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Exploring Rhizobacteria Associated with Pogostemon cablin for Sustainable Plant Growth

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ABSTRACT

Pogostemon cablin (Blanco) Benth., also known as patchouli, is an important aromatic and medicinal plant mainly used for its essential oil and various bioactive components. Its cultivation should be carried out sustainably, taking into account the necessity of improving the growth of the plants, the availability of nutrients, and the resistance to environmental stresses. Rhizobacteria that are inhabitants of the rhizosphere of *P. cablin* may become a great biological resource for sustainable plant production. These useful microorganisms may help in plant growth via several ways. One of the important functions of rhizobacteria consists in biological nitrogen fixation and phosphate solubilization. These bacteria are known for producing siderophores, growth hormones, and enzymes. By promoting the growth of the plant, they also fight soil pathogens and increase the plant's resistance to abiotic stresses. The interactions of rhizobacteria with patchoulli are likely to affect not only plant growth and nutrient absorption but also root growth, biomass build-up, and secondary metabolite production. This review aims to observe the structures and functions of rhizobacteria and their plant-growth-promoting mechanisms.

Keywords:- *Pogostemon cablin,* PGPR,

2.1 Description of Patchouli (*Pogostemon cablin*) Plant

Pogostemon cablin (Blanco) Benth., commonly known as patchouli, is a perennial aromatic herb belonging to the Lamiaceae family. Cultivated predominantly across tropical and subtropical regions of the world—including India, Indonesia, China, Malaysia, and the Philippines—this undershrub is characterized by soft, pubescent (hairy) stems and broad, serrated leaves. Economically, *P. cablin* thrives in warm, humid microclimates and demands highly fertile, moisture-retentive soils to sustain its vegetative biomass.

In recent years, global pharmacological research has shifted toward plant-derived bioactive compounds due to their low toxicity and profound therapeutic applications. *P. cablin* stands out as a highly valuable ethnomedicinal species with a deeply entrenched history in traditional Indian and Chinese medicine systems. Historically utilized as *Pogostemon herba* in Traditional Chinese Medicine, it has been prescribed to eliminate internal dampness, manifest anti-pyretic properties against exterior syndromes, alleviate emesis (nausea), and stimulate appetite. Beyond therapeutic remedies, its leaves have historically formed the basis for natural cosmetics and personal hygiene formulations. he pharmacological efficacy of patchouli leaves is governed by a complex array of secondary metabolites, including an extensive profile of terpenoids (monoterpenoids, sesquiterpenoids, and triterpenoids), flavonoids, lignins, phytosterols, organic acids, glycosides, aldehydes, alcohols, and pyrones. Among these, patchouli essential oil (PEO), extracted from the foliar biomass, remains the most industrially prized constituent. Celebrated for its complex, rich, earthy, and spicy aromatic profile, PEO serves as an irreplaceable fixative in high-end perfumery, cosmetic formulations, and therapeutic aromatherapy products.

The complex interactions between microorganisms living in the soil surrounding a plant's roots and the plant itself influence how well that plant grows and produces its fruit/vegetation. The area of soil immediately surrounding a plant's roots, known as its rhizosphere, provides a very rich and active environment for the roots excreting substances (called root exudates) that provide nutrients or signal to many different types of microbes that will grow abundantly and develop relationships with the plant. These microbes provide invaluable services to their host plant through either changing the way they can get nutrients from the soil or stimulating vegetative growth, as well as improving the ability of a plant to withstand abiotic (environmental) and biotic (living organisms) stressors.

Plants native to PGPR in the patchouli rhizosphere can either directly or indirectly help their host plant become healthier. For direct methods of phytostimulation, the PGPR microorganisms solubilize (break down) the mineral phosphorus in the soil using biochemical means, produce ammonia chemically, and create phytohormones and other metabolites that

stimulate root growth, and help to increase root surface area for taking up nutrients. In this way, through studying the native PGPR in the root zone of *Pogostemon cablin*, we can find plants that will make the best use of PGPR to maximize their growth potential and oil yield.

