MICROBIOLOGY RESEARCH ADVANCES

THE RHIZOSPHERE

STRUCTURE, ECOLOGY AND SIGNIFICANCE

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EDITOR

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Contents

Chapter 9

Diversity and Application of Yeasts in the Rhizosphere

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Abstract

The rhizosphere is a thin region of soil around plant roots where microbes, plant roots, and soil constituents interact and is supposed to be the microbial habitat. This region is rich in nutrients and chemical substances secreted from plant roots, which are substantially higher than those found in soil away from plants and serve as a major source of substrates for a variety of microorganisms. Yeasts are a group of unicellular eukaryotic microorganisms that inhabit the rhizosphere. Rhizosphere yeasts are rich in species diversity and population levels. The diversity of yeast in the rhizosphere varies depending on the season, soil type and depth, plant species, and location. Yeast species belonging to the genus *Cryptococcus*, *Candida*, *Torulaspora*, and *Meyerozyma* are the most frequently isolated from the rhizosphere. Presently, metagenomics, an effective method for phylogenetic identification and functional characterization of uncultured microorganisms, is currently being used to investigate the community and diversity of yeast and other microorganisms in the rhizosphere. The role of rhizosphere yeast in terms of plant growth promotion is well investigated and reported, such as organic matter mineralization, phosphate solubilization, nitrogen

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compound and inorganic sulfate transformation, plant root growth promotion, against root pathogens, and even acting as a food source for other organisms. However, information and examination of yeast ecology and interaction among yeasts, plants, and other microorganisms is limited. In this chapter, the diverse culturable and unculturable yeast species that inhabit the rhizosphere are reviewed. Furthermore, the interactions between yeast and plants are discussed with a focus on their mechanisms for promoting plant growth.

Keywords: rhizosphere, yeast, diversity, plant growth promoting

1. Introduction

The rhizosphere, a term that usually refers to the narrow region of soil surrounding plant roots, was described for the first time by agronomist Lorenz Hiltner in 1904 (Balandreau and Knowles 1978). This area is directly influenced by plant root secretions and has been recognized as a crucial habitat for microorganisms. Plant roots release an enormous variety of organic compounds, known as rhizodeposits, such as carbohydrates, protein, amino acids, lipids, vitamins, volatile compounds, and other secondary metabolites, which could stimulate the growth and activity of microorganisms and make the rhizosphere able to shape their rhizosphere microbiome (Prescott et al. 1999). Plant rhizodeposits vary greatly depending on plant species, age and development of plants, soil conditions, and seasonal environment (McNear and David 2013, Baetz and Martinoia 2014). Although the role of rhizosphere microorganisms is unclear, various rhizosphere microorganisms show their ability to promote plant growth, control plant pathogens, and may help plants become more tolerant to environmental stress (Pandey et al. 2019). Yeasts are single-celled eukaryotic microorganisms that are widely distributed in various ecosystems and natural resources (Yurkov et al. 2012, Canto et al. 2017, Hagler et al. 2017, Hoondee et al. 2019) as well as in the rhizosphere (Cecilia Mestre et al. 2011, Sarabia et al. 2018a). The diversity and activity of rhizosphere bacteria have been extensively studied, but only a few studies have focused on rhizosphere yeast. Yeasts are assumed to be one of the good competitors in the rhizosphere environment because they are able to consume simple carbon compounds quickly, grow fast and are able to produce spores (Hannula et al. 2020). Furthermore, various yeast species were able to assimilate the complex carbon sources derived from plant biomass such as xylose and cellobiose (Cecilia Mestre et al. 2011). Several studies have

reported rhizosphere yeasts' potential as Plant Growth Promoting Microorganisms (PGPM), which can produce chemical compounds capable of stimulating plant development via both direct and indirect mechanisms such as plant hormone production, mineral solubilization, pathogen antagonism, and plant resistance induction (Fu et al. 2016, Sarabia et al. 2018a). In this chapter, we presented the isolation, identification, and diversity of rhizosphere yeasts as well as their benefits for plants in terms of growth-promoting traits and others.

2. Isolation Cultivation and Characterization of Yeasts

2.1. Sample Collection

The rhizosphere soil samples were collected from soil attached to the root surface layer. The depth from the ground is typically 0-30 cm due to their ability to grow aerobically (Sarabia et al. 2018a). The first step in collecting a good soil sample is to draw a diagram indicating where you will collect soil samples. Soil samples were collected and dried. Stones, roots, and large debris were removed from the soil (Sarabia et al. 2018a). Sieving using 2-mm sieve was used to homogenize the samples and remove stones and other debris (Wang et al. 2021). Furthermore, to reduce the bias caused by soil composition variation, rhizosphere soil samples from each sampling site should be mixed (Sarabia et al. 2018a). Collected samples were tightly sealed in sterile packaging, transported to the laboratory, and maintained at 4°C until processed (Cecilia Mestre et al. 2011, Sarabia et al. 2018a). The samples should be processed within 2-4 h after collection Koricha et al. 2019) or no longer than 1 week (Cecilia Mestre et al. 2011). However, rhizosphere soil samples for metagenomic analysis should be stored in a freezer at -80°C (Hannula et al. 2020, Wang et al. 2021). Endorhizospheric yeasts, defined as yeasts that colonize internal plant root tissue, were also investigated. For plant root collection, the roots were cut from the shoots and removed the soil adhering. The roots were washed with tap water and disinfected. Weight of the fresh roots were recorded before further processing.

