarioti et al. (2008). These AOPs are defined by the use of good intensity and the creation of the hydroxyl radical (OH) as a particularly potent oxidant (Szabó et al., 2011). AOPs have been shown by researchers on numerous occasions to successfully breakdown pharmaceuticals in wastewater or other marine habitats. Because of photon-initiated carbon−halogen link breakdown and increased hydroxy free radical production, photochemical, ozone-based (O3/H2O2, O3/UV), and Photo-Fenton reactions are often more successful than ozonation alone (Ikehata et al., 2006). Furthermore, UV or visible radiation can cause medicines to become reactive excited states. The formation of harmful oxidation products which can linger in sanitized water is the principal issue connected with AOPs (Aloulou et al., 2020). For the removal of pharmaceutical active compounds, there are different processes and methods for synthesis of photcatalyst that are involved in removal of PHACs. In literature, the structure of the medicinal chemical, photocatalytic reactor designs and operation settings, and the effect of aqueous matrices on destruction have all been addressed (Friedmann et al., 2010). Many studies mentioned the elimination of paracetamol and acetaminophen ACE as NSAID medicines. For the removal of acetaminophen ACE, (Borràs-Ferrís et al., 2019) and (Gómez-Avilés et al., 2019) employed two completely distinct photocatalytic methods. Indeed, photo-electrocatalytic (PEC) breakdown of ACE using TiO2 nanotubes as photo catalysts showed that photolysis of ACE was faster at pH = 3 and that a higher level of mineralization was attained. In contrast, photocatalysis employing mixed Ti−Zr metal−organic structures under solar intensity resulted in complete elimination of ACE, and the photocatalyst was shown to have high reusability, allowing it to be used in real-world wastewater treatment. Using ferrioxalate at a concentration of 2 mg/L for 5 minutes of treatment once in an aqueous solution, photodegradation for paracetamol appeared to show 98% elimination under solar irradiation (Trovó et al., 2008). According to Jallouli et al. (2017), TiO2 heterogeneous photolysis with UV-LEDs proved effective in eliminating IBU from pharmaceutical industrial wastewater and distilled water at natural pH (between 5 and 5.3), but the same method proved less successful in municipal WWTP effluent treatment. After the treatment of about 40 minutes, they detected a percentage of degradation ranging from 89.83% to 100% in genuine urban wastewater. Monteoliva-García et al. (2019) employed light-driven AOPs to treat three different medicine items in real municipal wastewater: carbamezapine, ibuprofen, and ciprofloxacin. After 40 minutes of treatment in genuine urban wastewater, they were able to remove 80.4%−100% of carbamazepine, an antidepressant medicine, as well as 89.83%−100% of ibuprofen, and thorough removal of ciprofloxacin. He also emphasized the effectiveness of photocatalytic activity over photolysis, which was quicker and had a

greater rate of pharmaceutical product removal in wastewater: 74% extraction of carbamazepine and 100% elimination of propranolol and DIC. Calza et al. (2006) investigated an aqueous solution using TiO2 photocatalyst for the other NSAIDs. After 2 hours of irradiation, they observed 100% DIC breakdown and mineralization. Furthermore, it was demonstrated that photocatalytic removal of NPX pharmaceutical chemicals in ultrapure water is more effective than photolysis. In actuality, the TiO2-UV process eliminates NPX at a 98% efficiency and reduces chemical oxygen requirement COD by 25%. Furthermore, after 3 hours of photocatalysis at an initial pH of 6.5, the COD was 83% and 11%, respectively; whereas (Méndez-Arriaga et al., 2008) found that in an aqueous suspension 0.8 mmol/L, 3 hours of photodegradation method treatment results in 90% elimination of NPX with only 5% mineralization, and yet only 40% under TiO_2 photocatalysis. In the case of antibiotics, many studies (Long et al., 2018) agree on the efficacy of the method when Fe^{2+} and TiO_2 were added as catalysts in wastewater treatment plants; for example, the photocatalyst process for such degradation of different antibiotic drugs—such as Ciprofloxacin CIP—was successful in completely eliminating it from untreated wastewater before 20 minutes of treatment when Fe^{2+} and TiO_2 were introduced as catalysts.

13.14 Other methods for removal of PhAC from water

As many PPCPs have been detected in sewage water effluent and sources of drinking water, research is being done around the world to analyze the transporting process of PPCPs in wastewater cleaning strategies. The treatment procedures used to handle PPCPs, such as coagulation, filtration, deposition, flocculation, chlorination, degradability, ozonation, and activated carbon treatment, have a significant impact on their transit fate. MOFs and MOF-based nanostructured materials have high efficiency in adsorbing PPCPs. Several MOF-derived nanocomposites have recently been studied for a variety of applications, including storage of energy, gas filtration, energy storage, and environmental cleanup. MOFs and MOF-based nanostructured materials are utilized in a wide range of applications, including the removal of contaminants, especially PPCPs, from water and sewage. MOF-based micro adsorbents are gaining popularity because of their unique architecture, certain physicochemical characteristics, and excellent adsorptive performance. Few literature reviews have yet documented the removal of poisonous, organic, and other emerging contaminants using various MOFs. However, understanding the adsorption mechanisms of various MOF-based nano-architectures is essential for the removal of PPCP-based contaminants. It is also critical to understand the chemical study of dissolution conditions such as acid-base interactions, electrostatic interactions, hydrogen bonds, and - interactions. The physicochemical properties of the adsorbent, mineral, and water, on the other hand, pose a serious effect on the adsorption destiny of PPCPs. Some of the characteristics include charge, hydrophobicity, functionality, size or form, surface area (substance and

adsorbent), cations, solute concentration, pH anions, and NOM (Water). The aim of this research was to provide a comprehensive knowledge of the degradation of various forms of PPCPs utilizing different nanomaterials which are MOF-based.

Pharmaceutical removal is dependent on a variety of water treatment techniques, each of which has a varied efficiency. Pharmaceuticals are not entirely removed from wastewater treatment plants (Kumar et al., 2015). Adsorption offers advantages over other approaches due to its basic design, which can demand a low initial investment in terms of both money and area (Wang & Chen, 2009). Low investment, applicability at less dissolved substances, continuous processing, and the capacity to regenerate adsorbents and also their reuse are all advantages of adsorption processing for water cleanup. Adsorption is known as a low-energy technique that can be quite efficient and result in up to 90% elimination, although it has a very moderate operating environment. Large amounts of wastewater can be treated using the Fenton method. The use of the non-hazardous, environmentally acceptable, affordable, and stable semiconductor TiO_2 is the most significant advantage of photocatalytic activity. Indeed, under sunshine irradiation and using in situ cathodic generation of H_2O_2, degradation can be achieved quickly and at a high amount of organic matter. However, there are a number of drawbacks to this method, including the requirement for acidic environments with a pH approaching 3.0. As a result, after the treatment, a neutralizing is required, resulting in the formation of a substantial volume of sludge. The development of halogenated byproducts should be given special attention. An enhanced oxidation technique, according to (Kumar, 2019), can result in high pharmaceutical degradation, but it is hard to deploy on a large scale of WWTPs due to high operation costs. The high cost of all AOPs is a common issue, owing to the rising need for electric power.

13.15 Removal of PPCPs by advanced MOFs

MOFs have been widely used in a variety of applications in the last decade, including sensors, gas sorption, catalysis, and separation. MOFs have many advantages, including high porosity, plentiful functional groups, which include (e.g., −NH2, −OH), the conductivity of charge, etc., all of which enable good interactions such as electrostatic bond and hydrogen bond formation for PPCP removal. Furthermore, customized properties such as pore shape, its size, and hydrophobicity, as well as ease of manipulation, make MOFs good platforms for exploring structure-function correlation, leading to the development of better MOFs with higher catalytic activity. Zhao et al. (2019) investigated the properties and utilization of MOFs (UiO-66) for the specific and fast elimination of three commonly used PPCPs was investigated by investigating their relationship between structure-function and adsorption kinetics. The utilization of MOFs (UiO-66) for the specific and fast elimination of three commonly used PPCPs was investigated by investigating their relationship between structure-function and adsorption kinetics. PPCPs include

2,4-Dichlorophenoxyacetic acid (2,4-D), a very lethal, carcinogenic, and cytotoxic chemical, a nonsteroidal antiinflammatory medication, and clofibric acid (CLA), a widely used herbicide. The results showed that UiO-66-NH2 had the highest absorption capacity of these PPCPs, which was 3−4 times more than the UiO-66-COOH counterpart, which had the lowest absorption capacity. The structure-function investigation revealed that hydrogen bonding, electrostatic interactions, and interfaces between PPCP molecules and MOFs, all, had a significant impact on the adsorption process. Despite UiO-66's high PPCPs removal potential, it has been difficult to use in powder form due to difficult separation and recovery from aqueous environments. Sun et al. (2019) used microspheres made of calcium alginate and MOFs for the absorption of levofloxacin, a commonly prescribed antibacterial, from aqueous solution to solve this shortcoming. Individual adsorption performance of UiO-66 or calcium alginate was 86.43 mg/g, which was significantly higher than individual UiO-66 or calcium alginate adsorption performance. Furthermore, after five cycles of levofloxacin adsorption, the reusability study revealed that more than 70% of the levofloxacin had been absorbed. In a separate study, sodium alginate-chitosan-based aluminum MOF composites were used to reduce bisphenol A levels in contaminated water. In comparison to the Al-MOF/SA equivalents, the experimental findings indicated improved adsorptive ability. After five continuous batch cycles, as-synthesized beads were regenerated and recycled with over 95% adsorption efficiency. PAFs (porous aromatic frameworks) are particularly effective adsorbing agents for the elimination of PPCPs. PAFs in powder form, on the other hand, have significant drawbacks in larger-scale applications. To overcome these drawbacks, PAFs were covalently linked onto electro-spun polymer fiber membranes, which was a novel technology. PAF was modified by coating it with polyaniline, an aroma seed layer essential for the loading of PAFs onto the surface of the substrate. The resulting absorbent composite (PAF-45-PP FM) was used to remove three main PPCPs: chloroxylenol, ibuprofen, and N, N diethylmetatoluamide. For ibuprofen, chloroxylenol, and N, N diethyl-meta-toluamide, the newly synthesized PAF-45-PP FM composite had adsorption capabilities of 613.50, 429.18, and 384.61 mg/g, respectively. After 10 adsorption-desorption cycles, composites' adsorption abilities toward the tested PPCPs were slightly reduced. Micropore absorption, as well as hydrophobic and - interactions between PAF45-PP FM and PPCPs, were discovered during the adsorption mechanism analysis. Johannessen et al. (2021) identified bisphenol A, diclofenac, clofibric acid, CBZ, iopamidol, and musks as higher priority worrying pollutants in sewage systems while examining sources and implications of rising PPCPs that are present in urban water matrices. BPA, for example, is an endocrine disruptor even at very low concentrations (Clayton et al., 2010), but TCS is widely used as an antibacterial agent in numerous items. Caffeine and CBZ's toxic and carcinogenic effects on long-term bioaccumulation have been documented in several studies. The sorption of several PPCPs, including acetaminophen (ACP), caffeine (CAFF), BPA, CBZ, along with TCS, was recently investigated. According to estimates made by density - functional theory, adsorption is primarily due to diffusion,

with minor contributions from hydrogen bonding and electrostatic interactions (Delhiraja et al., 2019). Following pseudo-second-order kinetics, a new MOF (Basolite A100) demonstrated better removal efficiency for ibuprofen and CBZ when compared to a conventional powder activated carbon. The likely adsorption mechanisms of MOFs, (Jun et al., 2019) were attributed primarily to hydrophobic interactions, with contributions from hydrogen bonding, electrostatic interactions, and hydrophobic interactions. Furthermore, the regeneration and recycling properties of the MOF were tested for four consecutive cycles to confirm its suitability for wastewater remediation (Jun et al., 2019). Jun et al. (2019) used MOF as a sonocatalyst for ultrasonic procedures to accelerate the degradation of SA and CBZ. Under various processing settings, such as pH (3.5, 7.0, and 10.5), temperatures (293, 303, and 313 K), ultrasonic frequencies (28 and 1000 kHz), and ultrasonic power densities (45−180 W/L), the removal rates of the selected contaminants were recorded. The degradation rate of CBZ was found to be higher than that of SA under all of the evaluated settings. The oxidation process of OH• created by the dissociation of ions in water influences the degradation rate of both chemicals, which are strongly associated with the quantity of H_2O_2.

13.16 PCPs removal using magnetic MOF-nanocomposites

Magnetic nanoparticles have grown in popularity due to their simplicity of synthesis, modification, and recovery, as well as their environmental sustainability. They, too, have remarkable properties such as a huge surface area, small size, dispersibility, and a high adsorptive capacity (Li et al., 2019). Importantly, employing an external magnetic source, the solid-liquid recovery is simple. As a result, such nanocomposites might be used as magnetic adsorbents in environmental samples to efficiently separate and enrich a wide spectrum of trace elements. The alteration of these magnetic nanoparticles can be used to achieve adsorption selectivity in addition to the specificity of target molecules (Wei et al., 2017). Although SA is a phenolic acid that is frequently used in chemical synthesis, acetylsalicylic acid (ASA) is the most commonly prescribed nonsteroidal antiinflammatory medicine. Both pollutants reach the environment as metabolites of many other chemicals and as compounds of wastewater. In humans, the consumption could produce nausea and headaches, as well as interfere with the kidneys' and liver's natural activities. As a result, devising effective methods for removing these medications is an unavoidable issue. Zhang et al. (2019) created magnetic UiO-66-NH2 nanocomposites to see how well they worked as adsorbents for removing SA and ASA medicines from aqueous solutions. As-synthesized magnetic UiO-66-NH2 composite inherited the properties of MOFs and magnetic equivalents, enabling quick extraction efficiency and higher loading capacity. Electrostatic connections, hydrogen bonding, and the carboxyl group's affinity for Zr−O nanoclusters all played essential roles in SA and ASA adsorption. Surprisingly, even after three reuses, the

adsorption capacity of the synthesized composites was not diminished and reached up to 77% and 70% for SA and ASA, respectively (Rasheed et al., 2020).

13.17 Alternative treatment technique of AOPs/adsorption

Researchers have demonstrated the harmful impacts of PPCPs on the environment that we previously discussed, namely endocrine disruption and the danger of antibiotic resistance spreading. Different countries have set restrictions to stop the development of drugs in the environment, according to OECD (2019). For example, in the health sector, the United States has nationwide prohibitions on the removal of dangerous medical waste, and Germany has developed an environmental assessment for veterinarians and farmers with the goal of minimizing veterinary pharmaceutical use and discharge into the environment. In addition, for water quality monitoring, Korea uses suspect and nontarget screening to identify and prioritize pharmaceuticals. Australia also has a national pharmaceutical collecting and disposal scheme in the same order. We demonstrate in this review that a single method, such as conventional treatment, physical therapeutic options including coagulation and adsorption, or advanced oxidation methods, which include a combination of oxidizing agents (such as H_2O_2 and O_3), radiation exposure (such as ultraviolet light or ultrasound), and catalysts (such as Fe^{2+}), is insufficient to remove them. To ensure 100% medication removal in water, a combination of the two or even more mechanisms is required. According to (Kumar, 2019) the combined application of ozonation and sorption for the elimination of imidazole resulted in 90%−100% ozonation and 10%−20% mineralization. Toxicity is further reduced by 30% when an adsorbent is present.

For the elimination of medicines, catalytic ozonation combined with adsorption can be efficient. The medicinal products are destroyed by ozonation, and the adsorbent can extract any leftover pharmaceuticals and breakdown byproducts (Mojiri et al., 2019) also show that a combined ozone and adsorption process for the treatment of solutions containing acetaminophen and amoxicillin was effective in removing 84.8% and 82.7% of the acetaminophen and amoxicillin, respectively, with an initial ozone concentration of 0.17 and 0.16 mg/L. The combined Photo-Fenton and biological treatment was over 95% efficient, with the solar photochemical process accounting for 33% and the biological treatment accounting for 62% (Sirtori et al., 2009). Sulfamethoxazole was also removed satisfactorily utilizing catalytic ozonation and adsorption on modified powdered activated carbon (Fe2O3/CeO2-loaded activated carbon) at 2 g/L for just 50 minutes (Akhtar et al., 2011). Sui et al. (2014) investigated the removal of 13 pharmaceutical and personal care products (PPCPs) in a full-scale wastewater treatment plant using a sequential UV and ozonation procedure. The authors demonstrated that the majority of the target PPCPs were effectively eliminated and that the median extraction efficiency of individual PPCPs, which ranged from 13% to 89%, was dependent on their molecular ozone reaction rate constants. Salgado et al. (2011) also

Table 13.3: Removal of pharmaceuticals.

Drugs	Adsorption process	Matrix	References
Ibuprofen	copper nanoparticles green synthesis	Wastewater	Li et al. (2016)
Naproxen	Nano-composite film	Aqueous solution	Mondal et al. (2020)
Diclofenac	CTAC	Aqueous environment	Jodeh et al. (2015)
Salicylic acid	Pine wood biochar	Aqueous sol	Essandoh et al. (2015)
Naproxen clofidric acid	Ethylenediamide	Deionized water	Hasan et al. (2013)

investigated the elimination of 18 pharmaceuticals using UV photodegradation. They discovered that for 17 of the 18 most regularly detected PPCPs, up to 75% clearance was usually recorded, with the exception of diclofenac, which has frequently exhibited negative values for the rate of natural elimination in activated sludge and is mostly destroyed by UV photolysis. In reality, there are various options for combining sophisticated oxidation processes in treatment water, such as photo-Fenton/ozonation and photocatalysis/ozonation (Rivera-Utrilla et al., 2013). The combined procedures could result in transformation products that are more persistent and hazardous than the original medicinal medicines at the end of the treatment. The combined procedures of AOPs and adsorption resulted in nearly full pollutant removal. Adsorption can thus be used to remove low-molecular organic compounds produced by AOPs, resulting in a more cost-effective and advanced treatment. For this reason, numerous academics have recommended combining AOP and desorption to address the issues that arise whenever these two methods are used individually (Table 13.3).

13.18 Concluding remarks and future suggestions

Recently, several MOFs, along with some MOF-based nanocomposites, are being developed and have previously been developed and envisioned for use in applications of remediation of the ecosystem. Several investigations have shown that MOF-assisted composites are active and promising sorbents for a diverse variety of EPs, comprising active residues from hormones, pharmaceuticals, pesticides, surfactants, and personal care items, which are becoming increasingly prevalent in ecosystems. Among the multiple adsorption/removal associated processing variables and catalytic qualities, the high total pore volume, exceptional surface area, and customizable structure of MOF-based nano-adsorbents appear to be the dominant factor in obtaining the maximum adsorption capacity. Adsorption and removal efficiency is heavily influenced by major mechanisms such as electrostatic bonding, hydrogen bonding, metal effects, acid-base, hydrophobic, and - interactions/ stacking, according to mechanism-based research, i.e., calculations based on density

functional theory. One of the most important concerns for a sustainable environment is the regeneration and reprocessing capacity of MOF-based adsorbents during adsorption procedures because exceptional repeated use ability across several successive cycles can significantly reduce medical costs in the removal of various EPs based on personal care and pharmaceutical products. According to a survey of the literature, the majority of MOF-derived nanocomposites could be successfully regenerated utilizing a variety of solvents like acetonitrile, ethanol, and hydrochloric acid.

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CHAPTER 14

Bioprospecting microbial proteases in various industries/sectors

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14.1 Introduction

All living organisms have enzymes as their biological catalysts. As a result of their ability to perform reactions with high sensitivity and accuracy, enzymes have become the preferred tools in green chemistry and are increasingly being used in industrial processes. A large number of environmentally friendly industrial and chemical processes are of particular interest to enzymes for a variety of reasons. Proteases are a broad category of industrial enzymes, including almost 60% of the international enzymes marketed and 40% of worldwide enzymes sold. As well as having just one enzyme as a mandatory enzyme contained, proteases have a group of enzymes in them, such as proteinases, peptidases, and amidases. Industrial enzymes known as proteases are essential since they are likely to break the peptide links found in proteins, making them useful in a variety of chemical and biochemical mechanisms. Proteases do have many benefits, including stereo-specificity, specificity, and biodegradability, the capacity to make organic goods, and action during normal reaction conditions. Cell mechanism is cell processes and transition of a cell from one cell type to another is cell differentiations so during it foodstuff are those proteins resembles or drive from food sources must be digested by protease. Organism's cell division and proteins recirculations take place. During cell proliferation foodstuff proteins must be digested. These cascading actions are also important for cell repairing mechanisms like blood clotting and the life cycles of many infection-causing microorganisms, such as

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retrovirus replication. Intriguingly, the protease enzyme family comprises 2% of the human genome, making it the largest one. Because of their functional and structural variability, proteases do have a broad array of intracellular protein reprocessing and also a cascade of nutrient digestion and immune system attenuation activities.

Proteinases account for 60% of the enzyme market since they are utilized in so many industry sectors, including detergents, leather, food, and meat and cheese manufacturing, as well as paper and pulp. They can also be used in bioremediation processes to get the silver back from photographic films. Inflammation and harmful lesions can be treated with these enzymes. Alkaline proteases hold a 52% market stock share of the enzymes market, while enzymes used in detergents, textiles, and paper and pulp make up the rest of the market. There are numerous industries that use massive quantities of energy and raw materials in the production of life-sustaining products like textiles, paper, feed, food, pharmaceuticals, and chemicals and release large quantities of waste into the environment that negatively affect human health. Global consumption is increasing, as is the global population, and the economies of various countries are doing better, which puts more strain on the environment. As a result, meeting human needs while not depleting natural resources is an urgent priority. Industries all over the world are considering other options for current technologies which can deliver a variety of products while using fewer natural resources to meet yearly demand. Enzymatic handling is a feasible and long-term alternative to traditional processing methods.

Global sales of industrialized enzymes are now worth over $3 billion dollars. There will be a 6.1% CAGR in the global protease enzyme market by 2024, according to markets of US. The global market of protease enzymes is estimated to have cost $3 billion in US dollars before 2024. Nowadays, most industrial enzymes are hydrolytic and used to degrade various natural materials. Due to their widespread use, proteases are the far more broadly utilized enzymes in the dairy and washing industries. Furthermore, they are environmentally friendly and, after being used in industry, tend to reduce the amount of toxicity in the air. This chapter gives a comprehensive snapshot of proteases and their categorization, protease sources, industrial uses, protein engineering, and immobilization strategies to enhance protease–catalytic performance (Naveed et al., 2021).

14.2 Classification of protease

The wide variety and specificity of these indigenous enzymes can be attributed to their extensive characterization. Exopeptidases and endopeptidases are two types of proteases based on their active site positions (Fig. 14.1).

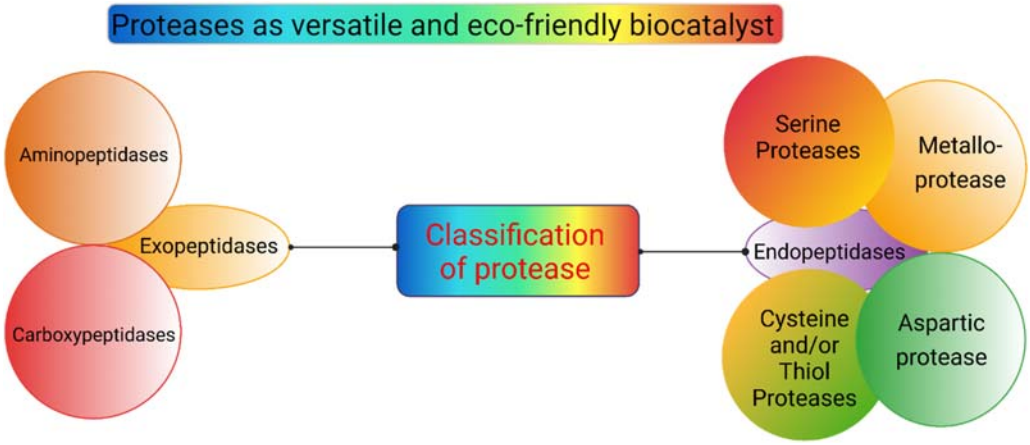

Figure 14.1
Simplified representation of the classification of proteases.

14.2.1 Exopeptidases

It is thought that exopeptidases are responsible for the cleaving of specific peptide linkages in proteins near the amino- or carboxyl-terminal parts of the substrate. N or C terminal precision determines whether or not they are categorized as carboxypeptidases or aminopeptidases, with the former being the more common.

14.2.2 Aminopeptidases

Aminopeptidases tend to release tripeptides, dipeptides, and isolated amino acid residues from the polypeptide chain's free N-terminus. Following identification, they attempt to eliminate any N-terminal methionine that may have been contained in heterologously produced proteins but is absent from several native (mature) proteins. Aminopeptidases are seen in microbes, such as fungi and bacteria, among the other species. Aminopeptidases are typically intracellular enzymes; however, another study discovered that Aspergillus oryzae aminopeptidases are extracellular enzymes.

14.2.3 Carboxypeptidases

Carboxypeptidases catalyze the dipeptides and a single amino acid freed from the polymer's free C-terminal. They are not considered endopeptidases because they leave the amino acid at the target protein. As an alternative, they can be used to remove C-terminal tags from the target protein. Type a carboxypeptidase, which is found in metallocarboxyprotease, first and foremost, deletes the aromatic side chain of the enzyme from the branch. When it comes to essential amino acids, type B is put to use.

14.2.4 Endopeptidases

Endopeptidases break off a long distance from the site of the peptide bond. Further subdivided into serine, aspartic, cysteine, and metalloproteases, endopeptidases are based on the active site's presence of a particular functional group.

14.2.4.1 Serine proteases

Serine proteases are found throughout the kingdom of cellular organisms, as well as in the genomes of numerous viruses. Among the known proteolytic enzymes, serine proteases account for one-third of them. Endopeptidases that cleave the bond in the middle of the chain are the most common. Several of these enzymes remove amino acids from the polypeptide chain's terminus before folding. Serine residue is a nucleophilic and located at the enzyme's active site that gave them their name. When the serine residue attacks the carbonyl end of the incoming substrate peptide bond, an acyl-enzyme intermediate is formed. The triad complex of "Asp," "His," and "Ser" residues is responsible for its nucleophilic activity.

14.2.4.2 Cysteine/thiol proteases

Cysteine residues make up the majority of this protease class's active site. They originate in both prokaryotic and eukaryotic organisms. The proteolytic activity requires a pH between 6 and 8 and temperatures between 50°C and 70°C which are considered optimum for the enzyme. Due to the regeneration of the SH group, hydrogen cyanide plays a significant role in activating this enzyme. Oxidizing agents can inhibit proteases, and sulfhydryl agents, such as p-CMB, can make them more sensitive. However, metal chelating agents have no effect on proteases.

14.2.4.3 Metalloprotease

Endopeptidases containing zinc predominate among metalloproteases. Fungi reactivation is aided by numerous metal ions, such as cobalt, calcium, and zinc. Zinc-containing enzymes are found in most of the fungus and bacterial metalloproteases. Their enzyme activity requires zinc, and protein structural stability requires calcium. EDTA is a metal chelating agent that can inhibit these enzymes, but cysteine inhibitors and sulfhydryl are not.

14.2.4.4 Aspartic proteases

Aspartic proteases are a minor subgroup of endopeptidases that cause little harm. These bi-lobed proteases have a core catalytic site composed of two aspartates. These acidic pH-dependent proteases have been reported in animals, plants, and microbes. Many organisms secrete them as virulence secretions, but these proteases can also be mutualistic in breaking the proteins in urea that yield nitrogen, demonstrating their dual nature. Aspartic proteases prefer hydrophobic amino acids near the dipeptide bond to hydrophilic amino acids

elsewhere in the protein. The first two endoproteases use nucleophilic residues in the active region to catalyze proteolysis. After that, both of these methods operate on the water molecules, activating them for nucleophilic attack. Some proteases, such as ATP-dependent proteases, require ATP to be activated and do not fit into the classification described above (Naveed et al., 2021).

14.3 Sources of protease

14.3.1 Plants

The use of plants as a source of proteases is dependent on a number of factors, including the availability of cultivable land and climate conditions that favor growth. Moreover, plants must go through a lengthy process to produce proteases. Papain, bromelain, and keratinases are examples of well-known proteases derived from plants. Papaya fruit yields papain, which is a digestive enzyme (Carica papaya). It aids in milk coagulation and protein digestion, as well as possessing a wide pH range. Pineapple leaves, juice, stems, and peel all contain bromelain, a plant protease that can be found in all four of these components. Tumor cell growth can be effectively curtailed with this treatment. Keratinases, which are used to remove hair, are derived from botanical groups of plants. As a result of the digestion of wool and hair, a critical aminoacid-like lysine is formed that helps keep sewage systems from becoming clogged. Cysteine protease was purified and characterized using maize leaves in Pakistan.

14.3.1.1 Animals

Among the most well-known proteases, trypsin, chymotrypsin, pepsin, and rennin were derived from animals and are among the most well-known proteases. Trypsin, a digestive enzyme, is present in the intestine and is in charge of the breakdown of dietary proteins. Chymotrypsin is detected in pancreatic excretions (animals). Pure chymotrypsin is a costly enzyme utilized in research and diagnostics. All mammals' stomachs produce protease (pepsin), which is the active precursor to rennet. When the pepsin enzyme is activated, it changes from its inactive state back to active. It's commonly utilized in the dairy sector to make consistent curd with a superior flavor. Pepsin is an acidic enzyme found in all vertebrate stomachs. Only employed in detergents until 1913, when it was replaced by a blend of microbial proteases (metal) and serine, pepsins are resistant to breakdown in alkaline environments, detergents, and high temperatures.

14.3.1.2 Microbes

Proteases that are currently accessible in the marketplace are derived from microbes. This is owing to a variety of factors, including their high rate of production, reduced cultivation area requirements, vast biochemical diversity, ease of genetic manipulation, and appealing

qualities that make them suitable for biotechnological applications. However, because animal and plant proteases are unable to meet the contemporary demands of the global industrial sector, the scientists discovered an alternative answer in the shape of microbial sources. Microbial-derived proteases account for roughly 40% of total global enzyme sales. Microorganisms are responsible for both internal and external protease synthesis. Intracellular proteases are required for many metabolic endproducts of cellular processes such as differentiation, protein turnover, sporulation, hormone and protein processing, and protein removal, whereas extracellular proteases are required for the consumption and hydrolysis of proteinaceous nutrients. Extracellular proteases play an important function and have several uses in a variety of sectors. Proteases are isolated from various microorganisms such as fungi, bacteria, and yeast.

14.3.1.3 Fungal

Proteases isolated from fungi pique the interest of researchers due to their broad substrate specificity, stability under adverse conditions, great diversity, and mycelium separation via simple filtration. Fungal proteases are enzymes that are utilized to modify dietary proteins. Proteases derived from fungal sources have benefits over proteases derived from bacterial sources, and they are generally recognized as safe (GRAS) strains widely. Fungi that produce protease include *Aspergillus niger*, *Aspergillus clavatus* ES1, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus melleus*, *Aspergillus nidulans* HA-10, *Aspergillus sojae*, *Aspergillus terreus*, *Aspergillus oryzae*, which are the most well-known fungal strains for protease generation; however, microbial strains such as *Myxococcus*, *Neurospora*, *Penicillium*, *Ophiostoma*, and *Rhizopus* are also frequent protease makers. *Cheotomuim globusum* is a new indigenous strain that produces high-quality alkaline protease.

14.3.1.4 Bacterial

Bacterial proteases, which are alkaline in nature, have economic importance in a variety of industries, including leather, food, laundry, silk, and detergent, due to their increased catalytic activities and production capacities. Bacterial proteases (alkaline) are differentiated by their greater activity at pH (alkaline) 8−12 and temperatures ranging from 50°C to 70°C. *Bacillus subtilis* is one of the microorganisms that produce protease. *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus clausii*, *Bacillus halodurans*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus circulans*, and *Bacillus safensis* (Naveed et al., 2021) are the other bacteria worth mentioning.

14.3.1.5 Bacillus thuringiensis proteases

Bacillus is a gramme + ve bacterium that is found the world over. *Bacillus* spp. are useful industrial tools for a variety of reasons, including their ability to release proteins into extracellular media and their GRAS status with the Food and Drug Administration. This genus contains a number of commercially important species that are responsible for the

production of a variety of products such as enzymes, fine biochemicals such as antibodies, and pesticides. Only a few diseases are known, and the majority of species are safe for people and animals. *B. thuringiensis (Bt)*, a highly studied bacterium, produces a powerful insecticidal protein, making it a successful biopesticide. Bt is also a great source of proteases; the principal sub-species of Bt (with several strains) capable of manufacturing diverse proteases include israelensis, kurstaki, and tenebrionis (Jisha et al., 2013).

14.4 Types of microbial proteases

Researchers in various microbial sources have successfully produced proteases. Microbes represent two-thirds of the world's trade proteases (Beg et al., 2003). Since the beginning of enzymology, microbial proteolytic proteases seem to be the most extensively studied enzymes. These enzymes have become more interesting not just because of their important role in metabolism but also because of their enormous use in industries (Sandhya et al., 2005). The proteases available in the marketplace seem to be of microbial origin and are appropriate for biotechnological processes on the market due to their high production, very little time, less space, high gene modification, and cost-effectiveness (Nisha and Divakaran, 2014). The presence of all required industrial characteristics means that, in contrast to plant and animal proteases, these microbial source proteases are preferable (Kumar et al., 2015). Microbial and mammalian systems have small, dense, and structurally spherical proteolytic enzymes (Oberoi et al., 2001). *Bacillus* sp. is of immense significance among various producers of alkaline proteases (Rifaat et al., 2021). The isolated proteases from these microbial sources are highly diluted in different industries (Beg and Gupta, 2003). Extracellular alkaline proteases are typically released to the liquid broth by the producer, which simplifies and purifies these proteases through downstreaming to produce the endproduct. In comparison, plant and animal proteases are more laborious than microbial proteases (Kaliappan and Sunitha, 2010). Microbial origin proteases are classified into groups based on their acidic or fundamental properties. They are also classified on the basis of functionality and peptide bond position (Panda et al., 2013). The majority of commercially exploited enzymes in the world is the class of microbial proteases. A huge number of intracellular proteases are produced in microbes that play important roles in differentiation, protein turnover, hormone regulation, and cell production; whereas extracellular proteases are crucial in protein hydrolysis in the process of photographic films (Adrio and Demain, 2014), for example (Jaouadi et al., 2008).

14.4.1 Keratin

Keratins are proteins that come in two varieties: hard and soft keratins. Keratins are widely used. Hard keratins mainly include structural proteins found in fingernails, horns, bugs, on top skin, and mostly hair. Keratin protein fibers are automatically assembled to form

compact follicles that form the hair structure. Multiple genes, cytokines, and growth factors are controlled by the protocol of combining keratin proteins in complex hair (Packianathan et al., 2008). In opposition to hard keratins, soft keratins are abundant in tissue, for example, epithelial tissues. The wool keratin structure has a great resemblance to hair keratin. There are three forms of keratin in hair that have been identified (Cheng et al., 1996). The first of these is the alpha keratins, which are 60−80 kDa in size. These include alpha-helical domains with low sulfur content. In general, alpha keratins constitute the protein structural class because they reside in the hair fiber cortex. The second category of keratins is the class beta keratins, a less studied, non-extractable keratin group. All those are frequently found in and perform protective functions in the hair cuticle. The third kind of keratin is the highly sulfurized gamma keratins; these keratins hold a dimension of approximately 15 kDa.

Gamma keratins is smaller in size than the other keratin categories. Through crosslinking disulfide links in the hair, these keratins help maintain the cortical superstructure (Cheng et al., 1996; Gupta et al., 2002; Reddy shetty et al., 2006). Keratinase is a protease enzyme that relates to the keratinase family and may degrade all types of keratin molecules. A number of different applications include the processing of detergents, foodstuffs, and leather (Adetunji and Adejumo, 2018; Calin et al., 2017; Kalaikumari et al., 2018; Suntornsuk and Suntornsuk, 2003). Protease accounted for 60% of worldwide specific enzymes. Keratinase (E.C.) 3.4.99.11 (E.C. 3.4.99.11) (Bohacz and Korniłłowicz-Kowalska, 2019; Cavello et al., 2015; Kalaikumari et al., 2018) are a serene hydrolase group disulfide bond present in keratin proteins so keratinase break that bond. UniProt's results show that one of the *Bacillus subtilis* protein keratinases includes two domains. The first has 59 long amino acids and inhibitor I9 codes; the second has 243 long amino acids and peptidaseS8 codes. The first domain contains aminoacid sequences ranging from 19 to 77, while the second domain contains aminoacid sequences ranging from 103 to 345. The enzyme also has a metal receptor site for calcium ions. This indicates that calcium ions behave as cofactors in keratinases, and the presence of calcium ions may increase the activity of keratinases inside the media. Because of its structure, it is very effective at degrading keratin proteins (Arora and Mishra, 2016; Moraga et al., 2019). There are plenty of keratins in our everyday life, for example, green waste and animal waste, that remain undegraded because of their complexities. Such insoluble keratins can lead, when left untreated, to environmental pollution. As a solution, keratinase enzymes are used to treat such waste, which makes it less complicated and more biodegradable (Cavello et al., 2015). Several microbes were successfully isolated utilizing a variety of fermentation techniques and optimizing environments such as pH, temperature, and the form of nitrogen and carbon sources, as well as microbe selection (Govinden, 2012; Lateef et al., 2014). Microbial keratinases are more

efficient, biodegradable, and cost-effective while delivering far remarkable outcomes in contrast to chemicals (Manirujjaman, 2020; Tamreihao et al., 2017).

14.5 Alkaline proteases

The *Bacillus* genus, which works for alkaline pHs of 9−11, is crucial for a commercially significant alkaline protease (CE3.4.21−2499) (Kocher and Mishra, 2010; Varela et al., 1997). These producers of alkaline proteases are distributed under high alkaline, soil and water conditions. Alkaline protease separation has been reported from a variety of sources, such as detergents pollutants (Singh et al., 1999). Other sources of alkaline proteases are dried like fish, sand, soil, and slaughterhouses (Kunamneni et al., 2003). The detergent sector uses the greatest amount of alkaline proteases which are alkaline-pH serine proteases. These alkaline serine proteases, quickly dissolved by Phenyl Methane Sulfonyl Fluoride (PMSF), represent a one-third share of the market of enzymes. Alkaline proteases are unique in their action and ability to maintain a consistent alkaline pH when used in medicinal products, food, and other related industries for various formulations. Wissenschaftlers are paying more attention to a broad array of applications of such alkaline proteases in the quest to find new strains with distinct properties and high function (Najafi et al., 2005; Saeki et al., 2007). *Bacillus* sp. has been shown to be hydrolytic, elastolytic, and keratinolytic when it comes to dehairing animal skin and covers (Bhaskar et al., 2007; Deng et al., 2010; Shankar et al., 2011). These Bacillus strains have commercially harvested huge quantities of enzymes produced naturally with high enzymatic activity taking place all over the world (Ito et al., 1998; Jacobs, 1995). Despite alkaline proteases being produced from a variety of sources (Ellaiah et al., 2002), and with growing demands in the market for proteases and economic efficiency, the current biotechnological development only accepts those strains that show a higher yield of hyperactivité (Kumar et al., 2012). *Bacillus* sp., which could be used as an enzyme for zein hydrolysates in industrial production, produces two crucial kinds of alkaline protease: subtilisin Carlsberg and subtilisin Novo (Miyaji et al., 2006). In halophilic sources, several microbial species have been found to secrete serine alkaline proteases (Gimenez et al., 2000). Ca2+ alkaline protease entomopathogens, *Photorhabdus* sp strain's EK1 (PhPrtPI), is classified as a metalloprotease. Alkaline protease gives PhPrtPl that is nematodes nutrient having wide range of specificity and different proteins and peptides by degrading their tissues. A metallotype protease with a decent thermostaticide and a vast range of pH (5.0−10.0) is produced from a Salinivibrio strain AF-2004 whose heat and halophile properties are highly recommended (Amoozegar et al., 2007). *Bacillus clausii* is also recommended for the commercial utilization of peptone, Cu, and fructose as the single most important source of energy for alkaline protease production. The recommended optimal pH and temperature are 8−9 and 37°C−40°C, respectively. *Bacillus* sp. strain, MPTK 712, an alkaline protease producing dairy slush extract, has a symbiotic relationship with marine shipworms. Alkaline proteases can also be produced by quite infrequent microbes such as *Kurthia spiroforme* (Amoozegar et al., 2007). In the goatskin metagenomic library, there are also some alkaline serine proteases that can be recognized as

peptidases are homologous, and *Cryptococcus aureus* exhibits high bioavailability at optimal temperatures (45°C−50°C) and pH levels (9−10). Also reported are the different mushrooms that produce alkaline protease (Pushpam et al., 2011; Steele et al., 1992).

14.6 Acidic protease

Acid proteases, which are balanced and effective between pH 3.8 and pH 5.6, are often used for the production of soy sauce, protein hydrolysate, and for digestive assistance. The ideal pH range is between 3 and 4.5, with a molecular weight of 30−45 kDa and an isoelectric point range of 3−4.5 kDa (Machado et al., 2015; Ravikumar et al., 2012; Zheng et al., 2011). Acid proteases are also used to clear beverage and juice drinks, enhance pasta crunchiness, and tenderize fibril muscle (Zhang et al., 2010). The extracellular acid proteases are mostly made by fungal species, like *Aspergillus oryzae* (Yongquan, 2001), *Aspergillus niger* (Sielecki et al., 1991), *Aspergillus awamori* (Ottesen and Rickert, 1970), *Aspergillus fumigatus*, and *Aspergillus saitoii*, in comparison to alkaline proteases. Most extracellular fungal acid proteases are referred to as *Aspergilla opepsins*. Aspartic proteases are acid proteases made up of 380−420 long aminoacid residue chains that are the active source of catalytic activity. These are endopeptidases and have been grouped into three families: pepsin (A1), A2, and Para retrovirus enzymes (A3) (Somkuti and Babel, 1967). The three families are classified as AA clans. A1 and A2, while A3 members show a relationship with A1 and A2 families, are found to be closely linked to each other. There is an active split between the lobes of a two-faced structure of the pepsin family (Pushpam et al., 2011). Acidic proteases with high specificity have aromatic aminoacid residues on both ends of the peptide chain. These aromatic aminoacid residues have peptide bonds. Acidic proteases are classified into two groups: the first one is pepsin-like enzymes; and the second one is rennin-like enzymes produced by Aspergillus, Rhizopus, Endothia, and Mucor (Tomoda and Shimazono, 1964).

14.7 Neutral proteases

Neutral proteases are described as being effective in neutral, less acidic, or less alkaline pH. The Bacillus genus is primarily composed of neutral proteases and has a pH range of 5−8 (Table 14.1). Because of their medium rate of reaction, they produce so little bitterness in food protein hydrolyzes as to make them more valuable in the food service industry. Due to its insensitivity to plant proteinase inhibitors, neutrase is incorporated in the brewing industry. Neutral proteases are identified and characterized based on their high affinity to hydrophobic amino acids. Due to low thermotolerance, the reactivity of neutral proteases is somewhat advantageous in the production of food hydrolysate. For the action of neutral proteases of metalloprotease form, a divalent metal ion is needed (Chavan et al., 2007). Metalloproteases are classified as I (neutral), II (alkaline), III (Myxobacter I), and IV (Myxobacter II), which is based upon action specificity. Neutral proteases for hydrophobic acids are shown to be a specific and

Table 14.1: A contrast between different kinds of proteases.

Types	pH	Classification	Sources	Uses	References
Alkaline	9–11	Serine proteasesubtilisin Carlsbergsubtilisin Novo	Manufactured mostly from bacteria like, for example, *A. salinivibrio* sp. strain AF-2004, sea worms, *Cryptococcus aureus*, mushrooms, *Bacillus* sp.	In the leather and washing industries	Dodia et al. (2008), Miyaji et al. (2006), Patil and Chaudhari (2009)
Neutral	5–8	Thermolysin and neutrase	Genus *Bacillus*	Brewing and food industry	Sodek and Hofmann (1970)
Acidic	3.8–5.6	Aspartic proteases, pepsin and Para retroviral enzymes	Mostly made from *A. niger, A. oryzae, A. awamori, A. fumigatus*, and *A. saitoi* fungal species	For hydrolysate of protein, making soya sauce, in digestive aids and for producing seasonal material for cleaning fruit juices and beer, and for enhancing the taste of the dough	Pushpam et al. (2011), Sielecki et al. (1991), Zhang et al. (2010)

chelating agent, like EDTA, and are inhibited (Ethylenediamine tetraacetic acid). Metalloproteases seem to be the broadest among various forms of proteases. *B. Stearothermophilus* produces thermolysin, a neutral protease with a single peptide without disulfide bridges. It weighs 34 kDa molecularly. The essential ZN atom and four Ca atoms, which have thermotolerance, are integrated into the twofold lobes of the protein. With half a life of 1 hour at 80°C, this thermolysin neutral protease is exceptionally constant (Dawson and Kent, 2000; Fitzgerald et al., 1990; Razzaq et al., 2019).

14.8 Applications of protease

Today, in many biotechnology industries, enzymes are widely used. Enzyme development is possible due to exceptional specificities and mild reaction conditions, economic, simple, and energy-saving processes in the use of Green-, Modern, and also in sustainable industrial chemistry. From $1.0 billion in 1995, the global enzyme market grew to $ 1.5 billion in 2000, and then to $2.2 billion in 2006. Almost 75% of the industrial enzyme market met with lipase, protease, and amylase hydrolytic enzymes; for instance, 75% of industrially generated enzymes have been used by major industries such as detergents (37%), textiles (12%), starch (11%), bakeries (8%), and feed (6%). There are five protease families in which serine, threonin, cysteine, aspartic, or metal groups are mainly catalysts in the form of proteinasis K/protease K/endopeptidasis K. Enzymes of protection break long protein chains into shorter fragments. Protease enzymes are omnipresent and are essential to cell

growth and differentiation in all living organisms (Naveed et al., 2021). While protease enzymes play an important role in one's life, extracellular proteases have commercial value and are used in several industries (Fig. 14.2). Proteases are a useful product from *Bacillus* spp., which has a wide range of physiological features for a host of enzymes, antibiotics, and other metabolites like proteases, esterases, and other exoenzymes, through many medical, pharmaceutical, agriculture, and industrial processes. Some of the top uses in various industries for protease enzymes are present. Proteases are enzymes that catalyze peptide hydrolysis in polypeptides and proteins. Proteases are used extensively in detergents and drugs and in the food industry. These account for 60% of the industrial enzymes on the market. The worldwide need for the protease enzyme market has increased by 5.3% over the 2014–2019 period at a compound annual growth rate (CAGR). Their demand is expected to continue as applications in leather and bioremediation processes can be found. Proteases can also be categorized in the catalytic site according to the catalytic activity, nature of the reactive group, and origin. Plants and animals, microorganisms are major

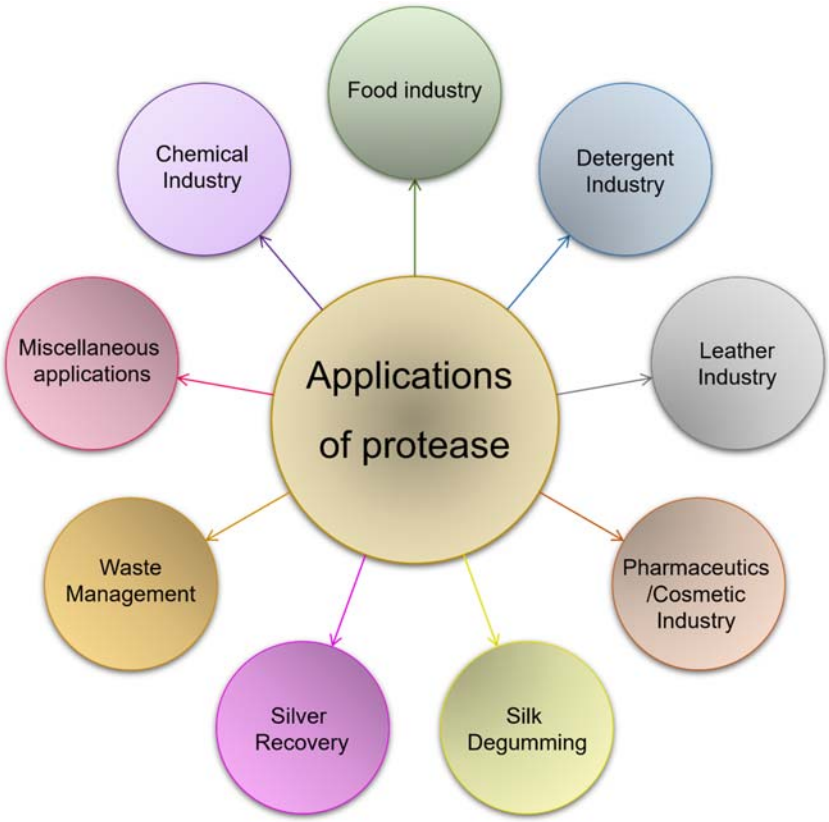

Figure 14.2
Biotechnological prospects of proteases in various industrial sectors.

protease enzyme sources (both bacterial and fungal). Proteases have two groups: exopeptidases and endopeptidases, which have been classified based on the polypeptide chain site. Exopeptidases act randomly on the ends of polypeptide chains while endopeptidases acts on the inner regions of polypeptide chains. Six groups of endopsis based on the catalytic residues in the active site are further classified as serine, aspartic, cysteine, metal, glutamic acid, and threone protease. In the food industry, plant protection products such as bromelain, ficin, and papain are widely used in a range of applications, including brewing, meat tenderization, milk coagulation, and as digestive help. Proteases also improve the nutritional value, flavor, solubility, digestible use, and functioning of food proteins, including coagulation and emulsification, by applying proteases. Proteases for the production of bread, baking food, crackers, and waffles are widely used in the baking industry. The enzymes needed for the mixing time, dough stability, and uniformity are reduced, the gluten strength is regulated in bread, and the texture and flavor are improved.