Botanical description

Taxonomy

Kingdom- Plantae
Family- Lamiaceae
Genus- Pogostemon
Species- *Pogostemon cablin*

Patchouli *(Pogostemon cablin* (Blanco) Benth.) belongs to the Lamiaceae family, which is the second largest family of flowering plants with 12 sub-families, 240 genera and about 7200 recognized species worldwide. The Pogostemon genus consists of around 80 species; mainly in Southeast Asia, 20 of the 80 species occur in India.

General habitat and distribution

Patchouli (*Pogostemon cablin Benth.)* is a tropical herbaceous plant that has adapted and flourished in warm weather. Originally from the Philippines, it has become established in various Asian nations—including China and Indonesia—and other regions—including Brazil, Singapore, Seychelles and West Africa. There are specific environmental conditions for optimum growth: Temperature between 24°C to 28°C; relative humidity of about 75%; annual total precipitation between 2000mm to 3000mm; and elevation from sea level to 1200 meters.

Morphology

P. cablin belongs to the family of plants known as dicotyledons. Morphologically, it is a bushy, herbaceous perennial with characteristics that allow it to thrive in tropical/subtropical regions. It typically reaches between one to one-and-a-half meters tall, has an upright stem, and long, somewhat oval leaves approximately ten centimeters long by two centimeters wide.



Fig: 2.1 - *Pogostemon cablin* (Blanco) Benth. (Patchouli) growing in its natural tropical forest habitat, displaying its characteristic pubescent stems and serrated foliar architecture.

The edges of the leaves have very slight lobes along with lobes with serrated edges. The lobes of the leaf as well as the tip of the leaf are both blunt. The underside of the leaves has a lot of epidermal trichomes along the mid rib, and are used as storage sites for PEO. The roots of the plant are extensive and branched. In a mature plant, they can penetrate to a significant depth and grow without any other competing plants. The flowers produced by the plant are small and typically pale pinkish white. The leaves, flowers, and seeds all produce strong aromas when disturbed.

2.2 Importance and Applications of Patchouli

2.2.1 Medicinal importance

Traditional Medicinal uses- Pogostemon cablin is considered an important plant in traditional Asian medicine (like Traditional Chinese Medicine and Ayurveda). Ethnobotanical studies show that the shade-dried leaves of Pogostemon herba were used traditionally for treating digestive issues, removing excess moisture from the body, alleviating nausea, and increasing hunger. In native populations throughout tropical Asia, leaf poultices are made from the leaves of

Pogostemon cablin to treat wounds and localized skin infections.

Therapeutic properties- As a result of numerous pharmacological studies, researchers have found that P. cablin has antipyretic, analgesic, and anti-inflammatory effects. Phytochemicals found in Pogostemon cablin also prevent inflammation by regulating cellular pathways that cause inflammation to happen; moreover, these chemicals are used in clinical aromatherapy to help patients who suffer from stress and anxiety disorders.

Biological activites- A wide range of bioactive properties associated with the patchouli leaf have been shown through both in vitro and in vivo tests. The potency of the leaf extract to kill many different types of bacteria shows high levels of antibacterial activity, including bacteria that are resistant to more than one medication (i.e., Staphylococcus aureus or E. coli). In addition, the secondary metabolites within the patchouli leaf extract possess very high levels of scavenging activity and can thus neutralize reactive oxygen species (ROS) or assess the amount of cellular oxidative stress.

2.2.2 Economic Importance

Commercial significance- *Pogostemon cablin* (patchouli) is an industrial cash crop with global economic significance. Its sesquiterpenoids and non-volatile secondary metabolites have highly complex structures that cannot be produced economically through purely chemical routes. As a result, industrial consumers around the world continue to rely on exportable production from agro-plantations, making the raw patchouli biomass a high-value export commodity.

Cultivation and market value- The global market value for patchouli has continued to increase significantly, which is partly due to the growing trend towards clean label, plant-based, and organic products. Major exporter countries, including Indonesia, India, and China, generate substantial economic gains from production. The price of commodity products is determined by the chemical markers present in the plant leaf tissue, which are highly regulated for volume and quality

Contribution to agriculture significance- Patchouli farming contributes significantly to the socio-economic structure of rural agricultural economies in tropical and sub-tropical regions. Patchouli is a high-input, labor-intensive crop, which means that it generates many job opportunities for smallholder farmers throughout both the crop's cultivation, the post-harvesting drying process, and primary production; therefore, it also creates much of the rural agro-processing infrastructure

2.2.3 Industrial application

Pharmaceutical Applications: In the biomedical world, several studies have been conducted with non-volatile leaf extracts, isolated flavonoids, and glycosides to develop new antimicrobial compounds, and create dermatological treatments for chronic inflammatory diseases such as acne and eczema, as well as create environmentally friendly botanical larvicides that are less toxic to the environment compared to synthetic products against mosquito larvae, such as *Aedes aegypti*.