2.2. Isolation and Cultivation of Yeasts

The diversity of rhizosphere yeast has been surveyed in many natural habitats. There were several techniques with various media used for yeast isolation (Table 1). The antibiotics (e.g., chloramphenicol, streptomycin, rose bengal) have been added into the medium to prevent the growth of bacteria (Ferreira et al. 2010, Cecilia Mestre et al. 2011, Chamnanpa et al. 2013, Sarabia et al. 2018a, Koricha et al. 2019, de Lima Targino et al. 2022). Meanwhile, the addition of sodium propionate, calcium propionate or lactic acid can be controlled the fungal growth (Chamnanpa et al. 2013, Fu et al. 2016, de Lima Targino et al. 2022). One of the most commonly used methods for isolating yeast is the enrichment approach. The rhizosphere soil samples were inoculated in the nutrient-rich medium, which was the general medium for isolation and enumeration of yeasts such as malt extract broth and yeast extract peptone dextrose (YPD) broth (Fu et al. 2016, Koricha et al. 2019). The resulting enriched culture was streaked or spread onto the agar medium that contained the same ingredients as the enrichment broth medium but was supplemented with agar. Yeast colonies of different morphologies were selected, purified, and maintained for further analysis. The dilution plating method was also used for both isolating yeast and quantifying their population (Cecilia Mestre et al. 2011, Ramos-Garza et al. 2016, Sarabia et al. 2018a). The rhizosphere soil samples were suspended in normal saline solution or sterile water at various ratios and then diluted as a series of sequential dilutions. The aliquots were spread on the surface of selective agar medium which is normally used for the enumeration of yeasts and molds such as malt extract yeast extract peptone (MYP) medium, yeast peptone dextrose (YPD) agar, rose bengal agar and sabouraud medium (Cecilia Mestre et al. 2011, Ramos-Garza et al. 2016, Sarabia et al. 2018a). After incubation, the yeast colonies were counted and expressed as colony forming units (CFU) g^{-1} soil weight. Then, single colonies were purified by repeated cross-striking. Isolation of endorhizospheric yeast was attempted from plant roots. Plant root samples must be washed with tap water to remove soil particles, followed by sterilization with a sterilization agent to remove epiphytic yeast and other microorganisms, and finally rinsed in sterile distilled water to remove the sterilization agents. Dilute sodium hypochlorite (NaClO) and ethanol are sterilization agents which are usually used for disinfecting surface microorganisms. The sterilized root sample is then cut into small fragments and plated individually on artificial agar media such as YPD agar and PDA agar (Deng et al. 2012, de Lima Targino et al. 2022), incubated at 28◦C for 5

to 14 days. Then, the colonies were purified in the same medium and morphologically characterized.

Table 1. Isolation media and their compositions and cultivation conditions

Media	Compositions	Cultivation conditions	Reference
Sabouraud medium	65 g sabouraud agar and 0.1 g chloramphenicol per litter of Millipore water	incubated at 28°C for 3–5 days	Sarabia et al. (2018)
Yeast extract peptone dextrose (YPD) broth medium	20 g glucose, 20 g peptone, 10 g yeast extract per litter of water and supplemented with 200 μg/ mL chloramphenicol and 1 mL 1M HCl	incubated at 25-28°C for 3–5 days	Koricha et al. (2019)
Malt extract medium	30 g malt extract and 5 g peptone per litter of water and supplemented with approximately 2-3 ml of 100% lactic acid	incubated at 28°C	Fu et al. (2016)
Potato dextrose agar (PDA) medium	4 g potato extract, 20 g glucose, 15 g agar per litter of water and supplemented with 50 mg of chloramphenicol	incubated at 28°C for 1–2 weeks	Deng et al. (2012)
GPYAc	20 g glucose, 5 g peptone, 5 g yeast extract and 20 g agar per litter of water and supplemented with 0.1 g chloramphenicol	incubated at 30°C for 3 days	Ferreira et al. (2010)
PDA medium	4 g potato extract, 20 g glucose, 15 g agar per litter of water supplemented with 500 μg chloramphenicol and 300 mg calcium propionate	incubated at 28°C in the dark for five days	De Lima Targino et al. (2022)
Yeast peptone dextrose (YPD) agar	10 g yeast extract, 20g peptone, 20 g dextrose and 15 g agar per litter of distilled water and supplemented with 20 mg streptomycin.	incubated at 28°C for 7 days	Ramos-Garza et al. (2016)
Rose Bengal agar	0.5 g $MgSO_4 \cdot 7H2O$, 1 g KH_2PO_4, 10 g dextrose, 0.05 g Rose Bengal, 5 g soy peptone and 15 g peptone per litter of distilled water and supplemented with 0.1 g chloramphenicol	incubated at 28°C for 7 days	Ramos-Garza et al. (2016)
Yeast extract malt extract (YM) broth	10 g glucose, 3 g yeast extract, 3 g malt extract and 5 g peptone per litter of distilled water and supplemented with 0.25 g sodium propionate and 0.2 g chloramphenicol	incubated at 25°C	Chamnanpa et al. (2013)
Malt extract yeast extract peptone (MYP) medium	7 g malt extract, 0.5 g yeast extract, 2.5 g peptone-soytone and 15 g agar per litter of water and supplemented with 25 μg/ml rose bengal and 200 μg/mL chloramphenicol	Incubated at 20°C for 3 days	Cecilia Mestre et al. (2011)

