CHAPTER 2

LITERATURE REVIEW

2.1 Mangroves

Mangrove forests are an important ecosystem that covers many millions of acres of coastline across the world. These are the most carbon-robust woods in tropical locations and typically located in the transition zone between land and water, making them potential sources of biotechnological resources (Castro et al., 2018). They serve a significant role in safeguarding coastal zones from erosion, floods, waves, and to some extent tsunami (Sakho et al., 2011). This is due to their adaptability to harsh climate, sea level rise, water temperature and salinity (Nur Anis Fadilah et al., 2015). In addition, mangrove ecosystems are well-known for their high productivity; they supply the coastal waters around with a wealth of organic matter, including detritus and living organisms (such as fish and shellfish). In this context, detritus is explicitly used as a source of nutrients and forms the basis of an all-encompassing food web that is participated in by a variety of organisms with varying levels of economic relevance. In addition, these mangrove ecosystems serve the purpose of providing a safe haven, a source of food, and a breeding ground for a wide variety of creatures, including crabs, mollusks, economically valuable fishes, and native as well as migratory birds. They are made up of a wide variety

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of species that belong to a variety of systems categories and include things like bacteria, fungus, microalgae, invertebrates, birds, and mammals (Holguin et al., 2001). However, estimate imply that just around five percent of the species in mangroves have been described due to the complexity of their microbial ecosystem (Thatoi et al., 2013). Scholars have therefore evaluated the mangrove microbial community by using metagenomic methodologies into determining the microorganisms significant in the metabolism of carbon (C), nitrogen (N) and sulphur (S) (Mo et al., 2023; Ghose et al., 2024)

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2.1.1 Mangroves Swamp in Sungai Lukut

Lukut is a district located north of Port Dickson that consists primarily of an estuary serving the fishing industry. The Lukut estuary may be found to the north-west of Port Dickson. The estuary receives freshwater discharges from the 22-kilometer-long Sungai Lukut, which flows through Seremban, Sendayan, and Lukut. The larger cities of Seremban and Sendayan may be found upstream on the Sungai Lukut, while the smaller town of Lukut relies on small-scale fishermen for its major source of income (Zainuddin et al., 2022). Moreover, Sungai Lukut is a significant river since it is used for both human consumption and the cultivation of crops. Mangrove forests surround a part of Sungai Lukut (Musa and Singh, 2018). Sungai Lukut is also surrounded by residential areas, aquaculture farms, and fishermen's jetties, all of which are known to engage in direct effluent discharge. According to Montagna et al. (2012), Sungai Lukut has a salinity gradient that ranges from fresh water to the water that is nearest to the open sea of the

Strait of Malacca. This salinity gradient gives the estuary the classification as being well mixed.

Types of sediment, physicochemical variables, and coastline topography all have an impact on the distribution of mangrove tree species in a particular area (Alongi, 2011). According to the findings of a study that was carried out by Nur Anis Fadilah and colleagues (2015), nine different species of mangrove trees were discovered in Sungai Lukut. These nine species are as follows: *Avicennia alba, Bruguiera gymnorrhiza, Ceriops tagal, Excocecaria agallocha, Nypa frutican, Rhizophora apiculate, Rhizophora mucronata, Sonneratia alba* and *Xylocarpus granatum*. Mangroves play an important role in the deposition of nutrients and other elements due to their location between fresh water and the ocean. A variety of secondary metabolites, including alkaloids, flavonoids, tannins, and phenol components, are generated and store in plant-specific tissues, and may be responsible for the therapeutic effects attributed to some plants (Busby et al., 2017; Purahong et al., 2018). *Avicennia* leaves may be a source of therapeutic properties such analgesic, antioxidant, antibacterial, and anticancer actions, as reported in the literature (Ganesh and Venilla, 2011). Morever, there are bacteria associated with the roots of the plant that are capable of converting the N in the air into nitrates and ammonium, both of which are used by the plant.

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2.2 Microbial Plant Probiotics

The term "plant probiotic bacteria" refers to a diverse group of prokaryotes, the majority of which are naturally occurring soil bacteria in the ecosystem. These bacteria exhibit favourable characteristics that are directly connected to fostering the growth of plants. Microbiomes from the rhizosphere and endosphere can be used to make probiotics for plants. Plant probiotics are, in essence, help the plants in the process of nutrient uptake through their interactions in the rhizosphere when they are provided to plants through seeds or soil. The technology used to create plant probiotics is pretty simple and the associated installation cost are remarkably low (Kour et al., 2020). Additionally, nutrient shifts in a mangrove environment are mostly driven by microbial activity (Holguin et al., 2001). This type of significant bacterium that acts especially as a plant symbiont can boost agricultural yields as well as the quality of products (such as grains, fruits, or others). Consistent and effective crop productions, as well as the production of nutritious food, are both critically dependent on the availability of soil nutrients in order to keep up with the needs of a growing population. The self-sufficiency of these organisms is a crucial part of agricultural sustainability (Kour et al., 2020). Moreover, microbiomes associated with plants and microbiomes that have been altered by inoculation also have the potential to possess pathogen-controlling properties (Sankar et al., 2017; Syed Ab Rahman et al., 2018; Saeid and Chojnacka, 2019). In spite of this, they are able to achieve great output because to the very efficient fertilizer recycling system that they have in place. The system selectively regenerates a new one while retaining the accessible, insufficient essential nutrients. Therefore, the rhizosphere plays a crucial role in plant growth by controlling the availability of essential plant nutrients including N, P, K, iron