Cosmetics and the Fragrance Industry: Raw plant material and processed leaf powder are widely used in high-end personal care products. They are used to create organic soap bases, specialized cosmetics, and wellness products. patchouli's structure acts as a natural fixative that slows the evaporation of volatile top notes in these products and contributes to their long-lasting earthy base scent.

Food and Flavors: In contemporary food technological applications, trace amounts of the patchouli leaf derivatives are used as natural flavoring agents to create unique herbal and spicy flavors in baked goods, jellies, and beverages, while their antimicrobial compounds may also be used as bio-preservatives to reduce foodborne pathogens and preserve the quality of goods.

2.2.4 Agricultural significance

The Role of Patchouli as a Medicinal Crop: Patchouli (*Pogostemon cablin Benth*.) is a high-value aromatic and medicinal crop (MAP) that provides a unique option as a strategic alternative for diversifying agriculture. When compared with other conventional cereal staple crops, economic analyses demonstrate that patchouli is capable of producing significantly higher net returns (per hectare of land) and therefore, represent an optimal land use option for smallholder farmers.

The Importance of Patchouli in Sustainable Agriculture: The incorporation of patchouli into agro-ecosystems contributes to paradigms of sustainable agricultural practices. This crop naturally produces secondary metabolite compounds that provide plants with insecticidal and nematicide properties. As such, the incorporation of patchouli into crop rotation or intercropping systems creates a natural means of suppressing soil-borne insect pest populations, and thus, reducing an agricultural system's reliance upon synthetic chemical pesticides.

Patchouli's Relationship with Beneficial Microorganisms: The successful agriculture of *P. cablin* is biologically linked to the biological dynamics of its rhizosphere. The roots of *P. cablin* produce specialized and carbon-rich root exudates that shape the surrounding microbial structure of the rhizosphere and recruit and establish complex associative networks of beneficial soil microorganisms that have evolved to optimize nutrient cycling.

2.3 Plant Growth- Promoting Rhizobacteria (PGPR)

Microbial communities occupy various habitats and niches around the world, including the lithosphere (land) and hydrosphere (water), and provide an essential role in the geochemical cycles of nutrients. Bacteria found in the soil are predominantly located in the rhizosphere (the soil surrounding plant roots), with a greater abundance than found in the bulk soil (100 – 1,000-fold higher). This high abundance is due to their metabolic diversity and ability to utilize root exudates (products released from plant roots), making up approximately 5% – 30% of photosynthesized materials. The interaction between bacteria and plants facilitates the movement and absorption of mineral nutrients and illustrates the mutualistic relationship which exists between them. Bacteria that live in the rhizosphere and stimulate the growth of plants are called rhizobacteria. Most of these have been identified as belonging to the genera *Acinetobacter, Agrobacterium, Arthobacter, Azotoracterium, Azospirillum, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Serratia, Thiobacillus, Psuedomomas and Bacillus*. Over the last 10 years there has been increasing interest from scientists in studying the ecological function of PGPR bacteria within the biosphere; this has led to researchers gaining greater insight into how PGPR bacteria function in the rhizospheres of plants. To achieve the status of PGPR, a putative rhizobacterium must have a demonstrable positive effect on a plant as a result of inoculation, thereby demonstrating its ability to out compete the resident microorganisms within the same rhizosphere environment. Approximately 2-5% of all bacteria in the rhizosphere could qualify as PGPR. The use of PGPR in sustainable agricultural practices is expected to grow in the future. The most common method of PGPR bacteria being immobilized onto soil particles is through the process of ion exchange. Fertile, productive soils are created when soil organisms release the required quantities of inorganic nutrients from the organic reserve of the soil to support rapid plant growth.

