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The Classification of Duckweed and Its Bacterial Community: A review

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Abstract — **Duckweed is the world underutilized tiniest aquatic plant which full of benefit especially as animal feed other than human consumption. However, usage of duckweed as food requires more understanding especially on its safety. This review paper describes classification of duckweed and bacteria diversity in duckweed plant which associated with various types of bacteria includes endophytic and epiphytic bacteria involving plant and human pathogen. Duckweed taxonomy can be divided into five genera according to their characteristics which are genera Spirodela and Landoltia, genus Lemna, genus Wolffiella, and genus Wolffia. Endophytic bacteria in duckweed plays a beneficial role which can help to improve the growth of duckweed such as Paenibacillus lemnae giving a high adhering ability in plant by promoting the growth of plant. Janibacter spp. is one of endophytic bacteria which can help the plant by increase plant nutrient absorption, Deinococcus spp. act as protector of a plant from pathogenic bacteria, and Acinetobacter spp. have potential to acquire anti-microbial by producing enzymes β-lactamases to protect the plant from disease. However, some bacteria presence in duckweed such as plant pathogen and epiphytic bacteria can cause disease in the plant includes Pseudomonas syringae and Xanthomonas spp. which can create bacterial canker on the leaf of the plant, and Erwinia spp. can cause fire blight. Since duckweed can also consumed in raw state, human pathogen bacteria presence in the plant which can lead to foodborne illness is also discussed in this paper. Source of bacterial contamination of duckweed plant is also reviewed. Water irrigation and improper handling and hygiene are sources contamination of bacterial transmitted into duckweed. Bacteria able to colonize duckweed through multiple mechanisms. Transmission bacteria into duckweed plant host through stomata which were aid by insects are called indirect contact and many other modes of transmission are also highlighted. Generally, duckweed can be divided into five genera and inhibited by various pathogen and non-pathogenic bacteria introduced naturally or through contamination.**

Keywords— ***bacteria, duckweed, endophytic bacteria, source of contamination, epiphytic bacteria***

I. INTRODUCTION

Plants are associated with different microorganisms during most of their life, which includes commensal, symbiotic, and pathogenic microorganisms [1]. This demonstrates that plants are connected with a wide variety of bacteria, ranging from beneficial to pathogenic bacteria. Plant-beneficial bacteria is one of type of bacteria which can provide their host plants with numerous benefit such as provide the plant with tolerance to different biotic and abiotic stresses that can hinder their growth [2]. Meanwhile, plants, particularly aquatic plants, have been linked to pathogen germs that can infect humans as well as the plant itself. Bacteria including pathogenic bacteria transmit into the aquatic plants to obtain nutrition as a source of nutrients [3] for their growth and survival. Aquatic plants are more exposed to bacteria, making it prone to bacterial contamination rather than terrestrial plants due to the growth environment. Higher bacteria count with various bacterium types are found in water due to the variety of environmental factors which accommodate the bacteria growth such as availability of nutrients and trace metal, pH or temperature [4].

Plants that serve as food and other human-animal related usages perhaps such as medicine must contain a safe level of pathogenic bacteria. It has been mentioned some aquatic plants are useful in medical treatment. *Bacopa monnieri* and *Cynodon dactylon* which are types of plants act as medical treatment in diabetic disease which contains anti-diabetic properties [5] while *Oryza sativa, Ipomea aquatica* (Water spinach), *Nymphaea nouchali* (Water lily), and *Spirodela polydetergentrhiza* (Duckweed) act as food for human consumption [6]. Besides that, the duckweed plant is also beneficial for animals as a feed supplement for the monogastric and ruminant animals due to high protein content [7]. These arguments can be used to support the importance of aquatic plants, such as duckweed, for humans and animals. As a result, it’s critical to assure microbiological safety, as bacteria can cause sickness in humans, animals, and aquatic plants [8].

Duckweed contains high protein which equivalent to the same protein content as meat [9]. Therefore, duckweed is consumed fresh and cooked to enhance the protein content in human body [10]. Apart from consumption, duckweed, and its associated microbial communities in some cases, can neutralize water contaminants into clean water [11]. Contaminated waste, irrigation water, vegetation, animals and various factors impacting the occurrence, fate, transport, survival and propagation of pathogens are the causes of bacterial infection [12]. To understand and tackle the safety issues including the utilization of the beneficial bacteria in duckweed, review on the types of bacteria presence and its source of bacterial contamination in duckweed are discussed.

II. DUCKWEED (LEMNOIDEAE)

Duckweed is a small and free-floating aquatic plant that originated from *Araceae* family able to grow and propagates quickly, accumulating biomass [13]. This section is divided into two subsections which are (A) duckweed classification and characterization, and (B) importance of duckweed towards ecosystem and human consumption.

A. Duckweed classification and characterized

Originally, duckweed is established as the family of *Lemnaceae Dumort,* but a study claimed the family of duckweed is more closely related to *Araceae* and it is not suitable to merge into *Lemnaceae* family of *Araceaeae* as subfamily *Lemnoideae* [14]. In addition, this group may be regarded as a plant family, such as *Lemnaceae Dumort*, in keeping with taxonomic laws [15]. Furthermore, duckweeds are not only smaller variations of larger angiosperms; they are also a widely transformed structural organization that has resulted from multiple morphological and anatomical characteristics being distorted, condensed, or destroyed [16]. Duckweeds consist of 37 species and are categorized into five genera which are *Spirodela, Landoltia, Lemna, Wolffiella*, and *Wolffia* based on taxonomy laws as shown in Figure 1, but for those native species, these marine angiosperms are cosmopolitan in distribution [14].

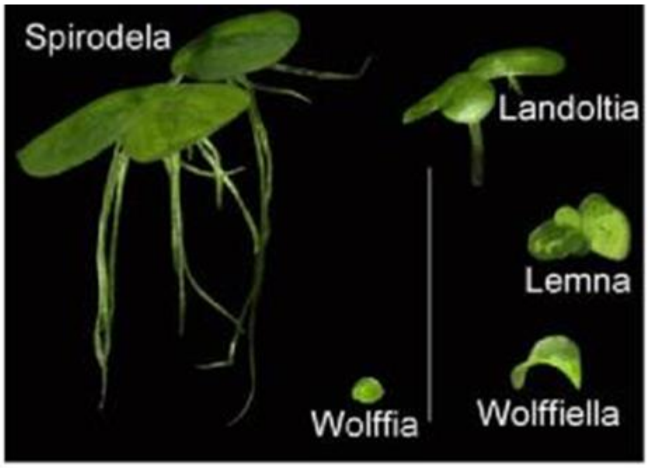

Fig. 1. The variety of duckweed species based on their genus and sizes. Source from https://www.researchgate.net/figure/Morphology-of-five-representative-species-for-duckweed-genera-Spirodela-Spirodela_fig1_338428099.