2.3. Identification and Characterization

Identification of yeasts is traditionally performed based on phenotypic characterization: morphological, physiological, and biochemical characteristics such as agar-grown colony morphology, asexual cell morphology, sexual state reproduction, mating type formation, carbohydrate fermentation, carbon assimilation, nitrogen assimilation, and other growth ability. The phenotypic tests were determined exclusively relying on the method and standard description of type strains as described in The Yeasts, A Taxonomic Study, 5th ed. (Kurtzman et al. 2011).

Recently, the identification of yeasts has been transformed by genotypic characteristic studies through DNA sequence analyses and other DNA-based methodologies. The D1/D2 domain of the large subunit (26S) of ribosomal DNA (rDNA) and the internal transcribed spacer (ITS) region, comprising the ITS1, 5.8S, and ITS2 regions, are the most widely used and accepted molecular targets for yeast identification. The structure of fungal rDNA and the sites of the universal primers are shown in Figure 1. For yeast identification, some studies began by grouping yeast isolates based on their colony morphology, e.g., color, surface appearance, margin, and elevation (Kurtzman et al. 2011, Sarabia et al. 2018a). Subsequently, the isolates with identical morphological characteristics were subjected to polymerase chain reaction (PCR) fingerprinting with the core sequence using the microsatellite specific oligonucleotides $(GTG)_5$ as primer (de Lima Tagino et al. 2022) and then grouped. The representative strain from each group was chosen for further identification by DNA sequencing.

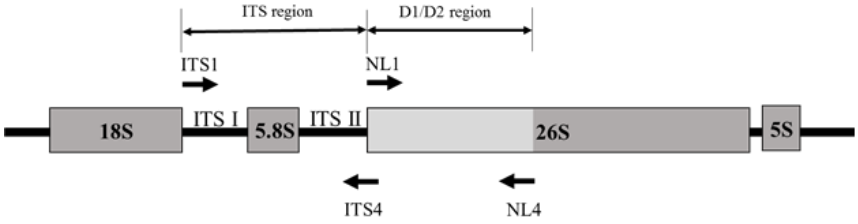

Figure 1. The location of the ITS region and the D1/D2 domain of yeast ribosomal DNA and the binding sites of the fungus-specific universal primers (short arrow).

The genotypic identification procedures usually involve DNA extraction, PCR amplification, and DNA sequencing. The yeast genomic DNA was extracted from 2-4 d old cultures grown on agar medium or in liquid medium.

Initially, the harvested cells were suspended into lysis buffer containing Tris–Cl, ethylene diamine tetra acid (EDTA), and sodium dodecyl sulfate (SDS), followed by removal acetic of cell debris by centrifugation. A mixture of organic solvents such as phenol, chloroform, and/or isoamyl alcohol in various ratios is used for denaturation and precipitation of proteins from nucleic acid solution, and denatured proteins are removed by centrifugation and wash steps. The undesired ribonucleic acid (RNA) can be eliminated by the ribonuclease A (RNAse A) enzyme treatment (Sarabia et al. 2018s). The extracted DNA was precipitated with ice-cold ethanol and then recovered by centrifugation. The DNA pellet is redissolved in TE buffer or sterilized ultra-pure water. The D1/D2 domain of 26S rDNA sequence was amplified by PCR using the forward primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) or ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and the reverse primer NL-4 (5′-GGTCCGTGTTTCAAGACGG) (Fu et al. 2016, Sarabia et al. 2018a, Koricha et al. 2019). For ITS region sequence analysis, this region was amplified using specific ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATAT GC-3′) primers (Deng et al. 2012, de Lima Targino et al. 2022). The PCR products were purified, and sequence analyzed. The DNA sequences of the yeasts were manually edited using the BioEdit program (Hall 1999) and were individually analyzed using the online database available in the NCBI GenBank with the search algorithm BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Generally, yeast strains that differed by six or more nucleotide substitutions (> 1%) in the conserved D1/D2 domain of the 26SrRNA were potentially considered to be novel species, whereas strains that differed by 0-3 nucleotide substitutions were considered conspecific or sister species (Kurtzman and Robnett 1998).

