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APPLICATION OF RHIZOSPHERIC BACTERIA FOR SUSTAINABLE AGRICULTURE PRACTICES, BIOREMEDIATION OF HEAVY METALS AND PRODUCTION OF BIOACTIVE COMPOUNDS

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Article Info

Abstract

Rhizospheric bacteria, inhabiting the soil closely associated with plant roots, have emerged as a vital resource in biotechnology due to their diverse metabolic functions. These microbes play a crucial role in promoting plant growth, improving soil health, and detoxifying polluted environments. Their unique abilities to tolerate heavy metals, produce siderophores, and synthesize pharmaceutically relevant bioactive compounds have attracted considerable scientific interest. Siderophores, in particular, are iron-chelating agents that not only facilitate iron uptake in plants and microbes but also show significant promise in medical applications, including treatment of iron overload and targeted drug delivery. For instance, siderophore-antibiotic conjugates such as cefiderocol have demonstrated enhanced antimicrobial activity against multidrug-resistant pathogens. Furthermore, contaminated soils can be bioremediated using rhizospheric bacteria that can withstand harmful heavy metals, lowering risks to human health and the environment. despite these benefits, large-scale applications are hampered by issues like the limited cultivability of microbial strains, variations in rhizospheric environments, and biosafety worries regarding gene transfer. By making it possible to discover, improve, and use effective bacterial strains, recent developments in microbial formulation, synthetic biology, and omics technologies present promising answers. Rhizospheric bacteria are set to play a pivotal role in achieving the growing global demands for sustainable agriculture, environmental restoration, and innovative therapeutics. Unlocking their full potential in the fields of ecology, agriculture, and pharmaceuticals requires present research and innovation.

Keywords:
Rhizospheric bacteria, Siderophore production, Heavy metal tolerance, pharmaceutical applications.

1. Introduction

Rhizosphere bacteria influence plant growth through several mechanisms. Beneficial interactions are often difficult to identify and isolate for study, and therefore favorable effects on plant productivity are not easily described in quantitative terms [1]. Major beneficial activities of soil bacteria include solubilization of minerals, fixation of nitrogen, production of growth-promoting hormones and competitive suppression of pathogens. Most recent research to improve crop responses has emphasized the study of nitrogen-fixing bacteria indigenous to rhizospheres of cereal crops and other grasses [2].The amount of nitrogen available to crops from fixation by these organisms is significant under some circumstances, but efforts to control and increase fixation activity have not been consistently successful. Increasing concentration of heavy metals (HM) due to various anthropogenic activities is a serious problem. Plants are very much affected by HM pollution particularly in contaminated soils. Survival of plants becomes tough and its overall health under HM (heavy metals) stress is impaired [3].Remediation of HM in contaminated soil is done by physical and chemical processes which are costly, time-consuming, and non-sustainable. Metal–microbe interaction is an emerging but under-utilized technology that can be exploited to reduce HM (heavy metals) stress in plants. Several rhizosphere microorganisms are known to play essential role in the management of HM stresses in plants. They can accumulate, transform, or detoxify HM (heavy metals) [4].The use of growing plants reduces the concentration of HM in the soil) and use of rhizospheric microbes have emerged as important alternatives to ensure high efficiency and better performance. Rhizospheric microbes in particular show abilities to protect the plant from HM stress as well as help in their accumulation from soil. Microbes have metabolic capabilities supported by molecular machinery to adapt and perform even in presence of high concentration of HM. This review is focused on current understanding of rhizospheric microbes in relation to remediation of HM contamination [5].

Siderophores comprise a high-affinity system for the uptake of iron from the environment, present in many microorganisms. This system involves the biosynthesis, exudation in the extracellular space, iron mobilization by competitive complexation or dissolution of iron-bearing molecules, and recognition and uptake of ferric siderophore complexes by highly efficient transport systems or liberation of iron from the siderophore complex and uptake of iron [6]. Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria associated with different plant tissues. They can enhance plant growth through different mechanisms, including siderophores production, hence, contributing to the plant nutrition and protection against phytopathogens [7].

Plant growth, soil fertility, and environmental health are all seriously threatened by heavy metal contamination in soils brought on by industrialization and agricultural practices. Even though a variety of physical and chemical techniques are used for remediation, they are frequently expensive and harmful to the environment. Because of their innate capacity to withstand heavy metals, generate siderophores that facilitate metal chelation, and produce bioactive compounds with pharmaceutical value, rhizospheric bacteria present a viable, environmentally friendly substitute. The diversity and functional potential of rhizospheric bacteria, particularly those from metal-contaminated soils, in concurrently displaying siderophore production, heavy metal resistance, and antimicrobial activities are not well understood, though. There is a lack of integrated research examining all three capabilities at once since the majority of studies concentrate on either bioremediation or pharmaceutical potential independently. Therefore, the goal of this research is to identify and isolate rhizospheric bacteria that have the ability to produce siderophores, tolerate heavy metals, and have pharmacological qualities. The goal of this work is to support the creation of sustainable environmental remediation and drug discovery initiatives.