1) Genera Spirodela and Landoltia

Spirodela typically consists of three species: *Spirodela polyrhiza, Spirodela intermedia*, and *Spirodela punctata.*

These three forms of species were divided into two genera, *Spirodela* and *Landoltia*, according to the taxonomic instruments of the *Lemnaceae* family, by modifying the nomenclature of the species, *Spirodela punctata*, to *Landoltia punctata.* This is because these species have different plastidic sequence analysis. According to the taxonomic instruments of the family *Lemnaceae*, these three types of species were classified into two genera, *Spirodela* and *Landoltia*, by changing the nomenclature of the species *Spirodela* punctata into *Landoltia punctata* [17]. The three species in an unweighted pair group method with arithmetic mean (UPGMA) tree were separated based on the atpF-atpH sequence by Wang et all., 2015 [16]. However, clone 9203 was misguided in this analysis as *Spirodela polyrhiza.* This clone was earlier known by Landolt as *Spirodela intermedia* (personal correspondence to KJAA). Seven *Spirodela polyrhiza* clones, eight *Spirodela* intermedia clones, and six *Landoltia punctata* clones, also pointed out this mistake. The combined cpDNA dataset relying on the intergenic spacers atpF-atpH and psbK-psbI specifically isolated all these three species with high bootstrap values for removing this error [18].

Spirodela polyrhiza of the *Lemnaceaee* family is known as giant duckweed. It is a small, free-floating aquatic species noted for its unusual vegetative reproduction mode [19]. Figure 2 shows the morphology of genus *Spirodela polyrhiza*. Petals are leaf-like structures produced from fused stems and leaves on each frond with a multiple root system. A mother frond several times during development develops daughter fronds from two meristematic areas, but turions are often formed as dormant overwintering structures within the mother frond near the end of the growing season [20].

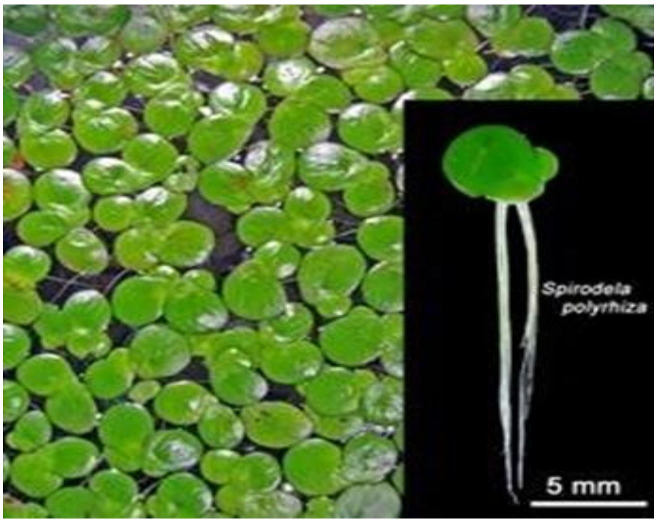

Fig. 2. Morphology of genus *Spirodela polyrhiza* with leaf-like structures. Source from https://onlinelibrary.wiley.com/doi/10.1111/plb.12171

2) Genus Lemna

The genus *Lemna* was introduced by Landolt in 1986 which was originally divided into five parts, but this arrangement was later rectified by decreasing the number of sections to four since the *Lemna. sect.* and *Hydrophylla Dumort*. It turned out that *Lemna trisulca* was a member of the *Lemna sect*. [21]. Well-supported monophyletic clades represent the *Lemna* groups; *Lemna Alatae Hegelm*., *Biformes Landolt*, and *Uninerves Hegelm*. Three species are found in *Lemna sect. Uninerves* such as *Lemna minuta Kunth*, *Lemna valdiviana Phil.*, and *L. yungensis Landolt* since 1988 [15]. Among angiosperms, the genus *Lemna* is the smallest and simplest species as shown in Figure 3. It has the attribute of multiplying with rapid vegetative proliferation every three days, which makes the organisms increase rapid growth. Therefore, the plant is suitable for measuring air emissions and checking for toxicity [22].

Fig. 3. Image of Lemna minor species. Source from https://www.researchgate.net/figure/The-common-duckweed-Lemna-minor-L-the-type- species-of-the-genus-Numerous-fronds-are_fig5_261141916

3) Genus Wolffiella

Wolffiella is biogeographically unique among the Lemnaceae genera in being confined to warm-temperate, subtropical, and tropical regions in the Americas and Africa, with a comparatively recent entry into India of one species (Wolffiella hyalina) [23]. As reviewed by Landolt (1986), the taxonomic history of Wolffiella has been mixed with Wolffia, the other Wolffioideae subfamily genus as shown in Figure 4. Phylogenetic studies based on morphological features found that the sections of Wolffiella is monophyletic, and the genus is paraphyletic. Wolffiella and the other genera may be overcome as being monophyletic by a tree five steps longer.

A study stated *Wolffiella* contains whole, symmetrical, ovate to narrowly oblong non-vascular, long as wide, green on both sides, two cohering (sometimes solitary) with one triangular flat pouch on the upper surface. It has a lower pouch wall elongating into a ribbon-, bent down and vertically hanging at 45-90 ° to the front [23]. Figure 5 shows the illustration of *Wolffiella lingulata* compared with *Wolffiella oblonga* with backlighting the shape of the budding pouch and relative position of the costa can be observed. In order to differentiate between *Wolffiella oblongata* and *Wolffiella lingulate*, we can observe the location and shape of costa within triangular budding pouch.