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(Fe), magnesium (Mg), and by decomposing organic materials in the soil (Thepbandit and Athinuwat, 2024)

The bacteria and fungus in tropical mangroves account for 91% of the overall microbial biomass, while the algae and protozoa make up the remaining 7% and 2%, respectively (Alongi, 1988; Ghizelini et al., 2012; Pramanik et al., 2019). Various kinds of microorganisms contribute to environmental health in distinct manners. There are several diverse approaches through direct and indirect mechanism in which plant growth-promoting rhizobacteria (PGPR) might affect plant development. N fixation, P solubilization, siderophore production, and alterations in the levels of phytohormones like indole-3-acetic acid (IAA) are all examples of direct mechanisms of plant growth promotion (PGP), while the production of antibiotics and lytic enzymes, the ability to survive competition, and the induction of systemic resistance in plants are examples of indirect PGP mechanisms (Iyer et al., 2017). Microbes capable of metabolizing plant exudates are attracted to the rhizosphere because of the abundance of amino acids, organic acids, carbohydrates, vitamins, and signals present there (Bais et al., 2006). For instance, the introduction of *Solidago canadensis* alters the number of N-fixing bacteria in the soil and raises the amount of available nitrogen, both of which aid in the propagation of the bacteria (Wang et al., 2018).

Liquid biofertilizers, peat formulations, granules, and freeze-dried powders are the forms that available as commercial biofertilizers. Recent developments in liquid formulations have increased their prominence due to their portability and simplicity of

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use (Allouzi et al., 2022). Many methods have been suggested for incorporating formulated biofertilizers into soil, including inoculating seedlings with dry biofertilizers or liquid bio- fertilizers (Kour et al., 2020; Sarbani and Yahaya, 2022). In order to be effective as a biofertilizer, a formulation must meet certain criteria, such as being safe for the environment, biodegradable, easily accessible, allowing for the addition of the nutrients, pH adjustments, consisting of low-cost raw material, and maintaining metabolically viable high numbers in unfavourable conditions.

2.3 Participation of The Bacterial Communities in Nutrient Transformation in The Mangrove Ecosystems

Bacterial plant growth promotion mechanisms often include the most widespread approach to provide plants with insufficient resources or nutrients (e.g. fixed N, Fe, P). Many agricultural soil lack an adequate quantity of one or more of these minerals, leading to poor plant growth. Thus, increasing reliance on chemical-based N and P sources is a reflection of farmers' resolve to address this problem and guarantee greater yields. Yet, the manufacture of these chemical fertilisers is costly, depletes non-renewable resources like oil and natural gas, and poses several risks to human health and the environment. Hence, the utilization of an efficient biological method in the provision of nutrients to plants may have the potential to compensate for a percentage of these chemicals' use to this point. The macronutrients, N, P, and K are essential to plant and microbial development and function, and deficiency in any one of them can have detrimental effects (Craig et al., 2021). Therefore, bacterial communities play an important role in nutrient

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transformation in mangrove ecosystems. This is what makes mangroves sustainable and productive, as it ensures that essential nutrients are continuously cycled and made available to support plant and microbial life.

2.3.1 Nitrogen-Fixing Bacteria

N-fixing bacteria are responsible for essentially fixing the atmospheric N_2 gas through the process of reducing the molecular N_2 to NH_3, which is subsequently utilized by plants and utilized in their biosynthesis of various N-containing substances including vitamins and proteins. Some types of bacteria and cyanobacteria are essential to the N cycle because they may reduce or fix atmospheric N gas (N_2). This makes the element accessible to other organisms, such as plants and animals (Saha et al., 2017). N-fixing and diazotrophic microorganisms dominate the microbial phyla because of their versatility and ability to colonize both terrestrial and marine habitats (Holguin et al., 2001). In theory, it is possible to meet the demands of practically all agricultural plants with nitrogen-fixing bacteria or the nitrogenase genes they carry (Saha et al., 2017). Nitrogenase, a complex enzyme, performs the N-fixation by combining the activities of dinitrogenase reductase (which requires Fe as a cofactor) and dinitrogenase (which requires molybdenum and iron as cofactors) (Mahanty et al., 2016). According to research carried out by Lindström and Mousavi (2019), the genes (nif genes) responsible for the process of N-fixation have been shown to be present in both symbiotic and free-living bacteria. Oxygen also acts as an inhibitor on the nitrogenase enzyme and is a negative regulator of the nif gene expression.

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Once N-fixation happens, soil microbes, which are controlled by soil N availability, can alter the terrestrial C cycle through decomposition and the creation of soil organic matter (SOM) (Cotrufo et al., 2013). When bacteria are given access to a high-N substrate, they may breakdown it quickly; this leads to a substantial accumulation of microbial products and the concurrent creation of stable SOM. Although C is held in soil as stable SOM for substrates with high N content, more C is respired for those with low N (Cotrufo et al., 2013; Xu et al., 2014). Extracellular enzymes are the primary agents of SOM decomposition, and their activity can serve as markers of microbial nutrient demand, soil nutrient cycling, and soil respiration (Zechmeister-Boltenstern et al., 2015; Jian et al., 2016). The production of extracellular enzymes is highly dependent on the community composition. For instance, fungal communities are predominantly responsible for the production of phenol oxidase and peroxidase (particularly white rot fungus), whereas bacterial communities are mostly responsible for the production of hydrolase (Cusack et al., 2011).