The *Aspergillus usamii* acid protease is successfully used to enhance wheat gluten's functional properties. Protease addition could emit enough peptides and aminoacids into the work to ensure that proper fermentation takes place. In improving fermentation of beer, acidic fungal proteases are used as they are efficient in balancing beer's aminoacid profile even at low pH. The dairy industry is associated with another major protease application. Proteases that occur naturally significantly contribute to the flavor of the cheese. They are used to speed up cheese ripening, change the functionality of dairy products, and reduce allergic properties. Proteases are also used for hydrolyzing the particular peptide bond in cheese production to generate paracasein and macropeptides (Raveendran et al., 2018).

14.9 Food industry

For over 40 years, proteases have often been used as an additive to enhance the quality of foodstuffs and mixed with the feed for the synthesis of protein hydrolysates. Patients with digestive disorders and food allergies may be administered this enzyme. It is available in various substrates such as whey, casein, meat, and also soy. Commercially available "SEB tender 70" proteases are widely used for the breaking of collagen in meat by the tenderization of animal proteins to make them more edible. Major applications of protease in food processing include cereal mashing, brushing, beer haze clearing, protein hydrolysate production, and cheese caping, as well as backing those changes with viscoelastic features of dough. Proteases are used to produce cheese in the dairy industry while breaking the peptide bonds to produce macropeptides with casein as the essential function of enzymes. In the bakery industry, it is used to produce cookies, baked wafers, and biscuits from flour in the degradation of the protein. Proteases are used in the brewing industry to remove enough proteins and to obtain a certain number of nutrients, like nitrogen, from both barley and malt. It plays an important role in changing the flavor and the reduction of lactose in

the dairy industry (Naveed et al., 2021). Proteases are used in the food industry to change, palate, and store protein sources from all available sources. The use of alkaline proteases results in large nutritional value production of protein hydrolysates. Alkaline microbial proteases are of immense importance in meat tenders (Razzaq et al., 2019).

14.10 Detergent industry

Proteases play one of the important roles in many applications in industrial biology in the detergent industries for 30% of global enzyme production. In the USA, 25% (powder), 50% (liquid), and about all powered detergents have enzymes that can assist with the removal/clearance of stains that are hard to wash alone with usual surfactants. The hydrolyzing of the larger protein molecules that are linked with tough strains play an important role in proteases. Peptide bonds—which hold together the protein molecule, involving the release of smaller polypeptides and amino acids—are broken down during hydrolysis. They function as scissors and cut pieces off from the tissue surface. In the laundry, detergents are used with protease from *Aspergillus niger* and *Tritirachium album*, *Aspergillus flavus*, and *Bacillus firmus* MTCC 7728. *Cheotomium globalusum* is also used in the industry as a protease produced from a new indigenous strain.

14.11 Leather industry

The leather industry takes different steps to obtain processed leather, such as soaking, calving, removal of hair, delimiting, bating, degreasing, and beating. Each step leads to pollution in the environment by the release of quite poisonous substances such as lemon, salt, solvents, and sodium sulfides. Collagen-like protein is the important protein in the making of the leather, which is in contact with different global proteins and fibrous proteins in the skins and hides. In leather processing, it is necessary to remove noncollagenous material; the final leather softness and durability are determined by the extent to which these constituents are removed. Leather-processing environmental pollution is controlled by replacing chemicals with enzymes such as proteases. In the leather industry, removal of undesired protein with the ecologically friendly and economical method is compulsory. *Bacillus tamarii*, *Streptomyces avermectinus* NRRL B-8165, *Bacillus* sp. are useful for dehairing of *Bacillus subtilis*.

14.12 Pharmaceutics/cosmetic industry

Proteases are always present in all living organisms and participate in the lifecycle of many infective organisms, which makes it important to increase the number of healing agents for severe diseases such as the tumor, the AIDS, malaria, and bacterial infections. With microbial sources, proteases are used to treat conditions such as dermal, cancer, cystic

fibrosis, heart failure, digestive, and inflammatory disorders. Proteases are drug amplifiers. Several protease medicines have been approved for preclinical and clinical trials by the FDA. The therapeutic properties of these effective protease drugs are strongly affected by protease engineering and advanced delivery systems. The specificity of such proteases for curative purposes is increased for physiological substrates. The proteolytic enzyme is used by heating at temperatures between 60°C and 100°C for cleaned and disinfected contact lenses in one step. Procoagulants, sepsis, thrombolysis, digestion, and neuromuscular medicines are FDA approved. Keratinases are used in cosmetics and pharmaceutics in the eradication of keratin in spots (acne) or in the depilation of junk, the removal of human calluses, keratin-covered skin degeneration, and dermatophytose, preparation of vaccine, and the improvement of drug ungula delivery. The *Bacillus subtilis* 316 M elastoterase enzyme is used to treat purulent wounds, deep abscesses, furuncles, burns, and carbohydrates. As digestive relief for the treatment of deficiency syndics of various lytic enzymes, oral administration of proteolytic enzymes manufactured from *Aspergillus oryzae* was utilized. In a study, Bacillus CK 11−4 protease with fibrinolytic activity was shown to be used as an agent of thrombolytics. Proteases were reported to use in medical industry. Studies have also shown the use of *Aspergillus niger* as alkaline proteases in the medical industry.

Proteases catalyze hydrolysis in the cosmetic industry of peptide bonds, which add keratin, elastin, and collagen to the skin. In skin softening and skin peeling, proteases such as bromelain, papain, and many others are used. This protease function is related to cell renewal, the promotion of the elimination of dead cells from the epidermis, and the restoration of closely related cells. The potential for use in the cosmetics industry for keratinases obtained from Bacillus licheniformis ZJUEL 31410 is well-known.

14.13 Silk degumming

Unprocessed silk threads should have been rubbed off to remove protein-like substances like sericin that are covered in silk fiber. It is usually carried out in a soap solution that is alkaline. Fiber has attacked itself, which is why it is harsh. Other disadvantages are the consumption of time, high energy utilization, and the destruction of silk luster because of its high moisture content. The use of “proteolytic” enzymes, on the other hand, is superior because they have an influence on the removal of the protein-like substance sericin without fiber. Tests are conducted at higher concentrations of enzymes, and results demonstrate that no fiber damage is virtual and silk threads are firm than is possible by conventional methods. The enzymatic activity process enhances the characteristics and strengths of the silk thread. According to one study, the degumming of fabrics by the *Bacillus subtilis* C4 protease through the RSM and Plackett−Burman design was optimized by statistical methods. *Bacillus firmus* proteases MTCC 7728 are utilized in the treatment of silk rubber.

14.14 Silver recovery

In the bioprocessing of the used x-ray films, in which silver is restored, alkaline proteases can be applied. The reuse of silver and polyester film bases is possible due to gelatinous hydrolysis on X-ray film. In 1987, in the Fujiwar and Yamamotto study, Bacillus sp. B21−2 alkaline protease decomposed the gelatin-coating of the x-ray films used to retrieve silver. Metallic black silver is the source of great silver re-energy from the waste photographic film by x-rays. The gelatin layer in the X-ray film contains approximately 1.5%−2% silver (by weight). The silver regained via combustion of X-ray films emits a bad odor that pollutes the environment. Stripping techniques for silver restoration can be applied to proteolytic enzymes derived from alkali hydroxides and various microorganisms. To breakdown a suspension layer, researchers used a proteolytic enzyme such as protease made up of silver which consists of protein gelatine. Protease product ATCC 6633 and Conidiobolus coronate were applied during silver rehabilitation from *Bacillus subtilis*. Cheotomium globusum isolated protease is also used to remove gelatin from the used X-ray films.

14.15 Chemical industry

Proteases have a unique capacity to make esters and peptides in a suitable solvent. Because the environment is inactivated and the enzymes are denatured, the proportion of peptide synthesis in organic solvents is slow. The use of protease-tolerant organic solvent microbes can alleviate the problem of less organic yield as well as oxygen deprivation and enzyme denaturation. The development of organic synthesis applications necessitates the use of highly stable alkaline proteases in organic solvents. For the construction of peptides in a nonaqueous medium, proteases from *Aspergillus flavus* and *Pseudomonas aeruginosa* are being utilized. SAPB and KERAB are serine alkaline proteases isolated from *Bacillus pumilus* CBS and Streptomyces. In lower H2O systems, AB1 strains have a high chance of using peptide synthesis. In the acetonitrile/sodium carbonate/sodium bicarbonate buffering system, alkaline protease, such as alkalassium, is used to synthesize Bz-Arg-Glyzin-NH2 (N-benzoylarginylglycinamide) [dipeptid originator of (Arginine-Glycine-Aspartic-Serine)]. *Bacillus licheniformis* protease (alkaline) production of 2H-1-benzopyran-2-one derivative product has been reported.

14.16 Waste management

Protease causes waste (protein) solubilization and also helps marine organisms decrease biological oxygen demand. The use of proteases in the waste control of multiple household activities and food handling industries has now opened a new era. Feather effluent, which is produced in large quantities as a byproduct of commercial chicken handling, provides a

good source of keratin protein and aminoacids, which can be efficiently used for animal feed and foodstuffs. Its poor digestibility, on the other hand, restricts its use. Feather digestibility, which requires high energy costs and the production of some toxic byproducts, can also be improved through thermal degradation, which restricts its practical use. The degradation of keratine-containing waste from different industries, such as domestic poultry and leather, by developing nonpolluting processes could have a significant role. Microbial proteases like keratinases used for feather degradation. The feather degradation process can be followed by the use of feather hydrolysates for the development and manufacturing of aminoacids like proline, cysteine, and serines like a film, glue, fertilizer, and feed improver. Feather degenerative proteases are used for *Bacillus licheniformis* ZJUEL 31410, *Bacillus pseudofirmus* FA30−01, *Streptomyces*, *Bacillus licheniformis* RV. B2.90, *Bacillus amyloliquefaciens*, *Pseudomomanas* sp., Ms21, *Aspergillus oryzae* NRRL-447, and *Bacillus* JB 99. *Chetomium globusus* proteases also have keratinase function, which is beneficial for feather breakdown (Naveed et al., 2021).

14.17 Miscellaneous applications of protease

Alkaline proteases obtained from *B. subtilis*, *Streptomyces avermectnus*, and *Conidiobolus coronatus* were reported to regain silver from X-ray films, making sure that the method is more environmentally friendly than it is with the utilization of chemicals (Godfrey and Reichelt, 1982; Wolff et al., 1996; Yang et al., 2000). Silver restoration using a thermally constant genetically altered alkaline protease produced by *Bacillus* sp. B21−2 has also been noted for its ability (Araújo et al., 2009; Dhawan and Kaur, 2008; Razzaq et al., 2019).

Protease was found to be used in the Anwar and Saleemuddin study in 1997 to remove blood from cotton clothes. The microbial protease properties include alkaline pH, thermostability, and collagen digestive ability to dehair. Alkaline proteases are also more likely to remove proteins and tissues from the cloth and are, therefore, used in powder and detergent solutions.

The uses of protease applications have been found mainly in the wool industry. Wool fiber is covered with overlapping scales that point to the tip of the fiber. Partial hydrolysis of scale tips with protease papain had been proved a successful approach to achieve this. A few years ago, mainly for financial reasons, the approach was abandoned.

14.18 Improvement of biocatalytic characteristics of proteases

14.18.1 Protein engineering

It is desired to have high catalytic performance as well as improved thermal stability features in order to use the proteases on an industrial scale. Because of their natural

constraints in hostile industrial environments, native biocatalysts are often obstructed in the industrial bioprocess. Temperature is particularly regarded as a key factor determining the catalytic effectiveness of an enzyme. In unfavorable circumstances, the catalytic activities of thermo tolerant enzymes can be preserved to encourage the creation of cost-effective large-scale strategies for enzyme use. In this respect, protein technology offers a promising solution for overcoming the inconveniences and for increasing protease stability to achieve goal accomplishments.

Catalytic features of proteases are often enhanced by four main types of strategies: explicit engineering of proteins, rational arrangement, the evolution that's also directed, and de novo design. Amongst them, the use of rational design, because of smaller variant libraries and higher processing time, is the superior choice for modifying enzyme properties. A wide range of reports shows that Enzyme Catalytic Performance and Thermal Stability have been improved through an in-depth structural analysis. Numerous studies have demonstrated that the design efficiency for large-scale applications is effective and cost-effective. The reasonable alteration of Bacillus pumilus serine alkaline protease resulted in increased biotransformation behavior and thermostability by producing a single mutant of N99Y. Similarly, unloaded aminoacid has replaced increased catalytic performance and specific substrate characteristics of alkalin protease Bacillus pumilus serine (L31 and T33). The modified variants were proposed as encouraging biocatalysts to be used in the leather-processing industry. Ashraf et al. recently noted the protein engineering of the Pseudomonas aeruginosa serine-peptidase in enhancing its catalytic action potential and thermal stability. Two mutants—V336I and A29G—have delivered the required effects among the eight variations. The recently discovered A29G and V336I mutants displayed a 14-fold increase in catalytic activity in comparison to the natural type. The remaining enzyme performance at elevated temperature improved dramatically after the Tm of the two variants was raised by 5°C. Previous studies have been made using Bacillus pumilus alkaline proteases using a similar technique. Rational engineering at sites with maximum solvent exposure of transformed aminoacids increased the resistance of trypsin to afatoxin-detoxifzyme and β-mannanase MAN47. After the introduction of N-glycosylation sections by rational molecular orientation design, the catalytical stability of trypsin and pepsin in β-mannanase MAN47 was significantly enhanced. Modification is a new biotechnology method that is being used for industrial biocatalysis in enzyme engineering but also to clarify the connection between protein sequences, structures, and functions. A library of mutants is built in a direct way, and the mutants that show the wanted characteristics are recognized by the appropriate method of screening and selection. The cycle continues until a variant with target characteristics is achieved. It has remarkable advantages over the use of a rational design so that careful pre-knowledge of the protein in mutation regions is not required. Although simple, significant efforts to develop a library, together with high-performance screening/selection methods, are generally needed. Adaptive and analytic

strategies are not mutually exclusive, and combined strategies have become increasingly popular in recent years. This method is known as the target or semirational mutagenesis of the design, and it entails using randomized mutagenesis or site-saturation on a subset of protein molecules rather than altering the complete enzyme. Recently, a guided evolutionary strategy was used to increase the resistance of organic solvents to the metalloproteases PT121. In the presence of acetone and acetonitrile, the transformed mutants (H224 F, H224Y, and T46Y) showed up exceptionally tolerant solvent capacity. The half-life of modified enzyme variants was 1,2−3, fivefold improved compared to the wild type after guided evolution. Out of note, the T46Y/H224 F and T46Y / H224Y mutual variants were extremely stable and caseine lytic.

14.19 Protease immobilization

Stability, recovery, and recycling challenges extensively use the free state of the enzyme in batch reactions. The many strategies proposed include enzyme immobilization as a promising way of using enzymes in industrial biocatalysis in a convenient and economical manner. The mobility, and attachment to the solid support of the biomolecule or enzyme molecule not only incorporates enzymes that enable reusability but also modifies their catalytic protease and stability to make it easier for industrial uses. The significant advantages of enzyme immobilization are process control, repeatability, cost reduction, and quick and easy enzyme isolation following the enzyme-catalyzed transition. Whilst there are many techniques, four methods are generally used for immobilizing enzymes such as adsorption co-polymerization, entanglement, and covalent bonding. The choice of an enzyme coupling method depends on maximizing enzyme activity, durability, and stability preservation. Among other methods used for immobilization of enzymes are physical adsorption and covalent attachment. The covalent connection provides the strongest connection, establishing bio-conjugates that support the most stabilized enzyme without leaching the enzyme into the compound. However, this approach sometimes results, because of the aggressive conditions, in drastic changes in the enzymes' catalytic and conformational properties. Adsorption can be an appropriate technique to fix enzymes because it takes little time and effort to achieve this easy task. Following enzyme desorption, the catalytic supports may also be recirculated, so the process cost could be considerably reduced. CL-4B, 50−4,40-dimethyl-thymidine-succinyl controlled porous glass and glutaraldehyde functionalized silica to enhance their catalytic properties were recently CL-4B octyl sepharose purified tripsin immobilized by Poonsin et al. The efficiently immobilized biocatalyst showed thermal suppression and better resistance in polar solvents in comparison to the enzyme in its free form. The inhibitor of trypsin was also less amenable than the non-immobilized enzyme form. The biocatalytic system as developed demonstrated a high rate of reprocessing capacity that keeps after four continous rounds, i.e., it retains roughly 60% of its original activity. Increased activity recovery of about 675% showed immobilized bromelain on glutaraldehyde cross-linked nanofiber membranes. A second study demonstrated improved thermal stability and a half-life increase in the protease enzyme,

successfully immobilizing it onto surface modified $_T$-carrageenan gel beads at high temperatures (from 24.06 to 79.95 min). Upper 80% of its residual activity was stored after eight weeks of storage by $_T$-carrageen gel beads encapsulated protease at 4°C. It also demonstrated promising silver removal activity for six consecutive cycles from x-ray films. Benucci et al. developed and protease enzyme is used for the first time to produce gluten-reduction beer, a feasible and revolutionary biocatalytic mechanism through the stabilization of Aspergillum niger endopeptidase chitosan beads. The chitosan-immobilized enzyme showed increased thermal stability with the same catalytic performance at 20°C or 50°C. The newly designed system in a fluidized bed reactor has effectively brought gluten content reductions from barley malt to commercial beer (Naveed et al., 2021).

14.20 Conclusions and perspectives

The critical role of proteases in a wide range of industrial areas has been summarized. Proteases are a major multifunctional class of enzymes. It is a particular enzyme for various biotechnological applications due to the unique features of proteases like quick action, work with moderate operational state, biodegradability, high specificity, and low waste production. With the compound annual rate of growth (CAGR) of 6.1%, the worldwide market for protease will grow by 3 billion USD by 2024. In washing leather, chemical, pharmaceutical, food, and wastewater processing industries, proteases have extensive applications. Proteases provide the enormous industrial potential to meet the challenges faced in the coming years owing to natural resource depletion and global population growth. Furthermore, new sources of efficient, stable, and reusable catalysts for a variety of industrial applications necessitate the use of enzymes with particular characteristics to be identified. In the future, protease engineering appears to be an addressed area for novel function and stability. Protease industries look for new fusion proteases in which all activities are combined into one that is engineered proteases. A fast-growing protein market always requires proteases that are fast-acting and active, and available at a low cost. The heterogeneities of proteases are their uniqueness that makes them even more versatile biocatalysts. However, it remains for the full array of benefits to be explored. In fact, the prospects for waste management, especially in urban areas, are underutilized. Proteases are less addressed in the engineering of new or combined catalytic capabilities with a long half-life. The protease-based sector expects to receive engineered fusion proteases combined in one activity. The growing market for proteases requires, therefore, efficient and rapidly acting proteases at a lower price.

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CHAPTER 15

Prospects of microbial phytases in the food and feed industry

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15.1 Introduction

The inclusion of plant-based feed ingredients in animal diets is gaining popularity (Allan et al., 2000) due to their local availability. However, the presence of non-starch polysaccharides and antinutritional factors in plant ingredients hinder their effective application at commercial levels. Among these, phytate is a major antinutritional factor (Francis et al., 2001) that restricts the availability of phosphorus in the animals fed on a plant diet. As two-thirds of phosphorus is stored in the form of phytate in plant seed meals, so it remains practically unavailable for absorption (Kumar et al., 2012). Besides, it acts as a chelating agent for positive metal ions due to its negatively charged phosphate group (Vohra and Satyanarayana, 2008). Also, it forms insoluble complexes after combining with vitamins, proteins, and amino acids which further reduces the digestibility and availability of feed ingredients (Baruah et al., 2004; Sugiura et al., 2001), leading to nutrient deficiency. Moreover, the discharge of these insoluble complexes in natural water bodies cause eutrophication (Baruah et al., 2004; Liebert and Portz, 2005; Singh and Satyanarayana, 2011a).

Supplementation of exogenous phytase is an effective strategy for boosting the availability of phytate-bound phosphorus. For this purpose, a continuous search for novel phytase sources is being carried out (Singh et al., 2020). Phytase (myo-inositol hexakisphosphate phosphohydrolase) hydrolyzes the non-digestible phytate in plant feed (Lei et al., 2013), improving feed utilization, nutrient digestibility, and growth performance (Baruah et al., 2007; Cao et al., 2007). Moreover, better nutrient utilization results in less phosphorus discharge to water bodies reducing aquatic pollution (Baruah et al., 2004). Naturally, phytase production is either absent or very scarce in animals, so the phytate remains

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undigested. Supplementation of exogenous phytase is an efficient method to enhance phosphorus and mineral availability (Hassaan et al., 2013; Liu et al., 2014; Morales et al., 2016). Commercially, phytase is obtained from microbial sources, like bacteria, fungi, and yeast. Phytase production from plant sources requires extra cost for preliminary treatment, so microbial sources are preferred (Vohra and Satyanarayana, 2008).

15.2 Mode of action

Exogenous phytase releases inorganic phosphorus from plant-based phytate. Further degradation of this antinutritional factor produces myo-inositol phosphate (IP), including IP5, IP4, IP3, IP2, IP1, and free myo-inositol (Schlemmer et al., 2009). As no available phytase is known to fully dephosphorylate phytate in the plant diet (Greiner and Konietzny, 2010) so that the end-product of phytate dephosphorylation is myo-inositol. Myo-inositol is a cyclic sugar and is absorbed by a Na+ coupled transporter in the small intestine (Aouameur et al., 2007). It is distributed to different body tissues through the bloodstream. Fig. 15.1 represents the schematic diagram of the mode of action of phytase.

15.3 Microbial phytase sources

Bacteria, fungi, and yeast species are major sources of commercially available phytase. These phytase-producing microbial species can be isolated from different environments. Among these, bacterial and yeast origin phytase is cell-bound and produced intracellularly (Jain and Singh, 2017). In comparison, fungal phytases are extracellular (Singh and Satyanarayana, 2011b). Various phytase products of microbial origin are commercially available and used in animal feed for better plant-based feed ingredient utilization.

15.3.1 Bacteria

Several bacterial species are reported to secrete phytase (Fig. 15.2). Bacterial phytase is either nonglycosylated histidine acid phosphatase or alkaline phytase carrying a β-propeller structure in nature. Bacterial species including *Bacillus* (Jain and Singh, 2017), *Bifidobacterium* (Haros et al., 2005; Tamayo-Ramos et al., 2012), *Enterobacter* (Hussin et al., 2010), *Escherichia* (Priyodip et al., 2017), *Pseudomonas* (Tungala et al., 2013),

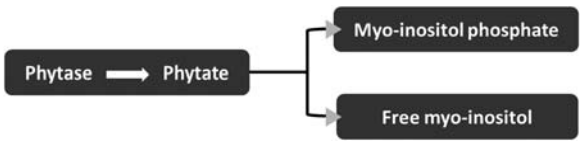

Figure 15.1
Schematic diagram of the mode of action of phytase.

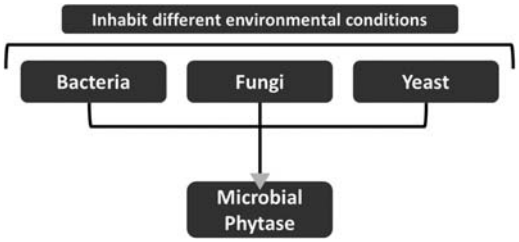

Figure 15.2
Sources of microbial phytase. All the species of bacteria, fungi, and yeast may inhabit different environments.

Lactobacillus (García-Mantrana et al., 2015), and *Serratia* (Kalsi et al., 2016) are famous as phytase sources.

Bacterial phytase production usually occurs through submerged fermentation (Jain et al., 2016) than through solid-state fermentation (Kammoun et al., 2012). Bacterial isolates are obtained from plants, animals, and soil. Phytase is mostly produced extracellularly by bacterial isolates (Singh et al., 2020). According to the study performed by Kalsi et al. (2016), bacterial isolates were obtained from degraded wood. Another bacterial species, *Bacillus subtilis* (*subtilis* JJBS250), was isolated from soil samples (Jain and Singh, 2017). It was identified based on morphology and molecular and biochemical traits. The bacterial isolates (*Serratia* sp.) produce more thermostable phytase. Other thermostable phytases were reported in *Bacillus amyloliquefaciens* and *Bacillus nealsonii*, tolerating a temperature range of 90°C−100°C (Rebello et al., 2017). Bacterial isolates of *Bacillus* and *Geobacillus* species obtained from Chilean hydrothermal areas could produce phytase both extracellularly and intracellularly (Jorquera et al., 2018). According to the study performed by Handa et al. (2020), *Bacillus aryabhattai* RS1 isolated from the rhizosphere produces extracellular phytase that is active at low temperatures. The β-propeller phytase in many rhizosphere bacterial species also has the ability to break down phytates (Hanif et al., 2015; Menezes-Blackburn et al., 2014). Other bacterial phytases have been isolated from *Obesumbacterium proteus* (Zinin et al., 2004) and *Klebsiella* spp. ASRI (Sajidan et al., 2004).

15.3.2 Fungi and yeast

Mostly, the phytases isolated from fungi and yeast are glycosylated, histidine acid phosphatases and are capable of reacting with various substrates (Wyss et al., 1999). The first commercial phytase was *Aspergillus niger* PhyA, having a higher affinity for phytic acid (Han, Wilson, and Lei, 1999). *Peniophora lycii*, another commercial phytase, had a dimeric conformation (Lassen et al., 2001) but was susceptible to temperature treatments, proteases, and low pH (Simon and Igbasan, 2002). Mostly filamentous fungi have been

detected to produce phytase (Singh and Satyanarayana, 2011a,b). Among these, *Aspergillus, Mucor*, and *Penicillium* are the most important. Fungal and yeast species including *Aspergillus oryzae* (Sapna and Singh, 2014), *Aspergillus flavus, Humicola nigrescens* (Gaind and Singh, 2015), *Sporotrichum thermophile* (Singh and Satyanarayana, 2006), *Penicillium purpurogenum* (Awad et al., 2013), *Pichia kudriavzevii* (Qvirist et al., 2017), *Saccharomyces cerevisiae* (Kłosowski et al., 2018), and *Zygosaccharomyces* (Lata et al., 2015) are famous for their phytase-producing ability.

Mostly fungal phytases are stable at high temperatures and at fluctuating pH (Simon and Igbasan, 2002). Phytase secreted by *Sporotrichum thermophile* is active at 45°C and pH 6.0 (Singh and Satyanarayana, 2006). Nampoothiri et al. (2004) reported that *Thermoascus aurantiacus* produced phytase in a medium containing organic and inorganic compounds (glucose, starch, and peptone). In another study, high phytase activity was seen in *Myceliophthora thermophile* using sugarcane bagasse substrate at 45°C and 70% moisture (Hassouni et al., 2006). Whereas the highest phytase production was observed in *Thermomyces lanuginosus* TL-7 in the presence of wheat bran in solid-state fermentation (Gulati et al., 2007). The presence of agricultural residues greatly affects phytase production (Bhavsar et al., 2008). Moreover, the agricultural residues in pretreatment enhance phytase production. There is much evidence in support of this claim. Phytase production was reported in *Aspergillus flavus* when the mustard cake is used in solid-state fermentation (Gaind and Singh, 2015). Phytase is usually produced extracellularly in fungi. Mostly *Rhizopus oligosporus* (Azeke et al., 2011) and *Saccharomyces cerevisiae* are used for fungal phytase production.

Yeasts involved in phytase production grow on solid and liquid media having phytate, a phosphorus source (Greppi et al., 2015). A mixed substrate containing wheat bran and sugarcane bagasse was used by *Sporotrichum thermophile* for enhanced phytase production in a study performed by Kumari et al. (2016). In another study, phytase production was enhanced in *Aspergillus niger* 7A-1 by using triticale waste as substrate (Neira-Vielma et al., 2018). The highest catalytic activity through phytase production was observed in *Saccharomyces cerevisiae* using sucrose, aspartic acid, and phytate as carbon, nitrogen, and substrate sources, respectively, in submerged fermentation (Kłosowski et al., 2018). Due to the increasing industrial and environmental applications of phytase, there is an increasing interest in the isolation, screening, optimization, and characterization of their source species. The industrialists have focused on fungi and yeast, as they produce potent extracellular phytase (Farhat et al., 2008).

15.4 Application

The characteristics of phytase vary as their origin varies. Changes in phytase sources may also change its ability to degrade phytate (Onyango et al., 2004). Its effective phytate

degradation ability is related to the inability of monogastric animals to properly digest the plant source phytates. The main source of phosphorus is basically phytate in a plant-based diet, including wheat, bran, maize, cottonseed meal, soybean meal, and rice bran (Kumar et al., 2012). As the inclusion of exogenous phytase has proven to be an effective strategy to boost the efficiency of plant feed ingredients, it's by effectively breaking down the phytates which leads to a better growth performance as the nutrients are readily available for digestion and absorption.

15.4.1 Aquaculture

15.4.1.1 Growth performance

A significant improvement in growth performance demonstrates that phytase supplementation degrades the negative effects of a plant meal-based diet (Kumar et al., 2012). In unsupplemented plant diet, phytate acts as an antinutritional factor (Francis et al., 2001) by restricting the availability of phosphorus in the animals fed-plant diet. As most of the phosphorus in plant-based ingredients is stored in the form of phytate, so it remains practically unavailable for digestion and absorption (Kumar et al., 2012). It also acts as a chelating agent for positive metal ions due to its negatively charged phosphate group (Vohra and Satyanarayana, 2008). Moreover, it forms insoluble complexes with important feed nutrients further reducing the digestibility and availability of feed ingredients (Baruah et al., 2004; Sugiura et al., 2001). This decreases the nutritional attribute of the feed, adversely impacting the growth performance of the fish. However, this effect may be eliminated by supplementing phytase that hydrolyzes the phytates and increases the bioavailability of minerals and nutrients (Shah et al., 2021).

The evidence for enhanced growth performance in fish fed phytase-supplemented plant meal-based diets are well documented. Debnath et al. (2005) reported the highest values for weight gain and specific growth rate at 500 FTU/kg of phytase in *Pangasius pangasius* (Pangas catfish). Nwanna et al. (2005) conducted their study on *Clarias gariepinus* (African catfish). According to their study, phytase supplementation significantly improved the growth parameters. Hung et al. (2015) noted a significant improvement in growth performance in *Pangasianodon hypophthalmus* (Tra catfish) fed phytase-supplemented diet. Similarly, Shah et al. (2021) observed enhanced growth performance in *Labeo rohita* (Rohu) fed a diet supplemented with 1000 FTU/kg of phytase; whereas the highest growth performance in *Labeo rohita* (Rohu) was noted at 750 FTU/kg phytase (Hussain et al., 2017). Also, phytase-pretreated diets improved the growth performance of *Oreochromis niloticus* (Nile tilapia) (Cao et al., 2008). Also, a significant difference in weight gain was noted in *Oreochromis niloticus* (red tilapia) fed a phytase-supplemented diet (Tudkaew et al., 2008). In another similar study on *Oreochromis niloticus* (Nile tilapia), a significant improvement in growth parameters was observed at the graded phytase levels of 75 and

150 mg/kg. However, further increases (225 and 300 mg/kg) in phytase levels did not significantly improve the growth performance (Tahoun et al., 2009). Phytase supplementation also significantly enhanced growth in *Salmo salar* (Atlantic salmon) (Sajjadi and Carter, 2004b). Studies conducted on carp species, including Liu et al. (2012), Phromkunthong et al. (2010) and Sardar et al. (2007) also demonstrated a significant improvement in growth performance.

In contrast to the positive results, some authors reported that phytase supplementation did not affect growth parameters. Vielma, Mäkinen et al. (2000) reported that *Oncorhynchus mykiss* (rainbow trout) fed a phytase-supplemented diet showed no effect on weight gain. Another similar study also reported that a phytase-sprayed diet did not affect specific growth rates in *Oncorhynchus mykiss* (rainbow trout) (Wang et al., 2009). Also, Pham et al. (2008) noted no significant improvement in the growth performance of *Paralichthys olivaceus* (Olive Flounder) fed phytase-supplemented diet. Phytase supplementation did not significantly enhance weight gain in *Salmo salar* (Atlantic salmon) (Sajjadi and Carter, 2004a). The contradictions in these results may be due to the variation in diet sources, the difference in fish species, and changes in phytase types (Da Silva et al., 2005; Hotz and Gibson, 2001).

15.4.1.2 Digestibility

Phytase supplementation improves the digestibility of nutrients by hydrolyzing the insoluble nutrient complexes, which increase the availability of these nutrients (Liu et al., 2013; Sugiura et al., 2001). As in an unsupplemented diet, phytates limit the availability of phosphorus in animals fed plant diet (Kumar et al., 2012). Furthermore, it also acts as a chelator (Vohra and Satyanarayana, 2008). Additionally, it forms insoluble complexes that are hard to digest (Baruah et al., 2004; Sugiura et al., 2001). This restricts the availability of the nutrients to be digested by the animal's endogenous enzymes. However, the supplementation of phytase solves the issue of restricted nutrient digestibility seen in phytase-unsupplemented plant diet.

Debnath et al. (2005) reported a significant improvement in nutrient digestibility in *Pangasius pangasius* (Pangas catfish) fed a phytase-supplemented diet. Similarly, Shah et al. (2021) observed a significant nutrient digestibility in *Labeo rohita* (Rohu) fed a diet supplemented with phytase. Nutrient digestibility was also significant in *Labeo rohita* (Rohu) fed phytase-supplemented diet (Hussain et al., 2017). Also, phytase supplementation improved the apparent digestibility coefficient of phosphorus in *Oreochromis niloticus* (Nile tilapia) (Cao et al., 2008). A significant difference in phosphorous digestibility was noted in *Oreochromis niloticus* (red tilapia) fed phytase-supplemented diet (Tudkaew et al., 2008). Liu et al. (2012) observed a significant improvement in crude protein and phosphorus apparent digestibility coefficient in *Carassius auratus gibelio* (gibel carp) fed phytase-supplemented diet. Liebert and Portz (2005) and Vielma et al. (2004) reported a significant

increase in protein digestibility in fish through phytase supplementation. Ashraf and Goda (2007) observed the highest apparent digestibility coefficient of crude protein, crude fat, and gross energy at 1000 FTU/kg phytase graded level. Liu et al. (2013) reported a significant improvement in apparent digestibility coefficient parameters with phytase supplementation in *Ctenopharyngodon idellus* (grass carp). Portz and Liebert (2004) found a significant improvement in the digestibility of crude fats with phytase supplementation. Also, Da Silva et al. (2007) observed an increase in gross energy digestibility in *Colossoma macropomum* (tambaqui) with phytase supplementation. In another study, gross energy digestibility was significant in *Oncorhynchus mykiss* (rainbow trout) fed a phytase-supplemented diet (Cheng and Hardy, 2002).

As the digestibility of nutrients is increased, a decrease in nutrient excretion is observed. Previously undigested phytate fraction of the plant diet is hydrolyzed by the phytase inclusion, so, the more nutrients that are digested, absorbed, and utilized, the less are the nutrients available for excretion. This ultimately minimizes the aquatic pollution that was seen in the case of an unsupplemented diet (Nwanna et al., 2005; Wang et al., 2009). On the contrary, some studies reported a nonsignificant effect on digestibility parameters. No effect on protein digestibility of a fish fed phytase-supplemented diet was observed in studies conducted by Cheng and Hardy (2002), Dalsgaard et al. (2009), and Yan et al. (2002). A nonsignificant effect of phytase supplementation on fat digestibility was observed in *Oncorhynchus mykiss* (rainbow trout) (Dalsgaard et al., 2009). The contradictions in these results may be due to the variation in diet sources, the difference in fish species, and changes in phytase types (Da Silva et al., 2005; Hotz and Gibson, 2001).

15.4.1.3 Nutrient absorption and deposition

Phytase supplementation improves the digestibility of nutrients. As the rate of nutrient digestion increases through the degradation of phytates and their insoluble complexes, so does the rate of nutrient absorption and deposition improve as the availability of the nutrients is increased for enzyme action (Liu et al., 2013; Sugiura et al., 2001). As net nutrient depositions in the body are an important tool for studying fish feed efficiency (Belal, 2005), so increase in the rate of nutrient deposition directly shows the efficiency of phytase in digesting and absorbing the plant-based feed ingredients.

Phytase supplementation increased the absorption and deposition of phosphorous calcium, magnesium, sodium, and potassium in *Labeo rohita* (Rohu) (Shah et al., 2021). Nwanna et al. (2005) observed a significant increase in calcium and phosphorus deposition with phytase supplementation. Also, Hung et al. (2015) reported that phytase supplementation increased phosphorus retention in *Pangasianodon hypophthalmus Sauvage* (catfish). Higher phosphorus absorption was observed in rainbow trout *Oncorhynchus mykiss* (rainbow trout) fed a phytase-supplemented diet (Vielma et al., 2004). Studies were also conducted by

Baruah et al. (2007), Cheng et al. (2016), Cheng and Hardy (2002), Debnath et al. (2005), Liu et al. (2013), Sugiura et al. (2001), Vandenberg et al. (2012).

Phytase supplementation reduces the quantity of body lipids. This was observed in grass carp and Nile tilapia fed dietary exogenous phytase (Cao et al., 2008; Liu et al., 2014). As higher levels of body lipids were found in Epinephelus coioides (grouper) fed a low phosphorus diet (Ye et al., 2006), phytase inclusion in the diet increased the release of phosphorus from phytates present in the plant diet. It was observed that higher phosphorus reduced the body's lipid content (Roy and Lall, 2003). So, the supplementation of phytase lowered the level of lipid in the body.

15.4.1.4 Feed utilization

As the inclusion of plant-based ingredients is a source of cheap dietary energy, it allows an economical source of nutrients to be used for animal growth. This is only possible if the feed-based ingredients are effectively utilized (Enes et al., 2010). As microbial phytase improves the break down of antinutritional factors (phytates), so it also increases the efficacy of plant diet by enhancing its nutrient digestibility, absorption, and deposition (Fig. 15.3) (Tengjaroenkul et al., 2000). Best FI and FCR at 1500 FTU/kg were observed in *Pangasianodon hypophthalmus* (Tra catfish) fed microbial phytase (Hung et al., 2015). Also, feed utilization was improved by phytase supplementation in *Labeo rohita* (Rohu) (Shah et al., 2021). Nwanna et al. (2005) noted a significant improvement in feed conversion ratio. Moreover, Hussain et al. (2017) observed a better feed performance with phytase supplementation. Additionally, an improved feed conversion ratio was reported by Cao et al. (2008). As an improved feed conversion ratio shows the effective ability of the animal body to change the feed into desired output (Shike, 2013), so phytase increases the ability of fish to break down the plant-based ingredients (containing phytates) into desirable products. Sajjadi and Carter (2004b) also observed a significant effect on feed efficiency

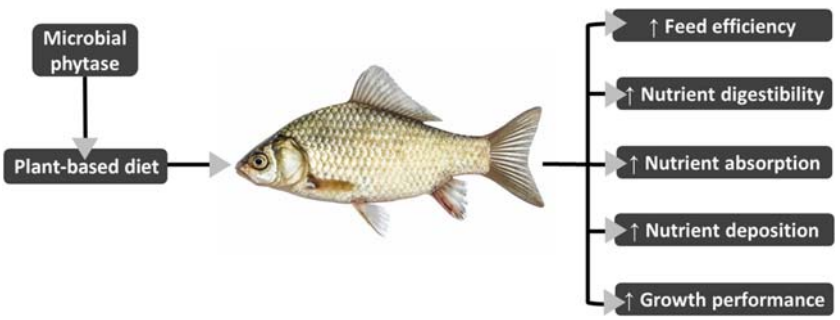

Figure 15.3
Microbial phytase supplementation in plant diet improved feed efficiency, nutrient digestibility, absorption, deposition, and growth performance in fish.

ratio. However, phytase supplementation did not significantly affect feed intake in the same study. This contradiction may be due to the variation in diet sources, the difference in fish species, and changes in phytase types (Da Silva et al., 2005; Hotz and Gibson, 2001).

15.4.2 Poultry

Phosphorus is one of the basic requirements for broiler feed as it is necessary for proper growth and development. Exogenous supplementation of microbial phytase has gained much importance due to its productivity-enhancing properties. Among the microbial phytases, bacterial phytase is proved to be more efficient in comparison to fungal phytase (Babatunde et al., 2020; Dilger et al., 2004). As phosphorous deficiency causes poor performance in broiler chicks due to its important role in growth and development, especially in the starter phase (Babatunde et al., 2019), so exogenous microbial phytase supplementation is an effective strategy for increasing the poultry output.

15.4.2.1 Growth performance and feed parameters

Microbial phytase improves poultry weight gain due to its phytate hydrolyzing ability that eliminates any possible phytate adverse effects (El-Hack et al., 2018). Basically, the phytase supplementation increases the utilization of plant-based feed ingredients leading to improvement in animal body weight. Shirley and Edwards (2003) observed weight gain in chicks fed a phytase-supplemented diet. Moreover, de Sousa et al. (2015) reported a significant weight gain increase in response to phytase supplementation in both male and female chickens. Also, phytase supplementation increased weight gain and decreased mortality in the broiler (Lim et al., 2001).

Phytase supplementation also enhances the weight gain of egg laying hens. As observed by Sukumar and Jalaudeen (2001), phytase supplementation significantly improved the layer body weight. Moreover, phytase improved the body weight in broiler fed phosphorous-deficient diet (Silversides et al., 2006). Phytase supplementation also improved growth performance in the broiler starter phase (Babatunde et al., 2019; Dersjant-Li et al., 2020; Kiarie et al., 2016). These evidence indicate that microbial phytase hydrolyzed the phytate complexes very efficiently, releasing nutrients necessary for growth and development. In contradiction, El-Deek et al. (2009), Hughes et al. (2008) reported a lower or nonsignificant body weight by broiler fed phytase-supplemented diet. This might be due to changes in dose level, age of the species, and experiment duration.

Phytase supplementation also plays an important role in feed intake and feed efficiency. According to Sukumar and Jalaudeen (2003), an increase in feed intake was observed by phytase supplementation. Ponnuvel et al. (2015) indicated that phytase supplementation at 500 and 1000 U/kg enhanced the feed efficiency without having any effect on feed intake. Çabuk et al. (2004) also noted a significant increase in feed consumption by phytase supplementation at the level of 300 FTU/kg diet. According to a different study performed

on hens, it was noted that feed efficiency declined when the animals were fed a low phosphorous-based diet than those fed higher available phosphorus (Snow et al., 2004). As the supplementation of phytase increases the availability of phosphorus, so feed efficiency of the plant diet is effectively increased. Birds fed 1000 U/kg of phytase showed a better feed conversion ratio than the birds fed a control diet (unsupplemented). The study performed by Nezhad et al. (2007) also highlighted the importance of phytase supplementation by showing that it significantly affected the feed consumption in the broiler. In another study, exogenous phytase supplementation at the level of 200, 400, and 600 U/kg diets also significantly improved the feed consumption and feed conversion (Hughes et al., 2008).

15.4.2.2 Nutrient digestibility

Phytate in the plant diet has an inhibitory effect on endogenous enzyme activity. This happens when phytate forms complexes through the chelation of cofactors involved in the optimum working of endogenous enzymes (Katayama, 1997). As the endogenous enzyme output decreases, so does the rate of nutrient digestion. However, phytase supplementation increases the plant ingredient utilization leading to an improvement in nutrient digestibility.

Zhang et al. (2000) reported that a broiler fed phytase-supplemented diet (250−2500 FTU/kg) showed a significant increase in dry matter digestibility in comparison to a basal diet (unsupplemented diet). Moreover, a significant improvement was observed in the digestibility of dry matter, crude protein, ether extract, nitrogen-free extract, and nitrogen retention in a broiler fed phytase-supplemented diet at the level of 750 FTU/kg (Abd-Elsamee, 2002b). As the optimum concentration of available phosphorus increases with phytase supplementation, it also enhances the digestibility of organic matter, crude protein, ether extract, and nitrogen-free extract in laying hens, whereas the digestibility of crude fiber did not vary significantly (Abd-Elsamee, 2002a). According to Liu et al. (2007), phytase supplementation significantly enhanced the digestibility of crude protein and ether extract. Salem et al. (2003) noted an increase in digestibility of dry matter in chicken fed high phosphorous available phytase-supplemented diet (600 FTU/kg). Selle and Ravindran (2007) reported a significant increase in the digestibility of calcium and phosphorous in the ileum. Thus, phytase is helpful in improving the digestibility of plant-based ingredients in broiler and layers.

15.4.2.3 Phosphorous utilization

Bioassays including growth and laying performance, blood phosphorous level, bone trait, alkaline phosphatase activity, and phosphorous utilization are used for estimating the efficiency of phytase supplementation. The amount of phosphorous released from the plant diet is dependent upon the supplementation level of phytase. Every phytase is unique based on its chemical features that vary as its derived source varies. This may change its activity which affects the release of phosphorous from the feed phytate (Maenz and Classen, 1998).

Waldroup et al. (2000) demonstrated that supplementation of phytase could release up to 50% phosphorous in a broiler fed plant-based diet. Studies proving the effective phytate−phosphorous utilization, total phosphorous, and phosphorous retention through phytase supplementation are available (Augspurger et al., 2007; Onyango et al., 2005). Moreover, as the utilization of phosphorus is increased, its discharge in the environment is reduced. Ahmad et al. (2000), Augspurger et al. (2007), Onyango et al. (2005) demonstrated that phosphorous excretion declined by 42%−51%, 32%−36%, and 37.5% through phytase supplementation. This decreased phosphorous excretion is effective in reducing phosphorous pollution. Similarly, application of reduced amounts of phosphorus in manure reduces the chances of excessive phosphorus pollution in soil when the manure is used as a fertilizer (Panda et al., 2010; Waldroup et al., 2000). As excess of phytate−phosphorus is retained by the animal, so less is excreted in the environment. As evident from the study performed by Żyła et al. (2011), it was noted that phytase supplementation increased the retention of phosphorus and calcium in hens. A *meta*-analysis performed on phosphorus retention suggested that it may increase by 5.02% when the laying hen is fed a diet supplemented with average phytase level (371 FTU/kg) (Bougouin et al., 2014). Vieira et al. (2015) observed that the bioequivalence of phosphorus was 40% higher in broiler fed 1000 FYT/kg of phytase than 500 FYT/kg. Most of the literature supports the role of phytase in the increased phosphorous availability with slight variations.

15.4.2.4 Availability of dietary energy and amino acids

The effect of phytase supplementation on energy utilization is well documented. Supplementation of phytase improved the apparent metabolizable energy (Newkirk and Classen, 2001). However, in another study, no effect was observed on apparent metabolizable energy by phytase supplementation (Onyango et al., 2005). This contradiction may be due to the variation in the source of phytase, bird age, and diet ingredients. Moreover, Selle and Ravindran (2007) noted a significant improvement in amino acid availability and energy utilization in a poultry fed phytase-supplemented diet. In a study performed by Rutherfurd et al. (2010), it was observed that phytase supplementation enhanced the digestibility of isoleucine. Moreover, it was reported that phytase supplementation reduced the loss of amino acids, thus improving their availability for body processes (Liu and Ru, 2010). So phytase supplementation is an effective strategy not only to increase dietary energy availability but also to enhance amino acid utilization.

15.4.2.5 Egg production

Phytase supplementation is proven to improve egg production performance, especially in diets with low available phosphorus. Lower levels of phosphorus reduced egg production and its mass in the study performed by Snow et al. (2004). Microbial phytase supplementation improved egg production in hens (Sukumar and Jalaudeen, 2003) due to an

increase in phosphorous levels (Fig. 15.4). In another study, phytase supplementation (0 and 100 FTU/kg) affected egg production significantly (Maria Casartelli et al., 2005). Furthermore, Ponnuvel et al. (2015) demonstrated that diets with phytase supplementation (500 and 100 U/kg) significantly increased the production of eggs. However, Musapuor et al. (2005) reported that phytase supplementation at the level of 1000 FTU/kg did not significantly affect the production of eggs. This contradiction may be due to the variation in the source of phytase, bird age, and diet ingredients.

Similarly, diets with phytase supplementation significantly increased the performance and egg quality parameters, in comparison to the unsupplemented group (Ahmadi et al., 2007). Also, Kim et al. (2017) reported a positive impact on the production of eggs in laying hens fed phytase-supplemented diet. Additionally, Englmaierová et al. (2017) observed an improvement in the egg production and quality of the eggshell in older hens fed 300 FTU/kg of phytase. It might be the calcium mobilization ability of phytase that is responsible, as the higher concentrations of calcium at the termination of the laying period increase both the production of eggs and the quality of eggshell. Moreover, it was observed that the source of phytase directly impacts egg production (Gao et al., 2013). However, Hughes et al. (2008) reported a decrease in production performance in white Leghorn laying hens fed 0.15% non-phytate-phosphorous diet with phytase supplementation. Furthermore, a decrease in egg production, weight, and mass were observed by El-Deek et al. (2009) in a broiler fed phytase-supplemented diet. This contradiction may be due to the variation in the source of phytase, bird age, and diet ingredients.

Byproducts like Distiller's dried grains with solubles (DDGS) carry high levels of phosphorus and low quantities of phytate-phosphorus. So phytase supplementation of diets containing DDGS may further enhance the laying performance. As was seen in the study conducted by Świątkiewicz et al. (2013), where total egg number production was increased

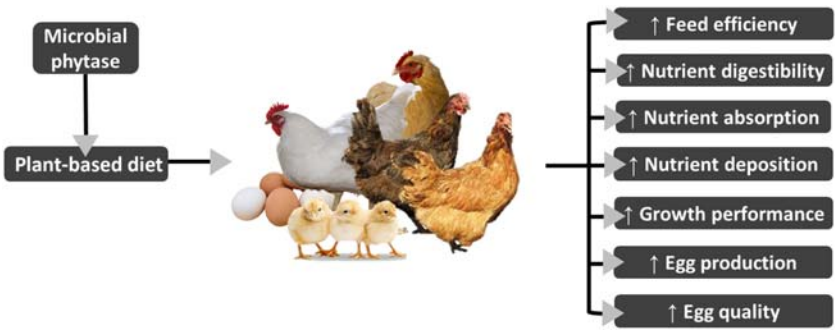

Figure 15.4
Microbial phytase supplementation in plant diet improves feed efficiency, nutrient digestibility, absorption, deposition, growth performance, egg production, and quality.

in broiler fed DDGS diet supplemented with phytase as compared to the unsupplemented diet. Similar results were reported by Deniz et al. (2013). Mostly, the studies have reported beneficial effects of phytase supplementation; however, the dose level was inconsistent.