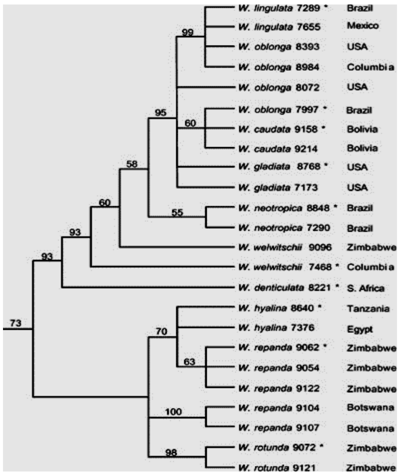

Fig. 4. Biographical of Wolffiella species in a different country. Source from https://onlinelibrary.wiley.com/doi/10.1046/j.1095-8312.2003.00210.x

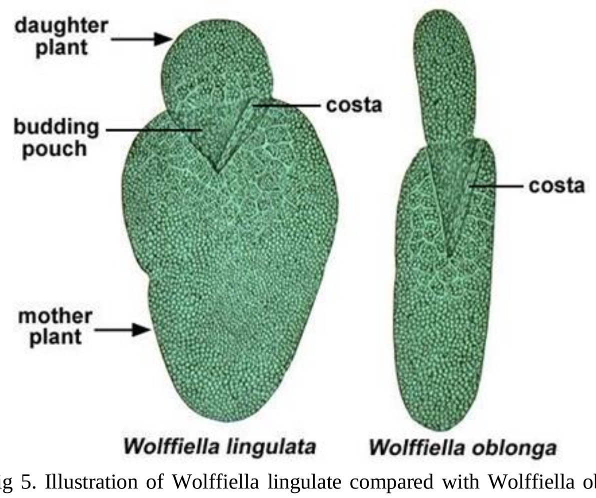

Fig 5. Illustration of Wolffiella lingulate compared with Wolffiella oblonga backlighting the shape of the budding pouch and relative position of the costa can be observed. Source from: https://www2.palomar.edu/users/warmstrong/1wayindx.htm

4) Genus Wolffia

The smallest flowering plants are found in the genus Wolffia of the duckweed family *(Lemnaceae*). Presently, 11 species are primarily recognized and classified based on morphology. Molecular methods are particularly needed for barcoding and identification of species and clones of this genus because of the extreme decrease in the structure of all species [24]. The 11 species of genus *Wolffia* includes *Wolffia angusta, Wolffia australiana, Wolffia brasiliensis, Wolffia neglecta, Wolffia elongate, Wolffia cylindracea,* and *Wolffia microscopica*. by using the sequences of rps16 (54 clones) and rpl16 (55 clones) to differentiate the type of genus *Wolffia* [25].

Moreover, the genus *Wolffia* is floating, rootless, and like the duckweed Lemna is their ancestor, the world's smallest flowering plant as shown in Figure 6. The genus *Wolffia* is recognized as dispersing by waterbirds, but through epizoochory such as attaching to the outside [26]. For the morphological of the genus *Wolffia*, it has been spread between underwater feeding sites and terrestrial loafing sites over an uncertain distance [27].

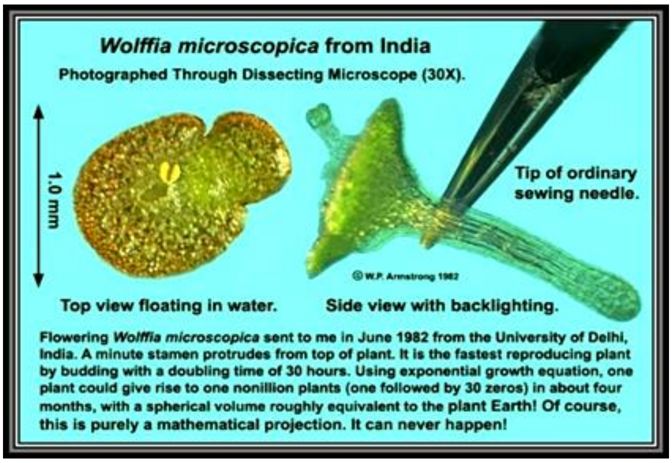

Fig. 6. Image of Wolffia microscopica from India with two types of view which are top view floating in water and side view with backlighting. Source from: https://www2.palomar.edu/users/warmstrong/plaug96.htm

B. Importance of duckweed towards ecosystem and human consumption

1) Ecosystem

Duckweed is beneficial for both for humans and the ecosystem such as food, biomass, bioremediation, hyperaccumulator of heavy metal. Beneficial bacteria able to degrade toxins or avoid waste helping in bioremediation. In other words, it is a technology to eliminate toxins from the atmosphere, thus preserving the natural environment and eliminating more emissions [28]. Bacteria found in duckweed which help in bioremediation includes *Acinetobacter*, *Aquitalea, Pseudomonas,* and *Exiguobacterium,* because of bacterial duckweed interaction for enhanced biomass production that can promote duckweed growth promotion capacity [29]. In addition, duckweed has air spaces that can provide buoyancy and can be a temporary reservoir for methane developed in anoxic water conditions that are critical for inhibiting the interaction between water and air (during an oxic environment) where methane oxidation occurs [30]. Reducing methane emissions is a good approach to take action on climate change by minimizing methane emissions, and the usage of methane will help local economies with a source of renewable energy that raises income, encourages investment, increases security, and contributes to cleaner air [31]. A study [32] reported that duckweed had the highest chemical oxygen demand (COD) elimination efficiency of around 80% in the system's surface wetland. Besides that, studies have proposed that duckweed contains rich biological duckweed proteins that can grow N-doped active centers to improve the pH-neutral status of oxygen reduction reaction (ORR) activity [29].

Furthermore, duckweed also functions as heavy metal removal from polluted water. For example, a study conducted by Todd (2019) [33] shows the deposition of up to 19 mg of iron (Fe) in dry tissue weight (DW) in *Lemna* minor of Fe-rich discharge from an abandoned coal mine in Portugal. Other than its contribution mentioned, Table 1 shows the additional importance of duckweed toward the ecosystem.