Sediments, rhizospheres, and root surfaces of several mangrove species harbour a wide variety of N-fixing bacteria, including *Azospirillum*, *Azotobacter*, *Rhizobium*, *Clostridium*, and *Klebsiella* (Sengupta and Chaudhuri, 1991). *Anabaena azollae,* a cyanobacterium, is regarded as one of the most effective biofertilizers because of its capacity to fix atmospheric N in terms of its N supply to rice. Besides, *Acetobacter* is an obligatory aerobic N fixer. *Azotobacter* is an example of a nitrogen-fixing bacterium that is predominantly found in neutral and alkaline soil. It belongs to the family *Azotobacteriaceae* and is an important free-living nitrogen-fixing bacterium. It does not

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require a symbiotic interaction to fix atmospheric N and does not require a specific host in non-leguminous plants, including cotton, rice, and vegetables. The use of *Azotobacter* as bioinoculants, as stated by Jaga and Singh (2010), has the potential to boost agricultural yields by 10–12%. In this manner, PGPRs that encourage N-fixation will accelerate the absorption of N by plants, which will ultimately result in an increase in the N content as well as the photosynthetic rate of leaves (Duan et al., 2021).

2.3.2 Potassium Solubilization Bacteria

Potassium (K) is a vital component for plant development and growth, as it is involved in many metabolic processes. It plays a crucial part in the plant's ability to withstand drought and diseases (Billore et al., 2009), but it also plays a role in starch production, controlling root growth, regulating stomata movement within plant cells, activating enzymes, maintaining cell turgor, and transporting sugars and starches (Meena et al., 2014) and ultimately contributing to the quality (Sardans and Penuelas, 2015). Rhizospheric bacteria called potassium solubilizing bacteria (KSB) are able to convert insoluble potassium to soluble forms through acidolysis, chelation, exchange reactions, and complexation (Meena et al., 2015; Sattar et al., 2019) and decompose organic matter and crop residues (Etesami et al., 2017) to promote plant growth and increase yield. KSB play an essential role in soil weathering by dissolving nutrients from insoluble minerals (Hu et al., 2018). It has been proposed that inoculating soil with KSB is able to stimulate the release of K (Khanghahi et al., 2018; Sarikhani et al., 2018).

In the soil, K is found at a concentration of 1–2% as a soluble compound, with the other 90%+ present as insoluble rocks and silicate minerals (e.g. mica, muscovite, feldspar, microline, and orthoklas). In this way, the latter type is mostly inaccessible to plants (Parmar and Sindhu, 2013). Bacteria play a vital role in maintaining soil fertility by secreting organic acid during the degradation of silicate minerals, which results in the release of K, silicon, and aluminium. There are a number of bacteria that can dissolve K in soil, such as *Bacillus sp*., *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *Paenibacillus sp*., *Paenibacillus glucanolyticus*, *Acidothiobacillus ferrooxidans*, *Burkholderia sp.*, *Enterobacter hormaechei*, *Pseudomonas sp.*, and *Sphingomonas sp*., (Sheng, 2005; Sugumaran and Janarthanam, 2007; Liu et al., 2012). KSB can provide benefits to tomato (Lin et al., 2002), cotton and rape (Sheng, 2005), chilli (Ramarethinam and Chandra, 2005), pepper and cucumber (Han and Lee, 2006), sorghum (Badr et al., 2006), wheat (Sheng and He, 2006), sudan grass (Basak and Biswas, 2009), khella (Hassan et al., 2010), banana (Archana et al., 2012), common bean (Kumar et al., 2012), tobacco (Zhang and Kong, 2014), rice (Bakhshandeh et al., 2017), tea (Pramanik et al., 2019), and apple (Chen et al., 2020). Among the soil microorganisms, KSB play the most significant role in plant K cycling (Sun et al., 2020). For this reason, using them as a biological fertilizer has been highlighted as a way to improve plant nutrition.

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2.3.3 Phosphate Solubilization Bacteria

Among the essential macronutrients, P plays a crucial role in the biological development and growth of plants (Soetan et al., 2010). In spite of the high quantity of P that is typically found in the soil (about 400 – 1,200 mg kg^{-1} of soil), a substantial percentage of it is insoluble in its natural environment and therefore unable to promote the growth of plants. The insoluble P content exists in the form of inorganic mineral (such as apatite, hydroxyapatite, and oxyapatite), which most often appears after the incorporation of chemical fertilizers (Shashidhar and Podile, 2010). Alternatively, P may also be found in organic matter in the form of inositol phosphate (found in soil phytate), phosphomonoesters, and phosphotriesters (Glick, 2012). Hence, phosphate solubilization and mineralization via phosphate-solubilizing bacteria (PSB) are critical features of plant growth-promoting bacteria and plant growth-promoting fungi (e.g. *mycorrhizae*) (Richardson, 2001). *Achromobacter, Agrobacterium, Aerobacter, Bacillus, Burkholderia, Corynebacterium, Erwinia, Escherichia freundii, Erwinia sp., Flavobacterium sp., Micrococcus sp., Mycobacterium sp., Pseudomonas sp., Rihizobium sp., Rhodococcus sp., Sarcina sp., Serratia phosphaticum,* and *Xanthomonas sp.*, has been reported as P solubilizers (Kour et al., 2020). Chittora et al. (2020) claimed that cyanobacteria such as *Anabaena, Nostoc, Scytonema, Calothrix brauni*, and *Tolypothrix ceylonica* can solubilize soil P. Among fungi, *Aspergillus, Paeciliomyces, Penicillium, Sclerotium rolfisi, Cephalosporium sp., Alternaria sp., Cylindrocladium sp., Fusarium sp., Rhizoctonia sp., Rhodotorula minuta, Saccharomyces cerevisiae*, and *Torula thermophila*, can solubilize inorganic phosphate.