15.4.2.5.1 Egg quality

Improvement in egg quality traits by phytase supplementation may depend on the concentrations of calcium and non-phytate-phosphorous in the diet. As noted in the study performed by Lim et al. (2003), a significant interaction was observed between phytase concentration, calcium, and non-phytate-phosphorous. Eggshell quality is improved in response to the phytase supplementation. Liu et al. (2007) reported that phytase supplementation at the level of 300 FTU/kg significantly improved the shell quality of the egg. Similarly, Ebrahim-Nezhad and Kandi (2008) reported that phytase supplementation in diets with low available phosphorus resulted in a significant increase in eggshell weight and thickness. Moreover, hens fed a phytase-supplemented diet at the level of 350 FTU/kg showed a significant improvement in the eggshell quality (Englmaierova et al., 2015). Also, Gao et al. (2013) reported that egg quality improved in hens fed a phytase-supplemented diet. In contrast, Kim et al. (2017) and Musapuor et al. (2005) reported that the egg quality traits in laying hens remained unaffected with phytase supplementation. This contradiction may be due to the variation in the source of phytase, bird age, and diet ingredients.

15.4.2.6 Bone mineralization

Phosphorous deficiency adversely impacts bone mineralization (Babatunde et al., 2020). Phosphorus and calcium are the main minerals in bone, and any deficiency in these is directly reflected in bone. Phytase significantly improved tibia ash in a broiler fed phytase-supplemented diet (Babatunde et al., 2021). Phytase inclusion at high or traditional doses significantly improved bone mineralization and broiler skeletal structure (Babatunde et al., 2020; Gautier et al., 2018; Olukosi et al., 2013). Also, a high phytase dose improves calcium and phosphorus absorption and utilization, resulting in enhanced skeletal development. Supplementation of phytase makes the bones strong due to an increase in the concentration of phosphorus and an increase in its deposition rate.

15.4.3 Pig feed

A plant meal-based diet contains phytate that contains phosphorus. However, the phytase, the enzyme that hydrolyzes phytates, is scarce in pigs. This reduces the digestibility of phosphorus (She et al., 2017). Moreover, phytate forms complexes with cations, proteins, and starch, negatively influencing proteins, minerals, and energy utilization (Woyengo and Nyachoti, 2013). Supplementation of exogenous microbial phytase at the standard 500 units/kg or higher levels enhances phosphorous release from phytase and improves nutrient digestibility (Holloway et al., 2019; Moran et al., 2019; Selle and Ravindran, 2008).

As a higher phytase dose results in lower ester production through phytate degradation, the lesser ability phytates have to form complexes with proteins and cations (Bedford and Walk, 2016). A higher phytase dose ensures enhanced energy and nutrient digestibility (Lagos-Muñoz Liz Vanessa, 2021). Phytase supplementation in the diet lowered the feed conversion ratio in growing pigs to the level of 1500 FTU/kg (Dang & Kim, 2021). Moreover, Lu et al. (2019) reported a decrease in feed conversion ratio with pigs fed a phytase-supplemented diet. Fig. 15.5 illustrates microbial phytase supplementation in plant diet improves *n*utrient digestibility, bone mineralization, and growth performance in pigs.

15.4.3.1 Growth performance

Supplementation of microbial phytase in diets degrades the phytates present in a plant-based diet. However, in the case of an unsupplemented diet, plant-based phytates act as an antinutritional factor. They restrict the availability of growth-related nutrients by making insoluble complexes and by acting as a chelating agent. Moreover, it also restricts the availability of phosphorus as the phytate is its major storage in a plant-based diet (Holloway et al., 2019; Moran et al., 2019; She et al., 2017; Woyengo and Nyachoti, 2013). This decrease in feed nutritional attributes adversely impacts the growth performance of pigs. Supplementation of phytase eliminates the adverse effects of phytase and increases the bioavailability of nutrients, thus improving growth performance.

Microbial phytase supplementation directly improves growth performance. Diets with phytase supplementation at the level of 2000 FTU in 21–49 post-weaning pigs improved growth performance (Lu et al., 2019). It was reported that phytase supplementation at a level greater than 1000 FTU in a diet containing proper levels of calcium and phosphorus showed significantly enhanced growth in comparison to the pigs fed an unsupplemented diet for 10 days (Moran et al., 2017, 2019). Moreover, studies by Holloway et al. (2019), Lu et al. (2019) noted that superdosing of phytase implemented a positive effect on growth

Figure 15.5
Microbial phytase supplementation in plant diet improves nutrient digestibility, bone mineralization, and growth performance in pigs.

performance. Dang and Kim (2021) reported a significant improvement in weight gain in pigs fed diets supplemented with 1500 FTU/kg of phytase. Furthermore, supplementing phytase at a high dose level significantly improved the growth performance of pigs (Jendza et al., 2005; Malagutti et al., 2012).

15.4.3.2 Nutrient digestibility

Microbial phytase significantly improves the phosphorous digestibility of plant-based ingredients. Improvement in digestibility also ensures a reduction in phosphate levels in manure. Moreover, reduced amounts of phosphorus in manure reduce the chances of excessive phosphorous pollution in soil when the manure is used as a fertilizer (Panda et al., 2010; Waldroup et al., 2000). As excess phytate−phosphorus is retained by the animal, so less is excreted in the environment. A standard phytase dosage level 500−1000 FTU and a high dosage level greater than 1000 FTU, both provide phosphoric benefits to pigs (Lu et al., 2019). Akinmusire and Adeola (2009) and Almeida and Stein (2010) reported a significant improvement in phosphorus digestibility in pigs by feeding phytase-supplemented diets at 500 FTU and 1000 FTU levels. The same results were also reported in the studies performed by Casas and Stein (2015), McGhee and Stein (2019), and Rodriguez et al. (2013).

Exogenous microbial phytase supplementation improves amino acid digestibility. However, the dosage of phytase for ileal amino acid digestibility is dependent on the weight of pigs. As evident from the study performed by Velayudhan et al. (2015), 2000 FTU of phytase in the diet was needed to increase the standardized ileal digestibility of amino acids in 25 kg pigs. Similarly, Zeng et al. (2016) noted that 1000 FTU of phytase in diets was not sufficient for ileal amino acid digestibility (Zeng et al., 2016). In contradiction, studies by Mesina et al. (2019), Rosenfelder-Kuon et al. (2020), and She et al. (2018) reported that 3000 or 4000 FTU of phytase in diet did not significantly affect apparent amino acid ileal digestibility. This contradiction may be due to the variation in the source of phytase, pig age, and weight in addition to plant-based diet ingredients used.

Graded levels of phytase from 0−1000 FTU and 0−2500 FTU in the diet significantly improved energy digestibility as a linear trend in pigs (Arredondo et al., 2019; Brady et al., 2003). Furthermore, gross energy digestibility positively improved in response to phytase supplementation in diets in pigs (Kiarie et al., 2016; Velayudhan et al., 2015). In contradiction, the studies performed by (Mesina et al., 2019; She et al., 2018; Zeng et al., 2016) reported no significant affect of phytase supplemented feeds in animals. This contradiction may be due to the variation in the source of phytase, pig age, and weight in addition to plant-based diet ingredients used.

15.4.3.3 Bone mineralization

The content of ash in the bones shows bone size, while its percentage represents the composition of bone (Lagos et al., 2019). De Cuyper et al. (2020) and Zeng et al. (2011)

noted an improvement in bone ash with phytase supplementation. The amount of mineralization depends on the maturity and age of pigs, as finishing pigs have higher mineralization in bone in comparison to younger pigs (Crenshaw et al., 1981). In the early developmental phase in pigs, maximum phosphorus that is released by phytase supplementation was absorbed by bones; thus, its level in blood plasma was less as compared to the pigs fed an unsupplemented diet. Moreover, in older pigs, the phosphorous requirement for bone mineralization was less. This indicates that the phosphorous concentration in plasma was higher in older pigs than in younger pigs (Lagos et al., 2019). Moreover, it was observed that pigs fed a phytase-unsupplemented diet excreted more phosphorus in urine (Lagos-Muñoz Liz Vanessa, 2021). This increases the chances of environmental pollution.

15.5 Conclusion

Phytase supplementation has proven to be the most effective strategy in degrading plant-based phytates. Due to its beneficial effects, it is being used worldwide in both research and cultural practices. Phytase improves the efficacy of plant-based feed ingredients by degrading phytates. The potential of phytase in improving feed for fish, poultry, and pigs has gained much attention. The search for a better phytase source is still ongoing to further enhance the feed production process and its utilization in animal systems. As all these animals are a source of food for us, so increase in the growth rate, and mineral composition in the vitals of these animals is directly beneficial to us. This enhances the cultural practices by improving the feed efficacy.

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CHAPTER 16

Gut microbial metabolites and colorectal cancer

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16.1 Introduction

In the view of Koch's pastulate, developed in 19th century, microorganisms must be secluded from harmful organism, grown in pure culture, and on re-inoculation into a susceptible recipient to result in the reoccurrence of a disease (Ehrlich, 1913). Based on Koch's postulate, the interaction of microorganisms with human mainly focused on a single pathogen; researchers have observed remarkable progress in developing an understanding of the development of different treatments against infectious diseases over time. Apart from this, it has been found that a few kinds of contagious agents increase the burden of cancer on the human society by approximately 18% of the global cancer rate (Balkwill and Mantovani, 2001; Trinchieri, 2012). Many pathogens, such as viruses, mainly target the human genetic make-up to promote cancer through genetic or epigenetic mechanisms (Moore and Chang, 2010). Other than genetic and epigenetic mechanisms, some pathogens, like hepatitis C virus and *Helicobacter pylori*, encourage cancer development by inducing epithelial injury and inflammation as major contributors to carcinogenesis (Balkwill and Mantovani, 2001; Trinchieri, 2012). However, these findings suggested that a single pathogen-induced disease in humans is attributed not only to the sense of pathogen but also attributable to changes in the microbiome (Turnbaugh et al., 2006). Scientists often use the term microbiome as "forgotten organ" (O'Hara and Shanahan, 2006); it has a metagenome that goes beyond 100 folds of our genome (Savage, 1977) and is involved in a number of critical functions, especially regarding human health (Kau et al., 2011). The laboratory method of culturing the pathogen successfully grows only 30% of our bacterial microbiota (Fraher et al., 2012). However, recent development in sciences to analyze the genome sequence by using next-generation sequencing resulted in refining our understanding of the bacterial metagenome and microbiome, especially to investigate its crucial role in

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inflammation and metabolism (Human Microbiome Project, 2012) as these two are a major contributor of carcinogenesis (Colditz et al., 2006). In the light of the above discussion and the importance of microbiota, especially bacteria, in the development of carcinogenesis, our primary focus in this chapter remains on host-microbiota interactions and effectors' mechanisms to induce cancer (Schwabe and Jobin, 2013).

About a trillion organisms, essentially bacteria, by and large, referred to as the gut microbiota, are a major resident of the human intestine. Reflecting a notable degree of co-evolution, the gut microbiota flourished in symbiotic equilibrium with the host (eubiosis). Microbiota in the human intestine develops a symbiotic relationship in which the human intestine provides micronutrients rich proactive and warm habitat for the microbiota, while in return, gut microbiota support humans in digesting complex carbohydrates and provides nonessential nutrient factors to provide great benefits of food and eventually take up ecological niches available in the intestine that otherwise might be occupied by other pathogenic microorganisms (Schwabe and Jobin, 2013). The human immune system can detect the incoming pathogenic invaders by activating its immuno-surveillance signaling system. In addition, massive evidence recommended that gut flora is required to correct the internal and external components of the intestinal immune system (Maynard et al., 2012). However, any disequilibrium between host and residing microbiota relationship (dysbiosis) affects the process of oncogenesis, tumor development, and therapeutic response, as well as to what extent gut microbiota can be managed for beneficial purposes (Maynard et al., 2012).

16.2 Colorectal cancer

Colorectal cancer (CRC) is still a major public health concern worldwide (Maynard et al., 2012). Among many others, CRC is the primary cause of cancer-associated death (Van Raay and Allen-Vercoe, 2017). CRC has a close link with the living styles and habits that clinically affect the large intestine and rectum (Pourhoseingholi and Zali, 2012). On the other hand, studies also suggested that any malfunctioning of the symbiotic association between host and microbiota resulted in the onset of complicated conditions, including type 1 or 2 diabetes, inflammatory bowel disease, and colorectal cancer (Jobin, 2018). Other than human genetic make-up and its associated environmental factors, dietary habits such as poor and imbalanced diet, alcoholism, and smoking tobacco, have a hand in gut bacterial dysbiosis and epigenetic changes linked to an elevated rate of CRC (Yang et al., 2013a,b). The word "dysbiosis" connotes a disturbance in the healthy human microbiome population (Petersen and Round, 2014), that is, the microbes that live in the skin, mouth cavity, lower respiratory tract, vaginal, urinary tract, and gut (Tarashi et al., 2018). The human gut is home to the highest and most diverse bacterial population, which interacts with the host in a symbiotic manner (Candela et al., 2011). Recent data linked the existence of particular

species in patients' microbiome to their response to anticancer immune checkpoint inhibitor therapy (Adachi and Tamada, 2018; Vetizou et al., 2015).

Furthermore, chemicals generated from microbes have an imperative role in host metabolism and CRC advancement (Brown et al., 2016). As a result, there has been rising awareness to determine whether there is a link between gut bacteria and CRC (Papastergiou et al., 2016). However, in the evolution of CRC, the causal genera are still poorly defined. Despite this, there remains a lack of knowledge regarding the impact of diverse gut microbial flora and its metabolites derivatives in CRC development, especially as modification of epigenetic factors plays a critical role in CRC development. As a result, the comprehensive mechanism of action behind CRC pathogenesis remains unknown. In addition, the current study gives a complete summary of the gut bacterial DNA as those that have been detected in the major types of CRC samples. Finally, the essential processes influencing CRC advancement are briefly described, including microbial-derived metabolites and epigenetic alterations.

16.3 Microbial flora and development of colorectal cancer

Human being has a supernatural inherited ability to cope with invading organisms such as microbial entities in addition to their cells (Walsh et al., 2014). The gut bacterial population includes a number of phyla classes (Qin et al., 2010). These microorganisms present significant effects in many ways on different perspectives of human physical condition, such as human physiology, food metabolism, nutrient absorption, defense system, and protection against invading pathogens. Recently, considerable numbers of studies have been done on gut dysbiosis to investigate its role in CRC (Walsh et al., 2014). Reddy et al. (1975) have investigated the involvement between gut bacteria and CRC progression (Reddy et al., 1975) and found that immune system dysregulation induced by bacteria has the potential to control the host metabolism. Therefore, investigating the role of microbial flora in developing CRC has been a hot area of debate. Recently, studies have suggested the role of bacteria as leaders or followers (Tjalsma et al., 2012), representing that pathogenic bacteria primarily colonize the epithelium of human intestinal and work as drivers to control several processes whereas self-assertive microorganisms called passengers to provide the conditions for cancer development. As a result, bacteria with carcinogenic potential, particularly self-assertive pathogens and anaerobic bacteria, are frequently found at CRC onset (Warren et al., 2013). Indeed, compared to healthy controls, a large percentage of bacteria from the *Citrobacter*, *Salmonella, and Shigella* genera were discovered in the early phase of CRC formation, although their number decreased at a later stage of CRC development (Oke and Martin, 2017). In contrast, passenger bacteria from the *Fusobacterium* ssp. and *Streptococcaceae* families have not been discovered in the early stages of CRC and took advantage of the altered tumor microenvironment to flourish (Lazarovitch et al., 2013) and play a role in CRC

development (Oke and Martin, 2017). Bacterial changes during the different stages of CRC (Marchesi et al., 2011) have been investigated. It has been reported an increased ratio of *Gemella*, *Parvimonas*, *Leptotrichia*, and *Fusobacterium*, while loss of *Blautia*, *Bacteroides*, *Alistipes*, *Collinsella* were detected at early stages (Stage I-II) of CRC. On the other hand, none of these strains have been identified at the later stages (Stage III-IV) of CRC. By another study, it's suggested that there is an increase in the population of *Peptostreptococcus* and *Fusobacterium* and thrashing of *Streptococcus* and *Eubacterium* at the early stages of CRC (Nakatsu et al., 2015).

16.4 Gut microbial flora and food metabolism

Numerous researches have compared the microbiota of CRC patients with healthy individuals as controls to analyze their tissue and stool samples. Several reports have evaluated the gut bacteria between tumor tissue and adjacent healthy tissue in CRC individuals (Warren et al., 2013). The prevalence rate of CRC in developing countries is much lower than the developed ones, which is greatly associated with dietary differences (Allali et al., 2018). Taking into account the global epidemiological data, researchers have found that high caloric intake and utilization of animal-based proteins and fat and little consumption of plant-based diet, multivitamins, and fibers, resulted in damage to the gut microbial flora that affected its metabolism that is followed by an increased risk of CRC (O'Keefe, 2016). The curative success of local CRC cases ranges from 70% to 90%, while advanced CRC cases have a significant mortality rate (Sears and Garrett, 2014). The worldwide incidence rate of CRC is about 4%−5%, while hereditary and physical traits are the most considerable risk factors (Marmol et al., 2017). Furthermore, prevalent gut bacteria present their key characteristics in CRC development (Marmol et al., 2017), though it is still needed to investigate the mechanism by which dysbiosis might promote CRC.

Some important gut bacterial pathways implicated with CRC are addressed in depth in this article. One of the earliest molecular investigations discovered a strong relationship between the Escherichia genus and CRC (Sears and Garrett, 2014). *Escherichia* is a gut bacteria, and its population is more in the colon of CRC patients than in individuals with good health. However, some strains, such as B2 and D phylogroups, are more frequently associated with CRC (Kohoutova et al., 2014). *E. coli* strains of the phylogenetic group B2 have polyketide synthase genomic island, *pks*, and are involved in producing genotoxin colibactin, which may lead to developing CRC (Cuevas-Ramos et al., 2010). Other *E. coli* strains have the ability to yield a cytotoxic necrotizing factor (CNF) or a toxin named cytolethal distending toxin (CDT) that may result in the induction of CRC (Buc et al., 2013). *Streptococcus* bacterium has also presented a tight linkage since 1951 with CRC when a case of enterococcal endocarditis from *S. bovis* was shown in a patient with CRC (Tsai et al., 2016).

About 25%−80% of cases with *S. bovis* bacteremia led to CRC, but the major mechanisms still need to be investigated (Tsai et al., 2016). However, *S. bovis* and its antigen can excite

the release of interleukin-8 (IL-8) in the colon (Ellmerich et al., 2000) and result in increased expression of NO and reactive oxygen species (ROS) that might contribute to colon carcinogenesis. Another biotype of *S. bovis* bacteremia, *S. gallolyticus* subspecies *gallolyticus*, has shown a closed link with CRC (Boleij and Tjalsma, 2013). *S. gallolyticus* has been identified in 20%−50% of colorectal adenoma (CRA) and CRC cases (Abdulamir et al., 2010). The latter is recognized as a not cancerous colon tumor that can change into CRC. *S. gallolyticus* carries a collagen-binding domain and produces a certain inflammatory signal (Boleij et al., 2012), making it more advantageous for CRC development (Abdulamir et al., 2010).

B. fragilis strains make up about 0.1% of Bactericides genus in healthy individuals and produce a toxin called enterotoxigenic *B. fragilis* (ETBF), resulting in CRC development (Sears et al., 2014), as it is detected in 38% CRC cases and 12% of healthy individuals (Toprak et al., 2006). *B. fragilis* strains cause E-cadherin cleavage, promoting CRC and Myc expression as a proto-oncogene. Furthermore, BFT activates NF-kB signaling and causes cytokine production, all of which contribute to mucosal inflammation (Sears and Garrett, 2014). Another possible bacterial species among CRC individuals is *Enterococcus*. Some *E. faecalis* strains can increase the generation of superoxide anions and ROS and cause genomic instability through DNA damage (Sears and Garrett, 2014). *E. faecalis* can provoke the generation of a chromosomal-breaking factor, a diffusible clastogen, which leads to DNA damage (Yang et al., 2013a,b) being found. Thus, these strains have been anticipated as activators and enhancers of CRC. Fig. 16.1 portrays the role and mechanism of intestinal microbiota in promoting colorectal cancer.

Fusobacterium has been known as a major class of bacterium that influences the CRC. This finding is more concerned about the role of *Fusobacterium* in regulating the expression of NF-kB genes, an inflammatory-driven gene, in human CRCs (Sears and Garrett, 2014). However, it is reported that *F. nucleatum* is related to augmented expression of pro-inflammatory cytokines, leading to activate the NF-kB pathway (Kostic et al., 2013). The carcinogenic characteristics of *F. nucleatum* strains are provoked by the exceptional adhesion, FadA (FadAc) (Rubinstein et al., 2013), in the course of E-cadherin binding with the resultant commencement of cell growth-related signaling pathways (Sears and Garrett, 2014). Furthermore, *F. nucleatum* can hold back tumor cell apoptosis by interrupting the NK cells and the Fap2 protein receptors interaction, which disrupts the cytotoxic effects of natural killer (NK) cells (Bashir et al., 2016). Moreover, *Salmonella* might increase the risk of CRC progression by releasing its pathogenic product, AvrA (Lu et al., 2014). The significance of *H. pylori* in CRC, on the other hand, is still debated, despite recent study suggesting that *H. pylori* cytotoxin-associated gene A (CagA), and the induction of ROS and nitric oxide sulfate (NOS), have a role in the stimulation of inflammatory pathways and CRC development (Shmuely et al., 2001). Based on a meta-analysis, it has also been suggested that there is a greater risk of CRC in *H. pylori*-positive persons than in healthy

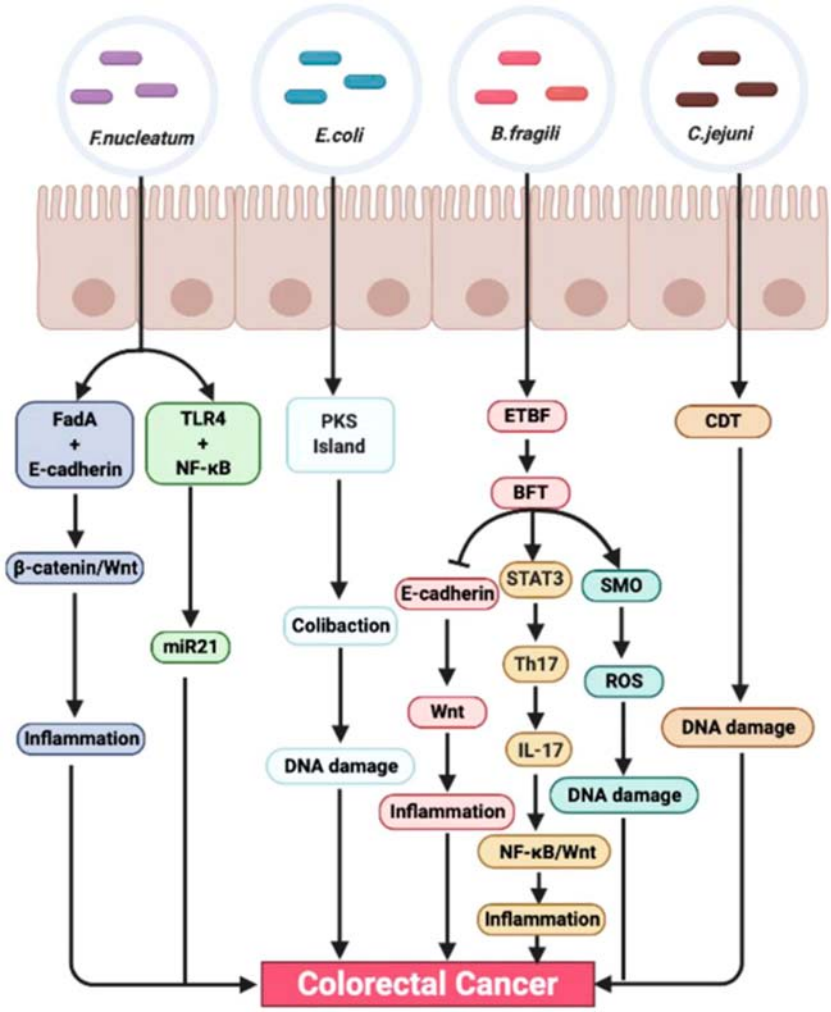

Figure 16.1

The role and mechanism of intestinal microbiota promoting colorectal cancer (Ren et al., 2021). *Reproduced from Ren, L., Ye, J., Zhao, B., Sun, J., Cao, P., Yang, Y., 2021. The role of intestinal microbiota in colorectal cancer. Frontiers in Pharmacology 12, 674807 with permission; This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY).*

individuals (Zumkeller et al., 2006). On the final note, *Clostridium septicum* infection leads to CRC (Mirza et al., 2009), but its fundamental mechanism remains vague, as there is no straight line to impede this direct association. So it can only be hypothesized that spores of *C. septicum* germinate and grow easily under hypoxic and acidic tumor microenvironments (Dylewski and Luterman, 2010).

Although the previous instances showed that gut bacteria have a negative impact on CRC advancement, some beneficial effects on CRC prevention have also been observed. In vitro studies are frequently conducted to investigate the mechanics of probiotic gut microorganisms (Mendes et al., 2018). However, the preventive impact of several probiotics in CRC patients has been studied in multiple human clinical trials (Drago, 2019; Rafter, 2003). Some microorganisms, such as *Lactobacillus acidophilus* and *Bifidobacterium longum* have been used to impede the induction of CRC (McIntosh et al., 1999). *L. acidophilus* seems to be used as a carcinogenic agent in the gut lumen of rats to affect the 1,2-dimethylhydrazine-induced CRC and decrease the chances of CRC development (McIntosh et al., 1999). To add up, we find that *B. infantis* and *B. adolescentis* also downregulated the effect of 3-methylcholanthrene-triggered CRC in mice models (Kohwi et al., 1978). Furthermore, L. acidophilus' protective impact in CRC patients appears to be due to its ability to bind to carcinogens in the human gut lumen and reduce intestinal cell propagation (Lidbeck et al., 1992). Some clinical trials have found that effect of *L. casei* presents its protective effect in reducing the chances of CRC recurrence (Ishikawa et al., 2005), whereas additional studies bring to light the protective effects of *B. lactis* Bb12 and *L. rhamnosus* GG in CRC patients (Mendes et al., 2018). Generally, the beneficial effects of gut bacteria include protective effects against CRC progression by reducing the DNA damage, intestinal cell proliferation, and interleukin-2 secretion, as well as interferon production, increased host immune responses, and modifying physicochemical parameters and bacterial metabolic activity in the gut (Mendes et al., 2018). Until the end of the last century, the link between gut bacteria and CRC was identified by the same methods. The progress in molecular techniques, primarily on the hypervariable region of 16S ribosomal RNA (rRNA) analysis, has provided a considerable amount of information and guided toward a better understanding and analysis of the various bacterial communities (Walsh et al., 2014). As a point of fact, highly efficient sequencing tools have greatly increased our understanding of the critical function of gut bacteria in developing CRC (Del Vecchio et al., 2017).

16.5 Role of microbial flora and its derivatives in colorectal cancer

New characteristics of gut bacteria as potential performers in CRC advancement are rapidly emerging. Different diets may influence the synthesis of microbial metabolites and their derivatives, having significant effects on host metabolism and CRC development (Qin et al., 2010). In general, consuming dietary fibers that are neither assimilated nor absorbed in the bloodstream is recognized as the best approach to transforming the gut bacterial metabolites, even for those launched as prebiotic elements (Holscher, 2017). The term "prebiotic" refers to food ingredients that cause certain positive alterations in an individual's gut bacterial flora (Lattimer and Haub, 2010). However, fiber consumption is directly associated with the type of gut bacteria. Based on the heterogeneous nature of fibers, it has been classified into the origin, physicochemical characteristics, and chemical composition, while their subclasses are based on carbohydrate chain length. The Codex Alimentarius Commission has classified dietary fiber as

edible carbohydrates in consumed foods, edible carbohydrates altered by enzyme reaction, physical or chemical induced modifications of edible synthetic carbohydrates, and raw food materials (Holscher, 2017). All the abovementioned types have demonstrated favorable physiological benefits supported by several types of research and can influence the decomposition of various types of the gut microbiome and, as a result, have therapeutic effects. Based on physicochemical characteristics of dietary fibers, it can be categorized based on solubility, fermentability, and thickness. Solubility appears to impact gut bacterial fermentation (Holscher, 2017) significantly. Soluble fiber, such as pectin and gums, is quickly digested in the upper part of the colon and considered a primary part of the food metabolism, resulting in lower glucose absorption, maintaining blood pressure, insulin, and the level of LDL cholesterol (Lattimer and Haub, 2010). While insoluble fiber, such as lignin and cellulose, is partially absorbed in the lower part of the colon where the bacterial population is higher, it's still important for gut health. Vegetable and fruit fiber is usually soluble, whereas grain fiber is mostly insoluble (Terry et al., 2001). In the large intestine, gut bacteria start fermenting undigested food fibers and create a wide range of metabolites (Belcheva et al., 2015). Short-chain fatty acids (SCFAs), such as propionate, acetate, and butyrate, altered by a fiber-rich food, are the primary metabolites of gut bacteria during the fermentation process in the colon (Belcheva et al., 2015). Butyrate and propionate regulate the gut functioning and defense system; on the other hand, acetate acts as a substrate in lipogenesis and gluconeogenesis (Macfarlane & Macfarlane, 2011). Apart from these, few species of phylum *Firmicutes* mainly produce butyrate, which put the basis of numerous controversial events in the colon (Donohoe et al., 2012). Butyrate is a compound with the potential to stimulate the colon epithelial cells' natural proliferation (Cardona et al., 2013). Moreover, they alter the gut bacterial community by reducing the expression of pro-inflammatory mediators (Cardona et al., 2013). Nevertheless, its potential to work together with the genetic backgrounds has raised questions about its role in CRC progression (Qin et al., 2010). Although many reports have confirmed the role of butyrate in cancer development, its role in CRC remains unconvincing. It is necessary to investigate the microbial-derived metabolite, but it is more challenging to find their interactions with genetic and epigenetic backgrounds so that the mechanism behind the development of cancer can be addressed better. Fig. 16.2 illustrates typical microbial metabolites involved in CRC pathogenesis.

Regardless of the advantageous SCFAs fermentation, amino acid fermentation may also generate possible malicious agents. Few of these compounds included p-cresol, ammonia, hydrogen sulfide (H2S), and some amines, which may lead to CRC and other metabolic-related disorders development (Chen et al., 2013). These harmful compounds may result in an increased rate of DNA modification, perforated gut, tenderness, and CRC development (Windey et al., 2012). For instance, a close association between gut bacteria and sulfate metabolism has been established, and it has been shown that sulfate metabolism results in the production of methionine, cysteine, and H2S, whereas higher concentrations of these compounds followed by colon cells proliferation

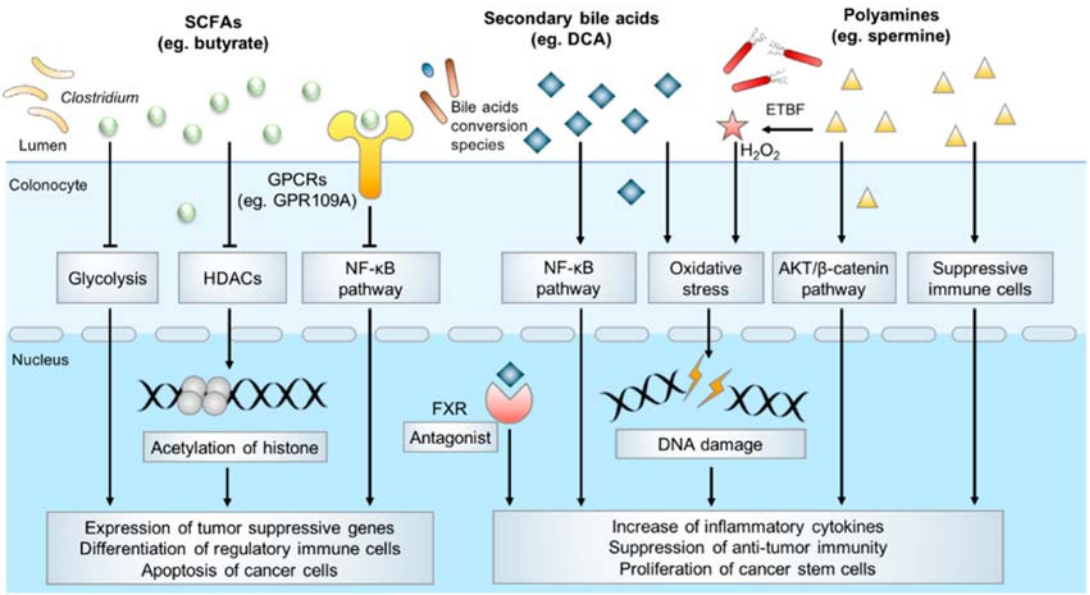

Figure 16.2

Typical microbial metabolites in CRC pathogenesis (Peng et al., 2021). *CRC*, Colorectal cancer. *Reproduced with permission from Peng, Y., Nie, Y., Yu, J., Wong, C.C., (2021). Microbial metabolites in colorectal cancer: basic and clinical implications. Metabolites 11(3), 159; This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).*

lead to CRC progression (Marchesi et al., 2016). *Desulfovibrio* spp. is responsible for sulfate metabolism and produces H2S in the gut. These bacteria can use available lactate to boost their growth and sulfide formation (Marquet et al., 2009) to accelerate the process of CRC development by inhibiting butyrate oxidation by the induction of gut blockade to break down. Hydrogen sulfide level is essentially dependent on bacterial activity, independent of their availability (Carbonero et al., 2012). *Desulfovibrio* spp., butyrate-producing bacteria, can mainly use lactate to compete with sulfate-reducing bacteria. Several bacteria, such as *Eubacterium*, *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, and *Enterococcus, can produce lactate, one of lactic acid gut bacteria's advantageous byproducts*, which is generally used by other gut bacterial genera in a symbiotic relationship (Duncan et al., 2004). Conversely, few studies suggested that increased amines, principally polyamines, are lethal and are coupled with CRC development (Louis et al., 2014). However, the virulence of some gut bacteria, such as *H. pylori, S. pneumonia*, and *S. flexneri*, increased by increasing polyamines (Di Martino et al., 2013). Because of their potency and antioxidant effect, phytochemicals played a critical role in cell detoxification, propagation, programmed cell death, and inflammation (Ramos, 2008). On the other hand, the antioxidative effect of phytochemicals can be neutralized by increased ROS levels that may damage DNA and increase the CRC risk (Louis et al., 2014). The nitrogen metabolites,

like N-nitroso compounds (NOCs), are also likely to stimulate CRC by inducing DNA damage (Louis et al., 2014).

Many studies proposed that bacterial inequity in the gut can promote the expansion of harmful bacteria and their cancer-inducing compounds (Arthur and Jobin, 2011). On the other hand, more studies are needed to prove this hypothesis. Bile acids present some cytotoxic properties and can promote the development of cancerous cells (Kahouli et al., 2013). Bile acids, such as lithocholic acid and deoxycholic acid, have been identified as possible carcinogens with a harmful association with anti-carcinogenic chemicals in the colon (Ou et al., 2012). Another microbial metabolite, uracil, is linked to ROS generation in the intestine (Lee et al., 2015). Trimethylamine N-oxide (TMAO), produced by gut bacteria metabolism, is closely associated with CRC (Xu et al., 2015). In addition, many gut bacteria also produced highly carcinogenic acetaldehyde (Homann, 2001) and suggested that some bacteria induce aerobic metabolic processes and glycolysis. Several organic compounds, such as nitrate, sulfate, and many other organic compounds, work as ion receptors in electron transport chain reactions (Sieber et al., 2012). Likewise, oxygen may be reckoned as an electron receiver of the facultative anaerobes such as *Bacteroides spp.* and *Faecali bacteriumprausnitzii* (Baughn and Malamy, 2004).

In addition to having a direct association between cancer and homeostasis, gut bacterial metabolites and their derivatives can have an indirect impact. Cross-feeding interaction, for example, is when bacteria exchange primary metabolites with other species (D'Souza et al., 2018). Dietary fibers significantly boost the metabolic interactions among the gut bacterial community significantly (Holscher, 2017; Marquet et al., 2009). Furthermore, only a small percentage of gut bacteria consume formate and hydrogen and mostly take part in anaerobic metabolism via cross-feeding (Carbonero et al., 2012). These interactions are considered critical in developing gut microbial populations (D'Souza et al., 2018). It may be summarized that a complicated bidirectional network mediates the regulating gut bacterial community by metabolites (Tarashi et al., 2019).

16.6 Gut microbes as an epigenetic modifier

Epigenetic modifiers, such as histone deacetylase (HDAC) inhibitor and DNA methyltransferase (DNMT) inhibitor, are the chemical entities that can induce microbial changes to modify the epigenetic status. These molecules control the biosynthetic pathway by suppressing the activity of the related enzyme to regulate further metabolic pathways (Seyedsayamdost, 2014). DNA methylation (addition of a methyl group to the DNA) in a promoter region of a gene may induce the gene transcription by regulating changes in DNA structure in the corresponding regions, blocking certain transcription factors from binding and suppressing their gene expression (Araujo et al., 2001). The most common DNMT inhibitor, 5-Azacytidine (5-AC), is used to transform the expression of microbial DNA,

followed by reducing the level of gene transcription. The chemical investigation has shown that culture growth media of *Aspergillus sydowii* produced three different bisabolane-type sesquiterpenoids by supplementing with 5-AC (Chung et al., 2013). Another entomopathogenic fungus, *Cordyceps indigotica*, brought a novel aromatic polyketide glycoside to PDB media, but the addition of 5-AC promotes another unusual glycoside (Asai et al., 2012a,b,c; Chen et al., 2016; Wang et al., 2010).

In microbes, adding or deleting an acetyl group to the histone alters its DNA binding. The chemical changes in the histone tail affect gene expression in various ways. The addition of a hydrophobic acetyl group to the N-terminal lysine residues of histone protein might boost electrostatic attraction where the steric barrier occurs between histone and DNA, making it easier for DNA to depolymerize and transcription factors to attach (Fukuda et al., 2006). Suberoylbishydroxamic acid (SBHA), suberoylanilide hydroxamic acid (SAHA), and nicotinamide are among the most frequent HDAC compounds for preventing deacetylation and facilitating gene transcription and expression in microorganisms (Moore et al., 2012). Many studies have shown that adding SAHA to a culture media can lead to new natural chemicals, such as the novel metabolite nygerone A produced by *A. niger* (Henrikson et al., 2009), two new aromatic norditerpenestied from *A. wentii* na-3 (Miao et al., 2014), three novel cyclodepsipeptides from *Beauveria feline* (Chung et al., 2013), and a novel chlorinated polyketide from *Daldinia* sp. (Ye et al., 2017).

16.7 Mechanism of bacterial metabolites and derivatives to develop colorectal cancer

It is a well-known fact that epigenetic modifications may regulate a number of cellular processes by modulating the gene expression, most probably independently of any variations in the DNA sequence of a genome. Till this time, a number of modifications at the epigenetic level have been identified, such as DNA methylation/demethylation, histone modifications, chromatin remodeling, and RNA-based regulation (Wang et al., 2017). However, the impact of epigenetic changes in the genesis of various diseases is overlooked mainly compared with genetic mutations and their leading effects. The growing interest in the potential link between epigenetic alterations and gene expression has led to increased efforts and research into evaluating epigenetic modifications in various illnesses (Yang et al., 2013a). In these scenarios, bacterially derived metabolites significantly impact the transcriptional profile of host cells by inducing epigenetic modifications (Hullar and Fu, 2014). These compounds play an essential role in communicating between the host cells and microbiota, and bacteria can help establish a variety of diseases by inducing epigenetic changes (Yang et al., 2013a).

Recently, the link between various epigenetic changes in CRC development and gut microbes has been a popular study topic (Fig. 16.3). However, epigenetic modification of certain genes such as *MLH1*, *GATA4*, *APC, LKB1*, and *p16INK4a* and their genetic pathways in CRC are well investigated (Bultman, 2017). SCFAs are the primary products of gut bacteria that cause histone modification (Tetro and Allen-Vercoe, 2016). Acetate, butyrate, and histone deacetylase blockers affect the epigenetic modification that may control CRC development (Lightfoot et al., 2013). However, propionate is a weak histone deacetylase inhibitor as compared to butyrate due to its bioavailability and low accumulation in colonocytes (Bultman, 2017). The major butyrate producers in the gut microbiota were *Faecalibacterium, Eubacterium*, and *Roseburia*. Other butyrate-producing bacteria, such as *Coprococcus, Clostridium, Peptoniphilus, Fusobacterium, Megasphaera, Porphyromonas*, and others, have also been discovered (Demehri et al., 2016) to influence CRC.

Studies have suggested that loss of histone H4 lysine mono-acetylation and H4K20 and H4K16 tri-methylation has been regarded as a hallmark in CRC (Fraga et al., 2005). A thorough assessment showed that H3K27 acetylation accompanied by H3K4 methylation is the probable reason for the upregulation of variant enhancer loci in tissue samples of CRC cases (Akhtar-Zaidi et al., 2012). Moreover, tri-methylation of H3K9, H3K4, and H4K20 has also been investigated in CRC (Benard et al., 2014). Methionine is produced by gut bacteria during the sulfate metabolism process and regulates bacterial metabolism by increasing the production of S-adenosyl methionine (SAM), a methyl donor for DNA methyltransferase (Ye et al., 2017). *F. nucleatum* also regulates DNA methylation using the innate immune signaling pathway as a target (Yu et al., 2017). In CRC cases, it has been reported that aberrant methylation of the cMyc gene has been observed (Sharma et al., 2010) to occur. *H. pylori* also cause methylation of genes involved in cell proliferation, adhesion, and DNA repair (Wen et al., 2010). Furthermore, miRNAs dysregulation is widely documented as a potential cancer biomarker in numerous studies (Fabbri, 2012). For example, upregulation of miR-106 and miR-21 has been noticed in stool specimens of CRC patients (Kong et al., 2012), while a bacterium *F. nucleatum* has shown a decreased miR-18a expression and regulates the innate immune signaling pathways in CRC (Wu et al., 2013). Besides, a large number of candidate miRNAs, miR-17−92, Let-7 family, miR-34b/c, miR-34a, miR-135a/b, miR-133b, miR-92a, miR-126, miR-145, miR-139, miR-143, miR-141, miR-192, miR-215, miR-144, miR-200c, miR-195, and miR-675 have also been linked to CRC (Kong et al., 2012). Overall, multiple relationships between distinct miRNAs and gut flora have been discovered to impact CRC formation (Yuan et al., 2018). In conclusion, various investigations have shed further light on the interplay between the microbiome and epigenetic changes in CRC (Yang et al., 2013a; Bultman, 2017). So far, the epigenome's current data suggested that epigenetic variables, rather than genetics, might serve as explicit disease pathogenetic indicators. In the light of this, more research is needed to fully understand the relationship between epigenetic changes and microbiota in CRC patients.

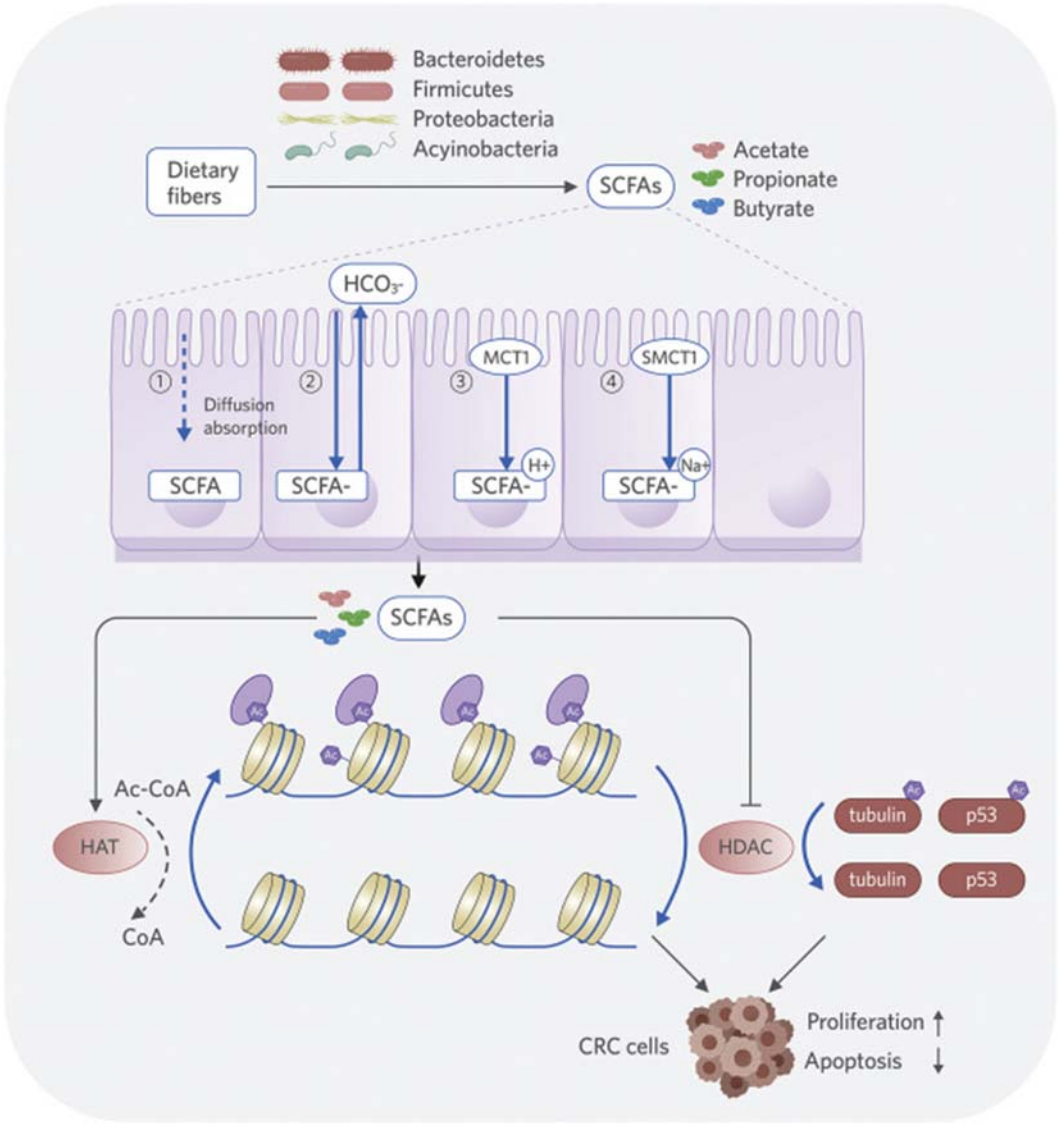

Figure 16.3

Epigenomic interactions between the gut microbiota and CRC via SCFAs. Gut bacteria in the colon or rectum produce a number of low molecular weight SCFAs, which can be absorbed by epithelial cells, and cause epigenetic modifications in DNA methylation and histone acetylation via activation or inhibition of certain enzymes such as DNMTs, HDACs (Zhao et al., 2021). *SCFAs*, short-chain fatty acids; *MCT1*, monocarboxylate transporter 1; *SMCT1*, sodium-dependent monocarboxylate transporter 1; *HATs*, histone acetyltransferases; *HDACs*, histone deacetylases; *CoA*, coenzyme A; *Ac-CoA*, acetyl coenzyme A. *CRC*, Colorectal cancer. *Reproduced with permission from Zhao, Y., Wang, C., Goel, A., (2021). Role of gut microbiota in epigenetic regulation of colorectal Cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1875(1), 188490. Copyright© 2020 Elsevier B.V.*

16.8 Conclusion

Developing scientific progress to understand the role of the gut bacterial community in the development of CRC needs to be explained. Given the current dysbiosis evidence in CRC and the association between gut bacteria and CRC progression, further biomedical research in this arena has become critical. We focused our efforts on examining the effects of gut bacteria and their metabolites in CRC patients and the relevant epigenetic pathways involved. Finally, it can be argued that combining epigenetic, microbiota, and metabolite investigations can be highly beneficial in developing targeted treatments and novel precision strategies for CRC. As a result, targeted manipulation of gut flora patterns, metabolic activity, or epigenetic alterations in reducing the risk of CRC progression could be a unique and helpful approach.

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CHAPTER 17

Microbial synthesized antibiotics in healthcare management

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17.1 Introduction

Antibiotic is derived from the Greek word "anti," which tends to mean "against," and "biotic," which means "life of microbes." Antibiotics are substances produced by microorganisms that have the potential to kill or prevent the growth of pathogens. Antibiotics are antimicrobial medications classified as antibacterial, antifungal, antiviral, or antiparasitic (Jindal and Goswami, 2020). They are substances that, at trace quantities, can suppress the growth of bacterial pathogens or germs (Donovick and Brown, 1965). Antibiotics work in a variety of ways to attack pathogens or restrict their growth. Antibiotics, relying on category, can halt protein and metabolite production, interrupt binary fission, or harm the cell wall's strength. Antibiotics were basically the sole medications used in the chemotherapy of harmful bacteria. These are characterized as low molecular mass organic natural products (secondary metabolites oridiolites) produced by bacteria that are effective against other microbes at low concentrations. Fig. 17.1 represents microbial synthesized antibiotics in healthcare management.