2) Human consumption

Duckweed is consumed in a raw state which is integrated with the salad. Due to the light flavor, the Dutch people have been consuming duckweed as fresh produce in their diet [12] as a protein source. They eat duckweed as a salad or additional ingredient in their cooks to make the food tastier. The source of essential amino acid is the main advantage of duckweed. Water plant protein is a source of essential amino acids [11]. The protein content in fresh produce like duckweed will yield major benefits for populations that are limited to vegetarian diets. Apart from that, this could also be alternative to protein source for those with a limited supply of milk or deficiency in vitamin A and phosphorous, as is the case in many of the dry countries of the world [34]. Figure 7 shows one of the examples of food develop from duckweed which called Mankai superfood. Apart from the protein, duckweed also contains starch about 15% of the total dry weight where it is important properties for binding, emulsifying, stabilizing, or thickening function in food and beverage [35] which makes it suitable for thicken food and beverage to meet the special needs for people who have swallowing difficulties. Duckweed is also consumed cooked as *Wolffia* spicy salad as shown in Figure 8 [36]. Table 2 shows other food application of duckweed for human consumption according to different location.

Fig. 7. Image of Mankai superfood as healthy drinks for human consumption. Source from https://www.foodnavigator- usa.com/Article/2019/10/30/Mankai-duckweed-a-fully-fledged-superfood-New-research-demonstrates-the-green-protein- s-health-benefits

Table 1. Application of duckweed in food for human consumption according to the different countries.

Roles of duckweed	Reasons	References
Test organism for Ecotoxicology	The ability for the accumulation of heavy metal	[90]
Bioaccumulation of nickel	The decreasing nickel concentration in wastewater	[94]
Phytotoxicity and degradation of antibiotic	The decreasing concentration of the antibiotics from the wastewater	[93]
Biomarkers in ecotoxicology	Counterbalancing the high concentration of toxicant (sodium chloride)	[64]

Table 2. Application of duckweed for human consumption according to different location.

Name of food	Type of food	Country	References
Mankai Superfood	Beverage	Thailand	[1]
Wolffia spicy salad	Fresh produce	China	[38]
Lentein food product	Protein bar, supplement, and beverage	USA	[91]
Meat substituted	Shrimp	America	[90]

Fig. 8. Image of Wolffia spicy salad as a healthy meal by using duckweed for human consumption. Source from http://www.baggenstos-rudolf.ch/project/Lemna/Fostering_Duckweed.html

III. TYPE OF BACTERIA PRESENCE IN DUCKWEED

Duckweed and other aquatic plants can thrive in natural habitats when they develop alongside other members of their ecosystem. Microbe associated with duckweed are thought to have important role in the growth of natural agricultural crops [37]. Plants have been widely known in the terrestrial sphere to establish intimate interactions with microbes that are important for their growth and survival such as endophytic bacteria [38]. Endophytic bacteria are the protective bacteria of plants that live within plants. It helps to promote plant growth in regular and difficult environments [2]. However, some bacteria can be pathogenic to plant and humans where it can cause diseases. A study stated that plant-pathogen occurs in aquaponic system due to humid or aquatic environment to obtain their nutrition from the root of the aquatic plant [39]. In the recent study, duckweed can be consumed by human as fresh produce or superfood due to the high protein content in duckweed [12][40] but it can be harmful for human because of food spoilage from water irrigation [41]. The type of bacteria present in duckweed are endophytic bacteria, plant pathogen and epiphytic bacteria, and pathogenic bacteria.

A. *Endophytic bacteria*

Endophytic bacteria are beneficial bacteria that flourish inside plants that can improve plant growth under normal and challenging conditions. Endophytic bacteria can directly benefit host plants by improving the uptake of plant nutrients by modulating phytohormones associated with the growth and stress of plants [2]. A study conducted by Lodewyckx, (2002) [42] observed that endophytic bacteria are also found on external surfaces together along with epiphytic bacteria, but mostly endophytic bacteria are found in internal tissues. Some endophytic bacteria identified in duckweed were *Paenibacillus lemnae., Janibacter spp., Deinococcus spp.,* and *Acinetobacter spp.* These types of endophytic bacteria were identified in duckweed acts as plant growth-promoting bacteria.

1) *Paenibacillus lemnae (P. lemnae)*

Paenibacillus is one of the genera originally included in the genus *Bacillus* that could form endospores that is rod-shaped, aerobic, or optionally anaerobic, allowing it to remain dormant under inhospitable conditions as shown in Figure 9 [43]. *P. lemnae* is one of the species from the genus *Paenibacillus* where it is designated strain L7-75 was isolated from duckweed and have cells that were motile with a monopolar flagellum (*Lemna aequinoctialis*) [44]. The benefit of *P. lemnae* is to improve the growth of duckweed plant (*Lemna* minor) about 2 times fold in 10 days because of their characteristics in high adhering ability and high growth-promoting activity [45].

2) *Janibacter spp.*

Janibacter spp. is one of the endophytic bacteria species in duckweed that has cocci-shaped cells, but irregular short rod or rodlike cells may occur when the cell of *Janibacter spp*. is unmatured as shown in Figure 10. In addition, morphology *Janibacter spp*. are smooth, circular, convex and vary in color from white to yellow where it is good growth on complex organic bacteria and growth in optimum temperature (23-35 °C) and it grows in aerobic condition [46]. *Janibacter spp*. also have their benefit towards duckweed plant by increase nutrient uptake, improves the hosts' tolerance to abiotic and biotic stresses, and shelter within the host in duckweed [47].

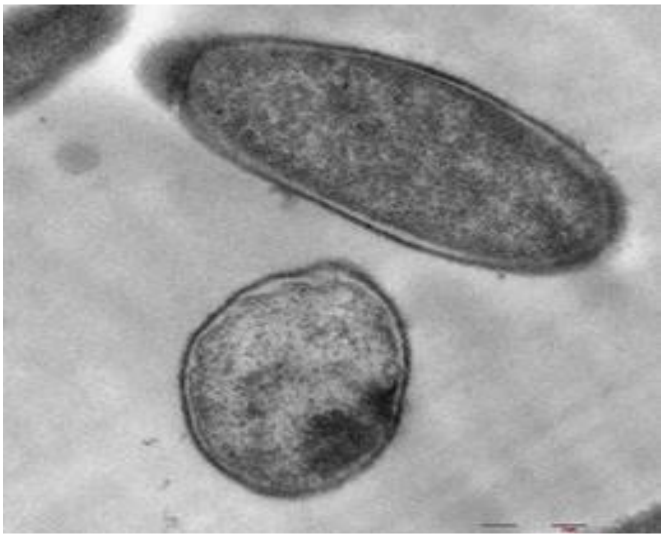

Fig. 9. Image of *P. lemnae* under the transmission electron microscopy (TEM) microscope with 30x magnificent. Source from http://hmi-us.com/research/applied-microbiology-programs/novel-bacterial-species/paenibacillus-sp-vt-400.html