The ability of phosphate-solubilizing microorganisms (PSM) to convert insoluble P to a soluble form is well recognized for plant development. Here are different mechanisms which are usually involved in this conversion which includes acidification, exchange reactions and chelation. P-solubilizers play a crucial role in the solubilization of soil P by secondary metabolites, producing enzymes (acid phosphatases and phytases) and generate phytohormones like IAA and siderophores that further boost plant yield (Kumar et al., 2014; Kour et al., 2020; Tariq and Ahmed, 2023). For instance, inoculation with these PSBs led to a considerable increase in chick pea plant growth compared to uninoculated controls, which may be ascribed to other PGP properties (Iyer et al., 2017). Hence, bacteria that are capable of phosphate solubilization is hugely beneficial for plant growth due to the potential function these bacteria play in the supply of soluble forms of P.

2.4 Interaction of Plant Growth Promoting Bacteria and Its Host

Plant health, crop yield, and the fertility of the soil are all determined by the interactions that take place between plants and microbes in the rhizosphere. Both plants and the microbiota that are associated with them have experienced evolution, which has led to the establishment of a mutualistic connection. This means that both entities will regard their associate to be advantageous. They engage in constant communication, which may subsequently be utilized for controlling the number and variety of microorganisms, changing plant development, and/or protecting plants from pests and diseases, all with the aim of increasing agricultural yield. As such, the microbiota species diversity is

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dependent on members of the same genera (i.e. horizontal transmission via the environment of the soil); yet, it may also undergo vertical transfer from seeds to succeeding generations (Compant et al., 2019).

One definition of the rhizosphere is the area of soil in which plant roots and soil microorganisms interact (Gray and Smith, 2005; De Souza et al., 2015). The area of the soil that is disturbed releases a broad variety of chemicals that are involved in the attraction of creatures that may be useful, neutral, or destructive to plants (Badri and Vivanco, 2009). Ions, free oxygen, water, enzymes, mucilage, and a wide variety of C-containing primary and secondary metabolites are all secreted by the root during the process of exudation (Bais et al., 2006). However, some of the environmental factor may disrupt the interaction between plants and bacteria (Cai et al., 2009; Carvalhais et al., 2013). In the soil, microbes use 10–44% of photosynthetically fixed C that plants excrete through their roots for various purposes, including as an energy source, signaling molecules, and antimicrobials (Guttman et al., 2014).

Rhizodeposition, the process by which nutrients are deposited in the plant's root zone, provides sustenance for a wide variety of microbes. Plant roots secrete several compounds in response to environmental factors including nutrient availability and soil pH. In addition, different microorganisms respond and interact with their host plants in different ways because of the differences in root exudation based on plant age and genotype (Aira et al., 2010; Ramachandran et al., 2011). Increased microbial activity relative to the surrounding soil is known as the "rhizosphere effect," and it is supported by rhizodeposition (Mougel et al., 2006). This extremely competitive root environment is the result of extensive microbial-microbial interaction facilitated by the strong molecular communication around the roots. Soil bacteria engage in a variety of competitive and active behaviors, such as motility (Capdevila et al., 2004), attachment (Cornforth and Foster, 2013), and growth (de Souza et al., 2015).

The growth of the host plant can be stimulated by a wide variety of microorganisms. Biological N fixation and P solubilization, stress alleviation via modulation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase expression, and the synthesis of phytohormones and siderophores are all examples of genetic processes of plant growth promoting bacteria (PGPB) employ to promote the plant development (Bhat et al., 2019; Lopes et al., 2021). Rhizobacteria can help in the synthesis of siderophores, which improves iron uptake (Bhat et al., 2019). The phytosimulator affects the metabolism of the plant hormones by stimulating the synthesis of auxin, cytokinins, abscisic acid, and gibberellins while suppressing the production of ethylene (Martínez-Viveros et al., 2010; Bhat et al., 2019; Khan et al., 2020). To further facilitate water uptake, microbial auxins help control cell division, shoot growth, vascular tissue differentiation, adventitious and lateral root development, root elongation, and root surface area. Growth-promoting bacteria like *Pseudomonas* and *Bacillus* stimulate plant development by upregulating auxin and ACC deaminase (Samaddar et al., 2019; Danish et al., 2020; Khoshru et al., 2020). Cytokinin production is stimulated by PGPM, leading to enhanced root growth, vascular cambium activity, cell differentiation, and apical dominance (Gouda et al., 2018; Khan et al., 2020). Moreover, plant growth promoting microorganisms (PGPM) has the

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ability to stimulate plant growth by elevating gibberellin levels, enhancing seed germination, and contributing to the development of stems, leaves, flowers, and fruits.