2.4. Metagenomics Study

Metagenomics is the study of the whole DNA of a microbial community for one time, allowing the identification of all microorganisms in an environment (Chen and Pachter 2005). Metagenomics technology is a powerful tool for identifying microorganisms that are unculturable or difficult to cultivate in vitro. Moreover, this technique can overcome the amplicon sequencing limitation (Wang et al. 2021). Previous studies have investigated the microbial community of various plant rhizospheres, but mostly focused on bacteria. Wang et al. (2021) evaluated rhizosphere soil fungal diversity using direct

isolation of the ITS2 rRNA gene region by high-throughput sequencing of cloned fragments. Meanwhile, Zhu et al. (2021) assessed rhizosphere soil yeast communities using the high-throughput sequencing analysis of the D1 domain of the LSU rRNA genes. The operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using the UPARSE (Zhu et al. 2021) and UCLUST (Wang et al. 2021) algorithms, and taxonomically classified using the UNITE dynamic database (Wang et al. 2021). The combination of DNA-SIP and next generation sequencing (NGS) was applied to investigate the rhizosphere fungi which actively assimilate plant-derived carbon. The $^{13}CO_2$ was provided to the plants in an intact soil core collected from a restored grassland in the Netherlands, followed by rhizosphere sampling in a time series to track the fate of carbon in the rhizosphere microbiome. The ^{13}C-enriched DNA was separated from the ^{12}C DNA using density gradient centrifugation and then individually subjected to ITS2 amplification. The sequencing was performed by pyrosequencing, which is a method of DNA sequencing that detects light emitted from nucleotide bases. According to Simões et al. (2015), pyrosequencing with the 454 GS FLX titanium method was used for sequencing.

3. Rhizosphere Yeast Diversity

Yeasts are eukaryotic unicellular fungi that are widely distributed in the rhizosphere of both natural (Cecilia Mestre et al. 2011, Fu et al. 2016, Koricha et al. 2019) and agricultural ecosystems (Sarabia et al 2018a, Wang et al. 2021, de Lima Targino et al. 2022). Diverse yeast species isolated from rhizosphere soil of various host plants were shown in Table 2. Sarabia et al. (2018a) isolated rhizosphere yeast from rhizosphere soil samples of six maize agroecosystems in Mexico which were sampling from three plant growth phases, including vegetative, flowering and senescence growth phases. Ascomycetous yeasts were dominant and the most two prevalent species were *Candida railenensis* and *Meyerozyma guiliermondii*. Meanwhile, *Cryptococcus flavus* was the one and only basidiomycetous yeast discovered in this study. Abundance of yeast during the full crop cycle in all six maize fields was in the range $6.3 \times 10^3 – 1.1 \times 10^6$ cfu g^{-1} soil dry weight. The highest abundance of yeast was observed in the flowering stages, which was significantly higher than in the vegetative growth stage. Moreover, researcher revealed that the abundance of rhizosphere yeast was negatively affected by soil pH and the amount of Mg. According to Botha (2006), pH and Cu

concentration in soil were the principal factors for decreasing yeast number. *Meyerozyma* are one of the most frequently yeast genera isolated from the rhizosphere. Previously, the halophilic yeast *Meyerozyma caribbica* MG20W was isolated from rhizosphere soil of Saemangeum reclaimed land on the west coast of the Republic of Korea (Kim et al. 2015). Their complete genome sequence was analyzed and published on Japan/EMBL/GenBank nucleotide sequence databases with the accession numbers BADS01000001 to BADS01000009. The phosphate-solubilizing yeasts, *M. guilliermondii* CC1, was isolated from the uprooted Ficus (*Ficus religiosa* L.) tree rhizosphere (Nakayan et al. 2013). Previously, *M. gullhermondii* has been isolated from Brazilian cassava (Manihot esculenta) root (Ferreira et al. 2010). According to de Lima Targino et al. (2022), two species of *Meyerozyma* genus, *M. guilliermondii* and *M. caribbica* were isolated from maize root collected from maize field in Brazil. The finding indicated that the genus *Meyerozyma* could be maize-associated yeasts. Koricha et al. (2019) studied the occurrence and frequency of wild yeast associated with rhizosphere soil samples collected from Belete-Gera forest in Southwest Ethiopia during the spring and summer seasons. Yeast abundance was $6.5 \times 10^4 – 2.15 \times 10^5$ cfu g^{-1} soil dry weight. The most two frequently isolated yeast were *Pichia kudriavzevii* and *Meyerozyma guiliermondii*. However, there was no significant seasonal variation in the diversity and frequency of yeast in the rhizosphere. In the *Drosera spatulate* rhizosphere, ascomycetous yeasts were dominant, whereas basidiomycetous yeasts were not found (Fu et al. 2016). Three known yeast species isolated from this rhizosphere were *Galactomyces candidum*, *Kazachstania jiainicus* and *Barnettozyma californica*. Meanwhile, four and one strains were assigned as potential new species in the genus *Dothideomycetes* and *Torulaspora*, respectively. Recently, *Torulaspora globose* were isolated from the sugarcane rhizosphere during the exploration for yeasts that can be used as biological control agents against phytopathogens (de Oliveira et al. 2019). Chamnanpa et al. (2013) reported that *T. globosa* was the prevalent yeast species isolated from vetiver rhizosphere soil.