2. Rhizospheric Bacteria and their Role in Plant Growth

Soil bacteria have been used in crop production for decades. The main functions of these bacteria (Davison are (1) to supply nutrients to crops; (2) to stimulate plant growth, e.g., through the production of plant hormones; (3) to control or inhibit the activity of plant pathogens; (4) to improve soil structure; and (5) bioaccumulation or microbial leaching of inorganics[8]. More recently, bacteria have also been used in soil for the mineralization of organic pollutants, i.e. bioremediation of polluted soils. In the era of sustainable crop production, the plant– microbe interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization, etc. of nutrients from a limited nutrient pool, and subsequently uptake of essential nutrients by plants to realize their full genetic potential. At present, the use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system. In this regard, the use of PGPR has found a potential role in developing sustainable systems in crop production [9].A variety of symbiotic (Rhizobium sp.) and non-symbiotic bacteria (Azotobacter, Azospirillum, Bacillus, and Klebsiella sp., etc.) are now being used worldwide with the aim of enhancing plant productivity [10]. Rhizosphere bacteria may improve the uptake of nutrients to plants and/or produce plant growth promoting compounds. They also protect plant root surfaces from colonization by pathogenic microbes through direct competitive effects and production of antimicrobial agents. These bacteria can indirectly or directly affect plant growth [11].

3. Heavy Metals and their Removal by using Microorganisms

The term “heavy metals (HMs)” represents a unique group of metals and metalloids existing naturally with high density and atomic weight. Among several HMs contamination of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) in environment are considered as highly toxic and are found in terrestrial, aerial, and aquatic eco-systems more than their threshold values (World Health Organization [WHO], 2010; Agency for Toxic Substances and Disease Registry [ATSDR], 2015). Metal contamination of food and water has been reported to result into several births related defects like cancer, lesion of skin, impairment of liver and kidney functions, and many more. Millions of people of Argentina, Taiwan, Bangladesh, India, Poland, China, Hungry, Japan, Belgium, North Mexico, Chile, and Mongolia are suffering from health-related issues mentioned above due to metal contamination of ground water [12]. Lead, cadmium and mercury have been given second, third and seventh rank, respectively, due to their highly toxic and widespread nature. A huge number of superfund sites were found to be HM polluted [13].HMs are present in different ecosystem naturally or due to anthropogenic activities like smelting of metal ores, fuel and energy production, sludge dumps, mine tailings, agricultural activities, and gas exhaust [14]. Physicochemical and biological methods are used for remediation of sites contaminated with HMs, both of which have their own pros and cons while use of microorganisms as a biological tool is the emerging technology for degradation and remediation of pollutants at contaminated sites. Microbial bioremediation is a simple, economic, ecofriendly, inexpensive, and efficient approach done for removal and detoxification of toxic pollutants using native microorganisms [15]. Removal of pollutant from any contaminated sites by employing microbial system could be achieved maximally by knowing the toxic concentration of pollutant and maintenance energy required by the microbial system. Several bioremediation methods are reported for the degradation of more than 50 pollutants, but very little innovative approach was found. Mechanisms involved mainly were biosorption, bio-oxidation and bio mineralization [16]. Microbial population associated with plant roots are efficient in remediation of polluted soils and promote plant growth by several direct and indirect mechanisms such as siderophore production, phytohormone production, phosphate solubilization, biological N2 fixation, antibiotic production, synthesis of lytic enzymes, etc [17].

In terrestrial ecosystems, soils are the major sink for metal contamination [18].Metal concentration may range in typical soil from 1 to 100,000 mg/kg of which a significant part is transformed by geo-active action of soil microbes. Soil microbes especially the rhizospheric population play important role in HM detoxification in contaminated soils. This input of the rhizomicrobial population is also referred to as rhizoremediation [19]. This involves higher metabolic activity of microbes including prokaryote and eukaryotes near the vicinity of plants' root. According to Pires et al. (2017) the bacterial population in HM contaminated sites is predominantly composed of Firmicutes, Proteobacteria, and Actinobacteria and most represented genera belong to Bacillus, Pseudomonas, and Arthrobacter. Rhizobia are also very important plant growth promoting (PGP) microbes found in the rhizosphere. In fact, nodulation and nitrogenase activities can be very sensitive to HM stress but HMT rhizobial strains have also been reported from contaminated sites effectively carrying out symbiotic nitrogen fixation. Legume–rhizobia symbiosis is widely known to detoxify HM and improves the quality of contaminated soils [20].