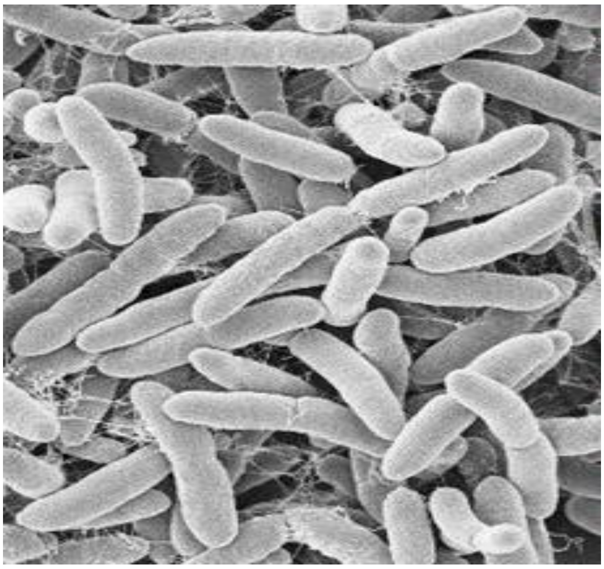

Fig. 10. Image of *Janibacter spp*. under scanning electron micrograph (SEM) with 1x 3.465k magnificent. Source from https://www.sciencephoto.com/media/873918/view/janibacter-hoylei-prokaryote-sem

3) Deinococcus spp.

Deinococcus spp. is a gram-positive bacterium which reddish in color and coccoid or rod shape as shown in Figure 11. In the recent study, *Deinococcus spp*. undergoes coordinated morphological changes at both the cellular and nucleoid level as it progresses through its cell cycle which has a highly condensed nucleoid where it can adopt multiple configurations and presenting an unusual arrangement in which oriC loci are radially distributed around clustered ter sites maintained at the cell center [44]. The special characteristics of is where *Deinococcus spp*. displays an amazing growth in the presence of high-level chronic irradiation so that it can protect the host plant from any substances that can damage the characteristics of the plant [48].

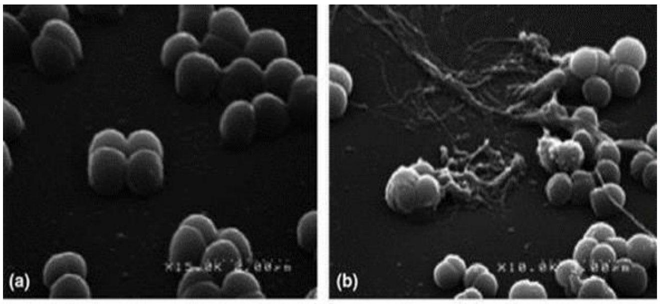

Fig. 11. Image of *Deinococcus spp* under scanning electron microscope (SEM) with 1x 15.0K magnificent. Source from https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/jam.12808

4) Acinetobacter spp.

The genus *Acinetobacter* tends to grow well on routine solid media, such as sheep blood agar at 37°C. Colonies are 1 to 2 mm, domed, mucoid, and nonpigmented as shown in Figure 12. *Acinetobacter spp.* identified as aerobic, gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli [46]. *Acinetobacter spp.* also has the potential to acquire anti-microbial genes rapidly by producing enzymes β-lactamases that can protect the characteristics of the duckweed plant from the disease [47].

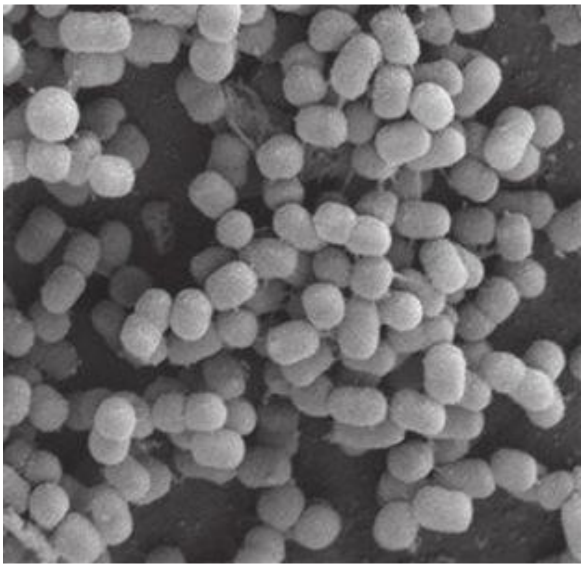

Fig. 12. Image of *Acinetobacter spp.* under SEM with scale 1x 12.0k magnificent. Source from https://www.researchgate.net/publication/276540205_Acinetobacter_baumannii_and_hospital_infections

B. Plant Pathogen and Epiphytic Bacteria

Epiphytic bacteria are the bacteria that reside non-parasitic on the surface of a plant on different organs, such as leaves, roots, bulbs, buds, seeds, and fruit. Current experiments have shown that usually, epiphytic bacteria do not damage the plant. However, if phytopathogenic bacteria live on plants in epiphytic process development on healthy tissues, the epiphytic bacteria are also associated with plant pathogen [49].

The plant disease occurrence depends on two conditions of agents which are biotic agents such as extreme environmental factors and abiotic agents such as microbial infection (plant pathogen). The interaction must occur between two-components which is the plant and disease cause to leads physical disorder towards infectious plants such as wilt and brown spot [46]. Example of plant-pathogen bacteria that have the potential for epiphytic growth on plant surfaces is *Pseudomonas syringae*, *Erwinia spp*. and *Xanthomonas spp.*

1) Pseudomonas syringae (P. syringae)

As a plant pathogenic bacterium that causes diseases in a multitude of hosts, *P. syringae* as shown in Figure 13 is mainly associated and has been used as a model organism to explain plant disease biology. Pathogenic and non-pathogenic *P. syringae* isolates, including water bodies such as rivers and precipitation, are often widely found living as epiphytes including in the broader of ecosystem [50]. The plant disease affected by *P. syringae* can cause plant symptoms such as necrotic leaf spots and shoot tip dieback appears as dead which called bacterial canker disease [51].