The diversity of yeasts inhabiting the rhizosphere and bulk-soil of a *Nothofagus pumilio* forest in Nahuel Huapi National Park (Bariloche, Argentina) was studied (Cecilia Mestre et al. 2011). They revealed that the total number of yeast isolates obtained from the rhizosphere (41 isolates) was slightly higher than in bulk-soil (37 isolates) but that the species diversity and community between them are similar. Basidiomycetous yeasts were predominant in both soil fractions. In the *Nothofagus pumilio* forest rhizosphere, twelve known yeast species (7 basidiomycetes and 5

ascomycetes) and 2 possibly new species were obtained. Yeast species belonging to the genus *Cryptococcus*, including *Cryptococcus podzolicus*, *Cryptococcus aerius*, *Cryptococcus phenolicus*, *Cryptococcus terricola*, and *Cryptococcus terreus*, are the most frequently isolated from the rhizosphere. In addition, the rhizospheric yeasts associated with plants grown in a seriously contaminated region were reported (Ramos-Garza et al. 2016). Yeasts were quantified and isolated from the rhizospheres of 5 plant species grown at 2 sites of a Mexican region contaminated with arsenic, lead, and other heavy metals. 31 yeast isolates were isolated and identified as 5 species in 4 genera of Basidiomycota and one species in genus *Exophiala* of Ascomycota. *Cryptococcus albidus* was the most abundant and widely distributed species, being isolated from all other sampling sites except for *Prosopis* sp. at the hill site. Other yeast species isolated from arsenic-contaminated rhizosphere soil were *Cystobasidium sloffiae*, *Cryptococcus uzbekistanensis*, *Trichosporon japonicum*, *Rhodotorula mucilaginosa* and *Exophiala pisciphila*. Furthermore, strains of *Cryptococcus albidus* were able to reduce the heavy metal, arsenate (As^{5+}), into arsenite (AS^{3+}). Previously, the *Cryptococcus* sp. CBSB78 (GIM2.180) isolated from rape (*Brassica chinensis*) roots collected from a heavy metal-contaminated site in China has been reported as Cd-, Pb-, Zn-, Cu-resistant endorhizospheric yeast (Deng et al. 2012).

To better understand the fungal communities and diversity in the rhizosphere, a variety of culture-independent techniques, such as metagenomics, have been applied over the past decade. Based on Illumina MiSeq high-throughput sequencing analysis of the D1 domain of the LSU rRNA genes, a total of 86 yeast OTUs identified from rhizosphere soil of Hami melon orchards in three different regions of Xinjiang, China was classified into 59 genera and 86 species (Zhu et al. 2021). Most OTUs (90.4%) were belonged to the Basidiomycota whereas only a few (9.6%) belonged to Ascomycota. *Filobasidium magnum*, *Solicoccozyma aeria* and *Filobasidium magnum* were the predominant species in the Southern, Eastern and Northern Xinjiang, respectively. There were noticeable differences in yeast diversity and community structure among regions. The results also demonstrated that both soil factors (conductivity, total phosphorus, and total potassium) and physical factors (average annual precipitation, relative humidity, and net solar radiation intensity) significantly influenced yeast community structure. Wang et al. (2021) characterize the fungal microbiome in *Argentina* (Syn. *Potentialla*) *anserina* rhizosphere soils of in three agricultural fields in different continuous cropping years, over four seasons, e.g., the germinating stage in spring, the flowering stage in summer, the vegetative stage of root

tuber in autumn, and the harvest stage of root tuber in winter using the ITS II high-throughput amplicon sequencing. The results showed that seasons were significantly correlated with both fungal diversity and fungal community. In addition, specific fungal groups and distributions were found to be related to the level of nitrogen, phosphorus, and potassium in rhizosphere soil. Among 35 identified fungal species, only four basidiomycetous yeast species, *Cryptococcus albidus*, *Rhodotorula* sp.3.23T, *Occultifur* sp., and *Sporobolomyces* sp., were detected. The functional role of different fungi was assigned by FUNGuild and the results revealed that *Rhodotorula*, *Occultifur*, and *Sporobolomyces* were belong to pathotrophs/saprotroph trophic patterns. Meanwhile, *Cryptococcus* has been reported as a competitive yeast capable of competing with bacteria and fungi. Previously, *Cryptococcus* found in arid soil demonstrated the ability to produce unique polysaccharide capsules that absorb nutrients from the soil, making them good competitors in the rhizosphere (Vishniac 2006). Hannula et al. (2020) investigated rhizosphere fungi which actively assimilate plant-derived carbon using combined technology between DNA-SIP and next generation sequencing and supported that yeast (both ascomycete and basidiomycete) OTUs were able to respond to plant-derived carbon quickly. Therefore, yeast may have the potential to modulate the food web interaction through competition with bacteria and each other. Simões et al. (2015) investigated the fungal diversity in the rhizosphere and bulk soil of gray mangroves (*Avicennia marina*) from the Red Sea mangrove shore in Saudi Arabia through a metagenomic approach and reported that rhizosphere samples had much higher fungal diversity when compared with bulk soil samples. The finding indicated that Ascomycota was the dominant phylum (76%–85%), while Basidiomycota was less abundant (14%–24%), which corresponded with previous studies on culturable yeast isolation (Fu et al. 2016, Sarabia et al. 2018a). *Candida albican*, *Candida glabrata*, *Candida maltose*, *Candida tropicalis*, *Cryptococcus neoformans*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Malassezia globose*, *Ogataea parapolymorpha*, *Saccharomyces cerevisiae*, *Scheffersomyces stipites*, *Schizosaccharomyces pombe* were the species identified in all four rhizosphere samples (Simões et al. 2015).