4. Mechanisms of hm remediation by hmt-pgp microbes

Alleviation of HM in soil largely depends upon their availability. However, bioavailability of HM can further impair the process of phytoremediation as HM toxicity causes inefficient plant growth. HMT-PGP microbes in the rhizosphere tackle these two major problems simultaneously by modulating plant growth as well as by altering physico-chemical properties of soil to enhance metal bioavailability which trigger rapid detoxification or removal of HM from soil. Figure 1 provides schematic representation of diverse mechanisms involved in detoxification and remediation of HM in contaminated soils. HMT-PGP microbes alter metal bioavailability in soil through acidification, chelation, complexation, precipitation, and redox reactions. Acidic pH conditions favor bioavailability and adsorption of HM in rhizosphere [21]. Organic acids released by HMT-PGP microbes lower soil pH and sequester soluble metal ions [22].Root exudates also play important role in changing metal bioavailability, as release of certain organic compounds not only mobilizes metals by forming metal complexes but also provide nutrient and energy sources to microbial communities which in turn support plant growth and survival. Root exudates contain organic acids, amino acids, and phytochelatins (PC) which perform as intracellular binding compounds for HM. Release of protons (H^+) and enzymes with root exudates helps in acidification and electron transfer in the rhizosphere which leads to enhanced metal bioavailability[23]. Changes in concentrations of exudate compounds in the presence of particular HM can also help in developing biomarkers. Recently, based on gas chromatography-mass spectrometry (GC-MS) and metabolomics methods, Luo et al. (2017) showed that Pb accumulating and Sedum alfredii can significantly change the types of root exudates, and 15 compounds were identified and assumed to be potential biomarkers of Pb contamination. Microbially mediated redox reactions also have profound effect on transformation of HM to less or non-toxic forms [2]. Outer membrane c-type cytochromes (OM c-Cyts), transouter membrane porin–cytochrome protein complex (Pcc), or MtrABC extracellular electron conduit play key role in microbial metal reduction processes (Shi et al., 2016). Such systems are well investigated in Shewanella and Geobacter species. Furthermore, HM may also be oxidized by specific enzymes. For example, multicopper oxidases such as

CueO or CuiD and/or CopR are essentially required in Cu efflux. Whereas ChrA of chromate reductase perform reduction of Cr^{+6} to Cr^{+3}. For Hg, the protein MerA reduces Hg^{2+} to lesser toxic Hg^0. These proteins are up-regulated under toxic HM stress. There are several instances where HMT bacteria provide substantial aid in detoxification of HM in plants. A study of Chatterjee et al. (2009) on Cr-tolerant bacteria Cellulosimicrobium cellulans showed transformation of toxic Cr6+ to non-toxic Cr^{3+} and also its enhanced uptake in the shoot and root of green chili. Majumder et al. (2013) reported biotransformation of toxic As^{3+} to less toxic As^{5+} by Asoxidizing bacteria Bacillus sp. and Geobacillus sp. isolated from As-contaminated soils. Bioaccumulation is also largely responsible for HM uptake and further detoxification by HMT-PGP microbes. There are two combined processes which are responsible for bioaccumulation of HM. Passive uptake or "biosorption" is metabolismindependent accumulation of metals by living or inactive nonliving biomass or biological materials, whereas "active uptake" occurs only in living cells, requires metabolism and energy for the transport of metals [24]. Biosorption may involve one or a combination of different processes including complexation, coordination, chelation, ion exchange, microprecipitation, and entrapment [25].Cell wall and associated functional groups like –SH, –OH, and –COOH, and other biomolecules have affinity for HM that helps in the biosorption process. Metal binding also involves chelators and metal-binding peptides, such as PC (glutathione-derived peptides) and metallothioneins (MT). PC and MT are produced by rhizospheric bacteria and fungi as well as by plants in response to HM stress and may result in the deposition of HM in microbial or plant cells [26]. MT are cysteine-rich metal peptides with high affinity for Cd, Cu, and Hg metals (Ahemad, 2014). In a study Murthy et al. (2011) found an increase in the MT biosynthesis in Bacillus cereus when it was exposed to increased Pb concentrations. Similarly, Sharma et al. (2017) also showed role of MT assisted periplasmic Pb sequestration by HMT Providencia vermicola strain SJ2A. Detoxification of HM via MT biosynthesis is also very well studied in HMT fungi. However, expression of the MT-related genes and their production in the presence HM has gained more attention in members of mycorrhizal fungi [27]. After entering into the cell final step of HM detoxification involves their sequestration or compartmentalization into different subcellular organelles. In mycorrhizal fungi, mainly vacuolar compartmentation of HM is noticed. Vacuolar compartmentalization of Zn, Cu, and Cd was observed in extraradical mycelium of Glomus intraradices renamed as Rhizophagus irregularis. Similarly, Yao et al. (2014) also showed vacuolar accumulation of Cd in Cd-exposed extraradical mycelium of R. irregularis in symbiosis with clover. Microbial communities in the rhizosphere also excrete extracellular polymeric substances (EPS) such as polysaccharides, glycoprotein, lipopolysaccharide, and soluble peptide which possess substantial quantity of anion functional groups and help to remove or recover metals from the rhizosphere through biosorption [4]. EPS production by certain PGP microbes induce biofilm formation in response to the exposure of toxic HM. Biofilm formation enhances tolerance of microbial cells by forming a protective sheath as well as transform toxic metal ions into non-toxic forms after adsorption. EPS produced by rhizobia and other PGP microbes with multiple number of anionic groups are reported to sequester several types of HM [28].