17.2 A brief history of microbial antibiotics

Herbal remedies were used to cure bacterial viruses until the early twentieth century. Penicillin's revelation totally altered the treatment of infections. The breakthrough came by opportunity after observing that blue mold (a fungus from the Penicillium genus) hindered the development of *Staphylococcus aureus* in culture dishes (Gelpi and Tucker, 2015), proving that some microorganisms can generate compounds that suppress the growth of other bacterial communities. The discovery of penicillin marked the beginning of a new period in the treatment of infectious diseases (Fleming, 1950). The number of antibiotics found increased exponentially from that period till the late 20th century.

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Microbial Synthesized Antibiotics

β-Lactam	Modified Tetracyclines	Streptogramins	Modified Erythromycins	Peptides	Linezolid
comprise imipenem, meropenem, and ertapenem, which are synthesized	recommended in the case of nosocomial pathogens	effective towards VRE, VREF, MRSA	effective against upper respiratory tract infections	used to treat VRE, methicillin-resistant *Staphylococcus aureus*	effective against vancomycin-resistant strains

Figure 17.1
Representation of microbial synthesized antibiotics in healthcare management.

Within years of the discovery of penicillin and the sulfonamides, health professionals were exposed to different types of other antimicrobial agents with different chemical and physical properties. In the 20 years pursuant to the medical advent of penicillin, 20 new groups of antibiotics had been developed, such as β-lactams, aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and cephalosporins. Altered β-lactams and β-lactamase antagonists were known to be successful in treating and controlling the *Enterobacteriaceae* family (Neu, 1992). One other novel antibiotic category was not presented till 1989. Each antibiotic category has a distinct structural property (scaffold). As a result of the artificial customization of scaffolds, numerous antibiotics have indeed been established. The synthetic scaffolds for many of the antibiotics used presently were ascertained by major discoveries between the mid-1930s and the early 1960s.

Available antibiotics were then altered to decrease toxic effects, broaden the spectrum of activity, or be cross-tested for enhanced bioavailability with several other antibiotics. Between 1981 and 2005, scaffolds of cephalosporins, penicillins, quinolones, and macrolides accounted for nearly 70% of all new antibiotics found (Fischbach and Walsh, 2009). The golden period of new antibiotics ended in the 1960s, and resistant strains have now surpassed drug development. Fig. 17.2 depicts a timeframe of antibacterial drug execution and the increase of resistant strains. Antibiotic excessive consumption has led to large-scale acquired resistance among many species of bacteria. As a result, two important things took place concurrently

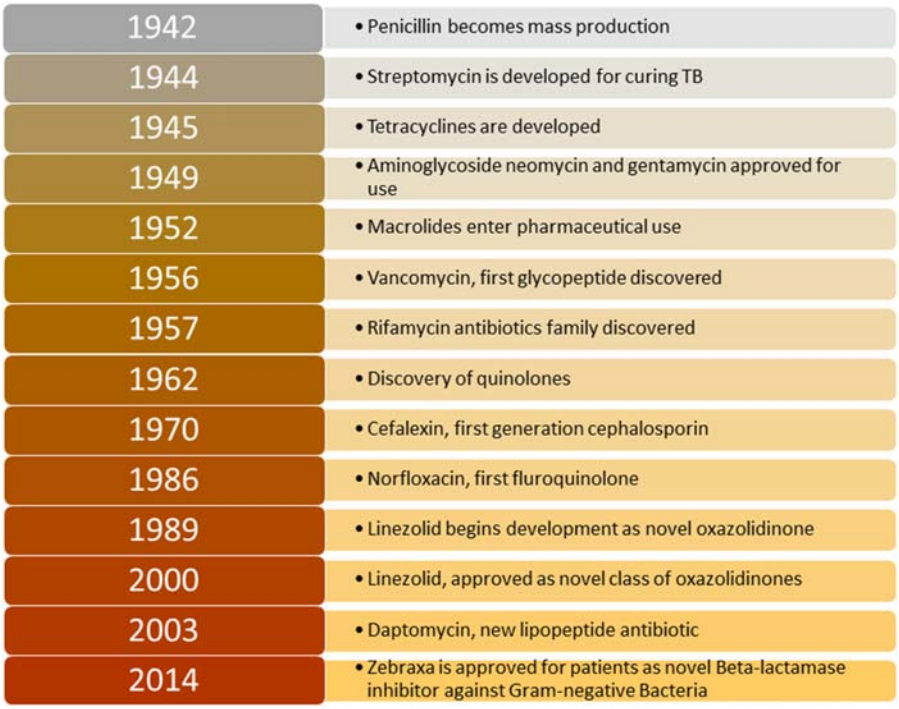

Figure 17.2
Timeline of antibiotic development.

over a century. Antimicrobials revelation has declined significantly to only a few antibiotics synthesized or found within the last century (Powers, 2004). Concurrently, resistance to antibiotics has skyrocketed, resulting in multiresistant life forms that are challenging to handle with existing antimicrobial therapy training regimes. The exploration of innovative therapeutic approaches is critical, and WHO considers it the most challenging task confronting medical research (Gyssens, 2018).

17.3 Antibiotic function

Antibiotics work in a variety of ways to attack pathogens or restrict their growth (Fig. 17.3). Antibiotics, relying on category, can halt protein and metabolite production, interrupt binary fission, or harm the cell wall's strength (Van Elsas and Bailey, 2002). Bacteria can show tolerance inherently or by acquiring impedance equipment from neighboring microbes. Bacteria use mobile resistance elements (MREs), such as transposons, plasmids, and integrons, to transport the hereditary materials necessary to cede opposition but not really the genetic makeup needed for cell function. MREs could be transferred among bacteria of various phyla, whether directly (conjugation) or indirectly

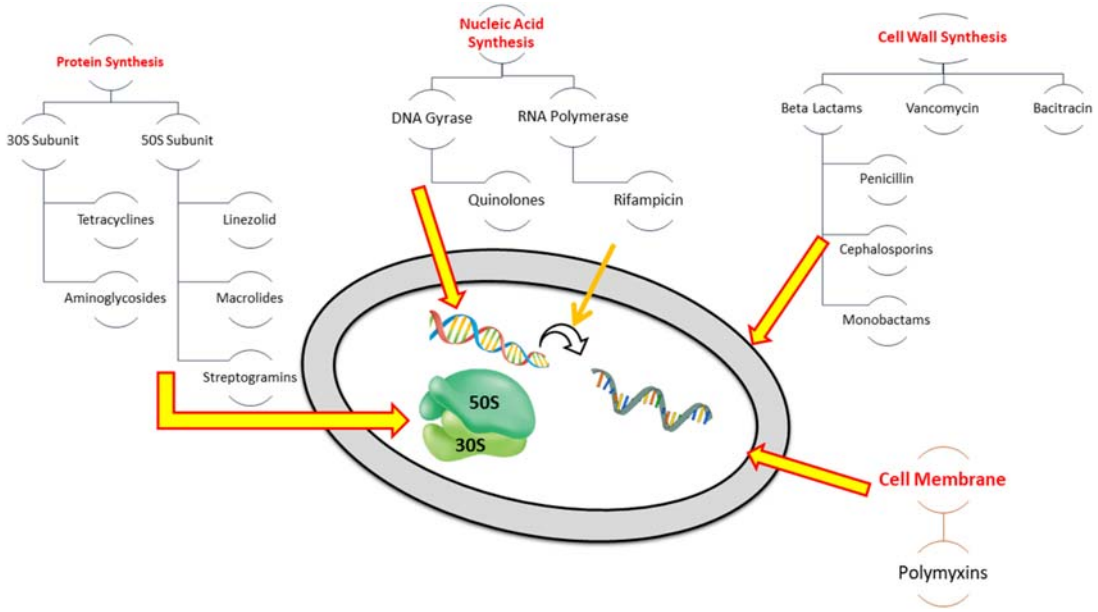

Figure 17.3
Antibiotics target to attack pathogens or restrict their growth.

(salvage of preserved components) (transformation). Evolutionary forces for MREs are required to survive to encourage the maintenance of drug-resistant processes in bacterial offspring (Holt et al., 2015).

17.4 Antibiotic production by microbial species

According to bacterial genomic sequence evaluations, just 10% of natural things manufactured by vetted isolates have indeed been found, but only 1% of the compounds manufactured by the global consortium of microbial manufacturers are recognized. Reemergence has always been a persistent issue in manufacturing antibiotic discovery programs. Yet another component is that so many manufacturing evaluations have centered on soil actinomycetes (Holt et al., 2015), which reflect a small proportion of microbial variety, both genotypically and environmentally. Provided perhaps only one actinomycete or myxobacterial genetic code transcodes 20−30 distinct genetic clusters for natural products (Liu et al., 2017; Schneiker et al., 2007; Udwary et al., 2007), another contributing factor would be an activity of a single known antibiotic in a raw or partially purified isolate that can disguise the action of new antibiotics. However, a third component is believed to be similarly vital: dissimilar bacteria frequently generate the very same antibiotics.

According to an estimate, 1 actinomycete generates streptomycin, 4 actinomycetes make tetracycline, 1.5 actinomycetes manufacture vancomycin, and 5 actinomycetes start

producing erythromycin. Actinomycetes weren't the only organisms with a global spread of antibiotic production. Andrimid and its comparative moiramide, antibiotics that inhibit acetyl-CoA carboxylase (Freiberg et al., 2004), were isolated from a tunicate-associated Pseudomonas strain from Alaska, an Enterobacter endosymbiont of a brown planthopper from Thailand, a marine Vibrio from southern California (Long et al., 2005), and a strain of Pantoea from upstate New York (Jin et al., 2006). The andrimid gene groups through the other two strains were decoded; these were almost similar, containing a transposase pseudogene, implying that these invaded hosts' lineages through horizontal gene transfer.

Several groups of naturally present substances make antibiotics, created from two or more microbial taxa, which diversified billions of years earlier. *Actinomycetes* and *Bacillus* spp. similarly manufacture complicated antibiotics such as aminoglycosides and thiopeptides, as well as their biosynthetic genes are identical and sometimes even partially syntenic (Brown et al., 2009; Liao et al., 2009). Cephalosporins are synthesized by actinomycetes, Gammaproteobacteria, and fungi in an illustrative case of cross-taxa synthesis of a unique antibiotic family and their biosynthetic gene groups are very identical. In the subsequent years, one key problem can be exploring the role horizontal gene transfer had in the cosmopolitan dispersion of antibiotic synthesis. Because Gammaproteobacteria and Firmicutes are typically possible to manage genetically than actinomycetes, strains of earlier taxa are indeed especially better adapted toward acting as hosts in the heterologous synthesis of well-circulated natural products categories (Zhang et al., 2008).

17.5 Biological activities of microbial antibiotics

The abundance of natural chemicals provides a plentiful source of antibiotic drug research scaffolds, although the polyketides, nonribosomal peptides, and aminoglycosides are probably the most therapeutically helpful of them (Wright, 2014). Polyketides, which are synthesized by polyketide synthases, are among the most highly varied natural product families and are one of the essential secondary metabolites for medical applications, farming, and manufacturing. Pikromycin, for instance, is the very first recognized polyketide antibiotic synthesized from *S. venezuelae* in 1950 (Vazquez, 1967). Pikromycin is shown to be extremely effective in multidrug-resistant respiratory infections (Woo et al., 2014). Erythromycin A (Table 17.1) (Fig. 17.4), a novel polyketide antibiotic with clinically important therapeutic applications, was introduced in 1952 as broad spectrum antibiotic made from the bacteria *S. erythraea*. In addition to treating a broad diversity of infectious diseases caused by bacteria, including respiratory infections, gastrointestinal infections, whooping cough, syphilis, as well as acne, the antibiotic is also quite useful in those who have had negative reactions to penicillin in the past (Cobb et al., 2013). Tetracyclines (Table 17.1) (Fig. 17.3), on the other hand, are effective against both Gram-positive and Gram-negative bacteria, in contrast to many natural antibiotics that are ineffective against Gram-negative bacteria (Demain, 2009).

Table 17.1: Origin and mechanism of action of microbial-derived antibiotics.

Antibiotics	Origin	Mode of action	Target pathogen	References
Erythromycin A	*Saccharopolyspora erythraea*	Antibacterial	Skin infection, pneumonia, Sinusitis, urogenital and chlamydial infection	Sagar et al. (2019)
Tetracycline	*Streptomyces rimosus*	Antibacterial	*Arachnia, Actinomyces israelii, Bacillus anthracis*	Chopra and Roberts (2001)
Vancomycin	*Amycolatopsis orientalis*	Antibacterial	*Streptococci* and *enterococci species*	Geraci et al. (1956)
Streptomycin	*Streptomyces griseus*	Antibacterial	multidrug treatment of pulmonary tuberculosis	Schatz et al. (1944)
Nisin A	*Lactococcus lactis*	Antimicrobia	*Streptococcus sanguinis, Streptococcus mutans, Enterococcus faecalis*, and *Lactobacillus acidophilus*	Kappers et al. (2005), Gyawali and Ibrahim (2014)
Reuterin	*Lactobacillus reuteri*	Antimicrobia	against bacteria, yeast, fungi, and protozoa	Gyawali and Ibrahim (2014), Talarico et al. (1988)
Amphotericin B	*Streptomyces nodosus*	Antifungal	neutropenic patients, cryptococcal meningitis in HIV infection	Abu-Salah (1996), Tevyashova et al. (2013), Tareq et al. (2015)
Amoxicillin, ampicillin	*genus Streptomyces, Pseudomonas species*	Antibacterial	Septicemia, respiratory and genitourinary tract infections, Rhinosinusitis	Akhavan et al. (2020), Peechakara and Gupta (2021)
Cephalosporin	*mold Acremonium*	Antibacterial	Gram-negative bacteria, respiratory and urinary tract infection	Bui and Preuss (2021)
Gentamycin	*Micromonospora purpurea*	Antibacterial	Gram-negative bacteria infections, Urinary tract infection	Waters and Tadi (2021), Habak and Griggs (2021)
Metronidazole	*Streptomyces* spp.	Antibacterial	*C. difficile* infections, Protozoal infections, Gram-negative bacterial infections	Rineh et al. (2014), Weir and Le (2021)

As earlier said, penicillin is a well-known antibiotic secondary metabolite formed from the bacterium *Penicillium notatum* that is effective against Gram-positive bacteria that cause diseases such as scarlet fever, pneumonia, gonorrhea, meningitis, as well as diphtheria, among others (Tan, 2015). Penicillin, like vancomycin, is an antibiotic that does not need the presence of ribosomes. In addition to having bioactivity that may be utilized for therapeutic reasons, nonribosomal peptides, which are produced by the enzyme nonribosomal peptide synthetase, also happen to be some of the most common and chemically varied secondary metabolites on the planet. In addition to vancomycin (Table 17.1) (Fig. 17.3), there are many additional nonribosomal peptides that are efficient

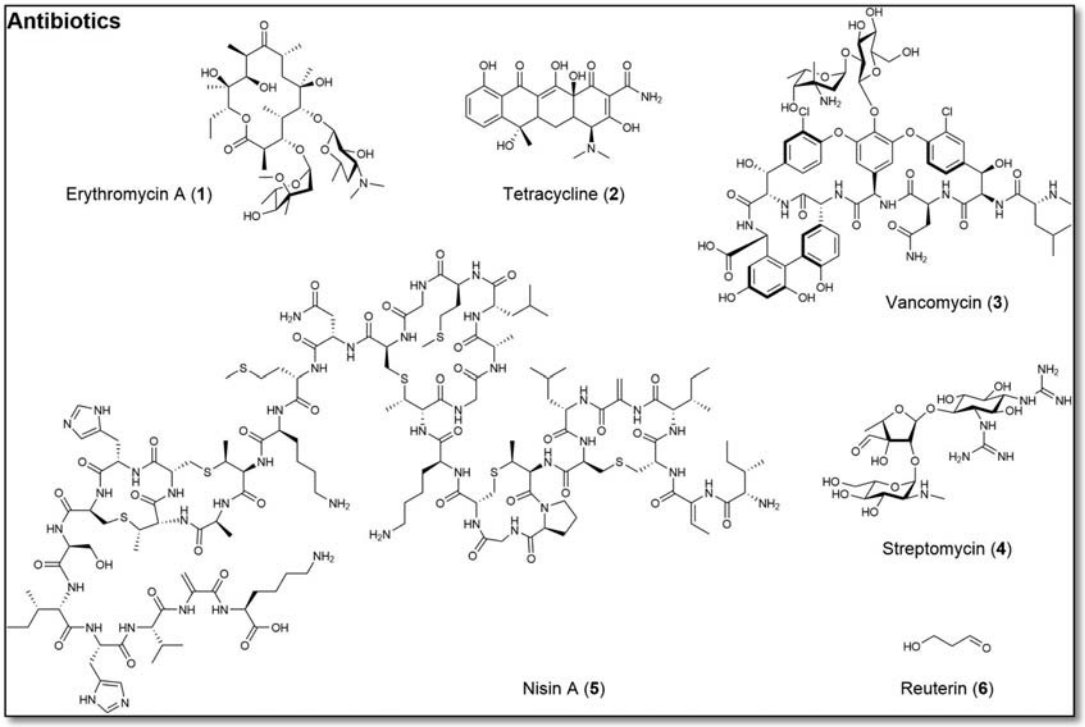

Figure 17.4
Structures of antibiotics with antibacterial activity (Pham et al., 2019).

toward disease-causing bacteria such as *Clostridium difficile, Streptococcus pneumoniae, Staphylococcus epidermidis*, *Listeria monocytogenes*, as well as *methicillin-resistant Staphylococcus aureus* (MRSA) (Dasgupta, 2012).

Aminoglycosides, a whole other group of antibiotics, impede protein synthesis by binding to a rRNA subunit of the 30 S bacterial ribosome. Streptomycin, (Table 17.1) (Fig. 17.3), the very first aminoglycoside developed around 1944, is helpful in pulmonary tuberculosis (Schatz et al., 1944). Seeing as streptomycin's development, aminoglycoside antibiotics, including kanamycin, gentamicin, sisomicin, but also lividomycin, were identified and frequently often used to cure pathogenic agents which have already evolved tolerance to streptomycin following continuous usage (Park et al., 2013). Notwithstanding its remarkable antibacterial action, aminoglycosides have encountered resistance in microorganisms. Semisynthetic aminoglycoside antibiotics are particularly intended to act toward such enzymes in an attempt to face antibiotic resistance to aminoglycoside antibiotics, which is a common occurrence (Pham et al., 2019). Semisynthetic aminoglycoside antibiotics like amikacin, netilmicin, dibekacin, or even isepamicin, have been generated from natural materials such as plants and animals.

Natural antimicrobials have also proved beneficial to the food sector in ensuring food security against infections found in food. A number of chemicals produced by lactic acid bacteria, for example, have been shown to inhibit the formation and growth of a broad range of other microbial species. It is highly active against Gram-positive bacteria, which are resilient to traditional antibiotics. Nisin A (Table 17.1) (Fig. 17.3), a *Lactococcus lactis bacteriocin*, recognized for preservation of food in more than 50 countries, is also highly active toward Gram-positive bacteria that are resistant to many typical antibiotics (Kappers et al., 2005). It was discovered that the antibacterial compound reuterin (Table 17.1) (Fig. 17.3) from the bacterium *Lactobacillus reuteri* had antibacterial action toward foodborne pathogens when it was tested in dairy products that include milk and meat too (Gyawali and Ibrahim, 2014).

Amphotericin B (Fig. 17.4 and Table 17.1) is a traditional polyene antifungal product of *Streptomyces nodosus* that is used to treat life-threatening fungal infections caused by *Aspergillus* species. It is especially effective in patients who have had organ transplants, aggressive chemotherapy, or have acquired immunodeficiency syndrome (Tevyashova et al., 2013). Isolated from the marine bacteria *Bacillus licheniformis*, the glycolipids ieodoglucomide C (Fig. 17.5 and Table 17.1) and ieodoglycolipid demonstrated antifungal activity with a 21 g/L minimum inhibitory concentration (MIC) against *Rhizoctonia solani, Aspergillus niger, Colletotrichum acutatum, Botrytis cinerea, and C. albicans* (Tareq et al., 2015).

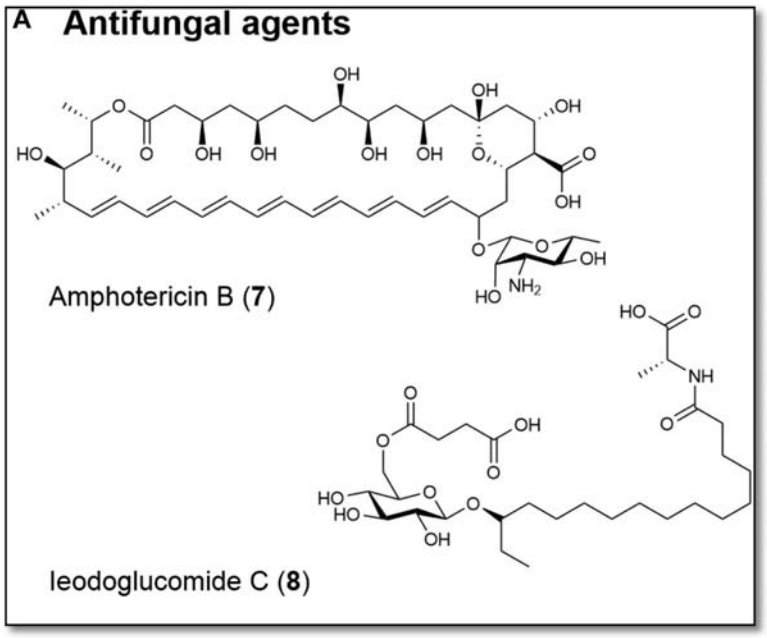

Figure 17.5
Structure of antibiotics with antifungal activities (Pham et al., 2019).

17.6 Antibiotics essential to human healthcare

It's indeed impossible to imagine life on this planet without bacteria. The biosphere is ruled by microorganisms, and Earth is a microbe-dominated planet (American Society for Microbiology, 2004). All life on Earth is descended from microbes. These are the most important system for studying evolution. They offer quick generation times, genetic flexibility, unrivaled experimentation size, and controllable research methods. These also resurrected the present system of all life on Earth's evolutionary relatedness, called the Tree of Life. Microbes were the first life form on Earth. According to estimates, there are 5×10^{31} microbiological cells having a total measure of 50 quadrillion metric tonnes. The process of photosynthesis by microorganisms is at a higher rate than by green plants. In fact, microbes are responsible for the world's biomes, and over 60% of the world's biomes are found in one place. Our body cells are made up of microorganisms to greater than 90% of bacterial cells. Those that have been sterilized are much less healthy than animals that have been colonized by microbes. Pathogens include a vast range of microorganisms (bacteria, fungi, and viruses) that have been identified. The presence of bacteria and viruses has been related to stomach ulcers and cervical cancer, according to recent study findings. It is possible that microbes are responsible for a variety of different diseases, including inflammatory bowel disease, diabetes, rheumatoid arthritis, sarcoidosis, Systemic Lupus Erythematosus, as well as cardiac disease. A natural microbial compound with a long and illustrious past, *Aspergillus oryzae* has been making koji from rice for about five thousand years, according to historical records. For the last four thousand years, the fungus *Penicillium roqueforti* has been utilized in making cheese. As far back as three millennia, people in Asia and Egypt making soy sauce from *Aspergillus oryzae* and bread from *Saccharomyces cerevisiae* are examples of traditional fermentation (Hölker et al., 2004).

Around the year 2002, biologically active chemicals numbering up to 22,500 that had been derived from microorganisms were discovered (Berdy, 2005). 20,000 antibiotics were contained. The percentage of bioactive components generated by single-celled bacteria (primarily *Bacillus and Pseudomonas*) is 3800 (17%), while 10,100 (45%) is through filamentous bacteria (*actinomycetes*), but also 8600 through fungi (38%). 20,000−25,000 substances are inactive microbial components. Organic chemicals have around five times the frequency of drug activity as microbial natural ingredients. Antibiotics and immunosuppressants are among them. Biofilms have yielded about 9000 physiologically active compounds, with over 60 of these being beneficial in health, agriculture, as well as research (Kieser et al., 2000). Species of the genus *Streptomyces* produce around 80% of all these. Although the majority of identified natural products come primarily from the dry area, 129 biologically active substances were separated from aquatic microorganisms between 2000 and 2003 (Xiao-hong et al., 2004). Bacteria can account for almost 40% of sponge biomass (Piel et al., 2004). Sponges, by far, are the most dominant contributor of

natural compounds, but associated bacterial symbionts are frequently the manufacturers. Prokaryotic genes, for example, encode the manufacture of anticancer polyketides classified as onnamides and theopederins in marine sponge *Theonella swinhoei*. The genes are comparable to some of those identified in terrestrial beetles *Paederus and Paederidus*, that generate the anticancer polyketide pederin; however, in this case, their synthesis is owing to an uncultured *Pseudomonas symbiont*. Therefore, it seems like polyketides were produced via symbiotic bacteria both in beetles and sponges. Many chemicals are frequently produced by a single microbe (Bode et al., 2002). Micromonospora strain that produces gentamicin produces 50 isolatable secondary metabolites. *Streptomyce ssp*. produces 12 chemicals as another instance of a bacterial product.

17.7 Microbial synthesized antibiotics and their economic history

In 1900, infection was the leading cause of death worldwide (Yoneyama and Katsumata, 2006). It is now the second most significant risk factor for mortality and the third in developed countries. The specific action of microbial secondary metabolites on dangerous fungi and bacteria brought in the advent of antibiotics, and we have profited after this astonishing feature of "wonder medications" for the past 50 years. Alexander Fleming identified the very first clinically usable antibiotic in 1928 from the fungus *P. notatum;* however, purity, separation, as well as composition also weren't recognized for the next 15 years. This has been done at Oxford University via the outstanding work of Howard Florey, Norman Heatley, Ernst Chain, and also Edward Abraham, who have all contributed to this accomplishment. In the 1940s, the Selman Waksman team at Rutgers University, consisting of Boyd Woodruff, Albert Schatz, and Hubert Lechevalier, found powerful antibiotics manufactured by actinomycetes, or filamentous bacteria, which were then used to treat a variety of diseases. As a result of these efforts, new results have been discovered. In 1948, the team of Benjamin Duggar at American Cyanamid's Lederle Laboratories (now Wyeth) achieved a breakthrough in the discovery of aureomycin, which was produced by *S. aureofaciens* in the laboratory.

The drug aureomycin, accepted for its utilization toward Gram-positive and Gram-negative bacteria, was discovered the same year it was approved. The discovery of oxytetracycline (OTC) at Pfizer was immediately followed by a federal investigation. Terramycin (OTC) is a completely different molecule from chloramphenicol, which was first researched by numerous researchers at Parke-Davis (now Pfizer), and that collaborating academics at Yale and the University of Illinois discovered. On the other hand, Chloramphenicol which was being manufactured from the bacteria *S. venezuelae* may also be produced chemically. This is due to the simplicity of the compound's structure. Aminoglycosides, like kanamycin, have been discovered by Hamao Umezawa and colleagues of the Department of Microbial Chemistry in Tokyo, while gentamicin has been discovered by Marvin Weinstein and colleagues at Schering-Plough Pharmaceuticals. These compounds, on the other hand, have

a wide range of action, are quite stable, and are bactericidal in nature. A number of microbiological antibiotics, such as penicillins and cephalosporins, also tetracyclines and aminoglycosides, chloramphenicol, macrolides, as well as glycopeptides, are among the most widely used. They had a significant part in improving the anticipated lifespan in the United States for 47 years in 1900, 74 years for males but 80 years for women in 2000, representing a significant increase (Lederberg, 2000). Antibiotics were, for the most part, the only drugs utilized in the treatment of germs that were hazardous to humans. These are organic natural products with a low molecular mass (secondary metabolites oridiolites) that are created by bacteria and are effective against other microorganisms when used in low doses (secondary metabolites oridiolites). One thousand two hundred and fifty percent of the 12,000 antibiotics identified in 1995 were derived from actinomycetes of Streptomyces genus, other actinomycetes comprising 11%, 12% from nonfilamentous bacteria, while 22% were derived from filamentous fungi (Berdy, 1995; Bull et al., 2000). In the late 1970s, new bioactive compounds derived from microorganisms became identified at an astounding rate, such as 200−300 per year. Nevertheless, there has lately been a significant decrease in this kind of findings. As antimicrobials, over 350 agents entered the global market (Bronson and Barrett, 2001). These are antibiotics that are either natural or semisynthetic in nature, as well as substances that are entirely synthetic in nature.

In the pharmaceutical industry, the most often used antibiotics consist of cephalosporins (45%), penicillins (15%), quinolones (11%), tetracyclines (6%), macrolides (5%), aminoglycosides (including ansamycins), glycopeptides (including polyenes), and polyenes (Strohl, 1997). Sulfa medicines, azoles, and oxazolidinones are examples of strictly synthetics (Linezolid). Furthermore, quinolones and fluoroquinolones, family of broad spectrum, systemic antibacterial agents were structured after the naturally occurring chemical quinine. In 1997, 42% of the overall 25 best-selling medications were natural/obtained from natural materials. Antibiotics accounted for 67% of total sales. In 1996, the worldwide economy for antibiotics was $26 billion (Erdmann, 1999), and it increased to $32 billion in 2001(Fig. 17.5) (Projan and Youngman, 2002). In case antiviral agents are included, the number rises to $55 billion in 2000. In 2001, the market for antibiotics derived from *Streptomyces* alone was valued at $25 billion (Hranueli et al., 2005). In 2002, the antifungal medication market was worth $4 billion (Fig. 17.5) (Connors and Pollard, 2004).

Several antibiotic companies achieved record-breaking revenue levels during their earlier days. Antibiotics were thought to be a powerful drug that produces immediate effects and are effective against practically all infections, including viruses. The cost of antibiotics was associated with their potency; hence, the costliest antibiotics were considered the most potent treatments (Nahar et al., 2020). Cephalosporins were purchased for 11 billion dollars, penicillins were purchased for 8 billion dollars, and carbapenems, β-lactams were purchased for 3 billion dollars, for a total purchase price of around $22 billion for antibiotics. It was estimated that macrolides, which mostly contained clarithromycin, azithromycin, and

erythromycin, generated marketing revenue of 7 billion dollars. Tetracycline marketings were 1.4 billion dollars, while aminoglycoside marketing totaled $1.8 billion, according to the latest figures. One billion dollars was generated by the glycopeptides, vancomycin, and teicoplanin together. The whole quinolone industry was worth 6.4 billion dollars, with fluoroquinolones accounting 3.2 billion dollars, with levofloxacin accounting for the lion's share of this figure (Levaquin). Sales of antifungal and antiparasitic medications totaled $4.2 billion, with synthetic azoles accounting for $2 billion of that total (Georgopapadakou, 1998). An estimated $16 billion was spent on antiviral drugs and vaccines in 2010. Specific antimicrobials with markets in excess of 1 billion dollars comprise augmentin, and a mixture of the semisynthetic penicillin as well as the beta-lactamase inhibitor, clavulanic acid (2 billion dollars), quinolones ciprofloxacin (5Cipro; 1.8 billion dollar), and also levofloxacin/ofloxacin (5Levaquin, Floxin; 1.1 billion dollars), and the semisynthetic erythromycins azithromycin (Wilson, 2002; Bull, 2004).

17.7.1 β-Lactam antibiotics

In relation to the availability, the β-lactams are by far the most significant antibiotic. They account for a sizable portion of the antibiotic industry. Penicillins and cephalosporins, clavulanic acid, as well as carbapenems are among the antibiotics mentioned. Penicillin G was being utilized in the following uses in the 1990s: 12% for direct healthcare, 65% in conversion to 6-aminopenicillanic acid (6-APA), 20% in conversion to 7-aminodeacetoxy cephalosporanic acid (7-ADCA) and intermediates, and 2% in agriculture. Semisynthetic penicillins are being created using 6-APA, while commercialized cephalosporins are being created using 7-amino-cephalosporanic acid (7-ACA) and 7-ADCA. There are over 50 cephalosporins in the marketplace (Schmitt et al., 2004).

The parts comprising the whole of penicillin V manufactured for different purposes are as follows: 19% for primary medicinal usage, 59% in conversion to 6-APA, also 23% in conversion to 7-ADCA, and several intermediates. From 6-APA derived via penicillins G and V, 48% is used to make ampicillin, and 27% is used to make amoxicillin. 7-ACA is a cephalosporin C derivative that trades for $100 to $200 per kg. Through the mid-to-late 1990s, the industry had presented a number of b-lactams: 9680 t 6-APA, 4730 t cephalosporin C, 2140 t 7-ADCA, 2360 t 7-ACA, and 2340 t additional cephalosporin intermediates (Data from Michael Barber and Associates, cited by Elander). By 2003, all β-lactam output was surpassed, which happened to be 60,000 t (Robin et al., 2003). Penicillin titers exceed 50 g/L, while cephalosporin C titers are around 25 g/L. The survival rate is greater than 90%. *S. cattleya*, *Erwinia carotovora sub sp. carotovora, Serratiasp*., and *Photorhabdus luminescens* produce natural carbapenems like thienamycin (Robin et al., 2003). Carbapenems are immune to most beta-lactamases. Market carbapenems comprise imipenem, meropenem, as well as ertapenem, which are synthesized. Thienamycin is the

most powerful as well as broad spectrum antibiotic currently available. Despite being a β-lactam, it is not a part of the penicillin or cephalosporin families. It is effective toward Gram-positive and Gram-negative bacteria, and also Pseudomonas. This unique component was identified in Spain from *S. cattleya*, a new soil species (Kahan et al., 1979). This culture, surprisingly, generates penicillin N as well as cephamycin C. Considering the limited level of synthesis and molecular instability, the structure of thienamycin was discovered in 1976. The key distinctions between normal β-lactams as well as thienamycin are the presence of a carbon atom rather than a sulfur atom in the ring linked to the β-lactam ring and the trans configuration of the β-lactam ring's hydrogen atoms. The chemical is immune to plasmid as well as chromosomal-β-lactamases, and also has antibacterial effects immune to topenicillins, cephalosporins, and aminoglycosides, in complement to its broad spectrum and key effects. It is reactive as a result of a form of self-destruction in which the b-lactam ring of one thienamycin molecule is destroyed with aminolysis—more by amine group of the next thienamycin molecule. Addressing the issue, chemists synthesized imipenem, a semisynthetic carbapenem, introducing a formididoyl group to the side chainamine. Ipenem was discovered not just as being of higher stability than thienamycin, but also active. The activity method of imipenem (and thienamycin) is bactericidal suppression of cell wall peptido-glycan production. In humans, imipenem was discovered to really be processed via renal dehydropeptidase-I, an enzyme that functions as a β-lactamase.

Enzyme seems to play no influence on human metabolism. Researchers could create a synthetic antagonist, cilastatin, that combined with imipenem to create the desired combination of the medication that was primaxin. Primaxin was first used in medicine in 1985. Thienamycin's distinctive action is due to multiple reasons: (1) it suffuses the Gram-negative outer cell membrane via porin channels at 10−20 twice the rate of classical b-lactams; (2) this isn't destroyed by periplasmic b-lactamases; and (3) if it impedes all of the penicillin-binding proteins (PBPs), then it is most energetic toward PBPs-2 and-1b rather than PBP-3, which Inhibiting PBPs-2 and-1b sequentially change pathogen cells to nongrowing spheroplasts that perish quickly. Inhibiting PBP-3, on the other hand, prevents septum development, and leads to longer viable filaments that take more time to perish. (4) There is a lengthy lag until the regeneration of any unkilled spheroplasts following thienamycin elimination. Unkilled filaments (containing 20 or even more cells per unit) septate and regenerate promptly in the presence of typical b-lactams.

17.7.2 Tetracyclines

Approximately 90% of natural antibiotics failed to suppress Gram-negative organisms such as *E. coli*. The causes for this included the outer membrane, which features (1) tiny porin channels which prevent relatively small hydrophilic substances from entering the cell, and

(2) the presence of a lipopolysaccharide moiety that prevents the intracellular absorption of lipophilic antibiotics (Nikaido, 1996). As a result of this, Gram-negative microorganisms usually consist of multidrug excretion systems that are capable of eradicating a wide variety of medications from their human host. Unlike Gram-positive cells, Gram-negative cells have an exterior membrane that is distinct from their inner (cytoplasmic) membranes. Even though it is rich in protein, it is made up of just a small number of protein-containing amino acids. Most of them function as holes through which tiny hydrophilic molecules may travel or as structural proteins. In addition to transporting diverse substrates, other proteins are involved. Colicins, for example, are phage and protein receptors that are found in many of its proteins. The outer membrane, except for phospholipase and protease, is devoid of enzymes, according to the literature. Lipopolysaccharide, the lipid structure, is present solely in the outer leaflet of the outer membrane bilayer. It is composed of the peptidoglycan and the inner membrane, which is the main permeability wall and includes enzymes involved in the production of fatty acids and cholesterol as well as active transport proteins and other components. Maltose-binding protein is a sugar transport protein that is found in the watery section between the internal as well as exterior membranes. The periplasm contains a diverse array of proteins, the majority of which are degradative (alkalinephosphatase, rNase) or binding proteins for the purpose of active transport, such as alkalinephosphatase and rNase. Noninfectious diseases such as malaria, Lyme disease, ulcer-causing bacteria *Helicobacter pylori*, anthrax, and other noninfectious illnesses are all treated with antibiotics such as tetracyclines.

Tetracyclines are effective against both Gram-positive but also Gram-negative bacteria, as well as intracellular pathogens such as mycoplasmas, chlamydiae, and rickettsiae (inhibition of angiogenesis in cancer). According to the Food and Drug Administration (FDA), OTC manufacturing industry titers are roughly 100 g/L, but chlortetracycline titers exceed 33 g/L after just 156 hours, indicating that the drug is toxic. Genetic research has been carried out on *S. rimosus*, the plant that produces OTC (Petkovic et al., 2006).

17.7.3 Macrolides

It takes around 10−13 g/L to create the macrolide erythromycin. The yearly production capacity is 4000 t. A significant quantity is used as erythromycin A, a total of 1500 t of azithromycin, and 1500 t of clarithromycin, including 400 t of roxithromycin were produced from the remaining quantity with the remainder being chemically converted into some other antibiotics.

17.7.4 Other important antibiotics

The glycopeptides include vancomycin, teicoplanin, aminoglycosides leave alone streptomycin, gentamicin, ansamycins containing rifampicin, fosfomycin, and chloramphenicol, mupirocin, quinolones, and some fluoroquinolones, as well as other antibiotics, have been around for decades.

17.8 Demand and need to develop new antibiotics

When the industry looked to be approaching a stage where it was in decline approximately 30 years back, complexity as well as increased cost of extracting unique antibiotic structures along with compounds having new different procedures of action for such a kind of applications were made clear, and the industry appeared to be on its way out. This is reasonable given the poor likelihood of discovering effective antibiotics in microorganisms. Below are the perspectives of field workers.

1. Just one out of every 10,000−150,000 molecules got made into clinical use (Demain and Lancini, 2001; Woodruff et al., 1979).
2. During the screening procedure for novel pharmaceutical products, almost 3600 active candidates were eliminated for each, and every one that is eventually approved for market release.
3. Only three viable antibiotics had been found following a 10-year screening of 400,000 microbe cultures (Demain, 2009).
4. Only one of 5000 chemicals tested reached clinical testing, but only one of them was FDA approved (Buchwald and Bodor, 2002).
5. Of the 5000−10,000 chemicals evaluated, 250 started developmental testing, 5 drug candidates advanced to Phase II, 4 advanced to Phase III, 1.5 advanced to Phase III, but one drug was authorized by the FDA. Plants revealed comparable findings (Malcolm and Jones, 1991).

While chemists had already been effectively enhancing natural kinds of antibiotics through the semisynthesis method for decades, novel diagnostic approaches were urgently needed to extract novel bioactive compounds from nature throughout the 1970s−1980s (DiMasi et al., 1994). However, from the 1960s to the late 1980s, the frequency of anti-infective investigational novel medicines (INDs) fell by half. Several corporations have scaled back existing attempts in natural product development in order to make big expenditures on recombinant DNA technologies. By the 1970s, most people felt that the golden period of antibiotic research had come to an end, and they were right. Nonetheless, several novel antibiotics were introduced to the market and later proved to be economically successful in the 1970s and 1980s, largely because of the production of special aim screening strategies through certain forward-thinking companies. Cephamycins (e.g., cefoxitin), fosfomycin, carbapenems (e.g., thienamycin), mono-bactams (e.g., aztreonam), glycopeptides (e.g., vancomycin, teicoplanin), aminoglycosides (e.g., amikacin, sisomicin), and semisynthetic variants of cephalosporins, as well as macrolides, were among them. Since thienamycin has such a high potency, it took over 25 years of worldwide screening before it was identified, which is rather remarkable. We now know that carbapenems are a prevalent kind of antibiotic. In labs all across the globe, other members of this family have been discovered; however, none of them have the intensity and scope of action as thienamycin has.

The answer is most likely found in the low-scale production through wild strains and the fragility of the plant. Traditional screening procedures clearly missed this activity, and it was only after the development of highly specialized and sensitive contemporary assay technology that this action was uncovered. Thienamycin was discovered using a sensitive technique of activity screening; however, the details of this process were never revealed. Additionally, carbapenems have greater tolerance and resilience to hydrolysis than dehydropeptidases which still remain intravenous, such as meropenem and biapenem, were added to the resultant commercial product, imipenem1cilastatin, to make it more effective. Faropenem is an oral carbapenem., CS-834 and MK-826 (Sader and Gales, 2001).

Considering the popularity of these subsequent advances in the 1970s as well as in the 1980s, the creation of novel antibiotics reduced considerably. Regrettably, the Surgeon General of the United States, William H. Stewart, announced to Congress in 1969: "The time has come to close the book on infectious disease" (Wilson, 2002). Yet, microbiologists recognized that, due to developing resistance to pathogenic germs, technology had still not fought the battle against microbial infections. Indeed, technology will never be able to defeat infections entirely, and we will have to be content with staying one point ahead of them for the foreseeable future (Lederberg, 2003). As a result, the hunt for novel antibiotics must never be put on hold.

The resistance to antibiotics is determined by

1. inhibition through the enzymes, such as β-lactamase;
2. trying to pump the antibiotic out of the cells;
3. altering the aim in order to limit antibiotic adherence to the target;
4. increased target production;
5. attempting to avoid the target's demand; and
6. reducing antibiotic absorption (Singh and Barrett, 2006).

New medicines are continually needed for a variety of purposes, which has led to the development of antibiotic resistance to hazardous bacteria:

1. the appearance of at least 30 emerging diseases in the 1980s and 1990s, including *AIDS*, the *Hanta* virus, the *Ebola* virus, *Cryptospiridium*, Legionnaire's disease, Lyme disease, *E. coli* 0157:H7, and others (DaSilva and Iaccarino, 1999);
2. the presence of innately resistant bacteria, such as *Pseudomonas aeruginosa*, which causes death from serious infections, burn infections, and chronic and fatal lung infections in cystic fibrosis patients, *Stenotrophomonas maltophilia, Enterococcus faecium, Burkholderia cepacia, and Acinetobacterbaumanni* (Tenover and Hughes, 1996);
3. the human toxic effects of some existing substances.

The formation of opposition is reflected by the remark, "hospitals are scary areas it is if you're sick, but even when you're not." In the United States, around 2 million nosocomial

illnesses occur each year (Levin and Bonten, 2004). *S. aureus* causes over half of all nosocomial pathogens and ends up killing 100,000 individuals in the United States each year. *Staphylococci* and other bacteria that create biofilms thrive on incisions, scar tissue, and surgical devices as kinds of joint prosthesis, spinal devices, vascular prosthetic grafts, and also include heart valves. Many bacteria do exist in nature as biofilms (Sauer et al., 2002; Johnston, 2004). Biofilms arise as free-swimming (planktonic) cells that connect to a substrate. Antibiotics have little effect on biofilms. *P. aeruginosa* lives as a biofilm in the lungs of cystic fibrosis sufferers. The cells in the film interact with one another via quorum sensing via autoinducers. AI-2, a tiny molecule containing boron, an unusual ingredient of microbial molecules, is one of the most important. During the first five phases of biofilm development, more than half of the *P. aeruginosa proteome*, or 4800 proteins, show a total sixfold or larger shift in its activity.

The stages are described as:

1. reversible adhesion,
2. permanent adherence (cluster centers form, mobility is lost, Las Quorum sensing regulon is triggered),
3. maturation I (rhlquorum sensing system activates),
4. maturation II, and
5. dispersal (pores and channels form, which release planktonic bacteria).

Among the proteins that are enhanced during the process are hypoxic processes and denitrification, efflux pumps, including quorum sensing proteins. Many commercially available antibiotics have little impact on the resistant microbes that have developed over time. *Enterococci* are immune to every antibiotic that has been discovered (Chu et al., 1996). Several microbes exist that are not generally pathogenic but may infect humans with weakened immune systems. In the years past, there has been a significant deal of concern regarding the development of resistance within Gram-positive infections, also referred to as methicillin-resistant bacteria. From 1987 to 1992, clinical isolates of penicillin-resistant *Streptococcus pneumoniae*, the most frequent cause of bacterial pneumonia, rose 60-fold in the United States (Breiman et al., 1994). Infections caused by *MRSA* are on the increase all over the world. Vancomycin, a glycopeptide antibiotic, has long been the medicine of choice for treating infections caused by such organisms; nevertheless, resistance to this glycopeptide antibiotic is on the rise, notably in nosocomial vancomycin-resistant Enterococcus infections. Having said that, some vancomycin-resistant enterococci (VRE) and *Staphylococcus aureus* are curable with the related glycopeptide antibiotic (VRSA) (Alekshun and Levy, 2007).

However, novel glycopeptides that are active against resistant bacteria have been produced. Oritavancin, tele-vancin, as well as dalbavancin are examples (Kim et al., 2007). Dalbavancin is more active than vancomycin against MRSA as well as VRE, except for

those with van A resistance. One essential feature is that it can only be taken once each week. Attempts are being taken to find or create chemicals that disrupt mechanisms of resistance (Wright, 2000). Clavulanic acid, a natural b-lactamase inhibitor, has long been used in this manner. Furthermore, it was discovered that the plant natural substance 50-methoxyhydrocarpin inhibits the NorA multidrug resistance pump, thus increasing norfloxacin action (Stermitz et al., 2000).

Increased natural product investigation is warranted due to unmet demands, the extraordinary variety of structure and behavior, their use as biochemical probes, novel and sensitive assay methods, advancements in separation, purifying, and characterization, and new manufacturing processes. Even though many new substances have been synthesized via genetic approaches involving gene alteration or transfer between organisms to form hybrid molecules (i.e., combinatorial biosynthesis) (Hutchinson, 1997; McAlpine, 1998), production titers remain rather low. Additional secondary metabolites for application in healthcare still are desperately needed. The huge variety of microbes is an important consideration for prospective medication research. Bacteria have been around for more than 3B years, and eukaryotes have been there for 1B years (Vicente et al., 1999). Because 95%–99.9% of the organisms in the environment still have not been cultivated by the laboratories, for secondary metabolite production, mainly a few fungi and bacteria were researched. As per estimations, 30 g of soil contains 20,000 common bacterial species and maybe 500,000 uncommon ones (Dykhuizen, 1998). During the period of 1983 to 1987, there were 16 antibiotics authorized, 14 from 1988 to 1992, 10 during 1993 to 1997, six from 1998 to 2002, and four in the period 2003 to 2007. There have been 17 novel natural antibiotics introduced between 2001 and 2005, despite the decline in the rate of new antibiotic discovery. Antibiotics and an anticancer agent were among the items on the list. Concerning the reason for the prescription of antibiotics, physicians' fear of dangerous bacterial infection and efforts to protect a patient's health are major factors in their excessive antibiotic prescribing. This emphasizes the importance of creating fast diagnostic tests/point-of-care testing to eliminate ambiguity and allow for a more appropriate prescription (Ashiru-Oredope et al., 2021).

17.9 Antibiotics' effectiveness since the late 1990s

Most antibiotics were produced between 1950 and 1980 (the "low-hanging fruit"), after which the pace of discovery dropped significantly. Between 1983 and 1987, 16 new antibiotics were authorized, 14 during 1988 and 1992, 10 from 1993 to 1997, six during 1998 and 2002, but four from 2003 to 2007. Considering the decrease in number, several major contributions to the listing of valuable products have occurred. There seems to be an intriguing connection between antibiotic resistance and the discovery and manufacturing of novel antibiotics (Silver, 2007). A variety of "old" chemicals became readily available that

had earlier been unavailable given the limited antibacterial spectrum, that was limited to the kind of Gram-positive bacteria. So, during the period (the 1970s, 1980s), the marketing aim was broad spectrum coverage, but rather more lately, the core objective is to block resistant Gram-positive organisms. Therefore, reconsideration of previous antibiotics that have not previously been investigated became beneficial in at least one case. One other recent innovation resulted from whole chemical synthesis. In addition, semisynthesis of medications from previous antibiotics resulted in numerous profitable ventures. It must be highlighted, though, that while semisynthetic antibiotics had proven clinically effective, the efforts to develop those were extraordinary. Exactly 23 semisynthetic penicillins and 24 additional semisynthetic antibiotics have been employed therapeutically out of 20,000 semisynthetic antibiotics (Béahdy, 1974).

17.9.1 New streptogramins

Vancomycin and teicoplanin have been the only antibiotics effective toward multidrug resistance Gram-positive bacteria for yet more than 35 years. Because of the rise in multi drug resistance, its use has been heavily restricted. Streptogramins, which are antibiotics generated from a single bacterial culture that work together effectively, are a type of narrow-spectrum drug. In both cases, the polyunsaturated macrolactone (Group A) has a rare oxazole ring and dienylamide fragment; however, the cyclic hexapeptide (Group B) contains a 3-hydroxypicolinoyl exocyclic fragment which is found in only one of the pairs. Streptogramins include the antibiotics valerianmycin and pristinamycin, to name a few. Pristinamycin is, in fact, a cyclodepsipeptide (pristinamycin I), as well as a polyunsaturated macrolactone derived from the bacterium Spiralis pristinae-spiralis (pristinamycin I) (pristinamycin II). Natural streptogramins were insoluble in water and could not be supplied intravenously, but by semisynthesis and mutational biosynthesis, new derivatives have been produced that are effective in the treatment of a variety of diseases. Synercid (5RP59500) is a combination of two semisynthetic water-soluble streptogramins, quinupristin (5RP57669) and dalfopristin (5RP54476), that are effective against resistant infectious diseases. Quinupristin (5RP57669) and dalfopristin (5RP54476) are effective against resistant infectious diseases (Nichterlein et al., 1996). The two Synercid components decrease protein synthesis synergistically (100-fold) and are effective toward VRE, VREF, MRSA, and b-lactam-resistant *S. pneumoniae* (Stinson, 1996). The FDA authorized Synercid in 1999 (Cimons, 1999).