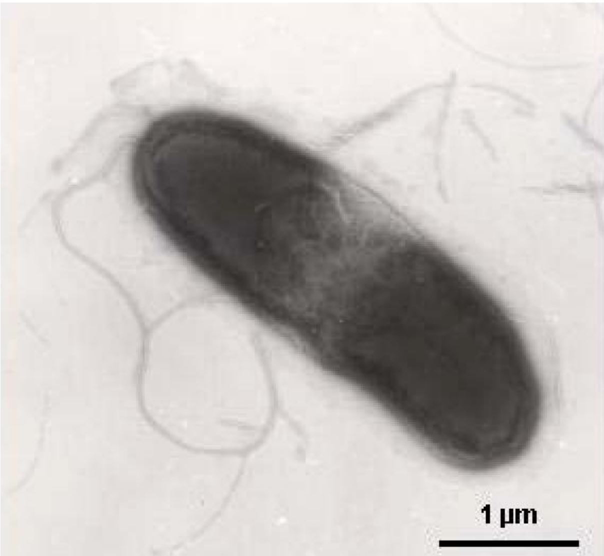

Fig. 13. Image of *P. syringae pv. aptata* with several polar flagella that reside on the plant under transmission electron microscope (TEM) (scale bar: 1 µm). Source from https://www.researchgate.net/figure/Transmission-electron-microscope-image-of-a-cell-of-the-plant-pathogenic-and-ice_fig1_230561354

2) Erwinia spp.

Erwinia spp. have a straight rod shape (Figure 14) and appears yellow colonies in nutrient agar [52]. The genus *Erwinia* is classified into *Enterobacter iaceae* and a part of pathogenic bacteria that make the disease of the plant such *as Erwinia carotorova*, and *Erwinia amylovora* [53]. The *Erwinia spp.* infect the plant and making the plant wilts and the bulb of the plant is soft where it can damage under the pressure of the finger which called fire blight disease [54]. *Erwinia spp.* can spread on the plant rapidly and cause crop losses within the infect production facility [55].

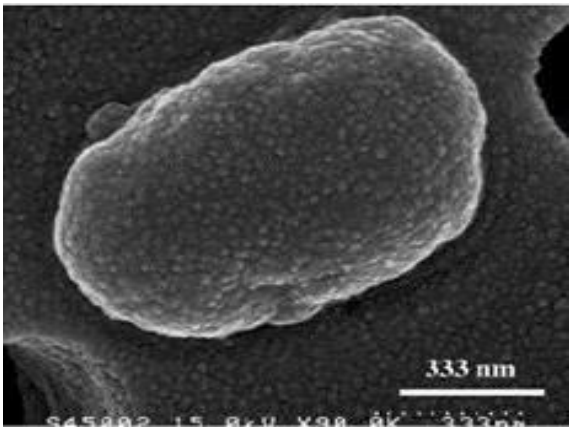

Fig. 14. Image of *Erwinia spp*. under scanning electron microscope (SEM) photomicrographs illustrating the effects of glidarc discharge on the morphology of *Erwinia spp*. after 1 min of glidarc discharge treatment. The surface of the bacterium is less regular. Source from https://www.researchgate.net/figure/Scanning-electron-microscope-photomicrographs-illustrating-the-effects-of- glidarc_fig2_6193289

3) Xanthomonas spp.

Xanthomonas spp. appeared in mucoid shape with yellow colonies on nutrient agar and has rod shape under the Scanning electron microscope (SEM) as shown in Figure 15 [56]. *Xanthomonas spp.* is one of the plant pathogenic bacteria that cause plant disease, and it is gram-negative bacteria such as *Xanthomonas campestris* [57]. *Xanthomonas campestris* can cause the plant to appear bacterial brown spot and it can kill the leaf of plants due to an increasing in the size of the brown spot on the leaves [58]. The plant that was affected by *Xanthomonas spp*. may cause vascular disease, infected plant stems, petioles, appear darker, wet, and slender and it is called bacterial canker disease [59].

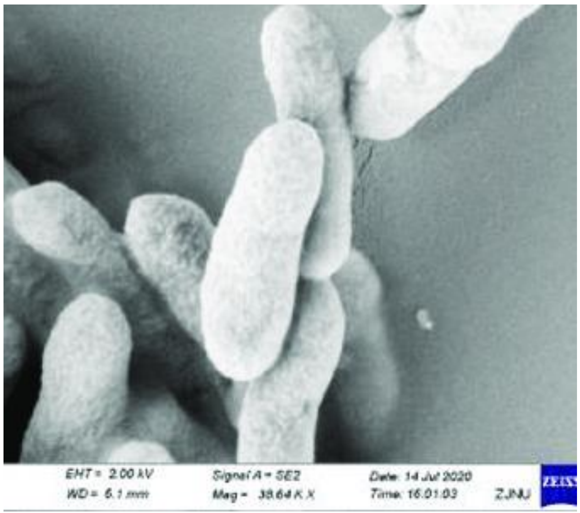

Fig 15. Photograph from scanning electron microscopy (SEM) of image *Xanthomonas oryzae pv. oryzae* with phosphate buffered solution for 4 hours. Source adopted from https://www.researchgate.net/figure/Scanning-electron-microscopy-of-Xanthomonas-oryzae-pv-oryzae-Xoo-treated-with_fig3_350352158.

C. Pathogenic bacteria that related to human consumption

Pathogens of duckweed is a member of the *Enterobacteriaceae* family, which is a human intestinal tract resident [60]. In addition, human intestinal pathogens are medically among the essential bacteria. *Salmonella spp*. and *E. coli* are often used as fecal pollution indicator organisms due to their constant presence in the feces of warm-blooded animals [61]. Pathogen that are commonly associated with fertilizer from animal waste are *Bacillus spp., Pseudomonas spp.,* and *Escherichia coli* which contribute to huge proliferation of total viable bacteria count in fresh produce [62]. In a recent study, *Escherichia coli* was detected in leafy vegetables after using poultry fertilizer [63]. Poultry fertilizer obtained from poultry manure by nature contains pathogens which could harm consumer if enter the digestion system. Pathogens originate from animal has potential risks to animal itself and possibly human health such as diarrhoea, abdominal cramps, and nausea [64].