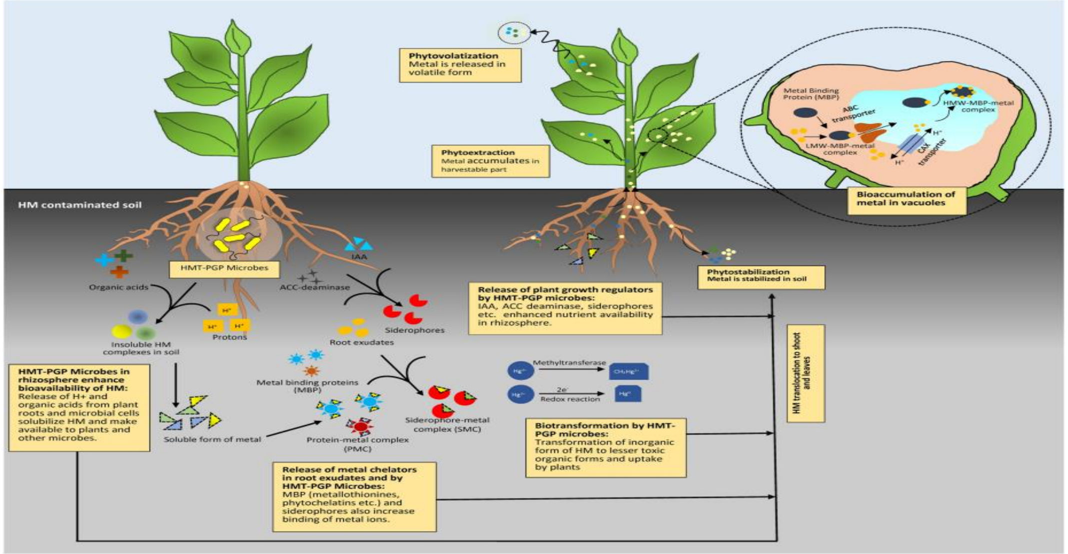

Figure 1: Mechanisms involved in remediation of HM contaminated soil by HMT-PGP microbes–plant interaction [1].

5. Enzyme production by Microorganisms

5.1. Proteases

Proteases, also termed as peptidases or proteinases, cleave the peptide bond present between the amino acid residues in a polypeptide chain. Based on the reaction, chemical nature of the catalytic site, and evolutionary relationships, proteases can be classified into two major types: (1) endopeptidases, which cleave internally the amino acids and (2) exopeptidases, which remove amino acids either from aminoterminal or carboxy-terminal end of the protein. Exopeptidases are further subdivided into (1) aminopeptidases that remove amino acid from amino-terminal or (2) carboxypeptidases that remove at carboxy-terminal, respectively. Some other types of proteases are also reported such as serine proteases (EC 3.4.21), cysteine proteases (EC 3.4.22), aspartic proteases (EC3.4.23), metalloproteases (EC 3.4.24), and threonine proteases (EC 3.4.25). These proteases are categorized based on the architecture of their catalytic site [29]. Apart from different applications in industries, proteases have also been recognized for their wide applicability in the field of agriculture. Earlier work on biocontrol demonstrated the ability of lytic enzymes including extracellular protease in antagonizing several plant pathogens [30]. There are several reports which show the role of proteases obtained from soil bacilli in biocontrol of phytopathogenic fungi and bacteria. An antagonistic strain of Bacillus amyloliquefaciens isolated from the rhizosphere of jute showed protease-mediated biocontrol activity against Macrophomina phaseolina, F. oxysporum, Fusarium semitectum, and Alternaria alternata (Majumdar and Chakraborty 2017). In a study, Essghaier et al. (2009) reported production of salt-tolerant protease from B. pumilus M3-16 strain responsible for biocontrol activity against phytopathogenic fungus B. cinerea.

5.2. Cellulases

Cellulases are glycoside hydrolase enzymes that degrade cellulose into glucose through the action of exoglucanases, endoglucanases, and β-glucosidases. These enzymes, commonly found in fungi, bacteria, protozoa, and actinomycetes, play a vital role in environmental biodegradation and biocontrol. Several rhizospheric and endophytic bacteria, such as Bacillus subtilis, B. pumilus, Paenibacillus polymyxa, and Pseudomonas poae, have demonstrated biocontrol capabilities against plant pathogens by producing cellulases along with other hydrolytic enzymes [31].The pharmaceutical and agricultural significance of cellulase-producing bacteria is heightened by their ability to inhibit phytopathogenic fungi, thereby contributing to sustainable disease management. Certain actinomycetes like Streptomyces rubrolavendulae also produce cellulases with antagonistic activity. These properties, along with their capacity for heavy metal tolerance and siderophore production, make rhizospheric bacteria promising candidates for biotechnological applications, including environmental remediation and development of antimicrobial agents [32].