Table 2. Diversity of culturable rhizosphere yeasts isolated from rhizosphere soil samples associated with various host plant species

Species	Sample type	Host Plant/Source	Country	Reference

Torulaspora globose	Rhizosphere	Sugar cane	Brazil	de Oliveira et al. (2019)
Myxozyma udenii sp. nov.	Rhizosphere	Mangifera indica	USA	Spaaij et al (1990)
Kazachstania jiainicus, *Torulaspora* sp., *Barnettozyma californica*	Rhizosphere,	Drosera spatulate	Taiwan	Fu et al. (2016)
Cryptococcus flavus, *Candida railenensis*	Rhizosphere	Maize	Mexico	Sarabia et al. (2017)
Candida albicans, *Candida humilis*, *Candida intermedia*, *Hanseniaspora uvarum*, *Kluyveromyces marxianus*, *Lachancea thermotolerans*, *Pichia kudriavzevii*	Rhizosphere	Belete-Gera forest	Ethiopia	Koricha et al. (2019)
Candida railenensis, *Clavispora lusitaniae*, *Cryptococcus flavus*, *Filobasidium globisporum*, *Meyerozyma caribbica*, *Meyerozyma guilliermondii*, *Solicoccozyma aeria*, *Symmetrospora coprosmae*	Rhizosphere	Maize	Mexico	Sarabia et al. (2018)
Meyerozyma gullhermondii	Rhizosphere	Ficus (Ficus religiosa L.)	Taiwan	Nakayan et al. (2013)
Cryptococcus sp. CBSB78	Roots	Rapeseed (Brassica chinensis)	China	Deng et al. (2012)
Debaromyces hansenii, *Kodamaea ohmeri*, *Candida glabrata*, *Candida haemulonii*, *Meyerozyma gullhermondii*	Roots	Cassava (Manihot esculenta)	Brazil	Ferreira et al. (2010)
Meyerozyma caribbica	Rhizosphere	Saemangeum reclaimed land	Korea	Kim et al. (2015)

Species	Sample type	Host Plant/Source	Country	Reference
Meyerozyma spp.	Roots	Maize (Zea mays L.)	Brazil	De Lima Targino et al. (2022)

Williopsis saturnus	Roots	Maize (Zea mays L.)	Brazil	Nassar et al. (2005)
Candida tropicalis, *Debaryomyces vanrijiae* var. *vanrijiae*, *Torulaspora globosa*	Rhizosphere	Vetiver	Thailand	Chamnanpa et al. (2013)
Cryptococcus albidus	Rhizosphere	Tithonia diversifolia in mine tailings	Mexico	Ramos-Garza et al. (2016)
Cryptococcus albidus, *Cystobasidium sloffiae*	Rhizosphere	Flaveria angustifolia in mine tailings	Mexico	Ramos-Garza et al. (2016)
Cryptococcus albidus, *Cryptococcus uzbekistanensis*	Rhizosphere	Sphaeralcea angustifolia in mine tailings	Mexico	Ramos-Garza et al. (2016)
Cryptococcus albidus, *Trichosporon* sp.	Rhizosphere	Prosopis sp. in mine tailings	Mexico	Ramos-Garza et al. (2016)
Cryptococcus albidus	Rhizosphere	Bahia absinthifolia at hill site	Mexico	Ramos-Garza et al. (2016)
Cryptococcus albidus, *Rhodotorula mucilaginosa*	Rhizosphere	Sphaeralcea angustifolia at hill site	Mexico	Ramos-Garza et al. (2016)
Rhodotorula mucilaginosa, *Exophiala pisciphila*	Rhizosphere	Prosopis sp. at hill site	Mexico	Ramos-Garza et al. (2016)
Cryptococcus podzolicus, *Cryptococcus aerius*, *Cryptococcus phenolicus*, *Candida maritima*, *Asterotremella albida*, *Cryptococcus terricola*, *Cryptococcus terreus*, *Cryptococcus* sp., *Guehomyces pullulans*, *Candida railenensis*, *Candida* sp., *Lindnera rhizosphaerae*, *Hanseniaspora valbyensis*, *Saccharomyces* sp.	Rhizosphere	Nothofagus pumilio forest	Argentina	Cecilia Mestre et al. (2011)

4. Mode of actions and Benefits for Plants

Plant growth promoting microorganisms (PGPM) are becoming more popular as the production of biofertilizers based on microorganisms gains attention in modern agriculture. This microorganism is a promising strategy to reduce dependency on agrochemicals. PGPM facilitate plant growth through direct and indirect mechanisms. However, in comparison to bacteria and mycorrhizal fungi, the use of yeasts as plant growth promoters is still relatively unknown. The modes of action in plant growth promoting traits and benefits for plants of rhizosphere yeast isolated from various host plant rhizospheres are shown below.