1) Escherichia coli (E. coli)

E. coli are gram negative bacteria, facultative anaerobic and also nonsporulating [65]. In addition, *E. coli* are always used as a measure of the contamination of mammalian faeces [66]. Some serovars play a major role in intestinal and extra-intestinal diseases, though the pathogen continues primarily an infectious agent. They are produced through ingestion of contaminated food and/or water. *Enteropathogenic E. coli* (EPEC) induces watery diarrhea like *enterotoxigenic E. coli* (ETEC). In their pathogenic mechanisms, *Enteroinvasive E. coli* (EIEC) closely resemble *Shigella* which causes clinical illness such as intestinal inflammation like vomiting and diarrhea [61]. Duckweed might be contaminated by E. coli since the duckweed is one of water-plant [59].

2) Staphylococcus aureus (S.aureus)

S.aureus are gram-positive coccus which colonizes the nasal mucosa and skin of healthy individuals [67]. This type of bacteria causes staphylococcal foodborne illness, a form of gastroenteritis with faster appearance of symptoms. *Staphylococcus aureus* is found widespread in the environment (soil, water and air) apart from in the nose and skin of humans [68]. Approximately 2.41 million foodborne disease per year in United State in which mainly caused by *Staphylococcus aureus* where six deaths per year of 1,000 patients [68]. These outbreaks occur because of consumption of contaminated raw vegetables [68]. Duckweed also is one of raw vegetables with full of nutritional value [11].

3) Listeria monocytogenes (L. monocytogenes)

L. monocytogenes is a gram-negative type of bacteria [69] which has been associated with foodborne illness in fresh produce [70]. Studies has reported that fresh vegetables like cabbage or other raw vegetables infected with *L. monocytogenes* have been relate for some cases of foodborne illness. Examples of food that has been mentioned related with this pathogen include raw celery, tomatoes, and lettuce [70]. Consumption of these vegetables could have caused the outbreak of listeriosis. Presence of *L. monocytogenes* on lettuce has also been documented in other study done [71]. Since duckweed is one of fresh produce consumed raw, we can also predict it may involve with contamination of *L. monocytogenes* causing listeriosis.

IV. TRANSMISSION BACTERIA INTO DUCKWEED

Transmission of bacteria into plant can happen in six modes which are direct contact, indirect contact, droplet particles, airborne particles, common vehicle, and vector-borne [72]. Figure 16 shows the mechanism used by the pathogenic bacteria to enter a host plant (duckweed) through insect. These six modes of bacterial transmission into the host plant have their own unique way in invading the plant. For example, dust or small particles floating in the air can aid bacteria transmission into the host plants – which is called airborne transmission [73]. Phytoplasmas and spiroplasmas are common type of bacteria which can infect plants due to lack of rigid cells walls by using indirect contact as vehicle to infect the plant. These bacteria can act as viruses due to fastidious characteristic of the bacteria which can infect the plants and produce disease to the plant [74]. These bacteria also show a typical symptom where phytoplasms can caused infection in plants showed with yellowing, decline and stunting of plants, and phloem necrosis [75]. On the other hand, spiroplasms can create abnormally bunched shoots and the growth of numerous axillary buds can contribute to the brooms of witches [76]. In addition, due to their vascular habitats, endogenous bacteria have a systemic spread throughout most of the plant, transmitted from plant to plant through corruption inoculation, and most of them are vectored by insects that supply phloem (leafhoppers, psyllids) or xylem (sharpshooters). These virus-like properties have long been used for virus diseases because of the diseases caused by endogenous bacteria [77]. This is because plants are hosted by different populations of endophytic bacteria where it is possible to colonies the interior of both below and aboveground tissues, whether horizontally collected with each new generation from the atmosphere or vertically transferred from generation to generation by seeds [78]. In general, pathogenic bacteria able to inhabit leafy vegetables by using various mechanism explained causing food outbreaks and cause diseases in duckweed plant depends on the source of bacterial transmission as shown in other leafy plant.

V. POTENTIAL SOURCE OF BACTERIAL CONTAMINATION IN DUCKWEED AS HUMAN CONSUMPTION

In recent studies, duckweed has been consumed as a superfood [79] or fresh produce due to the high protein content in the duckweed [80]. It is sometimes being used to replace other protein source such as soybeans and other legumes. However, consuming duckweed as fresh produce may cause foodborne illness because of possible presence of pathogenic bacteria contamination [81]. Pathogenic bacteria from plant could be transmitted into the human body through the consumption of fresh vegetables [82]. Pathogenic bacteria can enter the plant body and system through the following.