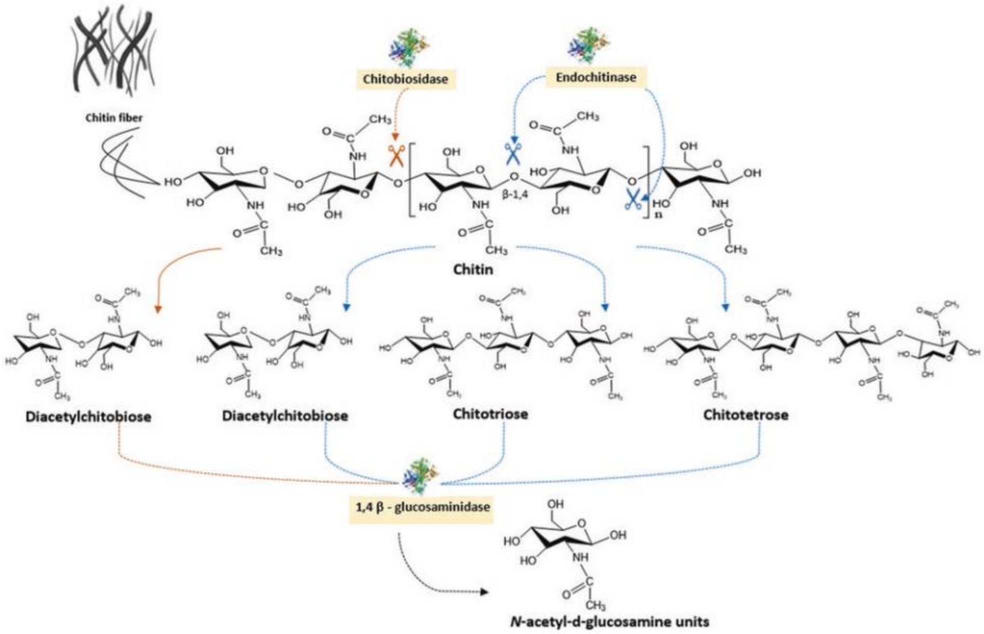

Figure 2: Mechanism of cellulases [33].

5.3. As Anticancer and immunomodulatory compounds

Medicinal plants are commonly associated with microorganisms, possibly as a result of the production of unique secondary metabolites [34]. These interactions occur in different regions of plants, such as the phyllosphere (aerial part), endosphere (inner part), or rhizosphere (area of the soil influenced by roots).The rhizosphere is characterized by high microbial activity and diversity and is inhabited by fungi, algae, protozoa, and bacteria.These microorganisms act as elicitors that may influence the production of bioactive compounds, such as alkaloids, steroids, and terpenes, by plants.They also have the potential to produce metabolites that help plants survive environmental or biological factors [35].

Among the microorganisms found in the rhizosphere, bacteria produce metabolites of interest in medicine , such as compounds with antimicrobial , antioxidant , and anticancer activities [36].However, scarce information on their potential to produce bioactive metabolites is available because the bacterial diversity is very large, and only a small fraction of the species are cultivable. Several studies have reported the biological activity of rhizobacteria that inhabit medicinal plants , including cacti [37]. However, this group of plants is still underexplored.

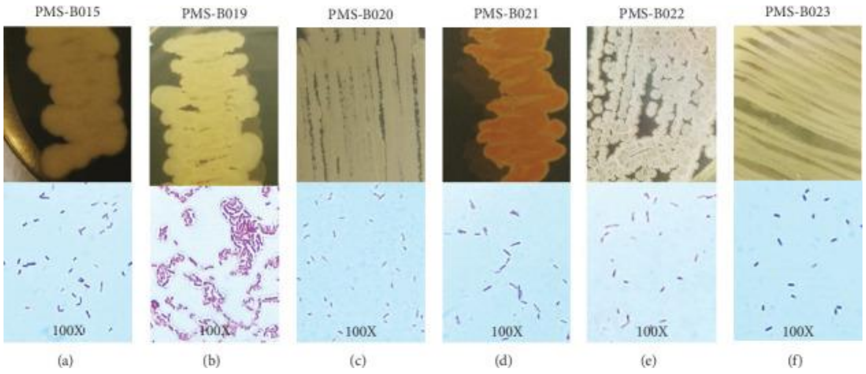

Figure 3: Rhizosphere soil bacteria from L. marginatus. (a) PMS-B015 strain. (b) PMS-B019 strain. (c) PMS-B020 strain. (d) PMS-B021 strain. (e) PMS-B022 strain. (f) PMS-B023 strain [38].