17.9.2 Modified tetracyclines

Glycylcyclines, semisynthetic tetracyclines, have indeed been produced to be used against tetracycline-resistant bacteria (Petersen et al., 1999; Livermore, 2005). The addition of the t-butylglycylamido group to minocycline resulted in the development of tigecycline, which was considered the first new antibiotic variant with diverse activity against bacteria that were

resistant to Gram-positive but also Gram-negative antibiotics and had acquired either the ribosome protection resistance or the drug efflux resistance mechanism. It is also effective against anaerobes. It is especially recommended in the case of nosocomial pathogens. Antibacterial activity against *P. aeruginosa* and other Proteus species is rather weak.

17.9.3 Modified erythromycins

Semisynthetic erythromycins reportedly had quite a great deal of success in clinical trials (Dougherty and Barrett, 2001). Clarithromycin, that is Biaxins, azithromycin including Zithromax, Pliva, roxithromycin, and the ketolide telithromycin (Taisho) are examples of modified macrolides (Keteks, Aventis). In addition to being effective against upper respiratory tract infections, each of these semisynthetic erythromycins is also safe and may be taken either topically or intravenously.

Even though the very first two have superior acid stability as well as bioavailability than erythromycin A, they are not much more effective against resistant bacteria than the latter. Ketolide antibiotics (6−0-methyl-3-oxoerythromycins, which are polyketides with a 14-membered macrolide ring and a C-3 keto group in lieu of the C-3cladinose in erythromycin A), but function against macrolide-resistant bacteria on the one hand (Kaneko et al., 2000; Yassin and Dever, 2001). Ketolides are also effective toward penicillin- and erythromycin-resistant S. pneumoniae, as well as a variety of many bacteria, including *Hemophilus influenzae, group A streptococci, Chlamydiaspp., Legionellaspp*., and *Mycoplasma pneumoniae* (Demain, 2009). Telithromycin and ABT-773 are examples of ketolides. ABT-773 exhibits broad spectrum efficacy against anaerobes as well as intracellular infections. It possesses a 10- to 100-fold high specificity for its ribosomal target than erythromycin A, enhanced absorption and/or reduced efflux, and is bactericidal. Ketolides were synthesized from erythromycin (Borman, 1998). The inability of the novel semisynthetic ketolides and glycylcyclines to operate as efflux pump substrates indicates that they have been only rarely bacterial cells that have been targeted and killed (Lynch, 2006).

17.9.4 Peptides

It was discovered by *S. roseosporus* that the cyclic lipopeptide daptomycin is an important antibiotic structure that may be used to treat VRE, *MRSA*, and penicillin-resistant *S. pneumoniae*, among other Gram-positive bacteria (Tally et al., 1999). Daptomycin was first found in the early 1980s by EliLilly and Co., but it was deemed too hazardous for human usage. Cubist Pharmaceuticals Inc. received a license in 1997, and the FDA authorized this in 2003. It does have a distinct mode of action (Eisenstein, 2004). It attaches permanently to bacteria's cell membrane, interrupting cellular activities and proving fatal. This does not enter the cell's cytoplasm. A by-product of this is that the target bacteria is no longer able to make ATP or absorb nutrients. Daptomycin is a bactericidal antibiotic that

is effective against Gram-positive infections within a short amount of time. It does contain a little amount of poison (rhabdomyelitis). It took several years before this calcium-dependent cyclic lipopeptide was approved for use in bacteria as the very first unique structure of a natural substance that has been authorized for microorganisms (Kirkpatrick et al., 2003).

17.9.5 Linezolid

It is important to add Linezolid, which includes Zyvoxs of Pfizer/Pharmacia, in the functioning of an antibacterial agent that is effective against vancomycin-resistant strains, despite the fact that it is not a natural substance. It was licensed for the purpose to use as anti-MRSA in 2000 and has been in use since then. Hexazolidinone antibiotics have Gram-positive action and do not show cross-resistance to any of the clinically important resistance mechanisms that have been investigated. It is effective against VRE, MRSA, *cephalosporin-resistant bacteria*, penicillin-resistant *pneumococci*, and *multidrug-resistant Mycobacterium TB*, including *Mycobacterium avium*, and also many anaerobes. They do not work against VRE. It has bacteriostatic properties (Bax et al., 2000) and orally active properties, as well as some Gram-negative properties. Oxazolidinones have no structural resemblance to any known antibacterial compounds. This time, they use a novel method of action to accomplish their goal: they interfere with the commencement of translation (Ballow et al., 2002). Their binding to the 50 S ribosomal submits and blocks the assembly of the 70 S ribosomal binding, which is necessary for protein production, prevents protein synthesis in growing bacteria from progressing to the next stage. A large number of analogs have been developed that have higher Gram-positive along with Gram-negative activity, respectively.

17.9.6 Antifungal antibiotics

Fungi that are pathogenic to mammals range in the two hundred species. Most of these infections are self-limiting, but in immunocompromised patients, they may be lethal. Systemic fungal infections, for example, are responsible for the death of 50% of leukemia patients. Fungal infections are becoming a big problem, with the number of cases tripling during the 1980s and 1990s, bloodstream infections increasing fivefold, and a reported death rate of 55%. Thus, according to current research, the prevalence of candidiasis, cryptococcosis, and aspergillosis is increasing, especially among AIDS patients. *Aspergillosis* is associated with a failure rate of more than 60%. Fungal infections are common following transplant operations: 5% for kidney transplants, but also 15%–35% for heart and lung transplants, as well as up to 40% for liver transplants. The most frequent pathogens are *Candida* and *Aspergillus* spp., which account for 80 percent of all fungal infections (Pham et al., 2019).

The five types of antifungal antibiotics presently in use include polyenes, synthetic azoles, allylamines, fluoropyrimidines, as well as cyclic lipopeptides, with natural polyenes being the most frequent (DiDomenico, 1999). The first three categories of drugs are directed toward ergosterol. Polyenes (e.g., amphotericin B) bind to ergosterol and cause fungal membranes to become destabilized. The azoles (e.g., fluconazole and flucytosine) work by inhibiting the cytochrome P450-dependent lanosterol 14-demethylase enzyme, causing ergosterol to be depleted. Squalene epoxidase is inhibited by allylamines (e.g., terbinafine). A fungal enzyme converts fluoropyrimidines (e.g., 5-fluorocytosine) to their deadly nucleoside counterparts in a controlled manner. However, because of the emergence of resistance to the azoles as well as the poisonousness of the polyenes, their use is becoming more restricted. The advent of cyclic lipopeptides, also known as candins, was a significant step forward and a watershed moment in science (or echinocandins).

Caspofungin is a powerful antifungal drug and effective against various Candida species, including Candida albicans, as well as Aspergillus and Histoplasma species. It is an aerosol that may be used to prevent *P. carinii* infection, which is a primary cause of mortality in HIV patients in Europe and North America (Georgopapadakou, 2001). In comparison to amphotericin B, it is more active and less harmful. Fujisawa's micafungin (FK-463), which was licensed in Japan, is an example of a semisynthetic candin. Anidulafungin (V-echinocandin and LY-303366A), is produced by *A. nidulans var. echinulatus*, (Debono, 1994). Aziles inhibit the growth of mold, whereas candins kill the mold that has grown on them.

17.10 Future perspectives

As a result, over the course of many decades, an increasing amount of natural materials and natural product-derived substances have been introduced onto the market. Around the year 2000, natural antibiotics generated exclusively by microbes accounted for 77% of all antibiotics approved by the FDA. According to current projections (Hasija, 2021), microbial biologics are anticipated to retain their dominance in the worldwide biologics market, which again was estimated at $277 B in 2015 and is predicted to expand to $400B by 2025. Microbial biologics are expected to maintain its dominance in the global biologics market. New results and views on microbial secondary metabolites and biologics are being created on a regular basis, despite the fact that the vast majority of their biological effects are well recognized. To meet the ongoing need for novel medications with antibacterial, anticancer, and immunosuppressive capabilities, as well as a variety of other pharmacological effects, chemical diversity obtained from microbial natural products is critical for future pharmaceutical research (Ong et al., 2022). Around the same period, microbial production of natural goods and biologics faces a slew of obstacles. Limited production titers, difficulties in product separation, and structural characterization are some of the difficulties of natural product-based medication development.

To guarantee the long-term viability of the desired behavioral change, the concerned authorities should see to it that rules and regulations forbidding the sale of antibiotics without a prescription are strictly enforced. Particular emphasis should be made toward the use of leftover antibiotics since this practice is difficult to avoid even with the adoption of legal regulations and necessitates additional measures such as adequate patient health education (Chang et al., 2022). While it is true that the patient chooses to take antibiotics on his or her own without visiting a doctor, healthcare workers, notably pharmacists, play an essential role in influencing the behavior of these patients. They play an important role in teaching people about the correct use of medications since they should advise their clients to a doctor before taking any prescription on their own. This is particularly essential since pharmacists are often the patients' final point of contact before they take antibiotics (Wilbur et al., 2010). These exchanges between patients and healthcare workers imply that greater study on healthcare professionals' perspectives is required (Poss-Doering et al., 2020). The same is true at the level of the healthcare system. There is still more to be discovered about the role that governments and other institutions may play in promoting antibiotic stewardship, including self-medication. Furthermore, the pharmaceutical industry's impact on healthcare professionals' prescribing/dispensing behavior and patients' medicine consuming behavior must be explored (Lescure et al., 2018).

Furthermore, there's tremendous space for advancement in regard to recombinant protein production in microbial platforms. The aggregation of the finished article in the microbial cell can lead to a widespread response to stress which restricts cell development. Constant attempts in natural product analog creation can open the doors toward the identification of molecules with superior biological activity when compared to their respective biological counterparts. Recently improved technology could be used to develop an area of natural microbial products that continue to become a reliable source of new molecules in pharmaceutical research.

17.11 Conclusion

Beyond its historical context, the creation of antibiotics has gone through a number of crucial stages. From the 1940s until the 1970s, one of the most prolific periods in history occurred. In those early days, countless unique substances were discovered via academic and pharmaceutical endeavors, and the world was a better place for it. It was during the 1980s that intensive soil microorganism research was conducted, as well as the rediscovery of some early compounds made. Several large pharmaceutical companies have decided to discontinue their antibiotic research as the consequence of which: (1) industries have significantly reduced the number of laboratories searching for novel antibacterial and antifungal; (2) Global capitalism's increasing earnings from pharmaceuticals given the public consume medicines each day for the entire lives but only when required for

infectious illness, as well as the state's limits on the use of perfect antibiotics to only the most gravely ill people; and (3) the global capitalism's increased profits from drugs in addition to using genetic data to discover new targets that are found in infections but not in humans, researchers are continuing to investigate methods for cultivating previously uncultivated microorganisms, and there are increasing attempts to improve novel antibiotics through combinatorial biosynthesis.

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CHAPTER 18

Microbial proteases—robust biocatalytic tools for greener biotechnology

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18.1 Introduction

Protease (EC 3.4) is the group of protein degrading enzymes that play important roles in industry and academia. They are also known as proteolytic enzymes or proteinases which perform the function of protein hydrolyzation. They are naturally ubiquitous playing a major role physiologically and commercially as well as they are distributed in all living organisms. Protease with vast diversity due to their mode of action and specificity has attracted researchers to know their physiological and biotechnological applications (Ahmed et al., 2011). On the industrial level microbial protease especially covers 60% of total enzymes traded worldwide due to their wide range of applications in food, pharmaceuticals, leather, paper, pulp, and different related chemical industries (Gupta et al., 2002; Najafi et al., 2005). Proteases with improved efficiency and a diverse range of activities are still the concern of researchers. Mostly protease-producing strains were identified based on morphological characteristics such as *Aspergillus niger* (Parvathy and Prabhakumari, 2017). Microbially-originated proteases possess different protein hydrolyzing enzymes. Microbes produce a wide range of intracellular and extracellular proteases. The structure and topology of proteases have been represented in Fig. 18.1. Different cellular and metabolic processes including differentiation, sporulation, protein turnover maturation of enzymes and hormones, and cellular protein maintenance by intracellular proteases. Extracellular proteases perform the hydrolysis of proteins from the environment to absorb and utilize hydrolytic products (Sawant and Nagendran, 2014). The protease market is expected to grow by 5.8% from 2020 to 2025

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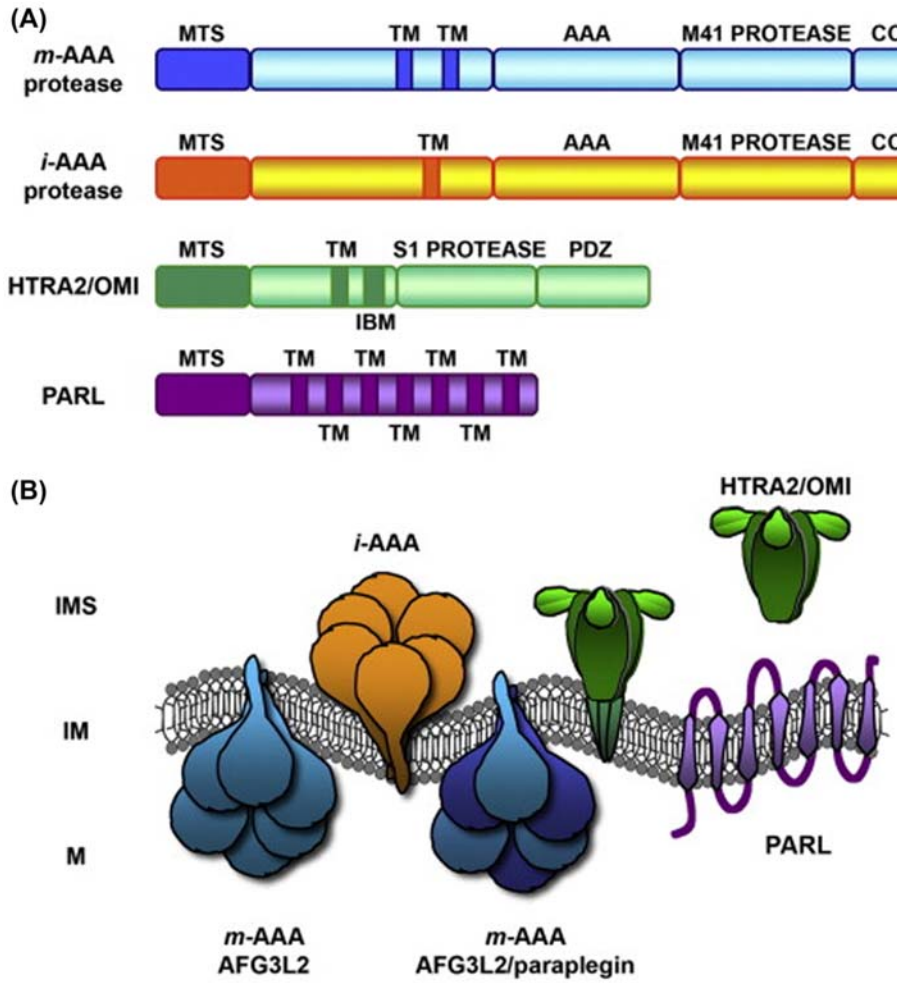

Figure 18.1

Structure (A) and topology (B) of protease enzymes. *TM*, transmembrane domain; *M41 protease*, metal binding proteolytic domain; *IBM*, an inhibitor of apoptosis (IAP)-binding motif; *MTS*, mitochondrial targeting sequence; *AAA*, triple-A domain; CC, coiled-coil, S1 protease, trypsin-like protease domain, PDZ, PDZ domain. (B) Illustration of localization and topology of human proteases in mitochondria. *IMS*, intermembrane space; *IM*, inner membrane; *M*, matrix (Martinelli and Rugarli, 2010). *Reprinted from Martinelli, P., Rugarli, E.I., 2010. Emerging roles of mitochondrial proteases in neurodegeneration. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1797(1), 1–10 with permission from Elsevier B.V.*

(Mordor-Intelegence, 2021). This chapter aims to provide updated knowledge on proteases, their types and mechanisms, microbial sources, protease engineering, and immobilization, and their role on the industrial scale.

18.2 Types of proteases

18.2.1 Serine protease

Serine proteases or alkaline protease is also known as serine endopeptidases are the type of protein hydrolyzing enzymes having serine that serve as nucleophilic amino acid at the enzyme's active site (Hedstrom, 2002). They exist both in prokaryotes and eukaryotes. Based on their structure, they are categorized into two main groups, (1) chymotrypsin-like, and (2) subtilisin-like serine protease (Madala et al., 2010). In our body, this class of enzymes performs different roles including digesting food, helping blood clot, helping sperm to enter the egg, and fighting against infections (Puente et al., 2003). Serine proteases catalyze proteolysis in two steps. The first one involves serine attacks substrate bond to be cleaved and form first tetrahedral intermediate and later form acyl-enzyme and release C-terminal (Fig. 18.2A). In the second step, the activated water molecule attacks the acyl-enzyme and forms a second tetrahedral intermediate, and later releases N-terminal (Radisky et al., 2006). Proteases were divided into families based on their catalytic mechanism and common ancestors. The name of the family starts with nucleophilic Ser-residue in the active site of the enzyme, which functions to attract the carbonyl group of substrates peptide bond to form an intermediate (Hedstrom, 2002). The nucleophilic capability of Ser residue is dependent on catalytic Asp, His, and Ser catalytic triad commonly called as charge array system (Blow et al., 1969). Four major groups of serine protease named trypsin, subtilisin, prolyl oligopeptidase, and ClpP peptidase use this triad for hydrolysis of the peptide bond (Page and Di Cera, 2008).

18.2.2 Aspartic protease

Aspartic proteases or acid proteases are a group of proteolytic enzymes that utilizes an activated molecule of water bound with one or more aspartate amino acid residue to cleave peptide substrates (Fig. 18.2B). At acidic pH, they bear two highly conserved aspartates in their active site. Almost all kinds of aspartic proteases can be inhibited by pepstatin (Fusek et al., 2013). They are generally grouped into two: pepsin and pepsin-like aspartic proteases produced by *Penicillium*, *Aspergillus*, and *Rhizopus* species and rennet and rennet-like aspartic proteases produced by *Mucor Miehei*, *M. Mucor pusillus*, and *Enthodia* species (Ward, 2011). The genome sequencing project started in 1990 and detected 354 members of this family until now. Within one specie different members do different jobs, for example in the human stomach two different enzymes are involved in the digestion of protein in the diet (Dunn, 2013). Aspartic proteases are highly specific in that they cleave dipeptide bonds which have hydrophobic residue and beta-methylene group at the same time. They perform proteolysis in a single step, unlike serine proteases which form a covalent intermediate. There have different mechanisms been proposed, but the most accepted one is the acid-base mechanism (Suguna et al., 1987; Brik and Wong, 2003).

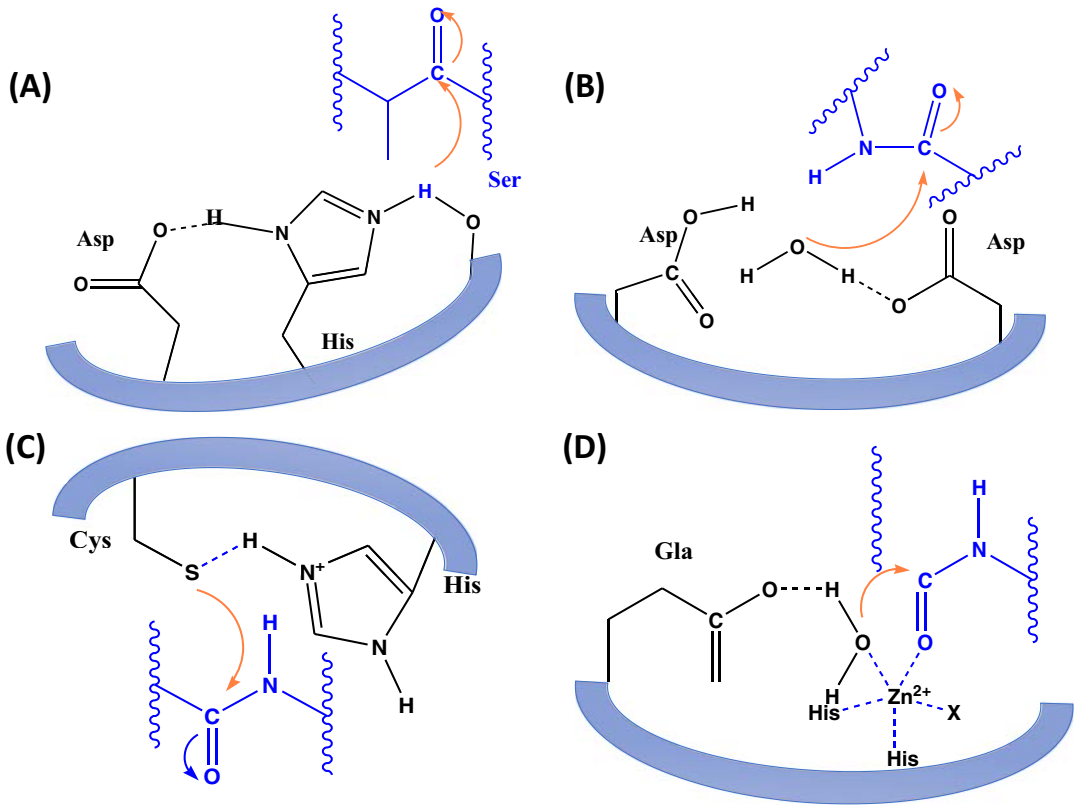

Figure 18.2
Proteases mechanism of action; (A) serine protease; (B) aspartic protease; (C) cysteine/thiol protease, and (D) metalloprotease. Metallo- and aspartic protease in the presence of aqueous moieties serve as the nucleophile. In cysteine and serine proteases' active site, the residues are commonly paired with a proton withdrawing group that lead to a nucleophilic attack on the peptide linkages. The overall mechanism of peptide cleavage is similar for all types of proteases (Neitzel, 2010). *Reproduced from Neitzel, J.J., 2010. Enzyme catalysis: the serine proteases. Nature Education, 3(9),21 with permission from Springer Nature.*

18.2.3 Cysteine/thiol protease

Cysteine proteases or thiol proteases are a group of enzymes that uses nucleophilic cysteine and thiol at the catalytic site to hydrolyze peptide bonds in a protein (Fig. 18.2C). For retention of their activity, they require reducing agents and sulfhydryl reagents can inhibit their activity (Ward, 2011). Their subgroups include papain and papain-like cysteine proteases (clostripain and streptozocin) produced from *Clostridium* and *Streptomyces* sp., respectively (Ward, 2011). Although cysteine proteases have a different evolutionary origin, their mechanism of catalysis is almost similar. Hydrolysis of peptide bonds depends on the thiolate-imidazole pair made by cysteine and histidine in their active site. Tetrahedral

oxyanion results after the nucleophilic attack on the carbonyl group which accepts a proton from the imidazole group and acyl-enzyme intermediate forms. This intermediate then releases the C-terminal of the peptide. In the next step, the acyl-enzyme intermediate gets deacylated by a water molecule and the remaining part of the substrate also gets released (Buttle and Mort, 2013).

18.2.4 Metalloprotease

Metalloproteases belong to a class of proteases that uses metal ions (Zn^{2+}) and a water molecule for their catalytic activity (Fig. 18.2D). Their diversity and importance become clear that they are present in all kingdoms and almost 2% of genes encode this enzyme. They have been proven as the most important class of enzymes in the biotechnology industry. They are highly thermostable and work best at neutral pH. Thermolysin is a well-known member of this family produced by *Bacillus stearothermophilus*. Another member of this clan includes metalloprotease collagenases (from *C. histolyticum*), elastase (from *P. aeruginosa*), and cell wall lytic protease (from *Mycobactor* sp.) (Ward, 2011). Metalloprotease involves Zn^{2+} tetrahedron which consists of the water molecule, two histidines (His), and a glutamic acid (Glu) at the catalytic site. Water molecule attacks carbonyl group of substrate acting as a base by interacting with Glu of the catalytic site and forms a tetrahedral intermediate leading to the first transition state. The leaving group gets protonated and the peptide bond gets cleaved by Glu which serves as acid and forms the second transition state and enzyme product complex (Wu et al., 2010).

18.3 Microbial protease sources

18.3.1 Fungal sources

Proteases have been produced from a large number of fungal strains like *Aspergillus, Rhizopus*, *Thermomyces*, *Thermoascus*, *Humicola*, *Cephalosprium*, *Penicillium*, and different other genera (Karuna and Ayyanna, 1993; Baoying and Jianmin, 1998). Interestingly many substrates have been employed to grow different fungi to get protease, but wheat bran was preferred among all of them. The fungal proteases from *Aspergillus* especially have been examined because of their potential to produce a high level of enzymes extracellularly. Many of their enzymes were produced on an industrial level by submerged fermentation and have been employed in the food and beverage industries for decades (Wu et al., 2006). Some species of *Aspergillus* have been employed including *A. flavus* (Kranthi et al., 2012; Macchione et al., 2008; Malathi and Chakraborty, 1991), *A. niger* (Donnell et al., 2001), *A. oryzae* (Vishwanatha et al., 2010). Among them, *Aspergillus oryzae* was a favorite because of its non-toxic nature with a long history of industrial uses till now. *A. oryzae* favorably grows on solid organic surfaces like steamed

rice, ground soybean, or agricultural byproducts like wheat bran, rice bran, sugarcane bagasse, and much other lignocellulosic waste.

Aspergillus species or filamentous fungi have been studied for the production of the protease in detail under solid-state fermentation. *A. flavus* and *A. oryzae* were reported to produce alkaline proteases under SSF. Filamentous fungi offer enzyme production over low substrates with high output, are less time-consuming, and are mostly extracellular which are easy to recover (Vishwanatha et al., 2010). *Penicillium* species also have great potential for protease production like *P. restricted*, *P. citrinum*, *P. roqueforti*, *P. camemberti*, and *P. griseoroseum*. Acid protease was reported from *P. camemberti* and *P. griseoroseum* under SSF and SmF (Ikram and Mukhtar, 2007). Mucor species *M. pusillus* and *M. miesha* have been reported to produce aspartate proteases which are also known as mucor rennins. Higher milk clotting than proteolytic activity enhanced their role in the cheese industry as a substitute for renin (Andrade et al., 2002). Mucor rennins have the disadvantage of spoiling the flavor of cheese after cooking because of their thermal stability after maturation time (Maheshwari et al., 2000). *M. bacilliformis* and *M. circinelloides* have been reported to produce aspartic protease with less stability over high temperature and produce milk clotting enzyme with such quality reported in *Penicillium oxacillium* and *Nocadiopsis* specie (Hashem, 2000; Fernandez-Lahore et al., 1999).

18.3.2 Bacterial protease

Different bacterial species belonging to the genus *Shewanella*, *Pseudoalteromonas*, *Microbulbifer*, *Psychrobacter*, *Bacillus*, *Photobactor*, *Halobacillus*, and *Vibrio* have been reported to produce proteases (Banerjee and Ray, 2017). *Bacillus* species have been dominant protease producing like *B. subtillus* RSKK96 (Pant et al., 2015), *B. licheniformis* (Banerjee et al., 2013), *B. sphaericus* (Surendran et al., 2011), *B. cohnii* (Tekin et al., 2012), *B firmus* (Jaiswal et al., 2014), *B subtillus* ATCC 14416 (Bhunia et al., 2012), *B. stearothermophillus* ap-4 (Qadar et al., 2009), *B. brevis* (Qamar et al., 2020). The optimum temperature for different *Bacillus* species has been reported by many researchers like *Bacillus licheniformis* with 50°C (Sareen and Mishra, 2008), *Bacillus subtilis* 50a with 70°C (Yusoff and Ibrahim, 2012), *Bacillus thermoruber* with 45°C (Manachini et al., 1988), marine *Bacillus* sp. with 70°C (Padmapriya et al., 2012), and *Bacillus amyloliquefaciens* Strain HM48 70°C (Mushtaq et al., 2021).

18.4 Strategies to improve the catalytic performance of proteases

18.4.1 Protease engineering

Almost all enzymes are made up of long peptide chains by link amino acids to one another. These amino acids play an important role at the catalytic site or at other sites to proceed

with biocatalytic reaction. Protease engineering is the modification of protease structure by changing amino acid composition which can lead to increased stability, catalytic activity, and specificity (Pogson et al., 2009). Proteases have been engineered for multiple roles in industries as summarized in Table 18.1. Presently, rational design, site-directed mutagenesis, and de novo design evolution are the methods for enzyme engineering. Rational design is considered to be better than others by researchers (Naveed et al., 2021). Catalytic activity and thermal stability can be increased by in-depth structural analysis. Many researchers have reported that rational design can be used for cost-effective enzyme mutation for industrial applications (Wang et al., 2012; Blum et al., 2012). For example, after rational design modification of alkaline protease produced from *Bacillus pumilus* by single N99Y mutant creation, its activity, and thermal stability got improved (Jaouadi et al., 2010). In the same strain and enzyme by replacing single neutral amino acid (Leu31 and Thr33), its activity and substrate specificity increased and have been proven to play their role in the leather industry (Zaraî Jaouadi et al., 2014). Another study has reported that transformed amino acids at the trypsin surface increased its resistance to detoxyfizyme and beta-mannanase MAN47 (Qiu et al., 2016; Li et al., 2013). When N-glycosylation sequence was introduced in trypsin and pepsin by rational design, their resistance to beta-mannanase improved (Hu et al., 2017). Site-directed mutagenesis is another biotechnological tool for

Table 18.1: Few recent studies report engineered protease for industrial use.

Microbial strain	Class	Modifications	Improvement	References
Bacillus pumilus	Serine protease	Site-directed mutagenesis	low-temperature proteolytic	Zhao and Feng (2018)
Bacillus subtilis	Serine protease	A higher proportion of the negative or positive charge on the surface	Halotolerance	Takenaka et al. (2022)
Pseudomonas aeruginosa	Serine protease	Site-directed mutagenesis	Increase in thermostability and catalytic activity	Ashraf et al. (2019)
Escherichia coli	Serine protease	N-terminal propeptide and site-directed mutagenesis in S-pocket	Increase in catalytic activity	Fang et al. (2019)
Bacillus amyloliquefaciens	Subtilase	Substitutions of amino acids with less stable counterparts	Increase thermostability and catalytic activity	Xu et al. (2015)
Bacillus licheniformis	Alkaline protease	Iterative chromosomal integration	Increase in production capacity	Zhou et al. (2021)
Escherichia coli	Keratinase	N-terminal propeptide replacement and site-directed mutagenesis	Improved catalytic activity	Fang et al. (2019)
Escherichia coli	Alkaline protease	3-nitro-l-tyrosine and 3-chloro-l-tyrosine at and near the catalytic site	pH stability	Osamura et al. (2019)
Bacillus clausii	Alkaline protease	Site-directed mutagenesis	improved activity and stability	Li et al. (2021a)

enzyme engineering but also for the structural and functional analysis of different proteins (Arnold et al., 2001; Porter et al., 2015). In this method, a wide library of mutants is created and mutants with desirable properties are selected. This method has several advantages over rational design because it does not require any knowledge about proteins and their structures. But it does require effort to deal with mutant libraries and study to evaluate mutants with required characteristics. Zhu et al. (2020) applied site-directed mutagenesis to improve metalloprotease resistance against organic solvents. The mutants presented high resistance towards solvents with improved catalytic activity by 3.5-times.

18.4.2 Protease immobilization

Enzyme immobilization was developed to solve the problems like recovery and reuse of enzymes because the initial price of enzymes was not economical for just single-use (Bilal et al., 2021a; Bilal et al., 2021b; Qamar et al., 2021). To reuse enzymes, the activity of the enzyme should be maintained (Sheldon and van Pelt, 2013). So, immobilization and activity of an enzyme are closely related to each other. Upon enzyme and supporting material interaction, this may cause the destabilization of an enzyme (Mateo et al., 2002; Noreen et al., 2022; Carballares et al., 2021). To have a stabilized-immobilized enzyme, support should not interact with the enzyme chemically to disrupt its structure and protocol that must support fast, but controlled biocatalytic reactions with appropriate buffers and composition of medium (Barbosa et al., 2013; Pedroche et al., 2007; Braham et al., 2021). Table 18.2 shows immobilizations of different enzymes on different supporting materials with the improvement of their different properties. Protease immobilization has bought positive effects with some special problems. When free proteases are used for food modifications, they will incorporate with food proteins and may cause some allergic reactions. Therefore, immobilization of protease can avoid this problem (Tacias-Pascacio et al., 2020; Cai et al., 2013; Siar et al., 2020). On contrary, immobilized protease must be properly oriented to catalyze the hydrolysis of large protein substrates (Tavano et al., 2018). Without proper orientation immobilized enzymes would be useless. Moreover, having a large number of immobilized enzymes in a small area could cause steric hindrance of big molecules to occupy the active site. Therefore, this problem should be overcome using the immobilization of biomolecules (Siar et al., 2017).

18.5 Emerging applications of proteases

Microbial protease has made its class because of its diverse role in the industrial sector including paper making, food, medical, detergent preparation, and leather making (Fig. 18.3). Among different types, alkaline and thermophilic serine protease are the most important. Some most important applications of proteases have been discussed in this section.

Table 18.2: Recent studies reporting protease immobilizations and their support with improved characteristics.

Protease type	Immobilization support	Improvement	References
Alkaline protease	κ-carrageenan gel beads	Enhancement in the thermal stability at high temperature with increased half-life	Awad et al. (2020)
Alkaline protease	Ca-Alginate Entrapment	Improved catalytic stability	Qamar et al. (2020)
Protease	Zinc oxide nanoparticles	Improved catalytic and thermal stability	Diyanat et al. (2018)
Protease	Chitin-starch material	Improved catalytic activity	Mehdi et al. (2018)
Aspartic protease	$Fe(OH)_3@Fe_3O_4$	Improved stability	Moslemi et al. (2018)
Alkaline protease	Graphene oxide nanosheets activated with glutaraldehyde	Improved catalytic activity	Ranjbari et al. (2019)
Aspartic protease	Iron oxide nanoparticles	Improved catalytic activity	Gao et al. (2018)
Protease	Magnetic metal-organic frameworks (MOFs)	Improved catalytic activity and thermal stability	Karami et al. (2022)
Serine metalloprotease	Magnetic chitosan nanoparticles	Improved catalytic activity	Khankari et al. (2021)
Keratinase	Fe_3O_4 nanoparticles	Improved catalytic activity	Lotfi et al. (2022)
Trypsin and chymotrypsin	Vinyl sulfone agarose beads	Improved stability and catalytic activity	Morellon-Sterling et al. (2021)
Cysteine proteases	Chitosan	Improved stability	Holyavka et al. (2021)
neutral protease	Alginate-Chitosan gel beads	Improved temperature and pH stability	Bai and Wu (2022)

18.5.1 Food industry

In the past few decades, microbial protease has found its application in the food sector tenderization of meat and cheese production (Shi et al., 1997; Adrio and Demain, 2014). Milk coagulation is the very first step in cheese making which can be performed with the help of protease. Cheese-producing industries use microbial-based proteases, but have a disadvantage because of their low output and souring upon storage because of their stability issues. Aspartic protease has proved to be the best cheese-producing protease (Claverie-MartÌn and Vega-Hernàndez, 2007; Feijoo-Siota et al., 2018). Food souring causes a major problem because microbes produce several kinds of protease. Alkaline protease is non-specific and can remove bitterness and add up sweetness (Adler-Nissen, 1986). Mamo et al. (2020) have reported milk-clotting aspartic protease from *Aspergillus oryzae* DRDFS13. On the other hand, protease obtained from *Bacillus amyloliquefaciens* has been used in making (methionine-rich) protein hydrolysate by using chickpea protein (George et al., 1997;

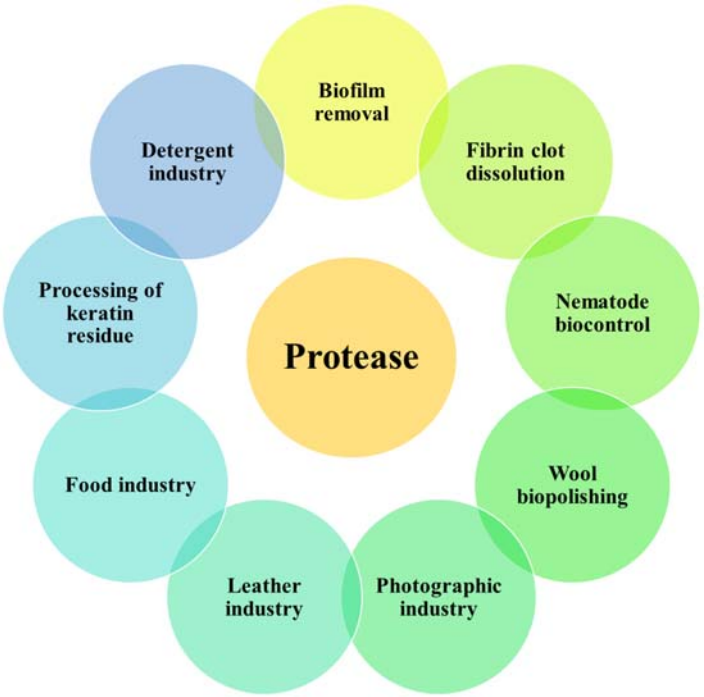

Figure 18.3
Emerging applications of microbial proteases.

Wang et al., 2016). Proteases also have been used in fruit juice fortification and the making of protein-rich diets for different therapies (Adamson and Reynolds, 1996; Amore and Faraco, 2015). Wheat flour contains gluten protein is insoluble and responsible for dough properties in the bakery. *Aspergillus orzae* produces protease that dissolves gluten to enhance product quality by increasing softness and texture and giving color (Sawant and Nagendran, 2014; Souza et al., 2015). By adding protease, fermentation improves as they release peptides and amino acids to use. Fungal Acidic protease has been reported to improve beer fermentation as they work better under acidic pH by balancing the amino acid content of fermentation media (Nogent-Sur-Seine, 2017). Recently, *Aspergillus tamarii* Kita UCP1279 has been reported to produce proteases for the beer industry with desirable characteristics (Alves et al., 2022).

18.5.2 Detergent industry

Enzymes like protease, amylase, and lipase are playing a major role in the detergent industry. Protease accounts for 60% of total sales in the detergent industry (Ibrahim et al., 2016). After that in 1956, the detergent company introduced enzyme-based detergent into the market. Chemical detergents contain chlorine, phosphates, and polycarboxylates which

are harmful and affect cloth's fiber quality. However, enzymatic detergent performance depends upon different factors like temperature, pH, and type of stain (Li et al., 2012; Hasan et al., 2010). Protease having the ability to work under alkaline pH and compatibility with detergent is best the choice. *Bacillus clausii* KSM-k16 and *Bacillus* sp. KSM-KP43 has been employed in the field to produce alkaline protease (Hasan et al., 2010; Białkowska et al., 2016). Thermophilic strain *Geobacillus stearothermophilus* (B-1172) has been reported to produce proteases having good washing results (Iqbal et al., 2020). Cold-active *Bacillus subtilis* WLCP1 has also been reported to produce proteases that can work best in cold water and alkaline conditions (Furhan et al., 2019). *Bacillus safenis* RH12 proteases have also been reported with good detergent quality (Rekik et al., 2019). Recently, *Geobacillus* sp. GS53 was reported to produce thermostable proteases up to 85°C and 10 pH and also with surfactant stability (Baykara et al., 2021). *Aspergillus ochraceus* BT21 protease explored which had a temperature and pH range of 20°C−60°C and 5−11, respectively (El-Khonezy et al., 2021). In another study, *Bacillus ruris* produced alkaline proteases that had stability up to 9 pH (Osesusi et al., 2021). Demand for detergent-based protease is still high so researchers are continuously screening for a better microbial protease with high stability over a wide range of temperature and pH.

18.5.3 Waste management

Keratinase is one of the most important types of protease in waste management. Generally, it degrades feathers, hairs, and wool to keratin. Keratin has high demand in poultry or other agricultural sectors as feed material. Keratin is an insoluble protein, but it can be converted into a soluble form by microbial keratinase under submerged fermentation (Suntornsuk and Suntornsuk, 2003). Keratinase has widely been employed in biofuel and fertilizer production from poultry waste and also as nematocide (Verma et al., 2017). Feather degrading enzyme by *Thermoactinomyces* sp. RS1 has been reported which can be used for feed production (Verma et al., 2017). Other keratins degrading enzymes have been reported for peptide production (Mótyán et al., 2013), recycling waste (Verma et al., 2017; Barman et al., 2017), food industry (Vanitha et al., 2014), and wastewater treatment (Jisha et al., 2013). Protease has also been reported to produce biochar from leather industry collagen-containing waste (Cao et al., 2020).

18.5.4 Leather and fabric processing

Leather processing involves soaking, dehairing, bating, and tanning which requires a significant amount of protease. Microbial alkaline proteases (keratinase) are very useful than hazardous chemicals to remove hairs from hides such as sodium sulfide which pollutes the environment (Ellaiah et al., 2002; Sawant and Nagendran, 2014; Souza et al., 2015). For leather processing, alkaline proteases also remove non-collagenous and non-fibrillar proteins from the hide like albumin and globulin (Fig. 18.4) (Jridi et al., 2014;

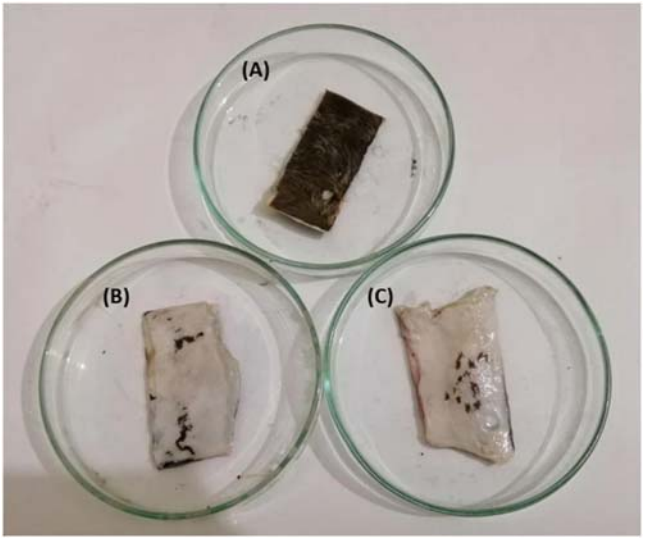

Figure 18.4
Dehairing of goat skin using both free- and immobilized proteases; (A) control; (B) treatment with free proteases; (C) treatment with Ca-alginate immobilized proteases. *Reprinted from Qamar, S.A., Asgher, M., Bilal, M., 2020. Immobilization of alkaline protease from* Bacillus brevis *using Ca-Alginate entrapment strategy for improved catalytic stability, silver recovery, and dehairing potentialities. Catalysis Letters, 150(12), 3572–3583 with permission from Springer.*

Qamar, Asgher & Bilal, 2020). Alkaline conditions are best for tanning hides and microbial alkaline protease is best for this (Ellaiah et al., 2002). After genetic modification strains have produced better results at the industrial level (Khan, 2013; Adrio and Demain, 2014). Proteases also have been used in silk processing. Silk sericin gives roughness to silk fibers and its removal through chemical meaning is expensive. Proteases produced from *Aspergillus* species and *Beauveria* sp. (MTCC 5184) have been employed to remove sericin which has saved time and is more economical (Freddi et al., 2003; More et al., 2018). *Bacillus* sp. C4 SS-2013 (C4) protease has also been reported to remove sericin completely with high specificity (Suwannaphan et al., 2017). *Bacillus cereus* has been reported to protease with good dehairing capability (Hashemabadi and Badoei-Dalfard, 2019). *Idiomarina* sp. C9−1 protease showed eco-friendly dehairing capability up to 70°C at 10 pH (Zhou et al., 2018). Recently, Li et al. (2022) have reported protease with good keratinase activity and showed no collagen dissolving.

18.5.5 Silver recovery

Silver is a valuable metal that is used in the film of CT-scan, MRI, and X-rays. About 1.5%−2% of silver is used to be present in the gelatin layer of the X-ray. This silver can be

recovered by several chemical and physical methods such as oxidation of silver and burning. Alkaline protease can degrade the gelatin layer to release bounded silver (Kumaran et al., 2013). Immobilized protease from *Bacillus brevis* has been reported to degrade gelatin to recover silver (Qamar et al., 2020). Alkaline protease from *Conidiobolus coronatus* recovered silver efficiently within 6 min at 10 pH (Shankar et al., 2010). Recently, a marine bacterium *Shewanella algae* have been reported to produce alkaline protease which could hydrolyze the gelatin layer effectively within 60 min (Javee et al., 2022). Protease obtained from *Bacillus sphaericus* has been reported to hydrolyze gelatin at 11−12 pH to recover silver in 8 min (Singh et al., 1999). Other proteases from *Bacillus* sp. 21−2 and *Bacillus coagulans* proved beneficial strains to recover silver (Fujiwara and Yamamoto, 1987; Jisha et al., 2013; Vanitha et al., 2014). Another *Bacillus* strain recovered from soil has been reported to produce silver recovery protease (Anju et al., 2014). *Bacillus cereus* has been reported to protease with good silver recovery (Hashemabadi and Badoei-Dalfard, 2019). As silver recovery proteases are eco-friendly and can be produced from cheap substrates, still have demand in this industry.

18.5.6 Cosmetic and chemical industry

Proteases can also play an impotent role in the chemical industry but their major problem is that their activity decreases with anhydrous conditions. Alkaline proteases have been reported to catalyze peptide synthesis (Mótyán et al., 2013; Anbu, 2016). Bacillus sp. Reported to produce alkaline protease for polymer synthesis using sucrose with anhydrous conditions and turning D-glucose to pyridine by transesterification (Patil et al., 1991). On the other side, *Conidiobolus coronatus* has been reported to be alkaline proteases that can replace subtilisin from many reaction mixtures (Sutar et al., 1991; Bhunia et al., 2012; Białkowska et al., 2016). Proteases have also played a role in cosmetics. They play their role in the exfoliation and breaking of other proteins which keep older and outer layers of cells and increase the renewal of cells. Clear the outer surface to penetrate the nutrients into the skin. Proteases like papain and bromelain proved to be beneficial for skin peeling and soothing. These proteases speed up cell renewal by the removal of old cells and exercise keratinolytic activity (Shin and Lee, 2000). On the other hand, elastase has been employed to treat purulent, carbuncles, deep abscesses, furuncles, and burns (Gupta et al., 2002; Liu et al., 2022). *Bacillus* sp. C4 SS-2013 (C4) has been reported to produce serine protease and demonstrated its potential in yellow color bleaching and cosmetics (Suwannaphan et al., 2017).

18.5.7 Biomedical industry

Apart from the wide range of applications of proteases in industry, they have also been accepted for medical purposes. Proteases demonstrated their capability for different diseases including cancer, inflammation, and infectious diseases. Protease from *Aspergillus oryzae*

Table 18.3: Few recent studies report fibrinolytic enzyme production.

Microbial strain	Enzyme	References
Staphylococcus aureus	Staphylokinase	Hachim et al. (2020)
Staphylococcus aureus	Staphylokinase	Deepa et al. (2019)
Staphylococcus aureus ASIA4	Staphylokinase	Alzahrani and El-Shenawy (2020)
Staphylococcus aureus	Staphylokinase	Mohammed and Al-Awadi (2021)
Staphylococcus aureus GH38	Staphylokinase	Noori and Aziz (2020)
Streptococcus agalactiae EBL-31	Streptokinase	Arshad et al. (2018)
Streptococcus equisimilis	Streptokinase	Naeem et al. (2018)
Streptococcus equisimilis	Streptokinase	Tanveer et al. (2021)
Streptococcus equisimilis	Streptokinase	Chaudhari et al. (2022)
Mucor subtilissimus UCP 1262	Streptokinase	da Silva et al. (2022)
Bacillus subtilis	Nattokinase	da Silva et al. (2022)
Bacillus subtilis 13,932	Nattokinase	Li et al. (2021b)
Bacillus subtilis JNFE1126	Nattokinase	Wang et al. (2021)
Bacillus subtilis TH9	Nattokinase	Minh et al. (2022)
Bacillus subtilis VITMS2	Nattokinase	Keziah and Devi (2021)

has been reported to treat different syndrome related to an enzyme deficiency (Palanivel et al., 2013; Gurung et al., 2013). In recent decades, the protease concept has changed its action. Proteases applications in several biochemical and physiological functions like apoptosis, cell adhesion, cell signaling, cell migration, and metastasis. Microbial protease can be used to inactivate proteases in our bodies to treat cancer (Pervaiz et al., 1998; Rakashanda and Amin, 2013; El-Sayed et al., 2019). The use of such enzymes has an edge over chemotherapeutic agents for toxicity comparison (Sawant and Nagendran, 2014). Sawant and Nagendran (2014) have reported the use of subtilisin or clostridial collagenase in many antibiotics, contrary to this, asparaginase proved to be effective for lymphocytic leukemia treatment (Kishore et al., 2015). Heart and cerebral stroke are the most common disease nowadays, which happens because of the clot formation in the blood vessel which supplies blood to these organs. Recently, many researchers have reported fibrinolytic enzymes from various microbial resources are mentioned in Table 18.3. Proteases also have been proven to be anti-inflammatory agents. *Serratia marcescens* have been reported to produce novel proteases with anti-inflammatory properties (Koul et al., 2021; El-Abd and Ibrahim, 2020; Chander et al., 2021). Wongputtisin and Supo (2021) have reported producing protease from *Bacillus* sp., which was able to degrade allergy-causing proteins from medical glove material.