Direct and Indirect mechanism of PGPR

To achieve better manipulation of the rhizosphere flora and therefore greater enhancement of plant productivity, it is essential that one has a good understanding of PGP (Plant Growth Promoters) processes. PGP processes can be divided into two categories; direct and indirect. The key difference between these two classifications of PGP processes is that indirect processes occur externally to the plant whilst direct processes occur internally within the plant and have a direct effect on the plant's metabolic processes.

Direct mechanisms include those processes that have a direct effect on plant metabolism by changing the balance of plant growth regulators as a result of; 1) micro-organisms who produce growth regulators that are ultimately assimilated into the plant or 2) micro-organisms that act as sinks for hormones secreted by the plant or 3) micro-organisms that induce metabolic processes of the plant which subsequently improve the plant's ability to adapt.

Indirect mechanisms are mediated through the plants defensive metabolic response to signals sent to the plant by the micro-organism that then influences the plant. Two main processes that are considered indirect PGPs, are; 1) induction of systemic resistance to pathogens (who are a biotic stress) and 2) the ability of plants to protect themselves from undesirable environmental conditions (that represent an abiotic stress)

2.3.1 Direct mechanism of PGPR

Biological nitrogen fixation

Bacteria that fix nitrogen can be divided into two types: bacteria that fix nitrogen in symbiotic association with the roots of legumes (e.g., Rhizobium) and free-living nitrogen-fixing bacteria (e.g., *Azospirillum, Azotobacter, Burkholderia, Herbaspirillum, Bacillus, Paenibacillus*). Although free-living nitrogen-fixers do not directly enter the host plant tissue, they will establish a loose symbiosis with the root of the host plant and then allow the nitrogen they have fixed to be used by the plant. Each of these bacteria has the potential to contribute between 20 and 30 kg of nitrogen/ha/year to a specific crop by fixing atmospheric nitrogen. *Azospirillum* and *Azotobacter* are two of the most commonly used free-living nitrogen-fixing bacteria for agricultural applications and have been documented since they were first reported in 1902. In addition to fixing nitrogen, these free-living nitrogen-fixing bacteria promote plant growth by producing phytohormones such as IAA, GA, and cytokinins. The use of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculants have resulted in substantial increases in yield in cereal crops.

Nitrogen-fixing capacity has proven to be among the most important features of beneficial microbial species that

contribute positively to plant growth. In addition to the aforementioned genera, *Azotobacter* and *Azospirillum*; the *Bacillus* and *Paenibacillus* genera are very important in demonstrating N2-fixing capabilities. This development can be attributed to the presence of the nif gene cluster that encodes for the enzyme nitrogenase. Several of the most important species that possess the capability to fix N2, such as *Bacillus azotofixans*, *Bacillus macerans*, and *Bacillus polymyxa*, have recently been reclassified to the genus *Paenibacillus*. Furthermore, according to the data, available to date, *P. azotofixans* is the most efficient N2-fixing species. The importance of the N2-fixing capabilities of these two genera of species is very significant for plant growth and has led to the commercialization of these species (as biofertilizer) and have supported agriculture for more than twenty years.

Phosphate solubilization

Phosphorus is a major limiting nutrient for plants after nitrogen. There are large amounts of phosphorus but they are unavailable for plant use in forms that can be absorbed. Only mono and dibasic phosphates may be absorbed. Organisms in the soil solubilize complex phosphates (such as tricalcium phosphate) by converting organic phosphorus into inorganic forms that can be taken up by plants. The main means by which this is done is by microorganisms secreting organic acids (material that has been metabolized from sugar) and these organic acids act as chelators of divalent cations and assist in the release of phosphates. There are many different organic acids produced by microorganisms (e.g., acetic acid, citric acid) and detection of organic acids during solubilization has been achieved using high-performance liquid chromatography (HPLC). The role of microorganisms in phosphate solubilization has been recognized since 1903.