5.4. Probiotic and therapeutic applications

Probiotics are defined as, ‘Live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host’ [39].Probiotic activity is associated with a variety of microbes and a range of hosts, including humans and other animals, fish, crustaceans, insects [40] and nematodes [41].The functions of probiotics in animals include the limitation of pathogens through varied mechanisms, such as reduced potential for binding and invasion, depression of growth by antibiotics and stimulation of host immunity. Probiotics in the gut tissue and on skin maintain a microbial balance that minimizes disease conditions. Thus, there is strong similarity in the roles of animal probiotics and plant-associated microbes, such as the root-colonizing pseudomonads, that have beneficial properties [42].

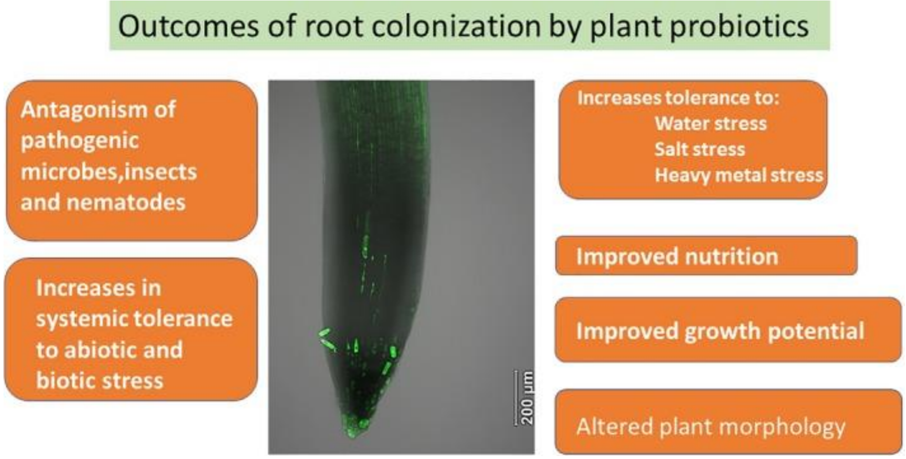

Figure 4: Summary of the potential probiotic-like effects associated with plant colonization by beneficial pseudomonads. The image of a wheat root tip is shown, colonized by Pseudomonas chlororaphis O6 and stained to reveal cells with accumulations of nitric oxide [43].

Beneficial interactions between eukaryotes and probiotic bacteria evolved early, in part to capture the extensive and varied capacities of microbial metabolic pathways for the benefit of the eukaryote [44].Mutual survival of the host and the probiotic occurs from the donation of a portion of the host metabolite to nurture microbial growth and permit microbial production of compounds that enhance host performance (Figure 5). For the plant, the chemical arsenal that reduces the viability of microbial pathogens, insects and nematodes and the metabolites/enzymes that stimulate induced stress tolerance are all examples of cross-kingdom interactions. The production of these types of metabolites that induce stress tolerance in the host is a key trait of a probiotic microbe.

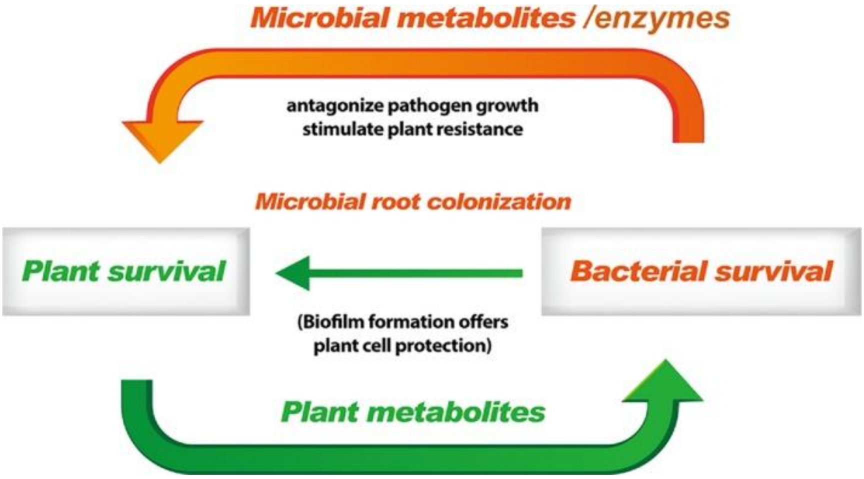

Figure 5: Schematic diagram of the basic positive interplay between the metabolism of the plant and its probiotic-like root colonizers [45].