18.6 Conclusion and perspective

In this chapter, the roles of microbial-based proteases for different industrial applications have been discussed. The protease industry has been predicted to grow by 5.8% from 2020 to 2025 and holds 3 billion dollars in sales in the market. Currently, Europe is the biggest

protease producer and Asia is becoming the fastest-growing market for protease sales. Proteolytic enzymes have been proven beneficial in a wide range of applications. Microbial-based enzymes have an advantage over plant or animal-based enzymes as they can be produced on large scale with less time and with easy recovery. Serine proteases are the most important type of protease industrially among others. Nowadays, researchers are looking for more efficient thermally stable properties from economical resources such as a substrate.

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CHAPTER 19

Applications of microbial biomolecules in sustainable agriculture

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19.1 Introduction

The world's population is growing at an exponential rate, i.e., 1.59% (Shabbir and Shahid, 2013), and by the end of 2050, it has been predicted to be around 9 billion people. Furthermore, with the current human population of 7700 million, and along with its rapid growth, food production is expected to increase by no more than 70% by 2050, so that feeding the population will be a major challenge (Shabbir and Shahid, 2013) while food insecurity is becoming a serious problem for developing countries (Hussein et al., 2019). The most widely grown and consumed staple food cereals by humans are rice, maize, and wheat (Boscaiu and Fita, 2020). Global water scarcity, pollution, and increase in soil and water salinity are the major issues of the twenty-first century. The shrinking of land available for cultivation and the growing human population are two potential threats to agricultural sustainability (Somasegaran and Hoben, 1994). The plant growth-promoting rhizobacteria (PGPR) are soil microbes that colonize roots and confer beneficial effects to their host plants Fig. 19.1. Such types of bacteria are applied to crops for enhancement of crop growth and of productivity. Several genera of beneficial bacteria aid in the acquisition of essential macronutrients such as N and P by secreting nutrient solubilizing compounds such as siderophore, exopolysaccharides, and other compounds (Bhattacharyya and Jha, 2012).

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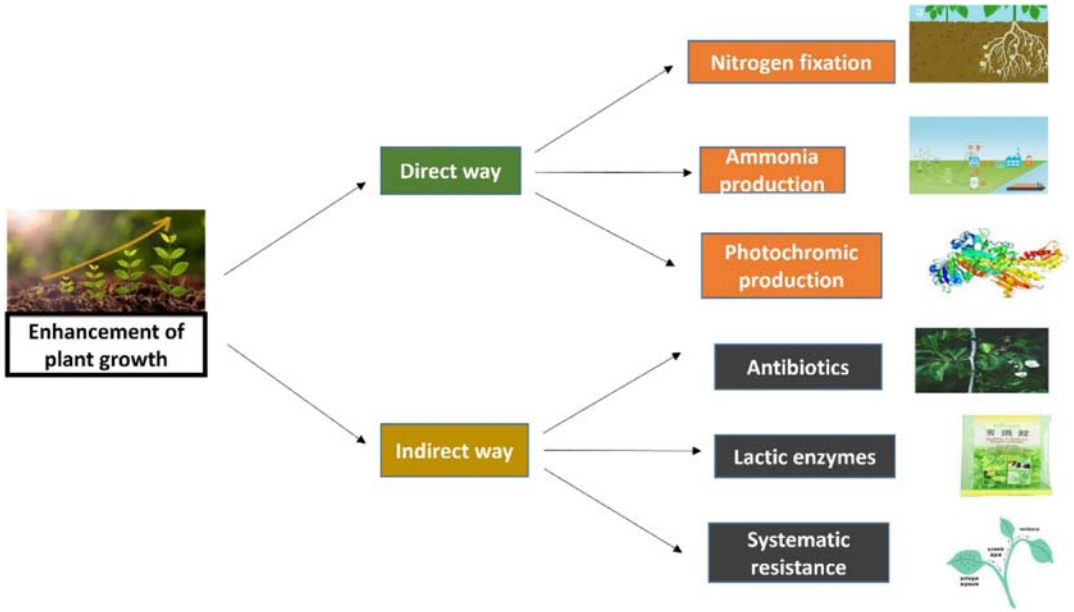

Figure 19.1
Representation of plant development via direct and indirect ways.

PGPR has the ability of:

1. Attachment to the root surface.
2. Survival.
3. Proliferation.
4. Competition with other microorganisms.
5. Plant growth promotion.

PGPR are characterized on the basis of different traits:

1. Biofertilizers may consist of bacteria or fungi that help the plants with nutrients taken from soil (Ramasamy et al., 2020);
2. Antibacterial compounds such as antibiotics, glucanase, siderophore, chitinases, and cyanides are produced by bacteria, which help the plants to control the disease;
3. Plant growth hormones such as ethylene, Indole Acetic Acid (IAA), gibberellic acid, and cytokinin are produced by bacteria that stimulate plant growth (Egamberdieva et al., 2017);
4. Organic pollutants decompose more quickly with the help of bacteria (Joutey et al., 2013).

Antagonistic bacteria protect against different pathogens in two different ways: by direct mechanisms or indirect mechanisms. In the direct mechanisms, the bacteria suppress the plant pathogens by producing defense-related enzymes, antibiotics, and siderophores, while in the indirect mechanisms, the biological agents compete with the pathogens for a niche or

nutrient site (Priyank Hanuman Mhatre et al., 2019; Köhl et al., 2019). The physiological state in which a plant's defense potential is increased due to a biological or chemical inducer is called Induced Systemic Resistance (ISR) (Romera et al., 2019). ISR protects the plant from severe pathogen attacks. And it does this with the help of microbes in the rhizosphere, causing systemic resistance to pathogenic invaders. Systemic aquired resistance (SAR) is one of the most studied types of host resistance, triggered by plant pathogens, whereas ISR is triggered by rhizosphere colonizing microbes such as *Bacillus, Pseudomonas*, and *Trichoderma*, among others (Pieterse et al., 2000).

Siderophores are produced by microorganisms that help the plants uptake the available iron from the soil. It will enhance microbial growth and limit the amount of available iron for other microorganisms, and as a result, limit the growth of microbes and suppress pathogenic microorganisms (Rajkumar et al., 2010). Auxins are plant growth regulator hormones that play a vital part in the interaction between the plant and the microbe. IAA processes have been proposed by various researchers as contributing to biocontrol activities (Mohite, 2013). IAA has been playing an essential role in plant defense reactions by inhibiting the growth of germinating spores of various pathogenic fungi. The application of IAA to potato leaves can prevent Phytophthora infestation. Gluconic acid is an important organic acid produced by phosphate solubilizing bacteria that is thought to regulate the production of antimicrobial metabolites. The plant pathogen is controlled by gluconic acids due to its antibacterial properties (Ezzanad and Brahim Oubaha, 2021).

19.2 Role of agriculture in developing countries

The agricultural sector is crucial to a country's economy (Ahmad Khan et al., 2013) and 60% of people in many developing countries like Pakistan, India, and Bangladesh depend on the agriculture sector for survival (Ben-Gal et al., 2008). The contribution of the agriculture sector to gross domestic product (GDP) growth of India was 19.9% and 17.8% during 2020−2021 and 2019−2020, respectively. The share of agriculture in Bangladesh's GDP was 12.92% in 2020. Pakistan is categorized as an agricultural country (Husain, 2012), with 22 out of 80 million hectares of land being used for agricultural production (Ahmad Khan et al., 2013). This particular sector generates over 20% of Pakistan's national income (Syed et al., 2021). It accounts for 43.7% of overall employment and contributes 21% to the GDP, as shown in Table 19.1 (Abdullah et al., 2015). Non-urban areas account for 66% of the country's population, and their income is largely dependent on agriculture, either directly or indirectly (Basharat, 2019). It generates more than 60% of the country's foreign exchange profits and employs about 3/4th of the population (Siyal et al., 2002). During the 2019−2020 fiscal year, the agriculture sector grew by 2.67% (Government of Pakistan, 2020). However, the agriculture sector mainly depends on irrigation due to arid and semi-arid climatic conditions (Priyank Hanuman Mhatre et al., 2019). The total

Table 19.1: Contribution of agriculture to GDP growth (%) of Pakistan Source: Economic Survey of Pakistan (Government of Pakistan, 2019, 2020, 2021).

Sr. No	Year	Agriculture as a proportion of GDP (%)
1	2011–2012	21
2	2012–2013	21.3
3	2013–2014	21
4	2014–2015	20.9
5	2015–2016	19.8
6	2016–2017	19.5
7	2017–2018	18.9
8	2018–2019	18.5
9	2019–2020	19.3
10	2020–2021	19.2

irrigated (through tube wells and the canal system) area is 17.65 million hectares, whereas 16.32 million hectares is the net cropped area out of a total of 21.99 million hectares of cultivable area (Siyal et al., 2002).

Water and energy shortages, as well as the rising cost of many important inputs, such as seeds, fertilizers, pesticides, and the rising cost of obtaining easy credit, are the challenges faced by Pakistan's agriculture sector (Abdullah et al., 2015). Small farmers, for the most part, face harsh conditions and are unable to survive on their own resources in the agriculture sector, so they need to obtain credit based on their socio-economic circumstances. They require credit to purchase seeds, fertilizer, pesticides, machinery, and other inputs. Agricultural credit comes from one of two sources: farmer savings or financial institution borrowing (Romera et al., 2019).

19.3 Microbial biomolecules

A biomolecule is any type of substance that cells and living organisms produce, vary in shape and size, and perform a variety of functions. The smaller molecules combine to form large molecules called biological macromolecules that are necessarily required for life to survive. Combining to form a major portion of cell mass (Pugh et al., 2018), the four major types of biomolecules are carbohydrates, protein, lipids, and nucleic acids.

Firstly, carbohydrates are among the most widely available biomolecules on the earth, and they are an important source of energy. They are mainly made up of molecules that contain oxygen, carbon, and hydrogen atoms. The four types of sugar units are monosaccharides, disaccharides, oligosaccharides, and polysaccharides. These molecules are commonly called sugars (Slavin and Carlson, 2014). The formula of carbohydrates is $(CH_2O)n$, where n represents the number of carbon atoms. Carbohydrate molecules have a ratio of 1:2:1 (carbon-to-hydrogen-to-oxygen). Carbohydrates can be very important that, compared to

other additional biological molecules, frequently form into extended chains by joining organized smaller components (Slavin and Carlson, 2014). Thus, a monomer is a general term for a single unit, while a polymer is a name for an extended filament of monomers. Illustrations of carbohydrates contain the sugars found in milk (lactose) and table sugar (sucrose). Carbohydrates have numerous functions in cells, such as being a great source of energy for the diverse actions that take place in cells. Carbohydrates can also serve a structural function. For example, cellulose is a polymer form of carbohydrates and is present in plants standing tall while providing timber its rough properties. Another example of carbohydrates is starch and glycogen are stored forms of energy made up of different sugar polymers (Cummings and Stephen, 2007). Glycogen is present in animals, whereas starch is present in plants. The functions include defense against and removal of foreign material, energy source, communication, structure, and cell adhesion.

Secondly, forming a diverse group of molecules that share a common characteristic, lipids are nonpolar fragments and hydrophobic ("water-repellent") in nature. Fat is an example of a lipid that stores energy for long periods of time. The main part of the outermost layer of the cell, lipids serve many functions in a cell. Fats, phospholipids, oils, waxes, and steroids are all lipids (Fahy et al., 2011). Thirdly, proteins are the most important organic molecules in biological systems and play a major role in structural, enzymatic, and functional roles (photosynthesis, transport, storage medium etc.) of cell. Different types of proteins are present in a cell and each protein has its own specific function (Watford and Wu, 2018). Chemically made up of twenty types of different amino acids that serve many functions in a cell, all enzymes are proteins in nature that act as a catalyst in biochemical reactions. According to the lock and key model, each enzyme has a specific substrate (a molecule that binds to an enzyme) on which it acts. Enzymes can break, rearrange, or form new molecular bonds. Salivary amylase, for example, is an enzyme that breaks down amylose, a starch component. Proteins play a number of important roles in the living, including assembly of cell mechanisms and cell membranes, transference, catalysis, and cell communication (Adams, 1993). Lastly, the nucleic acid is another type of macromolecule present in all cells and viruses. Deoxyribonucleic acid (DNA) encoded information to produce protein required by the cell. A related type of nucleic acid known as ribonucleic acid (RNA) transfers the message from the DNA to ribosomes, where proteins get synthesize (Crick and Watson, 1953).

19.4 Source of microbial biomolecules

19.4.1 Archaea

Archaea is a prokaryotic organism and can survive in extreme environments like cruel temperatures. The isolation and identification of different types of Archaea, especially extremophile archaea, has allowed for the study of their metabolic processes, which have

then been manipulated and used for industrial purposes (Raju and Nagaraju, 2014). Extremophile archaea are responsible for giving enzymes and molecules their thermostable nature like proteases, lipases, glucoamylases, glucosidases, amylases, xylanases, esterases, DNA polymerases, dehydrogenases, chitinases, pectinases, which helps microbes to survive in harsh environments like extremely acidic or basic solutions, extremely high or low temperatures, or when exposed to other harmful factors such as radiation (Egorova and Antranikian, 2005). The most important enzyme that has been isolated from Pyrococcus furiosus is thermostable DNA polymerase which is used for industrial purposes. This enzyme is highly used in molecular biology due to its stability at high temperatures in the polymerase chain reaction. The specific enzymes amylases and galactosidase isolated from Pyrococcus species are thermostable when used in food processing (Van de Vossenberg et al., 1998; Bowers and Wiegel, 2011).

19.4.2 Bacteria

Corynebacteria is a genus of bacteria and mostly are Gram-positive bacteria that can be present in a variety of ecological niches and are most commonly used in industry for large-scale production of amino acids and nutritional factors in the industry (Subramaniam et al., 1998; Bernard, 2012; Ikeda and Takeno, 2013). One of the most important amino acids produced by *Corynebacterium glutamicum* is glutamic acid used as food additives. The common name of glutamic acid is Monosodium glutamate. Corynebacterium is also involved in hydrocarbon degradation (it is critical for the breakdown and elimination of environmental toxins) and steroid conversion (in the development of pharmaceuticals) (Zhang et al., 2016). Xanthomonas is a type of proteobacteria that cause disease in the plant (Almeida et al., 2002). Xanthomonas produced an acidic exopolysaccharide called xyanthan gum, used as a stabilizing and thickening agent in cosmetics and food industry (Palaniraj and Jayaraman, 2011).

19.4.3 Fungi

Fungi are eukaryotic microorganism that is responsible for the production of different biomolecules like organic acids, antibiotics, enzymes, plant growth-promoting hormones, etc. Fungi produce important plant hormones like gibberellins, and ethylene in association with plant roots that are beneficial for plant growth and development. Fungi play a significant role in physiological processes, chemical changes, and biosynthesis of different compounds like biostimulants, ethylene, auxins, and lignins to enhance the capability of plants to cope with environmental stresses like cold, salinity, drought, and heavy metal stress. For example, *Fusarium moniliforme* produces important plant hormones called gibberellins. *Aspergillus* is another type of microorganism used in the food industry for large-scale fermentation. There are more than a hundred different species that are present in this genus. There are more than hundreds of mold species in this genus (Mojsov, 2016). *Aspergillus niger* plays a major role

in industrial microbiology, where it is used in the manufacturing of alcoholic beverages and pharmaceutical products. *Aspergillus niger* is used to produce citric acid that is used in a variety of products, including household cleaners, pharmaceuticals, foods, cosmetics, photography, and construction (Mojsov, 2016; Mandari et al., 2020).

19.5 Different biomolecules produced by microbes and their application in agriculture

Several microbial applications are well known for their use in mitigating major agricultural issues like crop productivity, plant health protection, and soil health maintenance, as shown in Fig. 19.2.

19.5.1 Microbial enzymes

In a polymerase chain reaction, high thermostable enzymes are used called Taq polymerase to produce DNA copies of particular segments. This enzyme was discovered in the Thermus aquaticus bacteria (Gelfand, 1989; Laws, 2021). The enzyme that has been obtained from a variety of bacteria and archaea as a natural defense against viruses is called a restriction

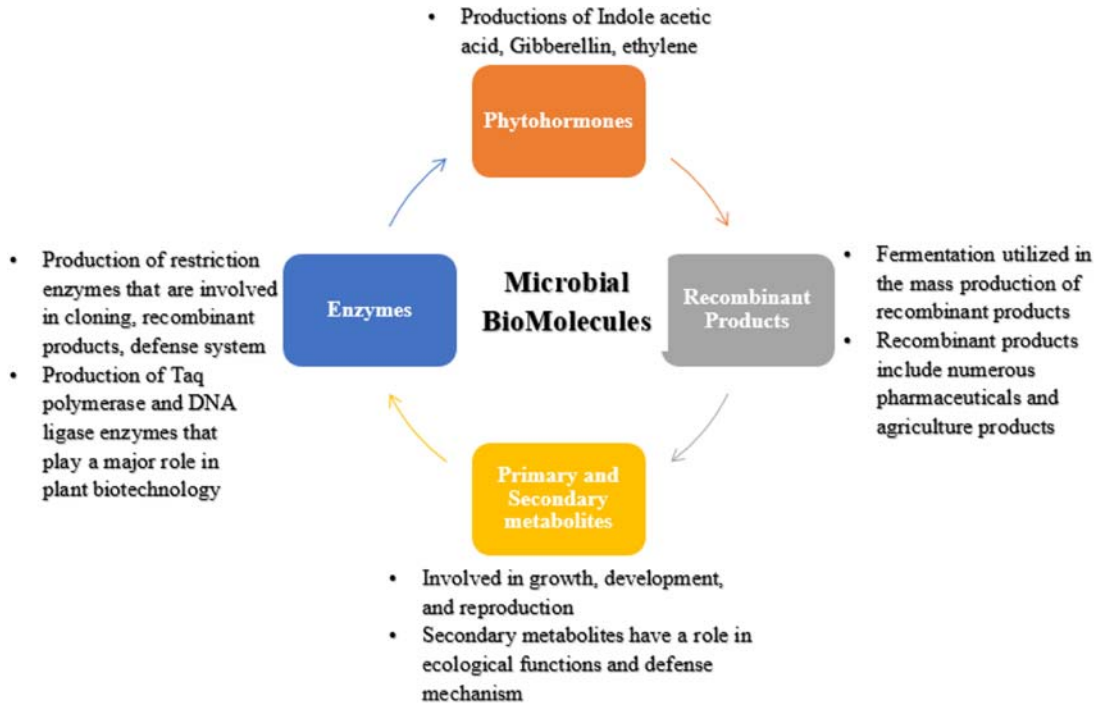

Figure 19.2
Types of microbial biomolecules and their applications.

enzyme. They have proven to be valuable tools in DNA manipulation or modification because they can cut DNA at specific sites. The enzymes can recognize and cut up foreign DNA and protect their own DNA sequences through the methylation process (Pray, 2008; Green and Sambrook, 2021; Loenen et al., 2014). DNA ligase—another important enzyme discovered in *T. aquaticus* bacterium—is highly useful in molecular biology for it is used to insert particular DNA segments into plasmids in the molecular field-related work. DNA ligase enzyme is used to covalently link the segment of DNA. Most commonly, T4 DNA ligase, which is produced from the T4 bacteriophage, is the most commonly used DNA ligase (Tomkinson and Della-Maria, 2013; Potapov et al., 2018; Chiuman and Li, 2002; Shi et al., 2018).

19.5.2 Primary metabolites

Responsible for reproduction, growth, and development, primary metabolites are produced as a result of energy metabolism during the growth phase, and it is essential for proper growth (Demain, 1980). Examples of primary metabolites are ethanol, amino acids, and lactic acid. Alcohol—the most common primary metabolite within the field of industrial microbiology—used in the large-scale production of spirits, like wine and beer, during the fermentation process. L-glutamate and L-lysine amino acids are primary metabolites that are commonly used as supplements in health and obtained through mass production of Corynebacteria glutamicum. Another example of a primary metabolite is citric acid, commonly used in industrial microbiology. *Aspergillus niger* is used to produce citric acid—the most widely used metabolite in food production, cosmetics, and pharmaceutical industries (Demain, 1980; Sanchez and Demain, 2009).

19.5.3 Secondary metabolites

The biomolecules that are produced by modified primary metabolite synthases are called secondary metabolites. Playing the main role in ecological function, antibiotics production, defense mechanism, and pigment production (Fouillaud and Dufossé, 2022), secondary metabolites are produced at the end of the stationary phase (Ruiz et al., 2010). Atropine and antibiotics are examples of secondary metabolites (Demain, 1992). Atropine is taken from a variety of plants that have clinical applications (Al, 2014), and between erythromycin and bacitracin antibiotics, erythromycin—derived from the Saccharopolyspora erythraea bacterium—has a broad antimicrobial spectrum, is mass-produced, and commonly consumed by mouth. Finally, bacitracin is an antibiotic obtained from the *Bacillus subtilis* bacteria that is commonly applied topically, is a nonribosomal synthetase enzyme that can synthesize peptides that occur naturally, and is used as an antibiotic in clinics (Barrios-González, 2018; Allison et al., 2020; Ahmed et al., 2020).

Kasugamycin and polyoxins are used as biopesticides. Some examples of bacteria and fungi producing secondary metabolites are:

Streptomyces spp.: Produced kasugamycin that is used as biopesticides.

Streptomycetes spp.: Produced polyoxins that are used as biopesticides.

Bacillus spp.: Produced lipopeptides nonribosomally that act with strong antifungal activity such as *Fusarium, Pythium, Rhizoctonia*, and others.

Bacillus thuringiensis: Production of nikkomycin and spinosyns used as insecticidal properties.

Lactobacillus plantarum: Produced various compounds like lactic acid for competing microorganisms.

Trichoderma spp.: Protect against pathogens such as *Pythium* and *Fusarium*, plant nutrient uptake, and the production of plant hormones.

19.5.4 Recombinant products

Recombinant proteins are produced by the fermentation process. The common examples of recombinant products are insulin and the hepatitis B vaccine. Insulin is a hormone produced by the pancreas that regulates glucose levels in the blood and acts as a regulator of fat and carbohydrate metabolism. Insulin is a drug that is used to treat people who have diabetes mellitus. Type 1 diabetes people cannot produce insulin, while type 2 diabetes people frequently produce insulin in which the hormone loses its effectiveness (Liras, 2008; Ferrer-Miralles et al., 2009). As the number of people diagnosed with diabetes mellitus rises, so does the demand for external insulin. Insulin is mass-produced using a combination of fermentation processes and recombinant DNA technology. Genetically modified *E. coli* is grown in fermentation to sufficient amounts of proinsulin. The proinsulin is then purified after the cell has been disrupted. The proinsulin is converted to crude insulin by enzymatic reactions, and then it can be modified to use a medicinal compound (Burnett and Burnett, 2020; Puetz and Wurm, 2019).

Another recombinant product—Hepatatis B vaccine—is produced using the fermentation process, is used against the hepatitis B virus, and was created using both fermentation and recombinant DNA technology. Hepatitis B virus (HBV) outer coat gene is inserted into the genome of the yeast organism for the development of HBV vaccine. The yeast is used to grow large amounts of the HBV gene before being harvested and purified. The genetically modified yeast in which the HBV gene is inserted is grown in the fermentation process to produce a sufficient amount of HBV protein (Sanchez-Garcia et al., 2016; Biswas et al., 2016; Jozala et al., 2016).

19.6 Phytase producing microbes and their influence on plant development

Soil microbes that convert insoluble forms of organic P to soluble forms are named plant growth-promoting microbes. Because plants acquire P as inorganic P, so organic P compounds are dephosphorylated by phosphatases formerly to their integration in the metabolism to covert into inorganic form (Singh et al., 2014; Singh and Satyanarayana, 2011; Balaban et al., 2017; Singh et al., 2020).

19.7 Uses of enzymes in agriculture

19.7.1 An organic way of farming-1

With the rapidly improving technology, farmers are finding it easier to get crops cultivated the right way. There are fertilizers and other chemicals that would help the plants with their growth aspects. However, these are not sustainable as far as the plant produce or soil fertility is concerned. These chemicals tend to devastate the piece of land and would render it useless and barren. Thus, the need of the hour is to go back to organic farming techniques. The primitive ways of organic farming include the usage of natural fertilizers such as compost and organic manure broadly. However, in the current times, these seem to be quite irrelevant because these are not only slow in replenishing the soil with all the nutrients and micronutrients but are also needed in bulk quantities to give an effective boost to the soil quality. The slow rate of replenishment, when set against the rapid rate of growth and development of the country, makes it not unusual for the producer to wish for a miracle (Wang et al., 2021).

19.7.2 An organic way of farming- 2: revolutionized phase

As it turns out for the real the producer's wish for a miracle, the niche of organic agriculture has received the benefits of such technological advancements. There was a need for it, and the ends have been provided for. The soil now lives up to the expectations of the landowner, and while reaping the crops, the farmer knows his efforts have been returned with a solid favor. Agricultural enzymes could be renowned in the coming years as the foundation of organic agriculture in this century. What is commonly known to the less knowledgeable persons as the process of replenishment of soil with nutrients is the work done by microorganisms. These microbes have one task to perform: the fixation of nutrients inside the soil granules. The microbes are responsible for several of the following outcomes (Satyanarayana et al., 2013; Singh, 2019; Kumar et al., 2021; Shah et al., 2021; Santi et al., 2013; García-Fraile et al., 2015; Yadav et al., 2020):

- Fixation of nitrogen in the soil gives rise to the nitrogen cycle.
- Kick-starting the oxygen cycle.
- Fixing other micronutrients in the soil.

- Helping the earth decompose dead plants and animals.
- Replenishing the soil with all the nutrients and micronutrients.

Therefore, when it comes to replicating the organic way of farming that was prevalent in the earlier times, the essence has remained the same, but surely the ways have changed. There are no longer any bulk investments in the form of manure and compost, but a simple spray full of natural microbes is more than enough to feed your garden. The natural way of doing farming has no side effects, too.

19.7.3 An enzyme in agriculture is the organic fertilizer

Enzymes are proteins that work as catalysts in the chemical reactions that take place. The catalysts are responsible for carrying out a particular reaction and expediting it. Hence, scientists need to get some catalysts to speed up the process and get things done. The biochemical reactions do need some organic compounds to hasten the processes, which is the basic function of the enzymes as catalysts in nature. Therefore, people and farmers should be aware of these mechanisms in nature so that instead of investing precious resources and time in primitive and old ways of organic farming, they could indulge in spraying enzymes over the fields of produce (Wang et al., 2021). Crops do require nutrition in the form of different nutrients such as phosphorous, nitrogen, oxygen, phosphates, and many more so that they grow unabated. The crop should be healthy to retain its growth rate, and the soil should return a flourishing yield to the farmer. However, to help in the sustenance of the crop by providing it with these nutrients, the farmer is responsible for everything, from ensuring a crop's health to ensuring the soil's quality for the best produce. The farmer has vested interests in the production concerning another aspect: to earn money by selling crops worth more than what had been invested in their cultivation (Wang et al., 2021).

To convince the farmer, one must list down all the possible benefits and merits of using enzymes are regular replenishers for the soil. A list is provided below as well (Shang et al., 2020):

- Enzymes act as natural herbicides, pesticides, and fertilizers, helping the crop to reach its best possible growth and yield.
- The toxicity in the compost is reduced to a much greater extent as enzymes help in the decomposition process.
- The barren land turns into a fertile haven for crops, relieving the farmer of unnecessary worries.
- The roots, the stem, and the fruit grow stronger and foster the farmer's expectations for lavish produce allaying fears of lost efforts and money.
- Enzymes acting as pesticides help in the growth of helpful organisms like earthworms allowing the soil to breathe fertility.
- Since no chemical fertilizer is being used, the soil would never deteriorate into an infertile wasteland.

- Enzymes could also be added to the fodder of animals, helping them with their proper digestive functions.

The merits are not limited only to these, as more research work in this direction is revealing exciting and interesting results.

19.7.4 Enzymes and agriculture go hand in hand

Enzymes are not chemicals that have been produced with certain man-to-man ideas, but it is the natural way by which the Earth has grown fertile since times immemorial but which have remained out of the repository of knowledge of common people. However, now, when it is known and cheaply procurable, there is no place for doubt or questions about whether to use these miracle resources. The farmer just needs to ponder over it, give it a go, and then reap the benefits following the investment. The farmers can now go back to the original organic farming techniques without indulging in chemical products. The soil is always natural and gives rise to the best products that a farmer can hope to grow. The farmer is happy with enhanced production in both qualitative and quantitative terms. The soil is happy too. There is betterment all around. With such positivity, there is no going back in any aspect for every stakeholder in agriculture. Thus, it would not be wrong to understand enzymes as beneficent for agricultural production and development.

19.8 Nanotechnology in sustainable agriculture

Protecting plants in two ways, nanoparticles act: (a) as crop protection nanoparticles or (b) as carriers for existing pesticides or other actives, such as double-stranded RNA (dsRNA), and can be delivered via spray application or drenching/soaking onto seeds, foliar tissue, or roots. As carriers, nanoparticles can give various advantages, including (1) increased shelf-life, (2) improved solubility of weakly water-soluble pesticides, (3) decreased toxicity, and (4) increased site-specific uptake into the target pest (Ahmad Khan et al., 2013; Nagula and Usha, 2016; Worrall et al., 2018). Another potential benefit of nanocarriers is an improvement in the activity and durability of nano pesticides under environmental stressors (UV and rain), resulting in a large reduction in the number of treatments, as well as lowering of toxicity and costs (Fig. 19.3). Microbe-based nanoparticles are synthesized against different pathogens. Details have been shown in Table 19.2.

19.9 Phytohormones

Phytohormones are key regulators of plant development and physiology under normal and stressed conditions. The ability to perceive and respond is integral to the immune system, and these chemical messengers are synthesized and translocated depending upon external/internal

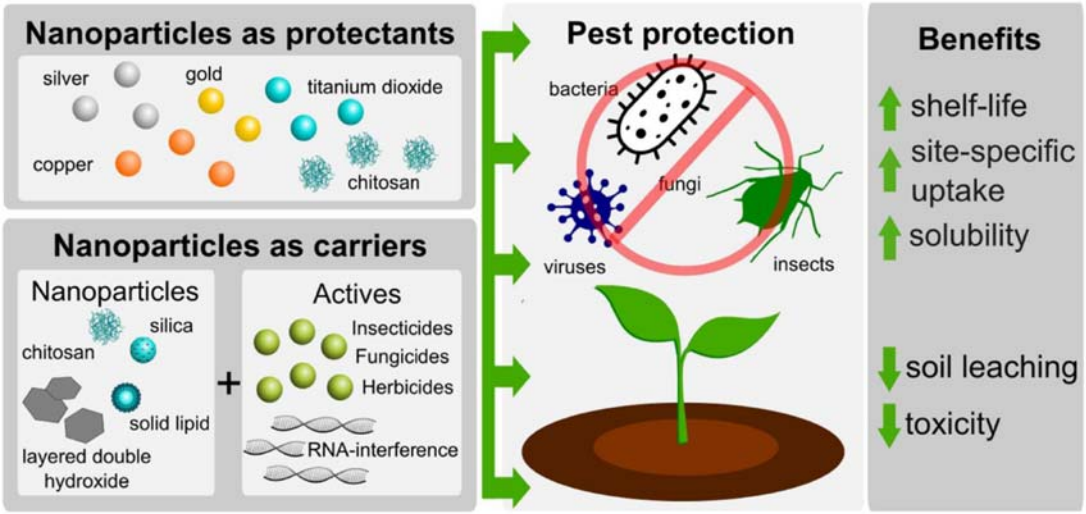

Figure 19.3

Nanomaterials as protectants or carriers to provide crop protection (Worrall et al., 2018). *This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).*

stimuli. Phytohormones such as auxin, gibberellins, cytokinins, ethylenes, and abscisic acid are synthesized in defined concentrations in defined parts of the plants under normal conditions, but stresses are known to modulate their endogenous levels. Recent reports showed major alterations in phytohormones, i.e., decreased levels of auxin, cytokinin, and gibberellin and increased ABA level by heat in a heat-sensitive rice variety associated with low yield components; while minor changes in phytohormones and associated changes in yield were displayed by the heat-tolerant variety. This clearly shows that the application of phytohormones producing PGPR strains might reverse the adverse effects of heat stress (Kudoyarova et al., 2019; Maheshwari et al., 2015).

19.9.1 Auxin

Auxin (IAA) is the most important plant hormone for sustainable agriculture, imperative for cell division and differentiation, and theirs having a vital role in seed germination, roots development, and apical dominance (Spaepen and Vanderleyden, 2011). The majority of the rhizospheric microbes ($>80\%$) can synthesize and release IAA in the rhizosphere, which elongates plant roots, thereby increasing the number of root hairs for enhanced uptake of water and nutrient. Inoculation of IAA-producing bacteria shows significant improvement in root and plant growth, yield, and stress tolerance of various crops, e.g., in drought conditions (Park et al., 2021). IAA-producing *Ochrobactrum sp.* and *Pseudomonas* spp. (volcanos isolates) have shown

Table 19.2: Applications of microbes in agriculture nanotechnology.

Bacteria	Nanoparticle	Size (Nano meter)	Shape	Application	Plant pathogens	References
Pseudomonas rhodesiae	Ag	20–100	Round	Antiseptic	*Dickeyadadantii*	Hossain et al. (2019)
Bacillus siamensis	Ag	25–50	Sphere-shaped	Antiseptic	*Xanthomonas oryzae pv. oryzae*	Ibrahim et al. (2019)
Bacilluscereus	Ag	18–39	Sphere-shaped	Antiseptic	*Xanthomonas oryzae pv. oryzae*	Ahmed et al. (2020)
Pseudomonas poae	Ag	20–45	Sphere-shaped	Antifungal	*Fusarium graminearum*	Ibrahim et al. (2020)
Bacillus sp.	Ag	7–21	Sphere-shaped	Antifungal	*Fusarium oxysporum*	Gopinath and Velusamy, 2013)
Serratia sp.	Ag	10–20	Sphere-shaped	Antifungal	*Bipolaris sorokiniana*	Mishra et al. (2014)
Stenotrophomonas sp.	Ag	12	Sphere-shaped	Antifungal	*Sclerotium rolfsii*	Mishra et al. (2017)
Pseudomonas sp., *and Achromobacter* sp.	Ag	20–50	Sphere-shaped	Antifungal	*Fusarium oxysporum f.* sp. *ciceri*	Cooper et al. (2007)
Aeromonas hydrophila	ZnO	57–72	Crystalline	Antifungal	*Aspergillus flavus*	Jayaseelan et al. (2012)
Streptomyces spp.	CuO	78–80	Sphere-shaped	Antifungal	*Alternaria alternata, Fusarium oxysporum, Pythium ultimum*, and *Aspergillus niger*	Hassan et al. (2019)
Streptomyces capillispiralis	Cu	4–59	Sphere-shaped	Antifungal	*Alternaria* spp., *Aspergillus niger, Pythium* spp., *and Fusarium* spp.	Hassan et al. (2018)
Streptomyces griseus	Cu	5–50	Sphere-shaped	Antifungal	*Poria hypolateritia*	Ponmurugan et al. (2016)
Bacillus thuringensis	Ag	10–20	Polymorphic	Antifungal	*Sun hemp rosette virus*	Yang et al. (2013)
Bacillus licheniformis	Ag	77–92	Polymorphic	Antifungal	*Bean yellow mosaic virus*	Elbeshehy et al. (2015)

improved root and shoot length, fresh weight, and biomass of maize under high temperature (40°C), drought (up to 60% Poly Ethylene Glycol 6000), and salt (500 mM NaCl) conditions. This indicates the suitability of IAA-producing PGPR in a harsh environment to induce heat stress tolerance in crops (Çakmakçı et al., 2020; Spaepen et al., 2007; Cox et al., 2018).

19.9.2 Gibberellin and abscisic acid

Gibberellins (GAs) are well-known plant hormones that regulate various developmental processes such as embryogenesis, leaf expansion, stem elongation, flowering, and fruit ripening. Abscisic acid is a classical phytohormone that regulates several aspects of plant growth and development, including cell division and elongation, seed dormancy and germination, embryo maturation, floral induction, and responses to stresses. ABA production by the plants is important to the plant themselves and the activities of root-associated microbes such as PGPR. Root-associated bacteria capable of synthesizing gibberellins are reported in several genera such as Rhizobia, Acetobacter, Pseudomonas, Azospirillum, Bacillus, and Herbaspirillum while aiding in stimulation of plant growth and improved stress tolerance (Shu et al., 2018; Li et al., 2016; Sah et al., 2016).

19.9.3 Cytokinin

Cytokinins are involved in various developmental and physiological processes such as seed germination, apical dominance, roots development, nodule organogenesis, development of vascular tissues, flower and fruit, and plant-pathogen interactions. Different bacterial genera (such as Bacillus, Escherichia, Agrobacterium, Methylobacterium, Proteus, Pseudomonas, and Klebsiella) inhabiting plant rhizosphere are capable of cytokinin synthesis and release and stimulating plant growth (Akhtar et al., 2020). The plant-beneficial role of cytokinin producing *B. subtilis* and *Micrococcus luteus* inoculation under drought stress is well-documented. Application of cytokine-producing PGPR can also be effective in mitigating the adverse effects of heat stress in plants because recently exogenous application of INCYDE-F (Prerostova et al., 2020), an inhibitor of cytokinin oxidase/dehydrogenase in Arabidopsis, revealed that it increased contents of cytokinin trans-zeatin and cis-zeatin in roots and auxin in all tissues after heat shock. It further reduced the level of ABA in leaves and ethylene in apices of roots. This shows that inhibition of cytokinin degradation helped the Arabidopsis to cope with heat stress (Großkinsky et al., 2016; Schmülling, 2004; Ha-tran et al., 2021; Liu et al., 2020).

19.9.4 Ethylene

Ethylene is a gaseous phytohormone that is responsible for various processes in plants, such as abscission, senescence, reproductive development, and a (biotic) stress response (Iqbal et al., 2017). In the reproduction phase, pollen development is most sensitive to the high temperature/heat stress;

therefore, regulation of ethylene signaling in reproductive tissues becomes significant to gain reproductive success (Giorno et al., 2013; Chaturvedi et al., 2021; Klee and Clark, 2010). Two transcripts are involved in the biosynthesis of ethylene (1) PsACS [encode enzymes that convert Sadenosyl-L-methionine to 1-aminocyclopropane-1-carboxylic acid (ACC)] and (2) PsACO (encode enzymes that convert ACC to ethylene (Wang et al., 2002; Pattyn et al., 2021). Various genera of plant-associated microbes, i.e., Methylobacterium, Bacillus, Alcaligenes, Enterobacter, Pseudomonas, Azospirillum, Rhizobium, and Bradyrhizobium have a vital enzyme,"1-aminocyclopropane-1-carboxylate (ACC) deaminase," which counters (metabolize) microbes (Houben and Van de Poel, 2019). The application of ACC deaminase-producing microbes mitigates abiotic stresses, including salinity, drought, heat, etc. While recently revealed *Enterobacter* sp. SA187 induced thermotolerance to wheat in field conditions without changing the microbial community composition. SA187-induced thermotolerance is mediated by ethylene signaling via the TF EN13 and constitutive H3K4me3 modification of heat stress memory genes, generating robust thermotolerance in plants (Andrés-Barrao et al., 2017; Andres-Barrao et al., 2021; Shekhawat et al., 2020). This guarantees that PGPR is a more sustainable and powerful tool to generate heat/temperature stress tolerance in the crops (Basu et al., 2021; Vurukonda et al., 2016) (Fig. 19.4).

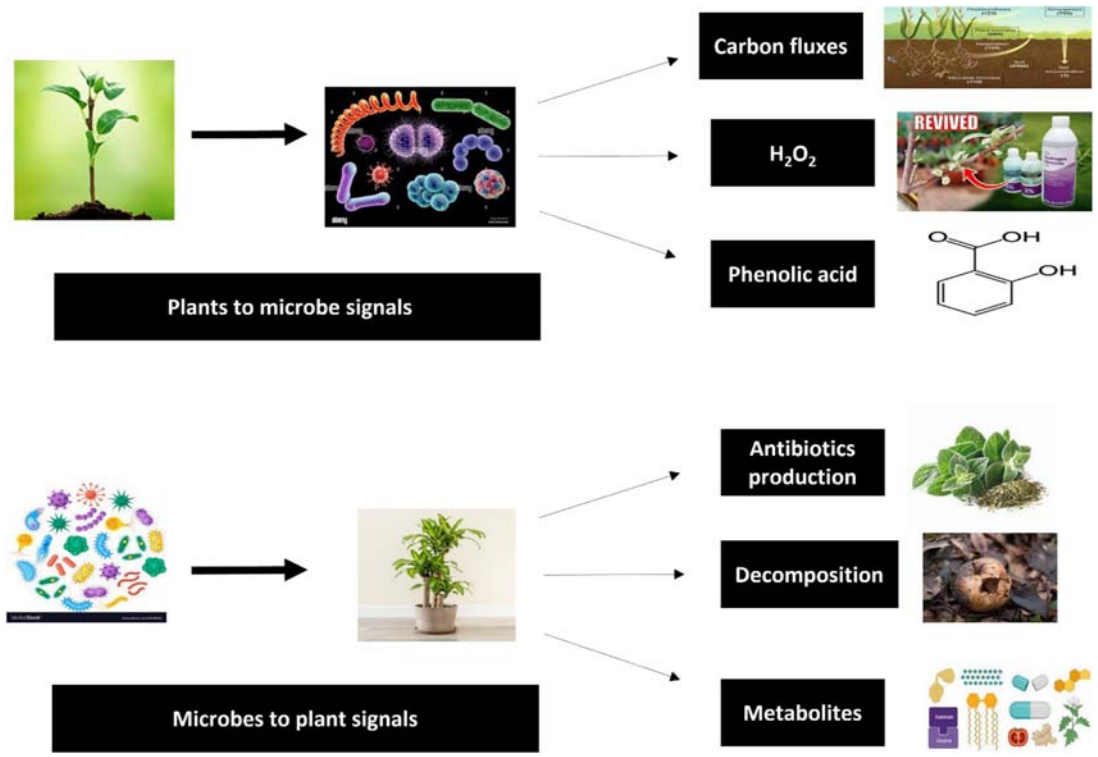

Figure 19.4
Microbial to plants, and, plants to microbe interaction in the form of various signals.

19.10 Conclusion

Finally, secondary microbial metabolites and bioactive compounds have been in high demand for sustainable agriculture in recent years. Microorganisms produce beneficial chemicals as well as industrially significant hydrolytic enzymes for a variety of biological processes. Plants rely heavily on bioactive chemicals and secondary microbial metabolites. The field of secondary microbial metabolites and bioactive compounds for use in the biotechnological and agricultural sectors has increased in recent years. A variety of such compounds have been reported from various groups of bacteria; however, the pace of discovery may be hastened by speeding up the pace of research and development in numerous microbial biotechnologies and strain enhancements via recombinant DNA technologies.

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CHAPTER 20

Prospecting bio-enzymes for a greener environment

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20.1 Introduction

Industrialization, rapid urbanization, and large-scale consumption of different products are significantly contributing to environmental pollution. All types of pollution, whether directly or indirectly, have a significant impact on overall human health and the environment. Many anthropogenic sources, mainly industrial and agricultural disposals, are the reasons for wastewater pollution (Rasheed et al., 2018; Ahmad et al., 2021; Muhammad et al., 2021; Rasheed et al., 2021). EPs are a subclass of organic pollutants called micropollutants, which have been found in many water systems (Teodosiu et al., 2018). Many important chemicals and other products related to transformation are used to make pharmaceuticals like antibiotics, analgesics, non-steroidal antiinflammatory drugs (NSAIDs), and many other personal care products (Lapworth et al., 2012). All of these can be seen in wastewater treatment plants (WWTPs), home and personal care products, hospital-related raw materials, cemeteries, landfills, water systems, industrial effluents, sewage of big cities, and pharmaceutical manufacturing plants (Sauvé and Desrosiers, 2014). Their values and concentrations are thought to pose major environmental and ecological risks, including interference with high organisms' endocrine systems, reproductive parts, physical deformities, and other congenital diseases in some species, and many other examples are known to exist (Bilal et al., 2019a; Bilal et al., 2020; Ali et al., 2021; Khan et al., 2021). In 2011, research found that the number

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of fluorinated compounds per unit of blood and other tissues of the body could be linked to the risk of breast cancer, blood cancer, and other types of diseases in the women of Greenland. Pollutants like perfluorooctanoate and perfluoro octane sulfonate, for example, have been associated with much lower human reproductive system capacity (Vélez et al., 2015).

Some contaminants, such as polyfluoroalkyl-containing substances, have been found in higher concentrations in our water supplies, with concentrations reaching μg/L and then mg/L and at last g/L levels, respectively (Kelly and Brooks, 2018; Nakayama et al., 2019). Just because they have unfavorable and detrimental effects on human health and the environment, these micropollutants have become the focal point of numerous important research groups. For example, N-diethyl-*meta*-toluamide can reduce the function of enzymes like acetylcholinesterase in many species of mammals and arthropods. This enzyme is primarily found in postsynaptic neuromuscular junctions and is involved in the hydrolysis of acetylcholine, a natural neurotransmitter.

It has been observed that there is a lack of understanding of ecotoxicological risk in the management of data, mainly in the sampling and analytical procedures and methodologies. There are three types of compounds of these micropollutants: newly developed compounds that are introduced to the environment; compounds that are only now being recognized but are present in the environment for a long time before their identification; and compounds that have been detected for a long time but whose significant impact on the environment and human health has only recently been recognized, such as antibodies, hormones, and other proteins (Kelly and Brooks, 2018; Nakayama et al., 2019). Researchers have classified these EPs into many types of classifications and systems including medicine, cosmetics, and other personal care products, pesticides, hormones, antibodies, and other proteins (Ben Younes et al., 2019; Bilal et al., 2019b).

Diclofenac is an antiinflammatory medicine in nature and is used to deal with pain and inflammation. Its accumulation can be seen in the food web and food chain and also found in drinking water and marine water bodies; it has recently been identified as an ecological pollutant of great concern in a study (Ali, 2012). Our water supplies have been found at various amounts that range from 0.5 to 1.3 g/L. Other physical processes like osmosis and filtration are also effective, but they are more expensive in terms of materials than the adsorption techniques (Bilal et al., 2019b, Unuofin et al., 2019). Although physical and chemical procedures are extensively used and can be effective, they have significant drawbacks, including high overall costs, inefficiency, high sludge production, and generating harmful by-products. As a result, it is widely acknowledged that new, creative, and environmentally friendly wastewater cleanup methods are urgently required. EPs have been successfully removed from wastewaters via biodegradation or bioremediation. This procedure uses microorganisms like bacteria, fungus, and yeasts to remove EPs that are organic compounds from water bodies (Alneyadi and Ashraf, 2016; Ahmed et al., 2017; Barrios-Estrada et al., 2018; Rasheed et al., 2019).

In biodegradation and bioremediation, microbes use the pollutant as a substrate to produce those enzymes, which subsequently transform and change the composition of the contaminants into many tiny compounds that are usually less dangerous to the ecosystem. There are many benefits of biodegradation methods over physiochemical techniques. They are safe, less disruptive, less costly, require less energy, are considered a greener catalysis technology, and use pollutants with very low concentrations, which physical and chemical techniques cannot achieve (da Silva Vilar et al., 2021; Kishor et al., 2021; Rafeeq et al., 2022a; Saeed et al., 2021). Biological treatments have the disadvantage and shortcomings of taking a longer time to achieve results, having undesirable results, and failing to achieve results as a series of microorganisms may not survive in the specific conditions provided to degrade persistent pollutants (Al-Maqdi et al., 2017). Laccases and peroxidases have garnered attention as industrially important catalysts. A large number of reports have demonstrated the EP degradation potential of these enzymes. Therefore, a comprehensive survey of laccase- and peroxidase-based biomitigation approaches can be a good addition.

According to the literature, a significant lot of progress has been achieved in the instrument's detection and removal analysis of numerous types of hazardous contaminants in the last several decades. However, a large range of environmentally related hazardous contaminants remains undiscovered due to considerable restrictions in sample handling, preparation, protection, and deprotection stages. It's also possible that undetectable transformation products, sometimes known as "micro-pollutants," with unknown toxicity and endurance, exist in the endproduct of various breakdown/degradation processes. Even in low quantities, these micropollutants may be mobile and/or persistent in air, soil, sediments, and water, making them mostly invisible. This is also due to the fact that the reactive chemistry of micropollutants is currently a mystery. Furthermore, reliable data on their fate after removal, persistence in various settings, and dangers to ecological and human health are currently absent and will continue to be the focus of future studies. Furthermore, using interdisciplinary methodologies, the effective and safe removal of various hazardous contaminants from any given environment might be made more predictable. Future studies and research devoted to EP are required to build strong, sustainable, and ecologically acceptable bioremediation techniques in order to ensure the entire eradication, detection, and degradation as well as removal of environmentally toxic new contaminants. According to current circumstances, a combination of multidisciplinary techniques such as molecular biology, bioinformatics, bioengineering, and protein engineering should be used to develop novel biocatalysts for biotechnological processes that could potentially be used to completely eliminate toxic environmental pollutants from any given environment. Given the long-term push for environmentally friendly processes, as well as health concerns about hazardous compounds, nontoxicity, and mild reaction conditions, the development and application of immobilized green catalysts is expected to continue to be a hot topic in the future (Rasheed et al., 2018). This chapter starts with a

brief introduction of laccases and peroxidases, followed by a detailed discussion on laccase- and peroxidase-mediated biocatalytic removal of EPs.