Indirectly, PGPM serve as biopesticides or biocontrol agents by competing with phytopathogens for nutrients, inhibiting them, and inducing systemic resistance (Abhilash et al., 2016; Bhat et al., 2019; Khan et al., 2020; Lopes et al., 2021). For microbes to colonize a host and persevere in hostile environments, they rely on a communication system called quorum sensing. Communication between cells and the management of microbial populations are essential to this process. Certain microbes, such as *Bacillus*, are capable of secreting volatile metabolites, which include alkyl sulfides, indole, and terpenes, amongst others. Soil pores provide a convenient pathway for volatile organic compounds (VOCs) to disperse and participate in microbial communication and interaction (Chandrasekaran et al., 2023). Some of the molecules that are secreted from the roots may have antibacterial effects on some species, while at the same time acting as stimulants for the development of mutually beneficial relationships with other organisms (de Souza et al., 2015). Several studies have shown that endophytic PGPB trigger stress and defensive responses, which in turn alter plant metabolites and finely regulate bacterial populations inside plant tissues (Lopes et al., 2021).

Although mangrove ecosystems rely on bacteria for nutrient recycling, the bacteria gain benefit from their connection with the mangrove plants as well. For instance, mangrove roots release nutrients that are used by bacterial populations which can be found both in the sediments and in the rhizosphere (Nedwell et al., 1994). Moreover, in

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terrestrial ecosystems, bacteria colonize plant root surfaces, producing root exudates and thus increasing microbial activity in the rhizosphere (Lynch and Whipps, 1990). Mangrove sediments notably show higher bacterial activity corresponding to the presence of plants. For instance, an Indian mangrove ecosystem revealed more bacteria used for N transformations (i.e., ammonification, nitrification, and denitrification) in soil with plants compared to those absent (Routray et al., 1996). Conversely, N fixation rates are often greater in sediments containing plants in Florida's mangroves than in areas without plants (Zuberer and Silver, 1978).

Plant microbiome services provided by beneficial plant microbes involve a complex interplay between various interactions and processes (Figure 2.1). When a plant is exposed to pathogens or certain Microbe-Associated Molecular Patterns (MAMPs), it activates its defense responses. Some beneficial microbes can also induce this response by interacting with the plant. As a result, the plant's defense system becomes more alert and primed to fend off potential pathogens. This priming is done through the release of defense hormones like salicylic acid. Induced Systemic Resistance (ISR) is a defense mechanism where the plant's immune system is activated by beneficial microbes. Certain PGPR and MAMPs can trigger this response in plants. These microbes release specific compounds that act as signals, alerting the plant to potential threats and preparing it for a faster and stronger defense response. Beneficial microbes in the rhizosphere can facilitate nutrient exchange with the plant. They may release enzymes that break down complex organic matter, liberating nutrients like nitrogen, iron, and phosphates, which can then be taken up by the plants. Overall, plant-microbes interaction involves a dynamic network of

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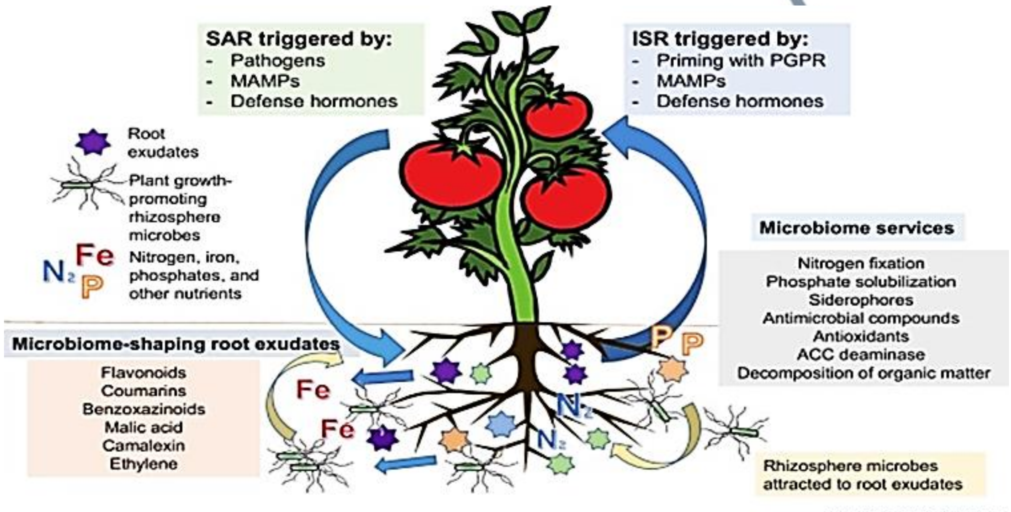
interactions (Figure 2.1) that benefit both the microbes and the plants, enhancing plant growth, health, and defense against pathogens.



Source: Arif et al. (2020)

Figure 2.1 : Plant Microbiome Services Provided by Beneficial Plant-Microbes

2.5 Methods of Studying Microbial Communities in Mangroves Rhizosphere

2.5.1 Metagenomic Whole Genome Sequencing (mWGS) and 16s rRNA Gene Sequencing

Whole-genome sequencing is a potent genomic technique used to investigate the genetic composition of complex microbial communities without isolating and cultivating individual organisms. The purpose of mWGS is to analyse the diversity and the functional capacities of the microbial community examined by sequencing all of the genomes

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present in an sample (Pérez-Cobas et al., 2020). This method provides insight on how a specific microbiome's taxonomy, biodiversity, evolution, and potential usefulness may alter in response to environmental factors and symbiotic connections with intact human, animal, or plant cells (Raes et al., 2007; Shami et al., 2022). Taxonomy can be assigned at the species and has become increasingly popular for taxonomic classification due to its advantages (Breitwieser et al., 2019).