6. Methods of Isolation and Characterization

Samples of rhizospheric soil are aseptically taken from the root zones of healthy plants, especially in regions where heavy metal contamination is known to exist. Aliquots are plated on nutrient-rich media like Tryptic Soy Agar (TSA) or Luria-Bertani (LB) agar, and serial dilution techniques are used to lower the microbial load. Certain concentrations of heavy metals, such as Zn^{2+}, Cd^{2+}, or Cu^{2+}, can be added to media in order to select for strains that are resistant to metals. To enable the growth of various bacterial colonies, incubation is usually conducted for 24 to 72 hours at 28 to 30°C. The morphological characteristics of isolated colonies, such as their shape, color, and texture, are evaluated. Bacteria are classified as either Gram-positive or Gram-negative using Gram staining [46]. Catalase and oxidase activity, citrate utilization, and carbohydrate fermentation profiles are examples of biochemical tests that shed light on the isolates' metabolic capacities. By calculating the Minimum Inhibitory Concentrations (MICs), the tolerance of bacterial isolates to heavy metals is assessed. This entails growing bacteria in media with escalating heavy metal concentrations and tracking growth inhibition. According to one study, Klebsiella variicola isolated from industrial effluents demonstrated a notable tolerance to nickel and cobalt, with MICs ascertained using these assays [47].

6.1. Siderophore Production Screening

The Chrome Azurol S (CAS) assay, which determines the presence of siderophores based on color change, is used to evaluate siderophore production. In this assay, bacteria that produce siderophores cause the CAS medium's color to change from blue to orange. Spectrophotometric methods can be used to obtain quantitative measurements. For instance, siderophore production by Achromobacter sp. RZS2, which was isolated from the groundnut rhizosphere, was influenced by a number of physicochemical factors and metal ions [48].

6.2. Molecular Identification

16S rRNA gene sequencing is used to identify promising bacterial isolates at the molecular level. Using universal primers, the 16S rRNA gene is amplified by PCR, and the resulting sequences are compared to databases like NCBI's BLAST for taxonomic identification. To comprehend the evolutionary relationships between isolates, phylogenetic analyses can also be performed.These accurate techniques make it easier to identify rhizospheric bacteria that have the capacity to produce siderophores, tolerate heavy metals, and possibly be used in pharmaceutical applications, which advances bioremediation and sustainable agriculture [49].

7. Applications and Biotechnological Potential

7.1. Bioremediation of Heavy Metal-Contaminated Soils

Heavy metals have harmful effects on the soil texture and its fertility, the number and activity of soil microbial population, and physiological and morphological features of plants that lead to crop yield reduction [50]. Also, heavy metals can easily be uptake by the plant roots and subsequently arrive into the food chain of animals and humans. Metals can cause severe diseases in humans through damage to vital biological substances, such as lipids, proteins, and nucleic acids through the production of free radicals. Among the heavy metals, which can cause environmental pollutions, lead and cadmium have become an important global problem, because they have no essential role in biological reactions [51]. Unlike other environmental contaminants, heavy metals are persistent and accumulative pollutants because they cannot break down to non-toxic elements gradually. So, it is necessary to use suitable techniques for cleaning up heavy metals from contaminated environments to reduce their toxicity. Numerous chemo-physical methods which are used for metal remediation have various deficiencies such as high expense, loss of soil fertility, and generation of secondary pollutants. Bioremediation is a simple, low-cost, and ecofriendly alternative method to remove heavy metals from polluted areas [52]. Microbial remediation, using heavy metal resistant bacteria can be applied as an effective and reliable type of bioremediation methods. Heavy metal-resistant plant growth-promoting bacteria (PGPB) can be used for enhancing plant biomass production as well as removing of heavy metals from the polluted agricultural soils [53]. These bacteria stimulate plant growth by different mechanisms such as increasing availability of essential nutrients through phosphorus solubilization, nitrogen fixation, and siderophore production for iron uptake. Also, they regulate many plant developmental processes by secretion of phytohormones like indole-3-acetic acid (IAA), and protect plants against deleterious effects of phytopathogens by the synthesis of antibiotics, HCN, and degradative enzymes [54].

7.2. Enhancement of Plant Growth and Stress Tolerance

Bioabsorption is one of the most important biological mechanisms which involve the ability of microorganisms to accumulate heavy metals from contaminated site through metabolically mediated pathway [55].

Every year, severe global economic losses to agricultural crops are encountered due to plant diseases caused by more than sixty pathogens leading to the loss of 30% crop yield amounting 416 million US dollars. Since agricultural fields due to the uncontrolled use of chemical pesticides and fertilizers are most contaminated, search for PGPR having potential of adsorbing heavy metals from agriculture field will have triple advantage of bioremediation, plant growth promotion and disease management. Biocontrol through siderophore-mediated competition for iron have merged as a sustainable approach for integrated plant disease management [56]. Siderophores are also found to complex with heavy metals like cadmium, lead, nickel, arsenic (III, V), aluminium, magnesium zinc, copper, cobalt, and strontium other than iron. Under iron stress conditions, rhizobacteria produce siderophores that chelate the available iron and prevent the iron nutrition of respective phytopathogen [57] and there by restrict the proliferation and root colonization by phytopathogen. Siderophore producing rhizobacteria are also known to impart induced systemic resistance (ISRs) to the plants and suppressiveness to the soil and have been implicated in the biocontrol of several plant diseases [58]. Siderophore based biological control agents (BCAs) are gaining commercial significance as they are safer, do not lead to biomagnification, their self-replication circumvents repeated application and target organisms do not develop pesticide resistance. They also provide iron nutrition to the crops thereby promote the plant growth [59].