20.2 Laccases, source, and biocatalytic features

Laccases (EC 1.10.3.2) are members of the blue multicopper oxidase (MCO) family that use electron acceptors to oxidize many organic substrates by different mechanisms. These enzymes are promiscuous biocatalysts with proven applications in various fields, such as biotechnology, biochemistry, agriculture, industry, environmental sciences, and also for biosensing of many analytes of interest (Gul et al., 2017). Laccases have been reported in a variety of organisms, such as plants, insects, bacteria, and fungi (Ahmed, 2017; Arregui Mena, 2019). The plant laccases are primarily involved in lignification, while fungus laccases have diverse roles, such as lignin degradation, pathogenesis, and morphogenesis (Rodríguez-Delgado et al., 2015). Many recombinant laccases have been produced from *Coprinopsis cinerea* to promote lignin oxidation at neutral pH. Similarly, a basidiomycete laccase has been used to remove dyes from contaminated waters. These characteristics make them an attractive tool for industrial applications (Nakazawa et al., 2017).

Several evaluations of laccase's properties and applications have been published in the recent decade. These articles, among other things, describe laccase's ligninolytic activity in lignocellulose degradation (Leonowicz et al., 2001), in vivo practice of laccase and its implications (Mayer and Staples, 2002), their chemical and physical properties (Solomon et al., 2001), and laccase inhibitors found in polluted environments (Couto and Herrera, 2006), thus providing a lists the substrates, inhibitors, and characteristics of pure fungal laccases that have been studied thus far (Baldrian and Šnajdr, 2006). Although novel bacterial strains producing increasing levels of laccase are continuing to be identified, the high expense of enzyme synthesis remains the most significant barrier to their practical application. Unlike nanotechnology, the biosensors are alike in that industrial and biomedical applications need vast numbers of laccase enzymes.

The utilization of zero or negative cost substrates, such as tertiary matter, agricultural and food wastes, or effluents from the food or pulp and paper industries, might lower the enzyme's cost. Furthermore, the majority of laccase research has been done in shaking flasks on a small scale. For industrial applications, these processes must be tuned and evaluated in large-scale bioreactors. The most frequent methods for determining activity in waste include measuring laccase breakdown of common dyes which is based on the idea that dye decolorization and aromatic pollutant biodegradation are significantly associated (Field et al., 1993). Nonetheless, investigations using R-478 dye and anthracene, both of which may be oxidized by peroxidases and laccases, led to this conclusion. In these tests, laccase activity was not measured. The disappearance of color is a quick and easy approach to check laccase activity; however, the catalytic characteristics and substrate range are still

unknown. Decolorization studies on two or three structurally distinct dyes at acceptable pH levels may provide more useful information regarding laccase's enzymatic activity in an extracellular liquid (Rodríguez-Delgado et al., 2015).

Laccases are found in many plants; however, they are rarely recorded. They're arranged into multigene families, and their function has never been shown. Laccases, which may oxidize lignin precursors and are present in xylem cell walls (Ranocha et al., 1999), are thought to be involved in lignin production. However, genetic modification approaches must be used to prove this theory. The phenotype of an Arabidopsis mutant for three laccase genes was changed, with impaired root elongation, early blooming, and varied seed color, indicating that laccase may be engaged in diverse roles in plants that are not genetically redundant (Cai et al., 2006). Prokaryotes have also been reported to have laccase and laccase-like enzymes. A nonmotile strain of the Gram-negative soil bacterium *Azospirillum lipoferum* was the first to be identified as a bacterial laccase (Givaudan et al., 1993).

The fungal sequence was compared to proteins implicated in copper resistance, manganese oxidation, and cell pigmentation in the bacterial databank (Alexandre and Zhulin, 2000). It has been discovered that it plays a role in melanin production, spore coat resistance, and morphogenesis (Endo et al., 2003). Bacterial laccases are distinct from other "real laccases" found in ascomycetes and basidiomycetes, according to phylogenetic study. Bacterial laccase-like proteins can be found intracellularly, periplasmically (Claus, 2003), or on the spore coat (as in Bacillus subtilis) (Hullo et al., 2001). Laccase activity may be inhibited by a variety of chemicals found in wastewater. Because laccase oxidation of pollutants is generally investigated in optimized synthetic media, pollutant degradation optimization must be done in real wastewaters to understand the precise function of inhibitors (Rodríguez-Delgado et al., 2015) offered lists of known inhibitors for the most frequent laccase-producing species. Various putative inhibitors have different effects on laccases from different species (Majeau et al., 2010).

20.3 Peroxidases, occurrence, and biocatalytic features

Most of the enzymatic browsing is done by peroxidases (Ozbek et al., 2021). Peroxidases consist of heme cofactors, and they have heme-binding proteins which contain iron ions. The iron of heme is replaced by copper, manganese, vanadium, or selenium and lead by some peroxidases (Backhaus and Karlsson, 2014; Dong et al., 2020). The world's population has been quickly increasing in recent decades, resulting in multiple ecological repercussions, and in this way, water and aquatic systems are the most affected regions because of their ability to retain compounds and chemicals (Romero-Guzmán et al., 2020).

Peroxidases, a type of important antioxidant enzyme, are found throughout nature and catalyze the oxidation of a variety of electron donor substrates while also decomposing H_2O_2.

Peroxidases are considered one of the essential catalysts for a variety of pharmacological, immunological, industrial, and biotechnological uses due to their capacity to catalyze the redox reaction for a wide range of substrates. Bioremediation of wastewater, including cresols, phenols, and chlorinated phenols, as well as degradation of synthetic textile colors, can help to minimize pollution. Peroxidases' generic nature allows them to break down a wide range of contaminants in the environment, including petroleum hydrocarbons, ordnance wastes, dioxins, polychlorinated biphenyls, pesticides, and herbicides (Marco-Urrea and Reddy, 2012). Peroxidases hold a prominent position among researchers and the scientific community as one of the most thoroughly investigated enzymes, thanks to a wide range of physiological functions and commercial uses. The current review article focuses on the unique capabilities of peroxidase enzymes in catalyzing the transformation of a variety of ecologically harmful chemicals with the goal of reducing their environmental effect (Rasheed et al., 2018).

20.4 Enzymatic treatment as a greener route for pollutants mitigation

It has been seen that the biological approach to degrade pollutants that uses oxidoreductase enzymes like peroxidases is the latest and hot topic for research. Many enzyme systems have been used to degrade a variety of organic contaminants, with prevalent results showing that the contaminants can be oxidized and degraded into smaller parts (Asgher et al., 2017; Amin et al., 2021a,b; Aslam et al., 2021; Singh et al., 2021; Noreen et al., 2021). Laccases are multifunctional enzymes that catalyze oxidation events that include the use of four electrons in the reduction reaction of molecular oxygen to water (Unuofin et al., 2019). Many species of higher plants and fungi have these types of multicopper enzymes. They can degrade lignin. Peroxidases, commonly known as catalases, are another type of oxidoreductase enzyme that catalyze oxidoreduction processes. The peroxidase enzyme catalyzes hydrogen peroxide to break down into the water and molecular oxygen (Al-Maqdi et al., 2017).

There are many uses and advantages of enzyme-based techniques to overcome pollution, which are as follows:

1. Ability to perform effectively and efficiently at high and low pollutant concentrations.
2. Reduction in sludge generation.
3. Microbial catalytic activities.
4. Mitigation of a large and wide range of spectrum of pollutants.
5. Energy input is very low, feasible, and reliable (Sheldon, 2011; Bilal et al., 2019b).

But there are also some disadvantages and drawbacks like:

1. Very high cost of catalysts that have been used to decrease the level of organic contaminants.
2. Many enzymes can't be reused.

3. There is the possibility that many enzymes can't function properly under rasping; hard and harsh environmental states like enzymes can lose their stability and optimum composition and shape.
4. Probable potential and possibility for the formation of hazardous and dangerous soluble by-products.
5. The concentration and quality of enzymes and pollutants also matter a lot because enzymes are very specific in their function (Zdarta et al., 2018).

Immobilization of enzymes on solid supports can be effective in mitigating and overcoming these drawbacks. Furthermore, efforts have been made to insolubilize certain types of enzymes to make them reusable and recyclable. Many other techniques have been developed to overcome the shortcomings related to the use of enzymes to decrease the levels of persistent organic pollutants (Alneyadi and Ashraf, 2016).

Laccase and peroxidases are two main enzymes that have been used to biodegrade EPs and in the process of bioremediation of polluted wastewater management industries. Organic compounds like chlorinated phenols, herbicides, pesticides, synthetic textile dyes, pharmaceuticals, and personal care products are examples of hazardous chemicals that the enzymes of this classification system can catalyze. Oxidases, peroxidases, dehydrogenases, and oxygenases are perfect examples of oxidoreductases. Peroxidases and laccases are the basic enzymes that have been used in enzymatic-remediation procedures because of their strong ability to degrade various types of organic pollutants. These enzymes produce oxygen radicals, which decompose the parent pollutant into more biodegradable, and less hazardous compounds (Al-Maqdi et al., 2017). The major classes of EPs that can be degraded by laccase and peroxidase are presented in Fig. 20.1.

20.5 Laccase as a biocatalyst to remove environmental pollutants

Laccases are MCO enzymes that can be found in many species, including fungus, plants, bacteria, and arthropods (Battistuzzi et al., 2010). Laccases from microbial sources have taken a lot of interest in research because of their ability to oxidize a wide range of contaminants. These enzymes are divided into three classes based on their copper centers: type 1 (blue), type 2 (normal), and type 3 (coupled binuclear) (Al-Maqdi et al., 2017). Types 1 and 2 have only one Cu atom each, but type 3 has two Cu atoms. The type 1 copper ion's oxidation-reduction potential is the base on which laccases' efficiency is dependent, and that occurs when the substrate is in an oxidized state of reaction. These microbial laccases have increased redox potential as compared to plant enzymes and show higher catalytic efficiency. Microbial enzymes have shown promising results as compared to plant-based enzymes (Chiong et al., 2016). Electrons move from T1 to the tri-nuclear site, type 2 and type 3, and create a tri-nuclear cluster (T2/T3) to reduce molecular oxygen to form H_2O molecules. In the case of laccase-mediated reactions, the ambient oxygen is

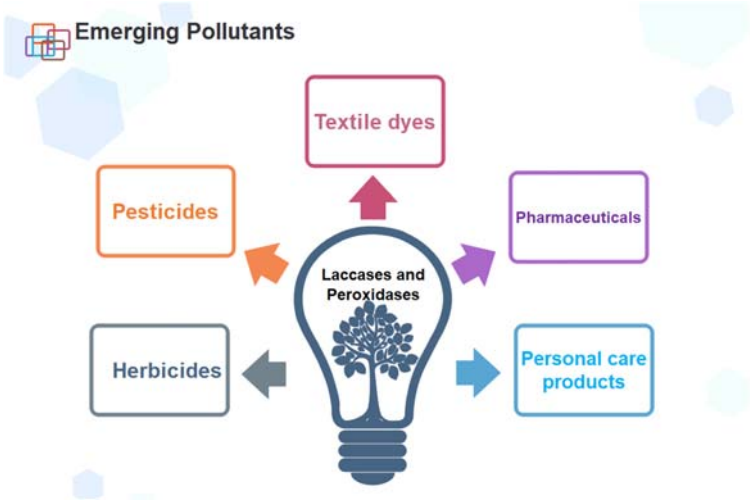

Figure 20.1
Emerging pollutants degraded by laccases and peroxidases.

used as an electron acceptor and is more reliable and feasible than using hydrogen peroxide through peroxidases. There is a paradigm shift that has been seen to occur from the use of laccases in lignin depolymerization to the oxidative removal of many EPs, MPs, xenobiotics hormones, PAHs, and many others, all of which can have a significant effect on the health of humans and aquatic biota (Alneyadi and Ashraf, 2016).

Laccase was seen to have the ability to break down phenol-related dyes into smaller, less harmful compounds. In another review, the hypothetical breakdown of the insecticide lindane into organic acids was explained as a greener and more environmentally friendly by-product (Mo et al., 2018).

The branching degree and location of the alkyl chain are rigorously correlated with the binding affinity between these isomers. Hydrophobic interactions between the examined isomers (NP or OP) and laccase are necessary; further, hydrogen bonding is an optional feature. Common amino acid residues involved in hydrophobic interactions in structurally similar isomers are regarded as residues that are crucial for enzyme-ligand patch up with each other and their reactions (Cabana et al., 2007). The phenolic hydroxyl ions are shown to be related to all the hydrogen bonds of specific NPs and OPs. Finally, this research contributes to a better knowledge of alkyl phenol degradation to lay a theoretical foundation for the remediation of these developing contaminants (Naghdi et al., 2018). Laccases have been used to degrade pollutants like Triclosan, Diclofenac, Bisphenol A, Carbamazepine, Phenanthrene, Benzo[α]pyrene, Tetracycline hydrochloride, Chlortetracycline hydrochloride, Oxytetracycline hydrochloride, Doxycycline hydrochloride, Nonylphenols, Triclosan, Sulfadiazine, Sulfathiazole, Ethinylestradiol, Naproxen, and Estrone (Naghdi et al., 2018).

Free enzymes cannot be reused and are vulnerable to denaturing chemicals. Laccase immobilization can protect them against denaturation by organic cosolvents, improve their overall stability (D'Annibale et al., 1998), make reaction product separation easier (Durán and Esposito, 2000), and keep their catalytic efficiency high throughout several reaction cycles (Palmieri et al., 2000; Brandi et al., 2006). Immobilized laccases have been shown to be efficient at removing phenol from synthetic and industrial wastewaters. Different catalytic characteristics, stability, costs, and handling features come from the type of support and the immobilization approach used. To improve immobilization, it's vital to consider the carrier's morphology but choosing the right pH and enzyme concentration during immobilization is also crucial (Rekuć et al., 2008). Laccase leaching is prevented by covalent grafting, which also enhances enzyme stability under severe circumstances. When utilizing adsorption, however, the frequent issues of decreased specific activity and inadequate laccase fixation are less common. Coating the immobilized laccase with polyelectrolytes was an innovative way to inhibit desorption (Rodríguez Couto, 2007).

Magnetically separable particles have also received a lot of attention as a way to make it easier to reuse and minimize mechanical stress during separation from the reaction mixture (Pich et al., 2006). Immobilization remains a difficulty in practical applications, despite recent advancements (Brandi et al., 2006) found that immobilized laccases' oxidation of nonphenolic substrates with the help of mediators is inefficient. Immobilization can also reduce enzyme activity by reducing the enzyme's structural alteration, limiting substrate contact with the active site, and producing nonproductive orientations. Natural immobilized laccases, such as the spore-coat-bound laccases of *Bacillus* spp. (Held et al., 2005) or *Trichoderma* spp. (Hölker et al., 2002) and membrane-bound enzymes, such as those from *P. putida*, can be used to get around fixation difficulties. Laccase coupled to spores may be readily removed from the reaction media and reused numerous times (Hirose et al., 2003). The potential role of laccase in pollutants degradation is presented in Fig. 20.2.

20.6 Peroxidases for removing environmental pollutants

Microbes, fungi, bacteria, mammals, and plants all have peroxidases, which are heme-containing antioxidant proteins. Because of the high selectivity, these enzymes can efficiently degrade contaminants (Chiong et al., 2016). It is crucial to use the proper amount of H_2O_2, as too much can cause the enzyme to become inactive. When an enzyme combines with an H_2O_2 molecule, peroxidase reactions occur. Peroxidases always bioremediate a wide spectrum of developing pollutants, and this is shown and explained in various research studies. Soyabean peroxidase, horseradish peroxidase and chloroperoxidases are examples of the most often utilized peroxidases for wastewater treatment. It can be concluded that peroxidases play a very important role in removing EPs. SBPs will react with Nonylphenols, Octylphenol, Triclosan, Sulfamethoxazole, Estrone, 3-Hydroxyquinoline, Sulforhodamine B dye,

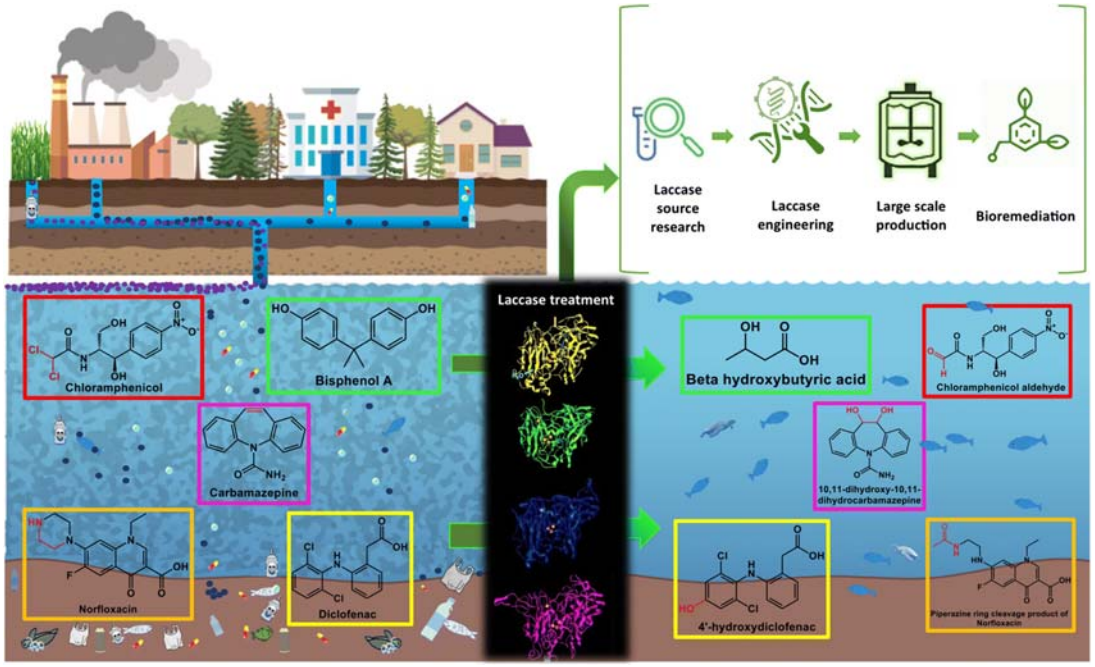

Figure 20.2
Schematic illustration of laccase-mediated biodegradation of water contaminants. *Reproduced from Arregui Mena, A.L, et al., 2019. Laccases: structure, function, and potential application in water bioremediation. Microbial Cell Factories 18, 200. An open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).*

o-Anisidine, Mercaptobenzothiazole, and Methyl Orange (Chiong et al., 2016). The potential peroxidases used for the removal of EPs are shown in Fig. 20.3.

20.6.1 Soybean peroxidase for removal of EPs

SBPs have conformational flexibility and, due to their interesting properties, they have been a hot topic in the recent era. It has wide applications in the treatment of wastewater and other industrial wastages (Morsi et al., 2021). It has shown the exclusive characteristics of oxidative polymerization by catalyzing the hazardous pollutants in water from various industries such as petroleum refining, coal extraction and conversion, metal casting, pulp and paper, dyes, adhesives, resins, plastics, wood manufacturing and preservation, and textile manufacturing. Many bacterial diseases have been treated using the antibiotic Sulfamethoxazole (SMX) and have been found in many water systems, such as sewerage provided to household levels and industries. Soybean peroxidase has the ability to degrade SMX, and it is used to catalyze 1-hydroxy benzotriazole (a derivative of benzotriazole). They have been used as a redox mediator in catalytic reactions. SBP was able to degrade >80% of SMX in the presence of

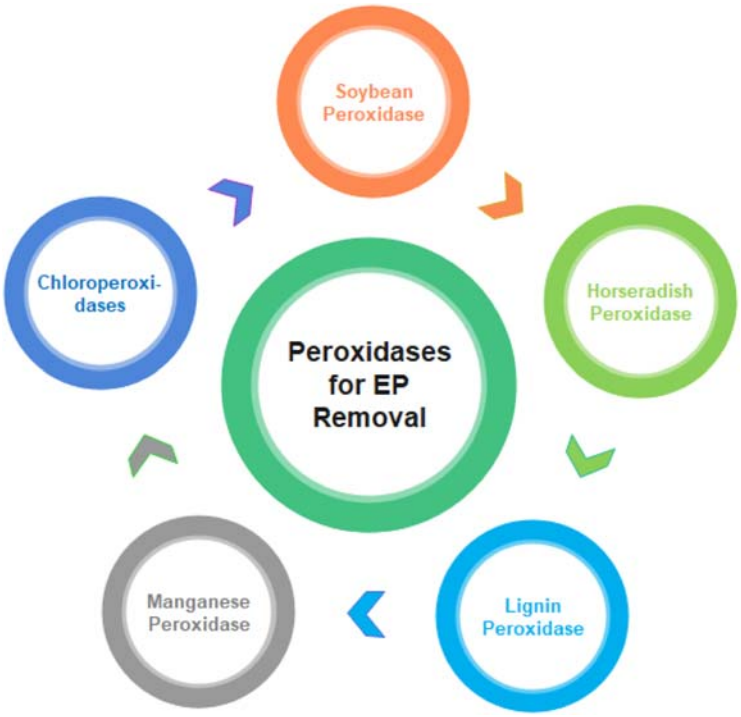

Figure 20.3
Peroxidases for removing EPs. *EPs*, Emerging pollutants.

H_2O_2 (Husain, 2019; Singh et al., 2019). Diclofenac is very difficult to remove from our water bodies by WWTPs; it shows problems and results in serious ecological difficulties. Organic pollutants in industrial contaminants, such as aromatic hydrocarbons, chlorinated phenols, and other polycyclic aromatic hydrocarbons, can be hazardous and even prove to be lethal to human beings, animals, plants, and the whole biosphere (Li et al., 2017).

The SBP-based system has been employed to break phenolic chemicals in refinery and coal tar wastewater. Thiazole-related chemicals are a growing family of persistent pollutants widely used in industrial, medicinal, and other personal care usages. MBT is an example of removal by SBP, and it is an organosulfur compound that is used in the manufacturing of rubber items such as tyers. MBT can be degraded by two different peroxidases, which are SBP and CPO (Subramanian et al., 2019).

20.6.2 Horseradish peroxidase of removal of EPs

HRP has been studied for over a century for its importance in waste management. It is a vital heme-containing enzyme with numerous applications (Veitch, 2004). HRP is a globular molecule with a predominantly α-helical secondary structure except for one short β-sheet

region. HRP has been produced in bacterial species like *E. coli* as a recombinant product. It has also been produced in plants commercially (Veitch, 2004). HRP peroxidase isoenzymes are plentiful in the root of the horseradish herb (Armoracia rusticana), with HRP-C being the most common isoenzyme. HRP has a role in the catalytic mechanism of plants and fungi. HRP has been isolated form the roots of plants. It has wide applications in life sciences, medicine, and biotechnology in bioremediation, biocatalysis, biosensor systems, and cancer therapeutics (Bilal et al., 2016; Rafeeq et al., 2022b). It has been proved to be the reporter enzyme in staining procedures and, in this way, has diagnostic applications. It has been used in enzyme engineering and detecting hydrogen peroxide as a bio-oxidant. Electrochemical biosensor systems have been shown that enzymatic activity of starch conjugated HRP was found promising for bromophenol blue decolorization, representing its deployment for wastewater remediation (Samavat and Kurzer, 2015). HRPs play an important role in removing pollutants like Phenol, Paracetamol, p-Chlorophenol, Catechol, 2,4-Dichlorophenol, Bisphenol A, 4-Methoxyphenol, Triclosan, 17α-Ethinylestradiol, Estrone, 17-beta-Estradiol, Estriol, Synthetic 17-alpha-Ethinylestradiol, and different type of Estriols (Mao et al., 2010).

20.6.3 Lignin peroxidase to remove EPs

Lignin peroxidase is a glycoprotein and one of the important enzymes in the biodegradation process that will decrease the stability of lignin and form free radicals in the lignin polymer (Bilal and Iqbal, 2020). LiP is an enzyme used to catalyze the depolymerization (through oxidation reactions) of lignin in the presence of H_2O_2. It's a monomeric hemoprotein that uses veratryl alcohol as a catalytic substrate and operates at an acidic pH (about pH 3.0). It has high redox potential; LiPs can oxidize refractory aromatic pollutants and other phenol and nonphenol-containing compounds. WRF produces veratryl alcohol, a natural redox mediator that aids in the oxidation of target chemicals. Just because of the negative impact on humans, animals, and plants' health and the overall ecosystem, a classified type of EDCs known as steroid estrogens has become a global concern (Kersten, 1990). The reason is that it can disrupt the growth, reproduction, and development of living organisms. Degradation and detoxification of lignocellulosic waste in the environment are done by ligninolytic enzymes. The activity of LiP with manganese peroxidase has been improved by various mediators to use them as a pollutant degrading enzyme. The overall reaction is done by 4 benzenediol with oxygen to form 4 benzosemiquinone, and a water molecule is released. Due to extremophilic properties of ligninolytic enzymes, they are being used in biodegradation and bioremediation of permanent and organic pollutants, but still, their production is limited. *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Klebsiella pneumonia*, and *Serratia marcescens* are examples of bacterial species that produce extracellular peroxidases to degrade lignin related waste. Lignin peroxidase can remove pollutants like Carbamazepine, Diclofenac, Oxytetracycline, and Tetracycline (Samavat and Kurzer, 2015). Fig. 20.4 portrays a mechanistic insight into catalytic oxidation of veratryl alcohol by LiP.

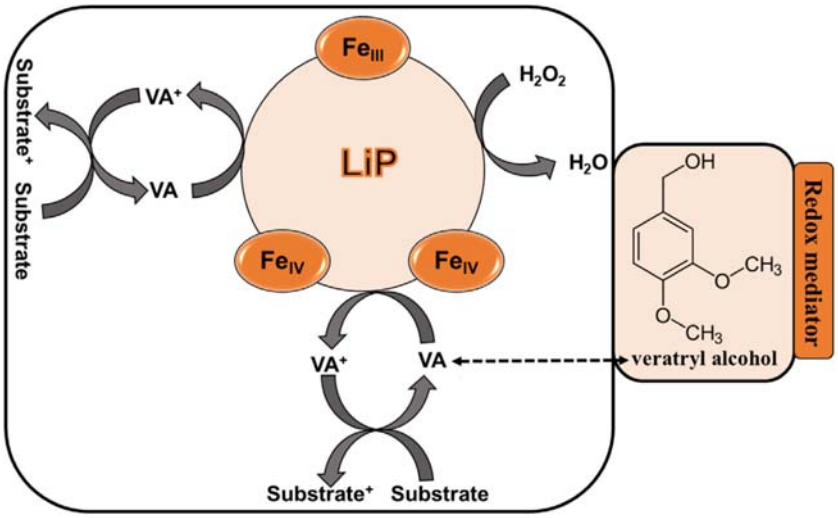

Figure 20.4

Mechanism representation of LiP catalytic reaction in the EP degradation process (Bilal et al., 2022). *Reproduced with permission from Bilal, M., Bagheri, A.R., Vilar, D.S., Aramesh, N., Eguiluz, K.I.B., Ferreira, L.F.R., et al., 2022. Oxidoreductases as a versatile biocatalytic tool to tackle pollutants for clean environment—a review. Journal of Chemical Technology & Biotechnology 97(2), 420–435. Copyright John Wiley and Sons.*

20.6.4 Manganese peroxidase to remove EPs

Manganese peroxidase that has been produced from Ganoderma lucidum in Pichia pastoris has wide applications and sustainability to degrade dyes and phenolic compounds (Hofrichter, 2002). Manganese peroxidase catalyzes the oxidation of Mn^{2+} to Mn^{3+} in the presence of hydrogen peroxide (H_2O_2) to catalyze the degradation of environmental pollutants (Fig. 20.5). Ganoderma can secrete lignin-modifying enzymes, e.g., laccase, lignin peroxidases, and manganese peroxidases that will aid in resisting and removing pollutants (Asgher et al., 2016). Recombinant GluMnP1 has a specific enzyme activity that will be able to decolorize the dyes and degrade phenol-related compounds (Camarero et al., 1999). Manganese peroxidase TP55 will degrade EPs like Poly R-478, Acid Blue 158, Direct Red 5B, Remazol Brilliant Violet 5 R, Methyl Green, Indigo Carmine, Remazol Brilliant Blue Reactive, and Cibacet Brilliant Blue BG. Manganese peroxidase will also take part in degrading Drimaren Yellow X-8GN, Tetracycline, Oxytetracycline, Nonylphenol, and Triclosan. MnP-Tween 80 system removes Miconazole and Antidepressant sertraline (Rekik et al., 2019).

20.6.5 Use of chloroperoxidases to remove emerging pollutants

CPO has oxidative biocatalytic properties to remove pollutants. *Caldaromyces fumag* has been used to produce chloroperoxidases in the presence of H_2O_2 to use as an electron

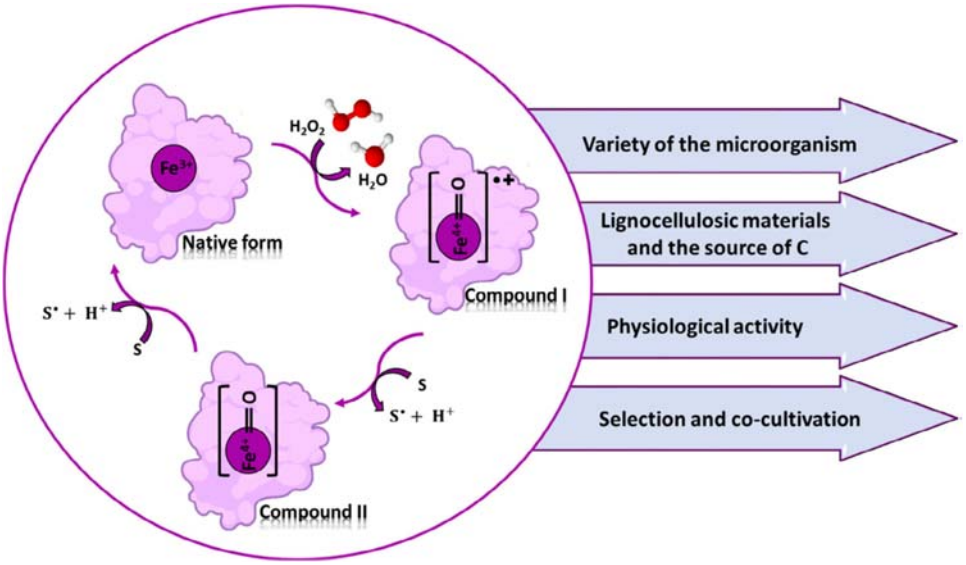

Figure 20.5
Catalytic cycle of MnP involving the formation of the intermediates (compounds I and II) for pollutants degradation and factors determining enzymatic production (da Silva Vilar et al., 2022).
Reproduced with permission from da Silva Vilar, D., Bilal, M., Bharagava, R.N., Kumar, A., Kumar Nadda, A., Salazar-Banda, G.R., et al., 2022. Lignin-modifying enzymes: a green and environmental responsive technology for organic compound degradation. Journal of Chemical Technology & Biotechnology 97(2), 327–342. Copyright John Wiley and Sons.

acceptor. Substrates for the oxidation of chloroperoxidases have been proved to be important compounds in pharmaceutical industries. The efficient catalytic activity of these enzymes has been used to remove wastewater effluents and other industrial wastes. So, the outcome is that these can remove pollutants like Mercaptobenzothiazole, Diclofenac, Sulforhodamine B dye, Ketorolac, Ketoconazole, Estradiol, and Tetracycline (Alneyadi and Ashraf, 2016).

20.7 Challenges and perspectives

Biocatalytic competency and adaptability for the change and biodegradation of many resistant and persistent pollutants such as polychlorinated biphenyls that are endocrine disruptors and environmental xenobiotics are efficient but, still, there are many shortcomings of a practical nature that can be observed like the use of oxidoreductases on a large scale has not been attempted (Bilal et al., 2018). The latest issues that arise during the real-time application of peroxidases were inspected and, in the case of peroxidases, augmentation of operative stability and strength, specifically stability and firmness of H_2O_2, remains to be seen; further, widening and broadening the substrate assortment and selection by amplifying and increasing the rate of enzyme's redox reaction potential, and formation

and development of heterologous expression systems for the production on a large scale are moderate examples of distinguished protein-engineering bottlenecks that have been examined (Nie et al., 1999). Low functional stability hampers and disturbs the large-scale application and utilization of peroxidases; it happens because, in most cases, enzymes are deactivated in the presence of their natural substrate (H_2O_2) that is applied in excess concentrations or in the absence of aromatic amines that are reducing substrates (Linares et al., 2011).

The mechanism of inactivating the specific compound is unknown; many incidents and events have been discovered and prospected that resulted in the loss of activity, that includes intermolecular cross-linkage and embellishment of designated linkages by using lower redox potential oxidation of amino acid residues occurring and heme instability during reduction (Conesa et al., 2002a) taking place. In this context, for the biodegradation of EPs and related hazardous pollutants, many artificial strong enzymes have been developed for efficient biocatalysts and reduction reactions (Conesa et al., 2002b).

20.8 Conclusion

Many technologies related to wastewater treatment and environment-friendly procedures are presented and depicted, focusing on peroxidases-based enzymatic approaches and laccases-based enzymatic activities for developing biodegradation and detoxification of pollutants in several elaborative ways. This study highlighted and put the spotlight on biocatalytic techniques, which have gained an outstanding reputation in reducing a mixture and amalgamation of developing contaminants normally present in wastewater effluents, based on an exhaustive literature scan and our research struggles. Even though several controlled studies on the enzyme's applicability have been conducted in the research field, a significant gap between laboratories and theoretical works and the usage of enzymes on large scales are the shortcomings that need to be addressed. Enzyme usage in a proper way is currently far from what is needed and has not yet been implemented in large-scale water treatment systems, despite being an effective technology with proven bioremediation potential. The key reasons for the absence of large-scale and commercial level uses of bioremediation are the cost of enzymes and other related products and their recycling activities. Furthermore, the immobilized enzymes can be retrieved and reused after applying many kinds of biodegradation procedures, given enzyme immobilization has been proved to be an effective technique to solve these challenges. Immobilization of enzymes has been shown to improve enzyme activity, stability, and substrate specificity in a number of studies. Immobilization has several benefits: continuous enzyme use, high stability, short reaction times, increased process control, multi-enzyme systems, fast product separation, environmental friendliness, and safety. Future research should expand the application of enzymes in real-world therapy settings.

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CHAPTER 21

Enzyme immobilization on alginate biopolymer for biotechnological applications

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21.1 Introduction

Enzymes are biocatalysts having the potential for use in biotechnology and industry (Aggarwal et al., 2021). They are preferred over chemical catalysts because of their higher catalytic speed in mild conditions, which include lower pH, temperature, lower level of energy use, and less occurrence of side reactions. These enzymes are ideal catalysts at the industrial level, but they have some drawbacks which sometimes make them less useful at commercial levels. These drawbacks include their lesser stability in organic solvents and at higher temperatures (Min and Yoo, 2014). They cannot be reused multiple times, and a slight change in pH can lead to their denaturation. Immobilized enzymes optimize the operational functions of various industrial processes, the reason behind this is immobilization that makes enzymes stable and usable in organic solvents. The process of immobilization of enzymes is carried out using three different methods which are:

1. Enzymes are bonded or fabricated to a solid support, e.g., nanoparticles, alginates, cellulose;
2. Enzymes are entrapped in the inorganic or organic matrix; and
3. Enzymes are cross-linked to enhance their stability (Sheldon, 2007).

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The process of immobilization of biocatalysts on solid supports increases their stability, and enhances their bioactivities, specificity, and reusability (Garcia-Galan et al., 2011). Various solid supports are used to immobilize enzymes such as nanoparticles, alginate, cellulose, and numerous other biopolymers. In this review, we will discuss how the alginate solid support interacts with enzymes to enhance their durability, stability, and reusability. Another significant advantage of enzyme immobilization is that these enzymes may show properties that can be employed in the reactions performed in nonaqueous environments (Jesionowski et al., 2014). Alginate is an ideal material to immobilize biocatalysts to get the optimal enzymatic functions at the industrial level.

Alginate is a carbohydrate and consists of two subunits, namely, D-mannuronic acid (M) and α-L-guluronic acid (G), which are connected through 1−4 glycosidic linkage. These glycosidic linkages generate three versatile blocks in alginate, which are PolyM, polyG, and polyMG (Bilal and Iqbal, 2019). These alginates have their applicability in enzyme immobilization due to their unique physiochemical characteristics. Alginates can be used in the form of beads or hydrogels to immobilize biocatalysts. Usually, alginate supports entrap (Anwar et al., 2009) and encapsulate (Zhao et al., 2015) enzymes to make it robust and fast working. The utilization of alginate as support for enzyme immobilization provides a remarkable benefit in terms of enhanced bioactivities. It makes enzymes more stable by giving them a biocompatible microenvironment which leads to their optimal catalytic activities. Alginate is a biopolymer matrix that is obtained from different types of algae; chiefly, it is obtained from brown algae (Khotimchenko et al., 2001). It is nontoxic, biocompatible, and its cost-effectiveness makes it an ideal material for fabricating enzymes (Broderick et al., 2006). Moreover, it has the ability to form gels under mild conditions with the use of divalent cations such as calcium ions, and these hydrogels can act as support for enzyme immobilization. Different derivatives of alginate have broader ranges of molecular weights, which make them promising materials for use as supports for immobilizing different types of enzymes. This chapter provides the use of alginate as support for the immobilization of enzymes, its advantages and disadvantages, and its potential applications at the industrial level.

21.2 Interaction of alginate and enzymes

Different methods are used to attach the enzymes to solid supports, but immobilization of enzymes using alginate supports is carried through the mechanism of entrapment and encapsulation.

21.2.1 Enzyme entrapment in alginate matrix

In the process of entrapment, biocatalysts are occluded in the matrix, such as alginate, and they disperse in the material and provide a way to substrates and products. Enzymes are dispersed in the beads, particles, and layers of material and form no covalent bond between

the enzymes and support, which ensures its higher enzymatic functions and elevated stability. This method is best used to immobilize isolated biocatalysts (Imam et al., 2021). Na-alginate was used to immobilize the lipase enzyme. Solution of Na-alginate and lipase biocatalyst was added dropwise in a calcium ions solution. The enzyme was entrapped in alginate beads, and it was observed that higher concentration and greater size of alginate beads reduce the efficiency of immobilization. Moreover, immobilized enzymes were more durable than their free counterparts (Won et al., 2005). Arthrobacter species produce lipase, and this lipase enzyme was entrapped in Ca-alginate beads by Bhushan et al. Na-alginate solution and calcium chloride solution were used to form Ca-alginate used in this process, and it was discovered that enhancing the amount of Ca-alginate matrix, the yield of immobilized biocatalyst can be enhanced up to a certain limit and its catalytic activity was same up to 10 times reuse (Bhushan et al., 2008). Kumar et al. employed a method of affinity precipitation to purify and get thermally stable α-amylase enzymes. In this affinity matrix process, they utilized alginate beads to entrap the enzyme, and it was precipitated using a $CaCl_2$ solution. 0.5 molar sodium chloride solution and 0.2 molar calcium chloride solution were used to recover the α-amylase. The specific activity of the enzyme was 1764U/mg, and it was homogenous with 76% recovery. This enzyme was thermally more stable than its free counterparts, and it was able to tolerate higher pH values. The optimum temperature and pH for free enzyme were 54°C and 5.5, and were 60°C and 6.0 for the entrapped enzyme, respectively, which proved the enhanced stability of the enzyme. The increase in activation energy was also an indication of higher stability (Kumar et al., 2006). In Table 21.1, the enzymes immobilized on alginate supports by using the process of entrapment are listed.

Although there is no covalent bonding between the solid support and biocatalyst in the process of entrapment, still sometimes, due to the formation of weak wander walls interactions among them, leaching of biocatalyst can occur. Due to this, the enzymatic activity of biocatalyst reduces, and alginate supports become less strong mechanically. To sort out all these issues, this is mixed with some other biopolymers, which makes it more compatible (Datta et al., 2013). Polyvinyl alcohol alginate was used to immobilize Mn peroxidase (MnP) enzyme. This catalyst was removed from the species *Ganoderma lucidum*, and immobilization was done on polyvinyl alcohol alginate beads. For immobilization, 1.5% Na-alginate, 10% polyvinyl alcohol, 3% boric acid, and 2% calcium chloride solution were used. This immobilized enzyme was used to detoxify and decolor the sandal dyes, and it was more thermostable and could withstand a wider range of pH. It had the potential for reuse up to 6 times consecutively with 60% retained activity (Bilal and Asgher, 2015).

21.2.2 Encapsulation of enzymes in alginate

In this method, the enzyme is immobilized by enclosing it in a membranous material which is often called the capsule. It is cost-effective and proved to be a very easy

Table 21.1: Enzyme immobilization by using the process of entrapment on alginate support.

Enzyme	Alginate support	Catalytic activity	Potential applications	References
Tyrosinase	Cu-alginate	67% yield of enzyme immobilization with higher range of pH tolerance and enhanced stability and reusability	Formation of bioreactors to yield L-DOPA	Munjal and Sawhney (2002)
Pectinase	Ca-alginate	1. Enhanced stability of enzyme with only 20% reduction in activity after 3rd reuse 2. Higher storage ability with less reduction of activity	Use in food industry to reduce viscosity and to clarify juices	de Oliveira et al. (2018)
Urease-glutamic dehydrogenase	Na-alginate	Lower sensitivity to inhibition in presence of heavy metals than its free form	For the detection of mercury (II) and copper in environment	Rodriguez et al. (2004)
Soyabean peroxidase	Ca-alginates	Increase in stability	It is used to oxidize environmental matrix and to remove azo dyes	Tummino et al. (2020)
L-arginine	Ca-alginate	Enhanced level of detection and storage stability	It is used in food industry to check the L-arginine in juices	Kumar et al. (2012)
Laccase	Cu-alginateCa-alginate	Enhanced thermal stability and wider range of optimum pH	It is used to decolorize various dyes in textile industry	Bagewadi et al. (2017)
Asparaginase	Ca-alginate	Prompt detection of L-asparaginine and increased storage stability	Used as a biosensor to check the level of L-asparaginase in leukemia patients	Kumar et al. (2013)

method to immobilize larger enzymes (Minteer, 2017). Urease enzyme was immobilized by using the process of encapsulation on xanthan alginate support. These immobilized enzymes were synthesized in two-step processes. In the first step, polysaccharides were coupled with calcium ions, and then it was cross-linked with amino groups of gelatins using glutaraldehyde. The thermostability of the immobilized enzyme was increased moreover, and it was capable of tolerating a wider range of pH. Its reusability was enhanced up to 20 times with only a 25% loss in activity (Elçin, 1995). Glucose oxidase biocatalyst was immobilized on Ca-alginate support (Blandino et al., 2001). *Saccharomyces cerevisiae* invertase was immobilized in alginate capsules. Optimal conditions for bioactivity were not altered by immobilization, and the maximum pH was 4.3, whereas the maximum temperature was 60°C for free and immobilized biocatalyst, and immobilized invertase was more stable at high pH and temperatures (Tanriseven and Doğan, 2001). Li Jian et al. studied the improvement in reuse and long-term storage capabilities of biocatalysts. Enzymes were immobilized by the method of encapsulation

on alginate beads. Firstly, β-Glucuronidase (GUS) was pre-adsorbed on calcium carbonate. It was then encapsulated on alginate. This inhibited the desorption of biocatalyst and reduced the swelling of the gel. Consequently, the reusability and storage stability of biocatalyst was enhanced. GUS would be used 7 times with 20% loss of activity and stored for 27 days with 67% retained activity (Li et al., 2009). Ca-alginate was used to encapsulate two biocatalysts, namely, glucose oxidase and catalase, for the formation of a co-immobilized catalytic system. Same catalytic inhibition was observed for alginate beads as well as capsules. But when alginate capsules were utilized to immobilize enzymes, glucose oxidase showed higher efficiency than beads, and it was related to the interaction of biocatalysts and capsule and to the structure of the capsule (Blandino et al., 2003). Kucharzyk et al. studied the effect of free ligninolytic biocatalyst and Ca-alginate encapsulated biocatalyst for the decomposition of crude oil. Changes in the oil were checked for about 70 days. The effects of enzymes on oil were monitored by checking the change in concentration of degraded products, e.g., resins and aromatic compounds. It was observed that when the concentration of residue oil decreased after the application of the enzyme, it was found to be enriched with organic degrading species of microbes. This demonstrated that firstly oil products were not available to microbes for degradation, and after the implementation of alginate-based enzymes, it was decomposed, and further decomposition was carried out by microbes producing laccase and Mn peroxidase (Kucharzyk et al., 2018). In Table 21.2, enzymes immobilized by the process of encapsulation on alginate supports are listed.

The encapsulation of enzymes in alginate beads sometimes provides less stability and reduced entrapment capability, especially when enzymes consist of water-soluble components. Laccase enzyme immobilized on alginate beads through process of microencapsulation caused 36% reduction of protein. Water leaching was 44% when Na-alginate was used. Water leaching was controlled by reducing the pH, and moreover, bentonite solution was added to reduce the water loss. Cross-linking also provided compatibility and stability to an enzyme (Dashevsky, 1998).

21.2.3 Immobilization of biocatalysts through adsorption

In this process, enzymes are adsorbed on the surface of the support and get stability (Guisan et al., 2020). β-galactosidase was immobilized by using the process of adsorption on sodium alginate. The enzyme thermostability was enhanced, and it was able to tolerate a wider range of pH (Souza et al., 2018). This enzyme has its potential applications in the nutrition and medicinal industry. Cheap, fast, and cost-effective methods are needed to separate and purify this biocatalyst. Li et al. studied the immobilization of lysozyme enzymes. Support for immobilization was made by

Table 21.2: Enzyme immobilization by using the process of encapsulation on alginate support.

Enzyme	Support	Catalytic activities	Applications	References
Glucose oxidase	Ca-alginate	Maintenance of 68% and 92% initial catalytic activity at pH 3 and 4, reusability up to seven times	Potential use to produce less alcohol containing wines	Ruiz et al. (2018)
Urease	Chitosan-covered alginate	Reduce the susceptibility of inactivity after exposure to proteases	It is used to protect enzyme from inhibition by chymotrypsin	DeGroot and Neufeld (2001)
Glucose oxidase	Ca-alginate	Reduction in biocatalytic leaching	Used to check the effect of concentration of Na-alginate and Ca^{+2} ions on the diffusional characteristics of capsule	Blandino et al. (2000)
α-chymotrypsin	Na-alginate	Higher physiological action up to 70%	Drug delivery	Tiourina and Sukhorukov (2002)
Glucoamylase	Alginate clay	Enhancement in reusability up to 7 times with retained catalytic action up to 51.77%	Used to hydrolyze cassava roots to yield glucose	Abd Rahim et al. (2013)
Lipase	Ca-alginate	Reusability up to 4 times with no enzyme leakage	Used in transesterification process	Yadav and Jadhav (2005)
laccase	Carbon-alginate	Improvement in enzyme loading, enhancement in half-life of adsorbed catalyst	Used in biofuel cell production	Khani et al. (2006)

combining graphene oxide with Na-alginate to yield a calcium ion cross-linked composite. The enzyme was adsorbed on this support. After immobilization, the enzyme was stable, and its reusability was enhanced up to four times (Li et al., 2018). Kurayama et al. immobilized the biocatalyst formate dehydrogenase on $C_9H_{23}NO_3Si$ (ATEPS) containing Na-alginate beads. They prepared an alginate hybrid of the enzyme by adsorbing the enzyme on it. The alginate was changed by cross-linking with calcium chloride solution and $C_9H_{23}NO_3Si$. After immobilization, the enzyme efficiency was increased up to nine cycles of reuse (Kurayama et al., 2020). The adsorption method was used to immobilize tannase biocatalyst on chitin alginate beads. The biocatalyst was extracted from *Bacillus subtilis*. The enzyme was immobilized by incubating for 4 hours and adsorbing for 1.5 hours using Na-alginate, calcium chloride solution, and chitin. The yield of the enzyme was eighty-two percent with 67% catalytic activity. After immobilization, the enzyme could be stored for up to 3 months at 4°C with the retention of 83% catalytic activity. Its reusability was enhanced up to 10 times retaining 79% activity (Jana et al., 2015).