The application of these techniques had significant implications across various fields. In environmental microbiology, metagenomic whole genome sequencing has been used to study microbial communities in diverse habitats such as soil and oceans providing insights into the roles of microorganisms in nutrient cycling, biogeochemical processes, bioremediation, and disease development (Nam et a., 2023). For instance, mWGS had been employed to identify novel microbial species, assess the impact of environmental disturbances, and understand the roles of microorganisms in biogeochemical cycles (Nwachukwu and Babalola, 2022). Research has shown the impact of land use and management practices on soil microbial communities (Liu et al., 2022), aiding in sustainable agriculture and soil health management. Similarly, the application of this technique has unraveled the complexity of aquatic ecosystems, contributing to insights into algal-bacterial interactions, and the response to pollution events (Nandy and Kaply, 2024).

The preliminary findings support the validity of the experiment and validate the classification of microbiomes. Bacterial genomes had the highest level of non-redundant

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genes, followed by archaeal and eukaryotic genomes, and finally by virus genomes, which had the lowest level. Overall, the results show that the most bacterial members are more strongly correlated with plant growth promotion in the rhizosphere than in the bulk soil, where eukaryotic members are more strongly linked with the presence of pathogens (Shami et al., 2022). Jeyanny et al. (2020) examined the bioinformatics analysis of the DNA sequence data, which revealed that *Proteobacteria* predominated in sediments, followed by *Firmicutes*, *Chloroflexi*, *Actinobacteria*, *Acidobacteria*, *Gemmatimonadetes* and others. *Desulfobacteraceae*, *Pirellulaceae*, and *Syntrophobacteraceae* were the most common families among all mangrove species, while *Proteobacteria* (especially *Deltaproteobacteria* and *Gammaproteobacteria*) was the most common phylum (Muwawa et al., 2021).

The application of metagenomic whole genome sequencing has also extended to agriculture and food safety, where it has been used to assess microbial communities in soil, plants, and food products, contributing to the optimization of agricultural practices and the detection of potential pathogens (Jagadeesan et al., 2019). This technique also has been applied to study the plant microbiome and its interactions with plants. Researchers have used this technique to identify beneficial microorganisms that promote plant growth and provide protection against pathogens, leading to the development of microbial-based biofertilizers and biopesticides (Iquebal, 2022). Furthermore, this approach has been harnessed in biotechnology and bioengineering, enabling the discovery of novel enzymes, metabolic pathways, and biomolecules from diverse microbial sources, with potential applications in industrial processes and drug development (Prayogo et al., 2020).

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In medical research, these techniques have been employed to investigate the human microbiome and its association with health and disease (Kim et al., 2024). Studies have demonstrated associations between specific microbial taxa and conditions like allergies (Fu et al., 2022), inflammatory bowel disease (Ning et al., 2023), and obesity (Hu et al., 2024). Moreover, metagenomics has proven valuable in tracking disease outbreaks by identifying pathogens and their genetic variations, enabling rapid identification, aiding in the development of targeted interventions (Dweh et al., 2023). This technique also has been employed to monitor the effects of dietary interventions, probiotics, and other treatments on gut microbial communities (De Castilho et al., 2023). Furthermore, mWGS has shown great promise in personalized medicine and the diagnosis of genetic disorders. Metagenomic sequencing has played a crucial role in tracking and characterizing microbial communities in response to epidemics and pandemics. During the corona virus disease 19 (COVID-19) pandemic, metagenomic sequencing has been instrumental in studying the severe acute respiratory syndrome coronavirus 2 **(**SARS-CoV-2) viruses (Goodrich et al., 2017). By analyzing the entire genome, including the human genome and the associated microbial genomes, mWGS can provide a comprehensive view of the genetic factors influencing an individual's health. This approach has the potential to revolutionize disease diagnosis and treatment, leading to more tailored and effective therapeutic interventions (Mall et al., 2019).

On the other hand, 16S rRNA sequencing focuses on a specific gene region of the bacterial and archaeal genomes. This gene is highly conserved across different microorganisms and contains variable regions that enable the differentiation of bacterial

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species. By sequencing these regions, researchers can identify and classify microbial taxa within a sample, providing a snaphot of the microbial composition and relative abundance (Caporaso et al., 2010). Using 16S rRNA sequencing, abundant populations of bacteria are discovered in urban mangrove forest included the phyla *Proteobacteria*, *Desulfobacteriota*, and *Chloroflexi*. By analyzing the 16S rRNA gene sequences, researchers can infer the phylogenetic relationships among different microorganisms and study their responses to environmental changes or perturbations. This approach has been widely used in characterizing microbial communities in various environments, including the human microbiome (Ames et al., 2018), aquatic ecosystems (De Lorenzi et al., 2022), and soil (Wutkowska et al., 2019). Studies on microbial community structure in mangrove ecosystems using 16S rRNA have focused on environmental changes such as (Muwawa et al., 2018), tropical monsoon (Khandeparker et al., 2017), across the aquatic ecosystems, and multiple mangrove regions. This includes across the countries who revealed the broad range of microbes that can provide ecological services, including oil spills, antibiotics resistome, genetic diversity, transmission patterns, and potential drug targets (Joye et al., 2014; Johnson et al., 2019; Hernández et al., 2023). However, studies on mangrove soil microbiome and their interaction with the mangrove trees are still scarce.