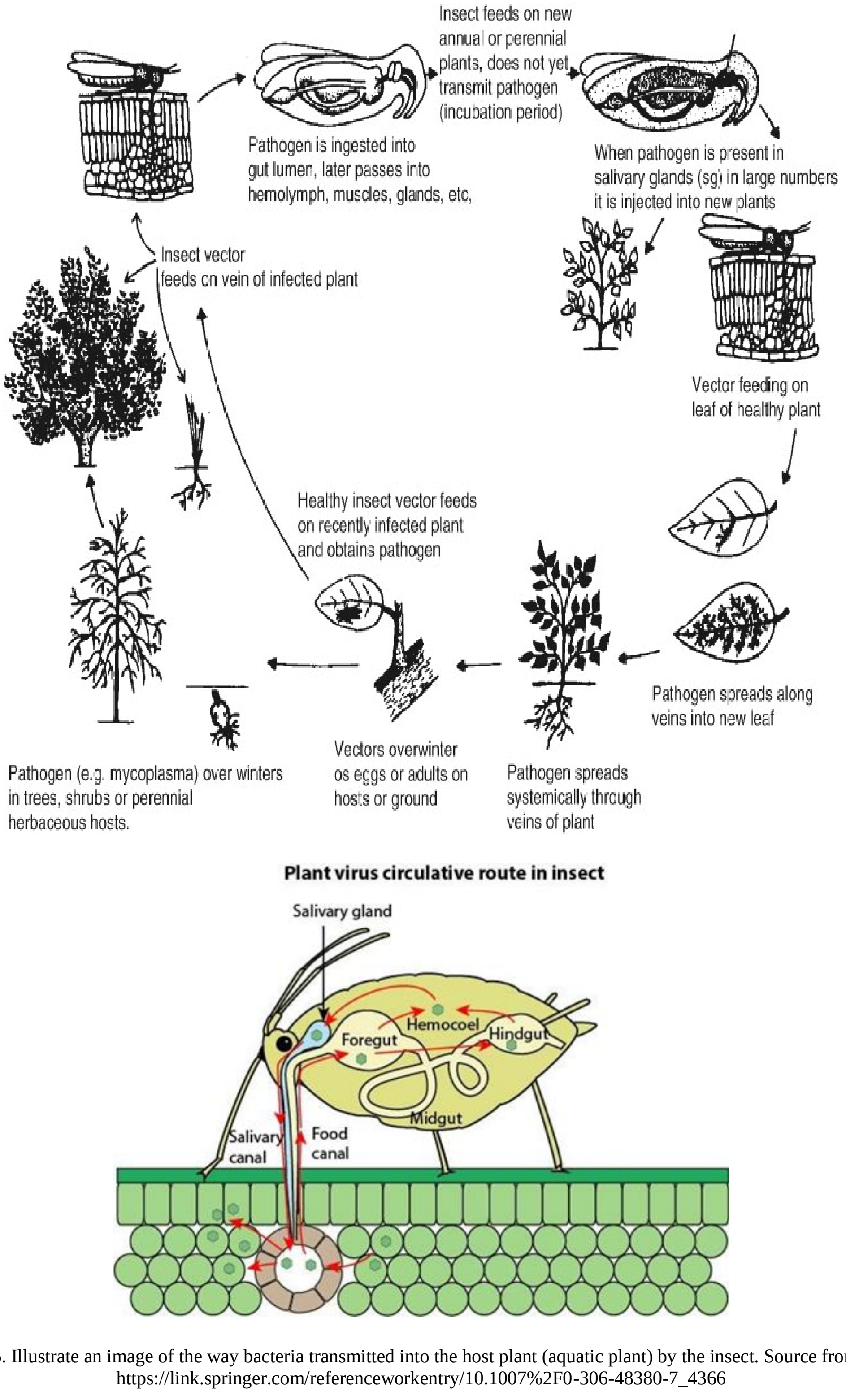

Fig. 16. Illustrate an image of the way bacteria transmitted into the host plant (aquatic plant) by the insect. Source from https://link.springer.com/referenceworkentry/10.1007%2F0-306-48380-7_4366

1) Water irrigation

Bacteria including pathogenic bacteria could contaminate fresh produce through water irrigation. For example, *Campylobacter spp.* was detected [83] in watercress grew in and harvested from contaminated water, while another study [84] mentioned eight percent of cabbages positive for *L. monocytogenes* after final irrigation in the field [85].

Contaminated water irrigation could possibly transmit pathogenic bacteria into duckweed, where the risk for microbiological degradation of fresh products can be affected [10] other than causing foodborne illness. Water irrigation could be contaminated when in contact with soil or plant debris which leads to increased opportunity of plant-pathogen entering and inhabit the plant [86]. Since duckweed is similar with watercress, which is water plants, therefore it might have higher chances of being exposed to pathogens.

2) Improper handling and hygiene

Improper handling practice during harvesting and processing can lead to bacterial contamination of fresh produce [84]. Duckweed contaminations might occur through transmission from the hand of the worker handling the fresh produce. It has been mentioned transmission of bacteria occur from the handler by direct contact with the fresh produce without proper hand washing [75]. Research found approximately 44 % of the bacteria presence from bare-handed without hand washing and shows hand washing able to reduce around 23 % of the bacteria by hand washing with water alone [87]. Besides that, the preparation of fresh produce from cultivating, harvesting, and within marketplaces also can lead to biological contamination even at the last stages in the kitchen of the consumer [88]. According to the author was said that *Salmonella spp*. and *E. coli* were detected on knives and grater during processing the fresh produce that can lead to contamination in raw vegetables [89]. It is also mentioned, prolonged exposure to high temperature during transportation and delay during storage upon arrival at retail store had been linked to high amount of total bacteria content in rocket salad [90]. On the other hand, organic fertilizers are one of the sources of pathogen which has been used widely in farming practices to grow plant. The most common types of organic fertilizers are animal manure, which contains high levels of human and plant pathogens [91]. Therefore, it could introduce bacterial contamination in the fresh produce during growing. Increasing trends of bacteria count has been observed in a supply chain study conducted using rocket salad. [92]. The study examined bacteria count at few supplies chain point of different rocket species from pre-harvest until seven days of storage in chiller. Results showed spiked in bacteria count in few supplies chain points especially post transport (PT) and during storage. Finding showed improper handling and storage could be important factors that contribute to fresh produce contamination [93].

Fresh produce can be contaminated by pathogenic bacteria since grown on soil and water where sometimes fertilized with treated animal waste and irrigated agriculture water, which could have a direct bacterial contamination effecting the safety of these products [94]. Wildlife interference into growing fields or faecal pollution originated from animal production facilities are main source of bacterial contamination. Apart from this, wastewater used for irrigation and improper manure handling in fresh produce production are also associated with food outbreaks [94]. Duckweed may have a high potential of being contaminated by pathogenic bacteria since the plant grows in pond [95] where pathogens originated from animal manure could enter the soil, accumulates and indirectly enter the water system in the field. In duckweed farm, animal manure from cow is sometimes used as fertilizers to enhance the quality of duckweed [96] thus increase the chances of bacterial contamination.

VI. RECOMMENDATION

Fresh produce that are eaten raw including duckweed are more prone to cause foodborne illness. Therefore, study on safety of the duckweed and method to control the proliferation of bacteria on the supply chain is recommended.

VII. CONCLUSION

Duckweed is a small floating plant which divided into five genera according to their characteristics. This plant is important towards the ecosystem, human and animal due to its because of its phytoremediation characteristic and high protein content. Duckweed is also associated with various bacteria resides in the plant such as endophytic bacteria, pathogenic bacteria, and epiphytic bacteria, and pathogenic. Bacteria uses multiple mechanism to invade duckweed plant causing foodborne outbreaks through both direct and non-direct contact. More thorough bacterial profiling using the molecular technique is required obtaining actual bacteria that resides in the duckweed plant. This information will be useful for the safe consumption of this plant.

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