7.3. In pharmaceutical applications

The remarkable metabolic versatility of rhizospheric bacteria goes well beyond interactions between plants and microbes, leading to novel possibilities for pharmaceutical research. Siderophores, which are low molecular weight iron-chelating substances with a remarkably high affinity for ferric iron (Fe^{3+}), are among the most promising biomolecules generated by these bacteria. Siderophores have been thoroughly studied in medical applications due to their dual function as highly effective vectors for targeted drug delivery and as therapeutic agents in the treatment of iron overload disorders. By creating siderophore-antibiotic conjugates, they have strategically taken advantage of their capacity to interfere with pathogens' iron acquisition systems. One well-known example is cefiderocol, a siderophore-cephalosporin conjugate that enters Gram-negative bacterial cells by imitating their natural iron transport systems. Cefiderocol greatly improves therapeutic efficacy against multidrug-resistant infections by using the iron uptake pathways to deliver the antibiotic directly and potently into the bacterial periplasm while avoiding resistance mechanisms like efflux pumps and porin mutations (Ito et al., 2016; Sato et al., 2020). This novel strategy is an example of how rhizospheric bacteria's metabolic characteristics, which were first developed for the purpose of acquiring nutrients in soil, can be adapted for precision-targeted treatments in medical settings.

8. Challenges and future perspective

Although there is a lot of promise in isolating and characterizing rhizospheric bacteria for pharmaceutical, siderophore, and heavy metal tolerance applications, a number of obstacles prevent their widespread use.

One significant drawback is the complexity and unpredictability of soil environments, where plant species, soil composition, climate, and contamination levels all have a significant impact on microbial diversity. As a result, it is challenging to standardize results across various conditions. Furthermore, many rhizospheric bacteria cannot be cultured using standard methods, which limits access to their potential metabolic potential. Excessive levels of heavy metals can suppress microbial activity, even in cultivable strains, compromising their ability to promote plant growth and perform bioremediation. The possibility of horizontal gene transfer, specifically the transfer of genes for antibiotic and heavy metal resistance to pathogenic bacteria, is another critical be concerned that raises biosafety concerns. Commercial challenges include production, formulation stability, and regulatory compliance when scaling up bacterial formulations for field use or pharmaceutical development.

The use of rhizospheric bacteria in biotechnology seems to have a bright future despite these obstacles. The identification of functional genes involved in siderophore biosynthesis, metal resistance, and the production of bioactive compounds, even from unculturable microbes, has been transformed by the integration of omics technologies—genomics, transcriptomics, proteomics, and metabolomics. The development of designer microbes with improved functions suited for particular uses like drug delivery, pathogen suppression, or detoxification is made possible by developments in synthetic biology and genetic engineering. Additionally, studies are moving toward the use of microbial consortia, which provide more ecological adaptability and synergistic advantages over single-strain applications. By lowering dependence on fabricated agrochemicals, these bacteria can be important parts of integrated nutrient and pest management systems in agriculture, promoting sustainable practices. In addition, as antibiotic resistance increases, the pharmaceutical potential of rhizospheric bacteria—particularly siderophore-based drug delivery systems and novel antimicrobial agents—is accelerating. In conclusion, rhizospheric bacteria have the potential to revolutionize environmental, agricultural, and medical biotechnology with sustained interdisciplinary research, responsible innovation, and supportive policy frameworks.

9. Conclusion:

In conclusion, because of their remarkable metabolic capacities, rhizospheric bacteria have enormous potential in the domains of environmental biotechnology, sustainable agriculture, and pharmaceuticals. Because they have developed defenses against heavy metals, the ability to produce siderophores, and the ability to synthesize bioactive compounds, these microbes have flourished in the soil environment associated with roots. As a result, they are useful agents for drug development, plant growth promotion, and bioremediation. As demonstrated by siderophore-antibiotic conjugates like cefiderocol, siderophores in particular are being studied for medical uses such as iron chelation therapy and targeted drug delivery systems. Their wider use is hindered by a number of issues, such as the difficulty of growing a wide variety of bacterial strains, environmental variability, and worries about horizontal gene transfer and the spread of resistance. Furthermore, overcoming formulation, regulatory, and financial obstacles is necessary to scale up these microbial solutions for commercial use. The future looks bright despite these obstacles. Key functional genes involved in siderophore biosynthesis, metal detoxification, and the synthesis of pharmaceutical compounds can now be identified and engineered through developments in synthetic biology and omics technologies. Furthermore, rhizospheric bacteria's practical efficiency in field settings is being improved through the use of microbial consortia and bioformulations. Global issues such as food security, antimicrobial resistance, and environmental health can be effectively and sustainably addressed by rhizospheric bacteria with responsible biotechnological development and going on interdisciplinary research.

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