In addition, metagenome data introduces functional genes that are active in the soil environment, in addition to microbial species identification. These genes are responsible for critical processes such as nitrogen fixation, phosphate solubilization, and the production of growth-promoting hormones (Kumar et al., 2021). Even though

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metagenome data has its benefits, biofertilizers have been developed for decades using more conventional methods of isolating and cultivating beneficial microorganisms. Nevertheless, these approaches have certain limitations as they frequently fall short in comprehensively capturing the complete range of microbial communities and their intricate interactions. For example, many microbes cannot be cultivated in laboratories, yet they perform crucial functions in natural habitats (Ghosh et al., 2022). This constraint is especially noticeable in the rhizosphere of plants cultivated in distinct habitats, such as mangrove soils, where the unculturable bacteria detected via metagenomics may not be detectable only from 16S rRNA sequencing (Jansson et al., 2022).

Metagenomic whole genome sequencing and 16s rRNA gene sequencing has revolutionized the study of microbial communities by providing a comprehensive and culture-independent perspective on their genetic composition and functional capabilities. These techniques continue to drive advancements in microbiology, ecology, and biomedical research, enabling us to unravel the complex interactions between microorganisms and their environments.

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2.6 Selection Medium Used to Isolate The Potential Biofertilizer from Mangrove Soil

2.6.1 Jensen's Agar

Selected nitrogen-fixing bacteria (NFB) were screened for their ability to fix N on Jensen's agar medium. The incubation period for NFB varied between 5 and 9 days (Zebua et al., 2020; Anggrainy et al., 2021; Reang et al., 2022). Colonies derived from the chosen isolates were evaluated for their form, texture, and overall appearance. Colonies of bacteria grown on Jensen's agar often take the forms of round-shaped that are milky yellow or white in colour (Sahoo et al., 2013). For instance, when incubated at 30°C for 72 hours, *Enterobacter cloacae* can increase soil N compared to without microbial application (Sembiring and Sabrina, 2021). In addition, incubation of soil from the mangrove rhizosphere for 5 days resulted in the isolation of *Azotobacter vinelandii* (Yu and Ullrich, 2018).

2.6.2 Aleksandrow Agar

Aleksandrow's agar is used to identify bacteria capable of dissolving potassium. Following incubation, colonies that have developed a distinct zone around them are regarded to be potassium-solubilizing microorganisms. After seven days of incubation at 27–30°C, the halo zone and colony diameter were determined (Meena et al., 2015; Oo et al., 2020; Backera et al., 2021). For instance, three different strains of *Agrobacterium tumefacien, Streptomyces sp.,* and *Rhizobium sp.* established a visible halo zone around

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their colonies after 7 days of incubation at 30 °C (Backera et al., 2021) and *Micrococcus varians* and *Corynebacterium kutscheri* were found to be the isolated KSB strain after plates were incubated at 27 ± 20 °C for 7 days (Verma et al., 2016). *Aeromonas caviae, Bacillus xiamenensis, Bacillus thuringiensis, Paenibacillus xylanilyticus, Aspergillus aculeatus, Aspergillus japonicus, Fomitopsis sp.*, and *Penicillium citrinum* were all found to be able to solubilize potassium and have been isolated by Mapile and Obusan, (2020). *Bacillus licheniformis* BHU18 and *Pseudomonas azotoformans* BHU21, two rhizospheric actinobacteria KSB strains, demonstrated considerably increased K-solublization (Saha et al., 2016). Oo et al. (2020) reported that the KSB strain *of Pseudomonas plecoglossicida* B3 and *Acromobacter insolitus* S3 could be used as biofertilizers individually or in a consortium as an alternative to chemical fertilisers.

2.6.3 Pikovskaya’s Agar

Phosphate solubilizing bacteria (PSB) can be identified morphologically on Pikovskaya’s agar medium. Indicative of phosphate solubilization is the formation of a clear zone around the colonies (Ghorpade et al., 2023). Calcium phosphate ($Ca_3(PO_4)_2$) is an insoluble form of phosphate that is present in Pikovskaya’s agar. While, dihydrogen phosphate (H_2PO^{4-}) ion is released from the molecule after being bound by bacteria. The discharge of ions and the phosphatase enzyme activity results in the formation of a cleared zone around the colony. In addition, it is shown that the clear zone develops when the pH of a medium drops as a result of the action of the phosphatase enzyme. Organic acids produced by these bacteria lower pH and react with P-binding agents including

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Al^{3+}, Fe^{3+}, Ca^{2+}, and Mg^{2+} to generate organic chelates, allowing free phosphate ions (PO^{4-}) to be released (Fatimah et al., 2021). According to research done by Maheswar and Sathiyavani, (2012), *Bacillus subtilis* and *Bacillus cereus* are capable of producing halozones in Pikovskaya's agar as a result of the solubilization of $Ca_3(PO_4)_2$ (Mohamed et al., 2018).

The incubation period varies depending on the type of PSB. After incubation on Pikovskaya's agar for 1–5 days at room temperature, the phosphate solubilizing activity of each bacterial isolate was calculated. The size of the clear zone that formed around the bacterial colony was a good indicator of its phosphate solubilization activity (Mohamed et al., 2018). Plates were incubated at room temperature for three to five days to cultivate possible KSB, for instance, *Pseudomonas*, *Bacillus*, *Alcaligens*, *Klebsiella*, *Serratia*, *Azotobacters*, and *Micrococcus* (Behera et al., 2016). *Bacillus subtilis* strain PH, *Serratia marcescens* strain PH1, and *Serratia marcescens* strain PH2 were identified as the strains with the highest solubilization index (SI) after 48 hours of incubation at 30˚C (Mohamed et al., 2018).