4.1. Indole-3-acetic acid (IAA) Production

Indole-3-acetic acid (IAA) is an indole compound belonging to auxin group that is commonly known as a source of endogenous plant growth hormone. IAA is known to have a direct effect on plant growth, primarily by stimulating the development of hairy roots and increasing root dry mass (Ludwig-Müller 2015). Naturally, IAA is synthesized by plants and various microbes including bacteria yeasts, actinomycetes and filamentous fungi. Diverse rhizosphere yeast species possess the ability to produce IAA; therefore, this ability is strain-dependent (de Oliveira et al. 2019). In addition, the production of IAA by yeast was dependent on tryptophan (Nassar et al. 2005). Tryptophan (Trp) is the biochemical precursor for IAA production. Eight yeasts isolated from *Drosera spatulata* rhizosphere produced IAA in both conditions: the presence and absence of Trp (Fu et al. 2016). The concentration of IAA produced when cultured on a YPD medium with Trp ranged from 295.77 $\pm$ 10.4 to 8.64 $\pm$ 1.4 μg mL^{-1}, which was higher than that produced on a YPD medium without Trp (which ranged from 180.54 $\pm$ 12 to 4.08 $\pm$ 1.2 μg mL^{-1}). Among them, *Dothideomyces* sp. JYC 396 had the highest IAA concentration at 295.77 μg mL^{-1}. Corresponding with (de Lima Targino et al. 2022), the maize-root-associated *Meyerozyma guilliermondii* strain ESA 34 and ESA 37 produced a higher concentration of IAA when Trp was supplemented in the medium. Therefore, *M. guilliermondii* strain ESA 35 produced a higher IAA concentration in the medium without Trp. de Oliveira et al. (2019) reported that the IAA production of rhizosphere yeast, *Torulaspora globose* CCA5S55 and *T. globose* CCA5S51, was maximum (669 and 641 μg mL^{-1}, respectively) after 48 h of incubation in the presence of Trp and then decreased about two-

fold after 120 h. Previous studies have reported that yeast can degrade IAA for use as a nitrogen source, and this incidence may also be a self-protection action of yeast to eliminate the excess IAA which could be toxic to the cell (Tromas and Perrot-Rechenmann 2010). Rhizosphere yeasts and endorhizospheric yeasts that produce IAA have been able to improve the growth of several crops. The co-inoculation of tomato seedlings with *T. globose* 5S55 under greenhouse conditions showed a significant increase in both root length and shoot dry weight (de Oliveira et al. 2019). Meanwhile, Cabrini et al. (2019) investigated the effect of *T. globosa* 5S55 inoculation on lettuce seedling development in the field. The results showed that inoculating lettuce seeds and seedlings with *T. globosa* 5S55 resulted in an increase in root dry mass and a significant increase in lettuce production. The endorhizospheric yeasts were also found to be capable of producing indole-3-acetic acid (IAA) and could enhance plant growth (Nassar et al. 2005, Deng et al. 2012).

4.2. Phosphate Solubilization and Mineralization

Phosphorus (P) is one of the major nutrients that are essential for plant growth and reproduction as it is part of nucleic acids, photosynthesis, metabolism, respiration, and energy transfer (Day and Ludeke 1993, Maharajan et al. 2018). The soil contains a large amount of P, but it is not in orthophosphate (Pi, inorganic phosphate) form, which is plant-accessible (Gupta and Sahu 2017, Malhotra et al. 2018). Various yeast species have been reported as P-solubilizers, which support plants in efficient P-uptake by solubilizing and mineralizing in both inorganic and organic soil (Nakayan et al. 2013, Fu et al. 2016). However, the capability of phosphate solubilization varies depending on the genus and species of yeast (Nakayan et al. 2013, Sarabia et al. 2018a). The most common mechanism for mineral phosphate solubilization by yeast is the production of organic acid and inorganic acid, which can bind with phosphate via their hydroxyl and carboxyl groups and thus release phosphorus in soluble forms (Nakayan et al. 2013, Gupta and Sahu 2017). Fu et al. (2016) evaluated the in vitro phosphate solubilizing ability of eight rhizosphere yeast isolates by measuring the clear zone around the colonies, which was caused by the inorganic phosphate solubilization and reported as solubilization efficiency (SE) units. *Torulaspora* sp. JYC 369 solubilized all three forms of inorganic phosphate, including dicalcium phosphate, calcium phosphate tribasic, and tricalcium phosphate, with SE units of 1.25, 1.63, and 1.17,

respectively. Moreover, *Torulaspora* sp. JYC 369 was also solubilized in zinc with an SE unit of 2.0. Zinc is as an essential micronutrient, which plays an important role as a cofactor of several enzymes in various plant metabolic pathways and also operates in the control of plant development through its indirect action on auxins (Castillo-González et al. 2018). Four of the eight yeast species isolated from maize rhizosphere soil, including *Candida railenensis*, *Clavispora lusitaniae*, *Meyerozyma guilliermondii*, and *Symmmetrospora coprosmae*, were found to be capable of solubilizing $Ca_2(PO_4)_2$ at various levels (Sarabia et al. 2018a). According to de Oliveira et al. (2019), *Torulaspora globose* CCA5S55 and CCA5S51 isolated from sugarcane rhizosphere could solubilize 47 and 35% of tricalcium phosphate in the medium, respectively. The phosphate-solubilizing yeasts, *M. guilliermondii* CC1, showed the significantly improved the dry weight, and nutrient uptake of maize and sword leaf lettuce when cultivated under greenhouse conditions and supplemented with half-dose chemical fertilizers (50 kg N, 25 kg P_2O_5 and 50 kg K_2O ha^{-1} for maize and 50 kg N, 37.5 kg P_2O_5 and 55 kg K_2O ha^{-1} for sword leaf lettuce and head lettuce) (Nakayan et al. 2013).