21.2.4 Covalent immobilization

Innulase enzyme was covalently bonded to modified alginate matrix. The alginate was combined with polyimines, and glutaraldehyde was used to cross-link them to immobilize crude enzymes. The pH sensitivity of the immobilized enzyme was enhanced from 4.5 to 5. Its thermostability was also increased. Immobilized biocatalyst tolerated the 60°C temperature for 120 minutes with only 23% loss in activity, while there was a 93% reduction in activity in the case of free enzymes. Moreover, the optimum temperature for covalently immobilized innulase was enhanced by 5°C. The immobilized catalyst was capable for reuse up to 20 times as compared to free enzymes which has reusability up to 8 times only with greater loss in activity (Elnashar et al., 2009). Glucoamylase biocatalyst was covalently immobilized on Cyclohexa-2,5-diene-1,4-dione stimulated alginate. This method was compared with the previously used entrapment method to immobilize the enzyme. No change was noticed in optimum temperature and pH sensitivity. However, retention of biocatalyst immobilized by the covalent bonding method was enhanced, and its storage stability and reusability were improved (Eldin et al., 2011). Li Tuoping et al. covalently immobilized pectinase enzyme. Na-alginate cross-linked with glutaraldehyde was used as support. Optimum pH was enhanced from 3 to 3.5. Thermostability and reuse capability of the enzyme were enhanced. The immobilized biocatalyst was reused up to 11 times with retention of 80% original activity (Li et al., 2007). The covalent immobilization method was used to immobilize aminoacylase on the biopolymer matrix. Alginate was covalently cross-linked with $C_8H_{17}N_3$ hydrochloric acid to form L-phenylanaline. The catalytic activity of biocatalyst was enhanced by the process of covalent immobilization on Ca-alginate beads. Enzyme reusability was also improved, and it was reused 4 times with a retention of 60% initial activity. The biocatalyst was able to tolerate a wider range of pH and had gained more thermostability (Lee et al., 1993). Similarly, a Na-alginate nanocarrier behaving like a super magnet of size 25−30 nm was made by using oil in the water emulsion process. These alginate nanocarriers were used to covalently immobilize lipase biocatalyst extracted by *Candida rugose*. The morphology and magnetic properties of the nanocarrier were checked by TEM, FTIR, and magnetometer. Enzyme reusability was increased, and support was also regenerated. This regenerated support can be reused to immobilize the enzyme (Liu et al., 2012). Pal Ajay et al. reported the comparison of immobilized biocatalyst to that of its free form. Covalent bonding was used to immobilize biocatalyst on activated alginate globules. Activation of alginate was done with the use of glutaraldehyde. Enzyme thermostability was enhanced, and it was stable at a low temperature. With the enhancement of enthalpy and reduction of negative entropy, the working efficiency of biocatalyst was improved (Pal and Khanum, 2012). In the same manner, the pullulanase enzyme was immobilized on alginate beads, and its catalytic activity was checked to hydrolyze the pullulan. Alginate -COOH groups were activated firstly using $C_8H_{17}N_3$ hydrochloric acid. Secondly, the enzyme was bound to support by the formation of an amide linkage. This has been illustrated in Fig. 21.1 The yield of immobilized biocatalyst

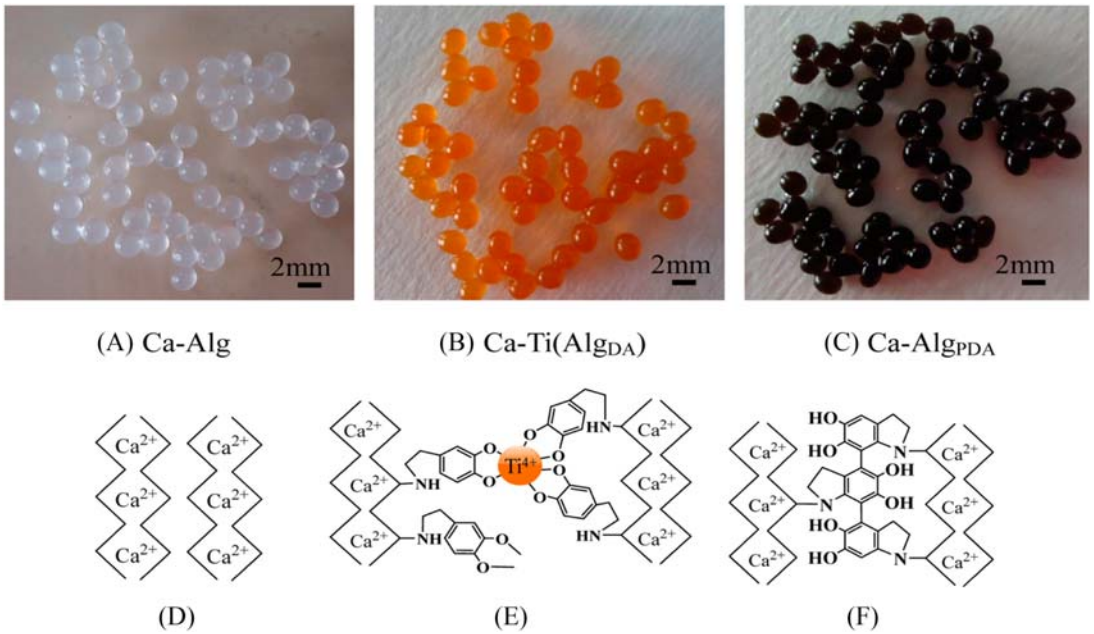

Figure 21.1
Cross linkage in alginate beads. (A) Calcium alginate beads, (B) Ca-Ti(Alg$_{DA}$) Beads, (C) Calcium-Alg$_{PDA}$, (D-F) Structural formulas of Ca-Alg, Ca-Ti(Alg$_{DA}$), and Calcium-Alg$_{PDA}$, respectively. *Reprinted from Wang, X., et al., 2013. Dopamine-modified alginate beads reinforced by cross-linking via titanium coordination or self-polymerization and its application in enzyme immobilization. Industrial & Engineering Chemistry Research 52(42), 14828–14836, with the permission of the American Chemical Society.*

was sixty percent. Their storage stability was improved as compared to free biocatalyst. The drawback of this method is that the interaction between the biocatalyst and substrate is decreased due to steric hindrance and chemical bonding between enzyme and support (Ali et al., 2015).

21.3 Factor affecting enzyme immobilization on alginates

Various factors affect the immobilization of enzymes in alginate supports. Some of them are summarized here.

21.3.1 Cross linkage

Cross-linking in alginate results in the creation of gels which is very useful for entrapping enzymes. Usually, metal ions are used for cross-linking within the alginate by the formation of bonds with the −COOH group of alginates. Alginates have a different attraction for

different metal ions. The interaction of biocatalyst with alginate depends upon the nature and conformation of the metal. This interaction can also be altered by varying the concentration of metal ions in the matrix (Smidsrød, 1974). The addition of metal ions to cross-link the alginate matrix changes its certain properties, which include porosity and swelling, etc. George and Abraham (2006). Wang Xiaoli et al. developed two types of dopamine-modified alginate matrix with the use of Ti bonding and self-linking among alginate strands. Dopamine was added to the alginate by forming covalent bonds with mannuronic acid of alginate. Ti (IV) was combined with it to form [Ca−Ti(AlgDA)] gel. The matrix was cross-linked with the aid of calcium ions. Two gels were developed: one of them only containing calcium ions along with dopamine, and the other containing Ti (IV) covalently cross-linked with Ca- DA alginate. It is shown in Fig. 21.2. The behavior was observed for both types of gels. It was noticed that the mechanical characteristics of Ti containing Ca-Alg gels were three times more than its free form, and it was useful to entrap enzymes for its immobilization. The yield of biocatalyst immobilization for Ca-Alg_{DA} and Ti- Ca-Alg_{DA} was 89% and 100%, respectively. It was only 67.4% for Ca-alginate beads (Wang et al., 2013).

Alginate cross-linked with calcium ions or glutaraldehyde has more stability and compatibility (Flores-Maltos et al., 2011). Bhushan et al. extracted the Xylanase enzyme from a fungus named *Aspergillus flavus*. It was immobilized on alginate. The biopolymer having enzyme on it was further cross-linked with the aid of glutaraldehyde to gain stability. This aggregate behaved like an enzyme and as well as support. The cross-linked biocatalyst had a remarkable reduction in leaching after continuous use and washing. The immobilized cross-linked xylanase had increased reusability up to twelve times (Bhushan et al., 2015).

21.3.2 Effect of pH and ionic strength

The main factor which has influential effects on the structure of biocatalyst and composition of alginate is pH. Decomposition in amino acids in the biocatalyst and disturbance in the structure of the active site because of variations in H^+ ions and imbalances in ionic concentrations of chemical species lead to the formation of globular and other structures of enzymes which has an effect on the catalytic activity of enzymes (Srinivas and Panda, 1999). The affinity between the enzymes and the alginate matrix can be changed with the change in the pH (Velings and Mestdagh, 1994). Neutrase enzyme was immobilized on the alginate matrix cross-linked with glutaraldehyde. The pH sensitivity of biocatalyst was increased (Ortega et al., 2009). Different concentrations of ions play a key role in the development of alginate support which in turn gives rise to beads of different shapes having numerous characteristics (Velings and Mestdagh, 1995). The ionic strength has an influential impact on the behavior of alginate solution, and it changes the viscosity

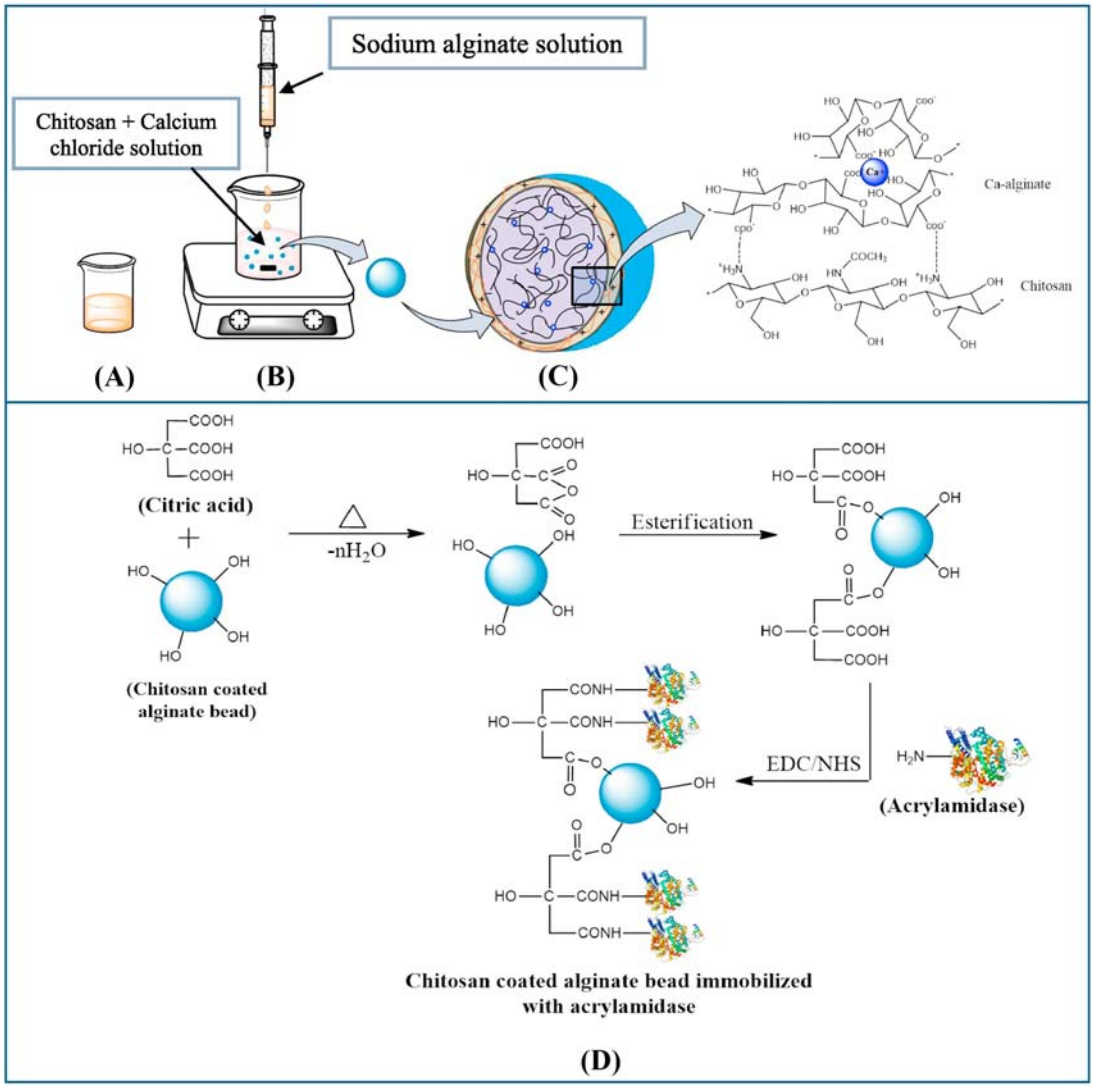

Figure 21.2

Mechanism of immobilization of acrylamidase on alginate beads. (A) Sodium alginate solution, (B) Preparation of chitosan coated calcium alginate beads, (C) Internal structure of chitosan coated alginate bead, (D) Reaction scheme for cross-linking of citric acid onto chitosan coated calcium alginate beads and subsequent immobilization of acrylamidase on it. *Reprinted from Bedade, D.K., Sutar, Y.B., Singhal, R.S., 2019. Chitosan coated calcium alginate beads for covalent immobilization of acrylamidase: process parameters and removal of acrylamide from coffee. Food Chemistry 275, 95–104, with the permission of Elsevier.*

of the gel as well. The alginate polymer chain extention is affected by altering the ionic strength of the solution. Precipitation and fractionation occurs in the presence of higher amount of salts like KCl in solution and salting out of alginates occurs. Hence, with the increase in ionic strengths, the solubility of alginate solutions is changed (Draget, 2009).

21.3.3 Viscosity

The viscosity of the alginate solution is changed by altering the concentration (Poncelet et al., 1992), the structure of the matrix, and by changing the weight and ionic strength of the solution (Donnan and Rose, 1950). Moreover, alginate, as well as biocatalyst, have charged groups that can interact with each other resulting in an enhancement in chain length, which in turn may cause an increase in viscosity. The change in phase and viscosity with the electrostatic interaction between similar groups affects the activity and yield of immobilized enzymes (Feng et al., 2017). The use of a more viscous alginate solution is very advantageous and suitable for the stability of mechanical characteristics of biocatalysts used in food processing and in the rapid bioavailability of drugs (Neiser et al., 1999). At lower pH, -COOH groups of alginates get protonated and hydrogen bonding occurs which, consequently, enhances the viscosity. Nevertheless, sometimes, higher viscosity of alginate solution makes it more susceptible to shear (Lee and Mooney, 2012).

21.4 Application of enzymes immobilized in alginates

Immobilized enzymes have numerous applications in day-to-day life as well as on an industrial scale because of their enhanced catalytic activity, robust ability, and reusability. Some of the applications are described here.

21.4.1 Food industry

Various enzymes are used in the food industry to enhance the storage capacity of food and to speed up the process used for the manufacturing of food items. Pectinase enzymes are one of them. Pectinases are used to clarify the juices of fruits by hydrolyzation of pectic compounds into smaller ones. Dai et al. immobilized pectinase on alginate and graphene oxide support. They observed that immobilized biocatalyst had increased catalytic activity, and its reusability was increased up to six times with only 27% loss in initial activity. The optimum pH for the immobilized enzyme was shifted to 4, which was 4.5 for its free form. The optimum temperature was enhanced up to 10°C compared to its free form. Furthermore, its storage capacity and thermostability were also improved (Dai et al., 2018). Roasted coffee contains a large amount of acrylamide in it. It is removed by using acrylamidase biocatalyst. Bedade et al. (2019) studied the catalytic behavior of enzymes and utilized the process of enzyme immobilization to increase their catalytic properties. To

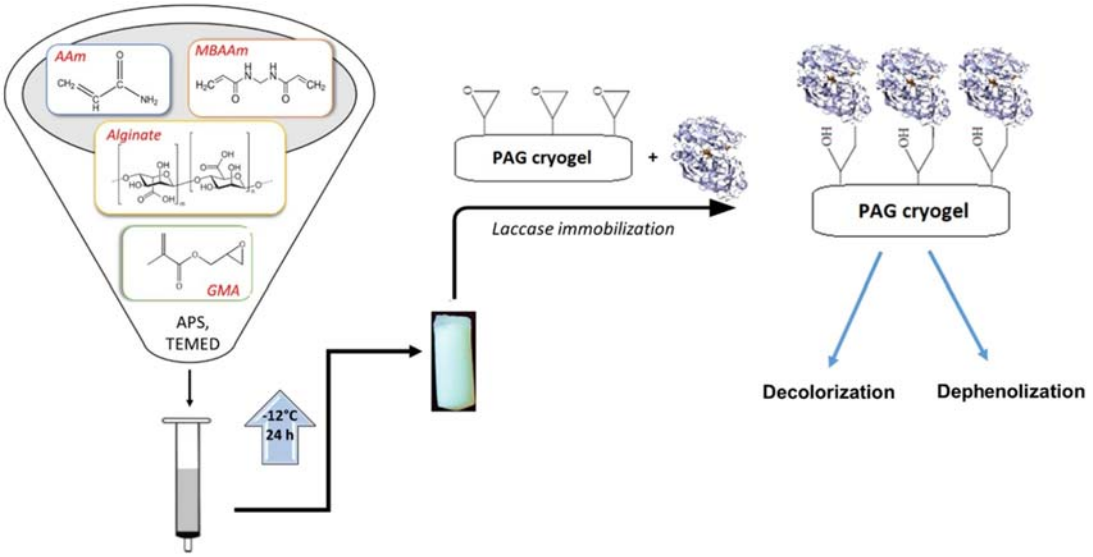

Figure 21.3
Immobilization of Laccase Enzyme on PAG cryogel support. *Reprinted from Yavaşer, R., Karagözler, A. A., 2021. Laccase immobilized polyacrylamide-alginate cryogel: a candidate for treatment of effluents. Process Biochemistry 101, 137–146, with the permission of Elsevier.*

enhance the catalytic activity of acrylamidase enzyme, researchers extracted the enzyme from *Cupriavidus oxalaticus* ICTDB921 and immobilized. For immobilization, chitosan-coated alginate beads were used as support. Na-alginate was added in it to enhance the mechanical strength of the beads. The support was nontoxically cross-linked with citric acid to make it functionalized. Furthermore, the beads were activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride ($C_8H_{17}N_3$) and *N*-hydroxysuccinimide, and it was covalently attached to the enzyme. The stepwise mechanism of this process is illustrated in Fig. 21.3. Frourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were used to characterize beads. After immobilization, thermostability, pH, and storage stability were enhanced, and the biocatalyst was capable for reuse up to four cycles.

Araujiain, which is a phytoprotease, was immobilized on the alginate matrix. Its characteristics were studied in aqueous and nonaqueous media. The entrapped biocatalyst showed enhancement in thermostability and storage stability. The immobilized enzyme was able to tolerate a wider range of pH, and its maximum temperature increased up to 70°C. After immobilization, the storage stability of the enzyme was increased. It was stored for 45 days at 4°C with only a 5% loss in initial activity. In nonaqueous media, the enzyme showed extraordinary performance in the preparation of peptides used in food manufacturing. The immobilized biocatalyst was more robust, thermostable, and capable of

bearing fluctuations in pH and ionic strength (Quiroga et al., 2011). Gupta et al. studied the effect of immobilization on β-glucosidase. The impact of free and immobilized biocatalyst on the properties of sugarcane juice was also investigated. The immobilized biocatalyst was able to withstand a wider range of temperature and pH. The enzyme hydrolyzed the glycosidic bonds between sugar and phenolic component present in the juice. The juice becomes darker in color when these components are released (Gupta et al., 2014).

21.4.2 Environmental applications

The environmental pollutants, such as manufactured and natural dyes, medicines, and phenol and its derivatives, have become a major concern nowadays, and they must be addressed in order to reduce the direct hazards these chemicals bring to the health and existence of living beings. The use of enzymes in the biodegradation of hazardous contaminants in wastewater and soils is one option. The use of immobilized enzymes for the treatment of effluents is a promising method to protect the environment. Laccase is a well-studied biocatalyst in the field of immobilization for environmental applications. Laccase enzyme was extracted from *Trametes versicolor* and immobilized on cryogel of polyacrylamide alginate (PAG). The gel was made functionable using glycidyl methacrylate. The gel was characterized using different techniques like FTIR, X-Ray analysis, and spectrometric methods. Immobilization of laccase was done on alginate gel via covalent bonding though epoxy groups. The whole process of enzyme immobilization is shown in Fig. 21.3. Catalytic activity of free and immobilized biocatalysts was observed using three substrates.

The stability of immobilized laccase biocatalyst was shown to be higher than that of free form when subjected to temperature changes, storage time, and repeated use. Moreover, immobilized enzyme was shown to successfully eliminate 70% phenolic components present in wastewater of olive mill. In addition, dye removal from textile wastewater was found to be 55.6%, with 93.3%−99.1% decolorization of certain dyes in solution (Yavaşer and Karagözler, 2021). Similarly, the laccase enzyme was immobilized on barium alginate beads. The biocatalyst was taken from *Lentinus polychrous*. The immobilized biocatalyst was utilized to eliminate the contamination of acetaminophen in water. Laccase that has been immobilized could be recycled up to five times. Removal capacity and enzyme activity were greater than 70%. The immobilized enzyme showed excellent activity and effective acetaminophen removal at 7 pH and 35°C (Ratanapongleka and Punbut, 2018). Peroxidase enzyme is used to treat textile sewage. Ginger peroxidase enzyme was immobilized on alginate mixed with guar gum. Its reusability was increased up to 10 times and was able to effectively remove or decolorize textile effluents up to 68% and 55% in a batch procedure (Ali and Husain, 2018). α-amylase, protease, and pectinase were coimmobilized on Na-alginate and chitosan beads. Multibiocatalysts were optimized using

2.5% solution of Na-alginate and 0.1 molar calcium chloride solutions. Chitosan-coated multienzyme showed greater specific catalytic activity as compared to those immobilized on alginate beads. These enzymes could be used to bioremediate the contamination of the environment (Gür et al., 2018). Glutathione-S-transferase was immobilized on Ca- alginate beads. This immobilized biocatalyst is used to detect captan in wastewater (Choi et al., 2003).

21.4.3 Medicinal applications

Tannase from *Aspergillus ficcum* was immobilized on Ca-alginate. Its potential application to treat boldo tea was studied. The immobilized biocatalyst reduced the tannin in tea and enhanced its antioxidant property and percentage of flavonoids. The reusability and storage capacity of biocatalyst was increased after immobilization. The enzyme was made capable for reuse for six times with a reduction in only 40% catalytic activity and could be stored for 3 months at 4°C (de Lima et al., 2018). Cholesterol oxidase was covalently immobilized on silica alginate beads. These enzymes are used to diagnose hypercholesterolemia (Prasad et al., 2011). The use of natural polymers in wound dressings is getting more attention because of their superior performance as compared to synthetic ones. Alginate is compatible with human tissues and biodegradable and due to its therapeutic properties, the alginate matrix is used for this purpose. Papain enzyme promotes the debridement of devitalized cells. Papain was immobilized on alginate. Its activity, stability, and performance were improved after immobilization (Moreira Filho et al., 2020).

21.5 Conclusion

Exploiting alginate as a support to immobilize biocatalysts is a developing field with enormous promises for developing unique and sophisticated functional enzymes having enhanced specific activity, reusability, and storage stability with the use of the world's most plentiful and sustainable resource. The immobilization by alginate is the key to effective stability and enhanced properties of enzymes. The increase in functionality of enzymes after immobilization can be tailored by appropriately designing processes. The immobilized enzymes can provide cost-effective paths at an industrial scale because of their better operational stability with vastly improved catalytic functions. The key difficulty in this regard is to design systems that can function continuously, lowering the operational expenses of immobilization processes. Despite recent significant technological breakthroughs in the immobilization of enzymes in alginate, much more work is needed to fully realize their promise for commercial applications.

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CHAPTER 22

Natural prebiotics and probiotics in use as an alternative to antiviral drugs against the pandemic COVID-19

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22.1 Introduction

The despair and gloom cast by Covid-19 pandemic, caused by the corona virus (which bears structural resemblance to the SARS virus) since December 2019, does not seem to wane in the near future, as the loss to humanity and the economy, including psychological stress and a paralyzed education sector, seem far from coming to normalcy and regaining its lost charm. The appeal to all the cohorts of the society, including researchers, scientists, policymakers, and public health experts is boisterous and clear and needs for an inter- and cross-disciplinary approach to address the aftermath of a pandemic of this colossal nature. Situations like the Covid-19 pandemic have shown that post the emergence of novel viral strains and the realization of the severity of the symptoms, the next step would be genome sequencing, followed by vaccine development. Organizations, both public and private, invested massively in R&D that eventually led to vaccine development. However, concerns persisted around issues related to bulk production, fair access, and distribution to the global communities irrespective of their financial status, caste-creed, sex, or socioeconomic status. The situation, however, reached a nadir due to the cost and economic concerns, vaccine unavailability, and inadequacy of means required for its administration, which had to be complied with, keeping in view the principles of social justice (Liu et al., 2020).

The global vaccination figures varied across nations, given where income was low, about 11% of the population received the required dose, while in sharp contrast, in affluent countries, more than 90% of the people were vaccinated. Still more astonishing a figure is

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that—which is relevant to note here—even a single dose was not administered to almost three billion people worldwide (Hunter et al., 2022). Thus, the administration of vaccines in Covid-19 has clearly witnessed a path of variable justness between developed countries where the vaccine was first administered and, only thereafter, the countries not so developed witnessed their vaccination drive. The moneyed nations hoarded vaccines in surplus of their requirement, and the innovators asserted that they would not share their IP, which created an alarm call that pointed to the fact that not less than 90% of people in 67 low-income countries had bleak hope of getting vaccinated by 2021 against the Covid (Dyer, 2020). Although populations worldwide as of now have been jabbed through mass immunization programs, not in their entirety though, the unvaccinated people are attached to the stigma of spreading the disease even in the vaccinated populations (Kampf, 2021). It was also essential to abide by the code of conduct like the use of facial masks, washing of hands, social distancing, etc., and it would be necessary to keep a close watch on boosting immunity by using food supplements, and this would be essential in poor countries until the public vaccination reaches—and the population gets immunized—to the maximum (Rasolabadi et al., 2022).

Precautionary measures had to be implemented realizing the fact that effective vaccine development could be arduous and pretty laggard—from vaccine development, clinical trials, approvals, and administration—so, ever since its affirmation by World Health Organization (WHO) as a global health emergency on January 30, 2020, the administration of natural therapeutics like probiotics and prebiotics is on the surge to boost natural immunity (Fauci et al., 2020; Velavan and Meyer, 2020). The symptoms of Sars-Cov2 include maladies of the gastrointestinal tract and pneumonia, wherein the patients' lungs are severely infected. With the intention to treat this, researchers have emphasized the role of herbal immune boosters, which contain steroids, alkaloids, diterpenes, triterpenes, aliphatic compounds, and glycosides, and also plants like *Azadirachta indica, Argemone mexicana, Ocimum sanctum, Tinospora cordifolia*, etc. having antimicrobial potential that can find the immense application (Chikezie et al., 2015; Khanna et al., 2021). Therefore, the severity of the pandemic can be lessened by the adoption of holistic approaches, and it is possible to combat systemic inflammation by augmenting host immunity (Yang et al., 2020).

Natural probiotics and prebiotics, and even synbiotics (probiotics and prebiotics) are good therapeutic options and could be potential immune boosters against prospective viral ailments of the respiratory tract, which they carry out by the modulation of gut microbiota. In elderly individuals or in those on controlled nutrition, pre- and probiotics can serve as a potential adjuvant by improving the health of the gut, enhancing the response of the immune system, and lowering the viral load of SARS-Cov-2 (Akatsu, 2021). Also, in hosts with weakened immunity, the modest intake of Omega3 fatty acids, Zn, Se, and lactoferrin, and vitamins of A, D, and E groups could potentially boost immunity (Rasolabadi et al., 2022). Dietary fibers, antioxidants, and bioactive peptides can also improve the viability of

probiotics and, here, special mention may be made of various overlooked fruits and byproducts from agro-industries like whey and pomace of plants, that could be valorized to yield probiotics and prebiotics, in compliance with the concept of sustainability and circular economy (da Silva et al., 2022). Additionally, neutraceuticals like quercetin, luteolin, glycyrrhizin, lactoferrin, hesperidin, and curcumin could also help in the treatment of Covid-19 (Alesci et al., 2021). Various nutrients like vitamin-C, A, E, and D, Fe, Zn, folate, probiotics, and prebiotics are abundantly present in fruits, vegetables, spices, herbs, seeds, nuts, cereals, millets, and single-cell proteins like *Chlorella* and *Spirulina* which are consumed worldwide as superfoods. Further, bioactive compounds containing fortified or functional foods of plant origin and also food that are encapsulated could be of immense help in boosting immunity against Covid-19. When Covid-19 patients were given vegan diets rich in probiotics in addition to vitamin D and C, and Zn salts, the severity of symptoms related to Covid-19 was found to be lessened (Vishwakarma et al., 2022).

The role of pro- and prebiotics in the regulation of neuroimmune processes is also important as they can interact with the GI tract, immune system, and nervous system, and it has been noted that the brain function is profoundly affected by communication along the gut−brain axis and it would be detrimental in boosting the overall wellbeing of the individuals. Another attractive emerging option are the postbiotics that can lead to gut microbiome alteration; however, it would be essential to undertake detailed studies to ascertain their efficacy and safety upon consumption. Postbiotics exert beneficial effects upon the host, given they are produced by microbial metabolism and also incur minimal risks upon consumption as they lack viable microorganisms. They exert pleiotropic benefits on the host, including modulation of the immune system, antiinflammatory role, as antioxidants, and also possess antineoplastic properties (Żółkiewicz et al., 2020).

22.2 Coronavirus structure and genome

Coronaviruses belong to the order Nidovirales, in the family coronaviridae (CRVD). Toroviruses and coronaviruses are two subfamilies of CRVD. Alpha coronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus have been classified based on rooted and unrooted genetic trees and partial nucleotide sequences of RNA-dependent RNA polymerase (Fig. 22.1). The first two (alpha and beta coronaviruses) primarily infect warm-blooded animals, while the latter ones (gamma and delta coronaviruses) infect birds, and few of them can also infect mammals (Thanigaimalai et al., 2020).

Coronaviruses are physically enclosed and possess positive sense RNA with a single strand RNA (+ss RNA)—a 30 kb genome with a 5′ cap structure and a 3′ poly A tail—which is one of the biggest known RNA genomes (Chen et al., 2020). In general, viral structures are more spherical in shape because they are pleomorphic, so they can shift or adapt their shape in different environmental conditions. Glycoprotein projections are common on the outer

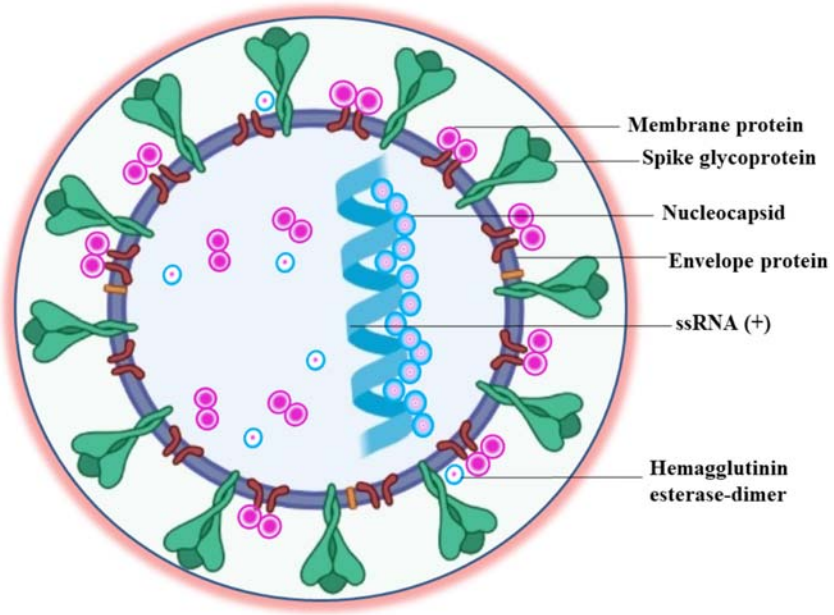

Figure 22.1
Structure of SARS-CoV-2.

capsular envelope membrane, which covers the internal organization and contains a positive-strand RNA matrix protein (Almaghaslah et al., 2020). The viral genome is identical to cellular mRNAs because it has ends that are 5′ capped and 3′ polyadenylated (Fehr et al., 2015). The structure is made up of hemagglutinin esterase, spike, small membrane, and nucleocapsid. Affiliation with the host cell is mediated by the envelope-containing glycoprotein, which contains the principal antigenic epitopes, primarily those that are recognized by anti-neutralizing antibodies. The spike protein undergoes structural rearrangement to make merging the virus outer membrane easier with the host-cell membrane (Luk et al., 2019). The angiotensin converting enzyme (ACE) is a membrane exopeptidase that works as a Covid-19 receptor and enters the human cell, according to recent SARS-CoV research (Zhou et al., 2020).

22.3 Transmission, symptoms, and prevention of Covid-19

Currently, SARS-CoV-2 or Covid-19, the causative pathogen of Coronavirus disease, is rapidly spreading across the world. The virus is zoonotic in origin and was transferred to humans via unknown intermediary animals (Singh et al., 2020a,b). One of the biggest concerns for Coronavirus disease is that it spreads primarily through direct contact with an infected person when they cough or sneeze (WHO, 2020). Indirect touching is also to blame

for this Coronavirus transmission is aided by respiratory droplets. Respiratory droplets might stay in the mouth or nose, or they can enter the lungs during respiration. However, given the current lack of knowledge about transmission systems, numerous governments have recommended aerial safety measures involving high-risk operations. It has been found that even persons who are asymptomatic or in their incubation period might act as coronavirus carriers (Hui et al., 2020; Sahin et al., 2020).

Infection with the Coronavirus causes a variety of symptoms in humans. Dry cough, fever, headache, and exhaustion are some of the most prevalent symptoms. Loss of smell or taste, sore throat, conjunctivitis (usually referred to as "red eyes"), nasal congestion, muscle or joint discomfort, various types of nausea or vomiting, skin rash, diarrhea, dizziness, and chills are some of the less prevalent symptoms in some patients (Fig. 22.2). Many people

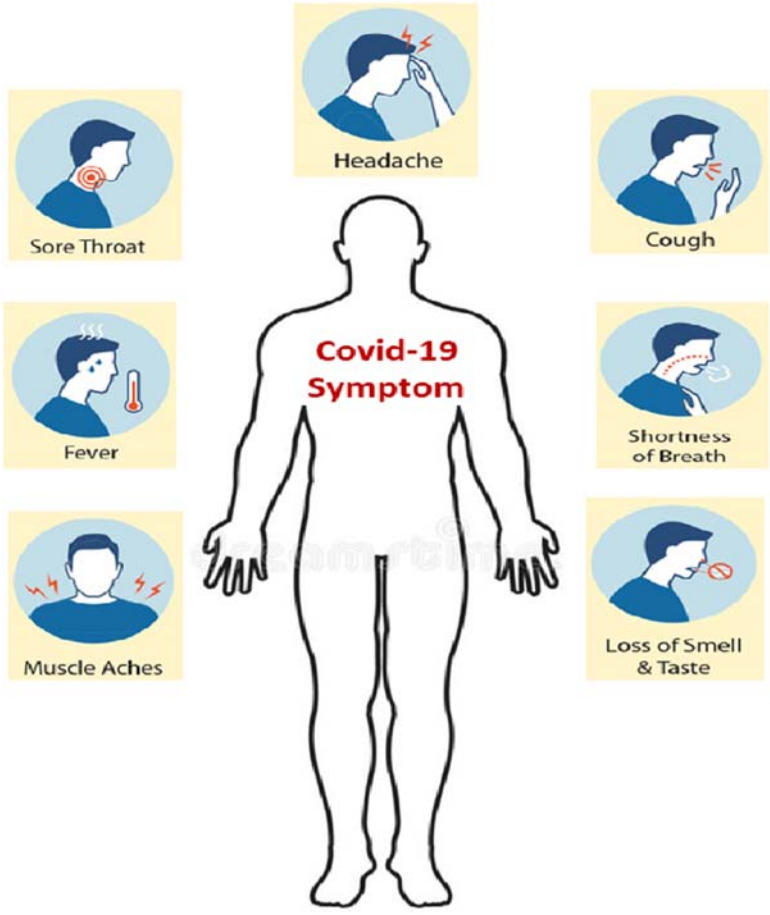

Figure 22.2
Symptoms of Coronavirus in humans.

can be asymptomatic carriers of this disease, spreading infections in society. Globally, the reported fatality rate is in the range of 6.8%−7% as of May 5, 2020 (WHO, 2020). Loss of appetite, shortness of breath, chronic pain, confusion, high temperature (over 38°C), and pressure in the chest have all been noted in individuals after a severe illness. Irritability, loss of consciousness (often linked with seizures), depression, anxiety, sleep difficulties, and more serious and rare neurological issues such as brain inflammation, strokes, and nerve damage are among the less common symptoms. Following recovery from the COVID-19, it has been seen that people experience a loss of appetite, long-term weakness, and other disorders. Mucormycosis (black fungus) was highly common after recovery from Covid-19 in India.

The RT-PCR test and antibody testing are the most often used diagnostic procedures nowadays. On the other hand, serological test kits for early detection are being developed (Singh et al., 2020a,b). For the time being, there are no effective treatments for Covid-19. Despite the fact that numerous vaccinations (i.e., covishield, covaxin, and sputnik V) have been introduced around the world, many cases of persons becoming infected with Covid-19 have been reported even after immunization. There are no specific drugs available to treat Covid-19. Covid-19 symptoms have been claimed to be combated by immune booster medications for health reasons. The WHO has proposed certain measures to reduce Covid-19 transmission. It includes hand washing with soap for 20 seconds, sanitizing with an alcohol-based cleaner (70% alcohol), avoiding direct touch with persons who are sick, and maintaining a social distance of six feet from anyone coughing or sneezing, not touching the eyes, nose or mouth, coughing and sneezing with a cloth and tissues, among other things. Wearing a face mask outside the house or in a crowded environment as well as wearing gloves can help avoid Covid-19. Plasma treatment and antiviral therapy with steroids, remdesivir, favilavir, and chloroquine phosphate have shown to be effective against Covid-19, although their use is limited because of side effects (Almaghaslah et al., 2020). Plants, animals, microorganisms, and other biodiversity aspects may be an excellent source of natural compounds that can help with Covid-19 and post-Covid-19 infection health.

22.4 Covid 19 and gut dysbiosis

Covid-19 is essentially a respiratory disease; however, the reservoir for SARS-Cov-2 is the gut of the organism (Giannoni et al., 2020). Bacteria like *Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes* are present in the mid-gut, and they provide protection to the host due to their secreted metabolites, and any change in the gut microbiota can result in various disorders like cardiovascular diseases, depression, diabetes, etc., which are collectively given the term gut dysbiosis (Dhar and Mohanty, 2020). Gut microbiota may be perturbed due to excessive usage of antibiotics, and the diversity of microbial flora goes

down with age. However, the presence of distinct microbiota in the lungs has also been implicated, and there is a cross-talk between the lung and gut microbiota due to various microbial metabolites (Groves et al., 2020). During Covid-19 infection, cross-talk between lung and gut microbiota and the central nervous system gets disturbed in both asymptomatic patients and those with fewer symptoms resulting in neurological afflictions and fluctuations in the gut immunity and gut dysbiosis is triggered (Andrade et al., 2020). The release of proinflammatory chemokine and cytokine has been implicated, and this leads to infection in the intestinal epithelia via ACE2 receptor (Angiotensin Converting Enzyme 2) and protease serine 2 residing in the membrane. The symptoms like acute intestinal inflammatory response are due to diarrhea and elevated levels of fecal caprolectin and serum interleukin-2 (Ceccaralli et al., 2020). Also associated with Covid-19 are menaces like stress, panic attacks, depression, anxiety, insomnia and sleep disorders, poor mental wellbeing, and suicidal tendencies among people, and these psychiatric ailments can be treated by psychobiotics, which are a class of probiotic bacteria having enhanced therapeutic potential (de Araújo et al., 2020). Hence, enhanced immunity along with a healthy mental state is important in Covid-19 management, and this could be boosted by the administration of prebiotics, probiotics, synbiotics, and psychobiotics.

22.5 Probiotics

Probiotics strengthen the immune system, and they aid in Covid-19 management by fomenting cytokine storms triggered by viral infections that result in persistent and damaging lung infections putting into use microbial strains of immense diversity (Aguila et al., 2021; Singh and Rao, 2021). The immense microbial diversity enhances the immunity of the host through probiotic administration that also adds to the host body's viable microorganisms in a fair amount (Akour, 2020). Probiotic bacteria include, majorly, strains of *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Streptococcus, Bacillus*, and yeast strains like *Saccharomyces cerevisiae* var. *boulardii* (Eskici, 2020). The principal source of probiotics is dairy items that have been fermented. However, due to growing lactose intolerance among individuals and the shift to strict vegan diets, the focus needs to be shifted to ingenious food items which are not based on dairy products and several food matrices like kombucha tea, herbal tea, baking mix, and cereal-based products that are devoid of dairy but are being included in the diet and are emerging as unconventional but potential probiotic sources (Cosme et al., 2022). Probiotics are antiviral and antiinflammatory in nature, and various trials in human beings, animal models, and in vitro systems have suggested that probiotics are helpful in reducing the risk and augmenting the treatment of Covid-19 (Mak et al., 2020). The clinical course and outcome of Sars-Cov-2 are improved by probiotic administration, and their wide use is attributed to them being safe and easily available too (Santacroce et al., 2021). Probiotic administration is effective in patients with comorbidities which effectively reduces the severity of infection

in them and also relieves Covid-19 patients with a history of infection by relieving gut dysbiosis (Hawryłkowicz et al., 2021). *Bifidobacteria* finds immense use as a probiotic, and it is known that it reduces the cytokine storm by restraining both pro- and antiinflammatory cytokines differentially (Bozkurt and Quigley, 2020). Bacteria that show probiotic effects are generally found in fermented food and have been in use for a long time. Probiotics can inhibit viral infections by the production of interferon, antiviral, and immune modulating activities. They can promote faster recovery and can complement therapeutics for respiratory tract infections. LAB (Lactic Acid Bacteria) is found in different fermented foods like fermented yogurt, cheese, pickles, wine, and kefir using *Lactobacilli, Bifidobacteria, Enterococcus, Streptococcus*, etc. and also finds use as probiotics (Eskici, 2020). They are known to promote lactose digestion by overcoming lactose intolerance, in addition to maintaining intestinal pH and inhibiting allergic reactions. Probiotics provide natural alternatives to drugs and provide supportive and prophylactic therapy (Sundararaman et al., 2020). Various symptoms like diarrhea, abdominal pain, vomiting, headache, sore throat, fever, and other snags are associated with viral infection complications, mainly acute respiratory distress syndrome, also known to be lessened by pro- and prebiotics (Batista et al., 2021). The secretion and release of various immunomodulatory molecules like butyrate, acetate, and propionate by the probiotic bacteria *Bifidobacteria, Lactobacillus, and Bacteroides* has been proposed and it has been suggested that these immunomodulatory signals lead to alteration in the functioning and metabolism of macrophages and dendritic cells (Delgado et al., 2020). These observations suggest that a gut-lung axis exists, and by modulating the activity of the gut microbiota, the prevention and treatment of Covid-19 can be one of the approaches (Bottari et al., 2021). Thus, the restoration of gut homeostasis by probiotics suggests that it could be used as an adjunctive treatment protocol in Covid-19 patients (Peng et al., 2021). The effects of probiotics have been favorable for patients whose immune systems have been compromised. Although the evidence is lacking in children, probiotic administration could be of immense help in the prevention of SARS-Cov-2 infection and also other viral infections like influenza, rhinovirus, respiratory syncytial virus, etc. (Parisi et al., 2021). Probiotics can regulate immune responses in the cells involved in both innate and adaptive immunity- the former includes NK cells (Natural Killer cells), macrophages, granulocytes, dendritic cells, etc., while the latter includes Th1, Th2, Th17, Treg cells, lymphocytes B, etc. (Hardy et al., 2013). The probiotics help in modulation of mucosal immunity, but it is still not certain how. The benign role of probiotics is due to Vitamin K formation, intestinal peristalsis, modulation of an immune response, and buildup of useful products generated by the dietary fibers upon fermentation (Parisi et al., 2021). The observation that Covid-19 patients can be treated with interferon has led to the surmise that probiotics can be used as rational adjunctive option for prophylaxis of Covid-19 by stimulating interferon production in addition to imparting antiviral and immunomodulatory activities (Prince, 2020). Probiotics also intensify immunoglobulin synthesis like IgA in the serum. Upon administration

of *Bifidobacteria* it was found that fecal matter contained B*ifidobacteria* in compelling numbers (Khanna et al., 2021).

22.5.1 Prebiotics as immune boosters

The gastrointestinal tract of human beings is lined by beneficial microbiota, and their composition and activity can be modulated by stimulating the immune system and inhibiting the pathogenic microbes by the administration of prebiotics. For the gut microbiota, prebiotics are a principal source of energy and are basically nondigestable carbohydrates in fruits and vegetables. It includes various oligosaccharides like fructans, oligosaccharides, arabinooligosaccharides, isomaltooligosaccharides, xylooligosaccharides, resistant starch, lactosucrose, lactobionic acid, galactomannan, psyllium, polyphenols, and polyunsaturated fatty acids, and they are administered orally so that they can reach into and colonize body areas like gut, vagina, or skin. These areas in the body are themselves rich in microbes, and then these prebiotics contribute to the health of the host due to the action of host microbiota on the food components that are nondigestible (Olaimat et al., 2020). Even the prebiotics can be immobilized and microencapsulated to achieve the enhanced capacity to penetrate and can also find application in diabetes and inflammatory bowel problems (Khangwal and Shukla, 2019). Thus, gut health is benefited by prebiotics in addition to the nondairy products formed upon microbial fermentation (Cosme et al., 2022).

The viable cells of probiotics or dead/inactivated cells of probiotics are called paraprobiotics and postbiotics that include useful metabolites produced by probiotics (Khaled, 2021). The inclusion of wheat, onion, banana, garlic, asparagus, peas, etc., can supplement the prebiotic fructooligosaccharides and promote the growth of *Bifidobacteria (*Eskici, 2020). The recovery and antiviral immunological responses against Covid-19 are enhanced by modest intake of prebiotics, fatty acids, proteins, and branched-chain amino acids. This is perpetuated by viral load reduction by enhancing levels of metabolic products having antiinflammatory responses and inhibiting the multiplication of microorganisms (Nejati et al., 2021). Nutrients, besides playing an important role in maintaining normal physiology of the human's body and healthiness, are also required for enhancing the immunity of the body and can be effective against viral infections. Nutrients enhance immunity by stimulating adaptive immunity or can directly interfere with the targeted viruses. When Covid-19 emerged, the world was lacking a medicine that could be used as a therapeutic; hence, it would be a corrective approach to incorporate macro- and micronutrients into the diet to boost immunity as a preventive measure (Thirumdas et al., 2021). Prebiotics and various foods used as nutritional supplements are obtained from marine sources like carrageenans, fucoidans, alginates, polyphenols, luminaries, carotenoids, phlorotannins, and they can trigger the microbiota of the gut in humans and help in

maintaining an efficacious immune system; for example, one of the reasons for the use of sea weeds could be due to their rich content of vitamins and minerals. Besides, the immune system may be modulated by carotenoids, phytosterols, vitamins, and fatty acids released by microalgal species (Pradhan et al., 2022). Specific bacterial species may serve as commensals like *Bifidobacterium longum* subsp. *infantis* and may utilize Human Milk Oligosaccharides (HMO) as their metabolic substrates. HMO may be considered as natural prebiotics and are found abundantly in milk and engage in cross-talk between the immune system and these commensal bacteria. Their ability is for the modulation of cells involved in immunity and cytokine release, and for epithelial cell maturation (Brink et al., 2021). When a comparison was made between dietary HMO and other prebiotic oligosaccharides, the former was found to be more effective immunomodulation in pigs. Microbial therapeutics could help in the prevention and treatment of Covid-19 by including prebiotics and probiotics along with a proper diet and could pose as a novel and pocket-friendly approach (Hu et al., 2021; Brink et al., 2021).

Immunomodulatory properties are also possessed by fructans that are widespread in plants and have a sucrose core surrounded by four-to-many fructose polymers and present as carbohydrate reserves in plants (Peshev and Van den Ende, 2014). Plants produce polyphenols which are regarded as the most important of the secondary plant metabolites; however, they have low bioavailability. Although it has been known that they modulate the microbiota present in the intestine and release metabolites, although the metabolic route is not known in much detail, still, the increase in beneficial bacteria and valuable metabolites has been noted by polyphenol consumption as supported by various studies; however, the effect in humans further needs investigation (Plamada and Vodnar, 2021). Leguminous plants are the source of pulses in our diet, rich in proteins, and it has been observed that the legumes themselves and the beverages based on them are rich in oligosaccharides, resistant starch, polyphenols, and isoflavones which have excellent prebiotic characteristics, including immunomodulation, antiinflammatory, antineoplastic properties, regulation of metabolism, and as a way to promote luxuriant growth of LAB. The fermentation of legume-based beverages and legumes themselves can be brought by LAB. Thus, legumes could be used as an excellent synbiotic with excellent prebiotic and probiotic cues (Cichońska and Ziarno, 2021).

22.6 Conclusion

It is essential to maintain immunity to prevent viral infections such as Covid 19, and the boost in immunity can be achieved by the modest consumption of prebiotics and probiotics and a healthy lifestyle. As viruses evolve, resistant strains develop, and to tackle these, it is necessary that a synergistic effort should be raised from the scientists, nutritionists, and regulatory bodies like WHO/FAO, pharmacists, and industries catering to manufacturing

food to achieve better health and prevent disease transmission. Good and healthy nutrition can enhance wellbeing of human beings and also serve as psychiatric remedies. Besides Covid-19, for the treatment of various diseases like congenital heart disease, diarrhea, inflammatory bowel disease, hypertension, genitourinary tract infection, colon cancer, immune system defense, mineral absorption, allergic disorders, and atopic dermatitis of the gastrointestinal tract, probiotics and prebiotics are promising therapeutics (Manzoor et al., 2022). It is, therefore, necessary that the consumption of various biotics—probiotics, prebiotics, postbiotics, and psychobiotics—be promoted to achieve a reinforced and strong immune system that will protect us not only from Covid-19 but also from other diseases.

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Microbial Biomolecules

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Compiles the latest research advancements and applications of microbial biomolecules in various fields, including human health, sustainable agriculture, pharmaceutical industries, as well as in the management of environment contaminants.

Key Features

- Explores eco-friendly and sustainable approaches to healthcare, agriculture, and environmental contamination management
- Compiles new aspects of microbial based biomolecules
- Proofs that the use of microbes or microbial products are suitable alternatives to manage the current challenges of health-care issues, chemical pesticides, and environmental contamination

There is an urgent need to explore eco-friendly and sustainable approaches to health care, agriculture, and environmental contamination management. The use of microbes or microbial products appear as a suitable alternative to manage the current challenges of healthcare issues, chemical pesticides, and environmental contamination. Focusing on these factors, in 21 chapters, Microbial Biomolecules explores and compiles new aspects of microbial biomolecules such as microbial enzymes, microbial metabolites, microbial surfactants, exopolysaccharides, and bioactive compounds, synthesized from microbes and their potential applications in the field of health related issues, sustainable agriculture, and environment contamination management.

This book is written for researchers, scientists, graduate, and PhD students in the areas of microbiology, biotechnology, environmental science, and pharmacology.

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