2.7 Duckweeds As A Model System For Plant Probiotic Testing

Duckweeds or *Lemnaceae*, are the smallest free-flowing aquatic plants with a widespread distribution in freshwaters. Duckweed species often live in the wild, like in ponds and lakes, and they grow significantly in tropical and temperate climates. They are also able to thrive in the wastewater of locals and industries (Baek et al., 2021). As a

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result of these qualities, duckweeds are an excellent candidate for a wide variety of applications, both theoretical and practical. Duckweeds have a basic plant body structure that consists of fronds, floating juvenile tissue that lacks a stem, and a single root, multiple roots, or no root at all depending on the duckweed species (Appenroth et al., 2013). They grow faster than other higher plants (Ziegler et al., 2015) and reproduce asexually (i.e., new daughter fronds emerge from a mother frond) (Toyama et al., 2022). There are five distinct genera that make up the duckweed family, *Lemnaceae* include *Landoltia, Lemna, Spirodela, Wolffia,* and *Wolffiella* (Van Hoeck et al., 2015). There are 12 different species of *Lemna* include *Lemna aequinoctialis, Lemna disperma, Lemna gibba, Lemna japonica, Lemna minor, Lemna minuta, Lemna obscura, Lemna perpusilla, Lemna tenera, Lemna trisulca, Lemna turionifera*, and *Lemna valdiv* (Baek et al., 2021). Duckweed is noted for its starch-rich biomass, high protein content, and high biofuels (bio-ethanol) production despite its diminutive size (Cui and Cheng, 2015; Xu et al. 2012).

In the duckweed ecosystem, both beneficial and deleterious microorganisms coexist for the growth of the plants (Ishizawa et al., 2017; Yamakawa et al., 2018). Similar to terrestrial plants, duckweeds interact with bacterial communities in aquatic habitats (Acosta et al., 2020; Iwashita et al., 2020). It has been demonstrated that the first PGPB strain of duckweed, *Acinetobacter calcoaceticus* P23, enhances duckweed production in both artificial medium and natural waters (Yamaga et al., 2010; Suzuki et al., 2014; Toyama et al., 2017). This raises the prospect of using this PGPB as a bioinoculant for duckweed cultivation (Ishizawa et al., 2019).

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The common duckweed (*Lemna minor*) is a valuable biological model to comprehend the fundamental of plant biology and host-microbe interactions (Ishizawa et al., 2017). The convenience of inoculating, manipulating, and maintaining the rhizosphere of duckweeds makes them an appealing model system. Besides, duckweed can be used as a phytoremediation plant (Kaur and Kanwar, 2021; Radulović et al., 2021). The synergistic interaction of microbiota with duckweed as a phytoremediation plant for cleaning up waste water or environmental toxins in water bodies has been the topic of significant study. Duckweeds also are suitable for biochemical research requiring isotope labelling because they are tiny, morphologically reduced, rapidly proliferating, and readily cultured under sterile condition (Demmig-Adams et al., 2022).

The complexity of plant growth and the necessity to modify soil conditions are the biological factors that make it difficult to employ reductionist techniques to get a mechanistic knowledge of plant microbiome activities in terrestrial plant model systems (Vorholt et al., 2017; Kremer et al., 2021). Comparatively, duckweed offers a simple model plant microbiome system for analysing the interactions between plants and microbes. In contrast to soil, the liquid growth medium used to cultivate duckweed allows for the development of an experimental system with little variability and easy access to exudates and potentially interesting microbes. To study these exudates and microbiota, researchers have created a technique for trapping exudates (Lu et al., 2014) and a method for capturing whole microbial communities (Ishizawa et al., 2017). The aquatic lifestyle of the duckweed and its tiny size make it ideal for the functional analysis of plant-microbe interactions in controlled environments (Acosta et al., 2021). Evaluation of the

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community dynamics revealed by inoculating several plant-growth-promoting bacteria onto duckweed showed that, despite the fact that these bacteria inhabit various plant niches, the colonization of bacteria can alter in the presence of other microorganisms (Yamakawa et al., 2018). Recently, a standard relative yield measurement for duckweed has been established. Both biomass (in the form of fresh weight and dried weight) and frond count can be used to quantify the duckweed growth (Ziegler et al., 2015).

Several researchers have studied the symbiotic interaction that exists between duckweed and microorganisms that promote plant growth (Appenroth et al., 2017; Ishizawa et al., 2019). Phytoremediation, industrial utilization, and the use of duckweeds as a source of food or feed, medications, and others have been extensively studied. Yet thus far, no research on the mangrove microbial community has been applied to the duckweed plant's rhizosphere. Despite significant progress in understanding the symbiotic interactions between duckweed and growth-promoting microorganisms, there remains a notable gap in the exploration of the mangrove microbial community's potential application to the duckweed plant's rhizosphere. Therefore, this study could help elucidate the broader ecological roles of mangrove-associated microbes, contributing to a more comprehensive understanding of microbial diversity and function that can contribute to more sustainable agricultural practices and environmental conservation efforts.

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