4.3. Siderophore Production

Siderophores are high-affinity iron-chelating compounds for ferric iron that are secreted by microorganisms. These compound support plant growth via iron chelating by reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) which is more easily adsorbed by plants. Moreover, siderophores indirectly inhibit plant pathogenic fungi by scavenging available iron from the environment (Ahmed and Holmström 2014). Fu et al. (2016) reported that siderophores were produced by 2 out of 8 strains of rhizosphere yeast: *Dothideomyces* sp. JYC385 and *Dothideomyces* sp. JYC383 with an AU of 1.78 and 1.33, respectively. The endorhyzosphere yeasts, *Meyerozyma guilliermondii* and *Meyerozyma caribbica*, isolated from maize root were also found to be siderophore producers (de Lima Targino et al. 2022).

4.4. ACC Deaminase Production

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme that can break the ACC, an immediate precursor of ethylene to ammonia and α-ketobutyrate (Glick et al. 1998). The ACC deaminase has been proposed to play a key role in microbe–plant association to decrease the stress generating ethylene in the plants (Danish et al. 2020). Four rhizosphere yeasts: *Galactomyces candidum* JYC 360 and *Dothideomycetes* sp. strain JYC362, JYC 383 and JYC 396 exhibited strong ACC deaminase activity (Fu et al. 2016). The ability of the endorhizospheric yeast *Cryptococcus* sp. CBSB78 to produce ACC deaminase and synthesize IAA and siderophore may be linked to its ability to increase the survival rate and biomass of *Brassica alboglabra* growing in metal-contaminated soils.

4.5. Plant Pathogen Antagonism

Fungi are among the most common pathogens of plants. Fungi on plants are classified into two types: pathogenic and saprophytic (Abdulkhair and Alghuthaymi 2016) Pathogenic fungi live in or on plant tissues and cause serious problems with the plant's vital physiological functions, whereas saprophytic fungi live in or on dead tissues. Ghodsalavi et al. (2013) revealed that the ability to synthesize fungal cell wall-degrading enzymes, which inhibit the growth of fungal pathogens, can promote the successful competition for plant nutrients with fungal pathogens. Thus, the ability to produce fungal cell wall-degrading enzymes of rhizosphere yeasts was investigated. All strains of *Dothideomyces* sp. produced the cell wall degrading enzyme including protease, carboxymethyl cellulase (CMCase) and Chitinase (Fu et al. 2016). 11 out of 13 isolates belonging to *Meyerozyma* sp. isolated from maize root had ability to produce the protease enzyme (de Lima Targino et al. 2022). Furthermore, some strains of rhizosphere yeast were discovered to produce an antifungal substance. The yeasts isolated from the rhizosphere of *Drosera spatulate* were investigated for antagonistic activity against *Glomerella cingulata*, a fruit fungal pathogen that causes ripe rot in grapes, bitter rot in apples, and several diseases in fruits and vegetables (Fu et al. 2016). The results show that *Galactomyces candidum* JYC360 and *Barnettozyma californica* JYC386 had significant antagonistic effects against the fungi tested.

4.6. Beneficial Interactions of Plant Growth Promoting Rhizosphere Yeasts

In the last few years there have been relatively few studies of combined inoculation with rhizosphere yeast and arbuscular mycorrhizal fungi for the purpose of plant growth promoting traits. Arbuscular mycorrhizal (AM) fungi are the obligate biotrophic fungi that form a symbiotic association with most land plants. They are commonly known as bio-fertilizers. Sarabia et al. (2017) examined the interaction between maize, either one of the native maize rhizosphere yeasts *Cryptococcus flavus* or *Candida railenensis*, and the community of AM fungi combined with and without phosphorus fertilization condition. It was discovered that *C. railenensis* inoculation without P fertilization improved the root colonization of AM fungi and that only AM fungi with phosphorus fertilization could promote maize plant growth. This result revealed that interactions among maize, rhizosphere yeasts, and AM fungi are highly influenced by P fertilization. Consequently, (Sarabia et al. 2018b) discovered that the cooperation between AM fungi *Rhizophagus irregularis* and the rhizosphere yeasts *Cr. flavus* and *C. railenensis* could increase the specific root length of maize and improve maize P nutrition and AM hyphal P transport. Moreover, they revealed that the improved P nutrition of mycorrhizal maize inoculated with rhizosphere yeasts is related to increased specific root length and AM hyphal P uptake.

Conclusion

The rhizosphere, which is hidden in the soil matrix, is a dynamic narrow zone of significant interaction between plant roots and soil microbiome. Yeast communities in rhizosphere soil are genetically diverse and play an important role in the ecological functioning of the soil and promoting plant growth such as phytohormone production, phytopathogen inhibition, phosphate solubilizing ability, siderophore production and stimulation of mycorrhizal-root colonization. As a consequence of their ubiquity and plant growth promoting properties, yeasts are potential microbes for selection as plant growth promoting microorganisms (PGPM) for use in sustainable agriculture that is a promising strategy to reduce dependency on agrochemicals.

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