Microorganisms that can solubilize phosphorus from various compounds account for between 20 and 40% of the population of culturable microorganisms present in soils, most of which have been isolated from the rhizosphere (root-soil) of plants. Compared to those found elsewhere, these microorganisms tend to be more metabolically active. In soils, phosphorus can precipitate when it interacts with metals such as calcium, iron or aluminum, thus rendering the phosphorus unavailable to plants. In acidic soils where the pH of the soil is less than 7, inorganic phosphorus is bound to aluminum and iron containing compounds. In alkaline or calcareous soils, the most common form of phosphorus is in the form of calcium phosphates. Approximately one-half of the total amount of soluble phosphorus in high organic content soils is as organic phosphorus (the other half being as inorganic). Approximately 80% of the total organic phosphorus present is as phytate, which is a very chemically stable form of organic phosphorus and because of this will generally not become available to plants. Although some microorganisms are known to produce enzymes known as phosphatases or phytases that will hydrolyze or solubilize phytate, phytate will frequently form insoluble complexes with aluminum, iron and calcium, thereby accumulating in soil over time. *Pseudomonas* and *Bacilli*, as well as rhizobia that are located inside plant roots and root nodules, are commonly known as being highly efficient in their ability to make phosphate bioavailable to plants. The principal strains that can be categorized as "bacterial phosphate solubilizers" include *Bacillus megaterium, Bacillus circulans*, *Bacillus coagulans*, *Bacillus subtilis*, *Pseudomonas polymyxa*, *Pseudomonas sircalmous*, and *Pseudomonas striata*.

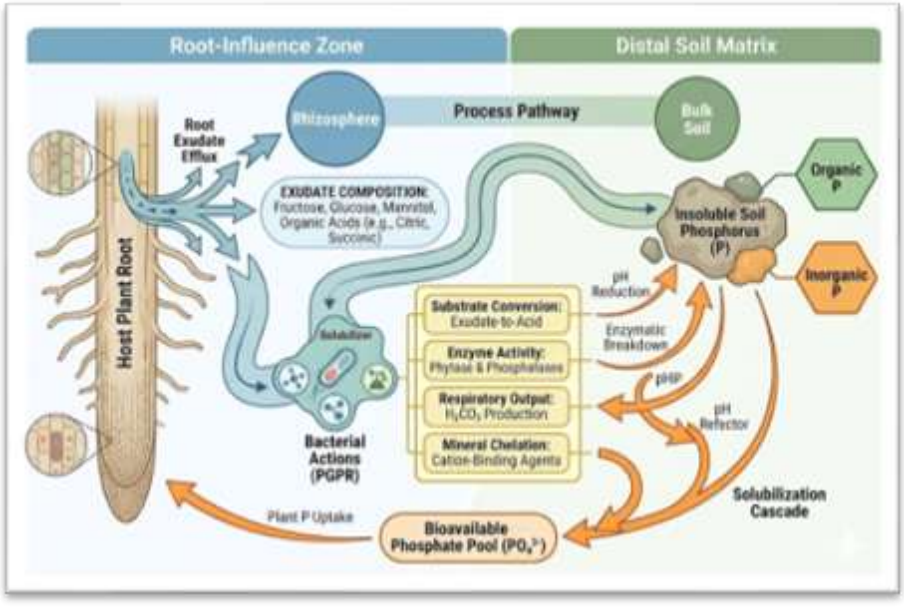


Fig-2.2: Schematic representation of solubilization of soil phosphorus by PGPR

Indole-3-acetic acid (IAA) – an auxin produced by several PGPR

Phytohormones (for example auxins such as indole-3-acetic acid (IAA)) have a very important role in plant processes, including things like cell elongation and division and differentiation of tissue and dominance apically. Extensive research done on how bacteria produce IAA and how that works with the mechanisms of plants. The amount of IAA that is produced by different strains of plant-growth promoting rhizobacteria (PGPR) is responsible for promoting the rapid response of plant growth through cell elongation as well as the long-term response through cell division and differentiation. Long-term treatment with IAA produces a well-developed root system that helps to enhance the uptake of nutrients to produce a greater healthy plant.

Approximately 80% of all rhizosphere bacteria produce IAA and provide plants with additional endogenous levels of IAA; this increased concentration in turn stimulates root development and creates a greater exchange of nutrients with the soil.

There are multiple pathways used by PGPRs to produce IAA. Most use L-tryptophan, which roots release into the soil, as a precursor but some, like *Azospirillum brasilense*, do not exclusively use this pathway when producing IAA. The details of the other pathways and enzymes used in the IAA alternative pathway (to L-tryptophan), will likely not be known until the functioning of the indole-3-pyruvic acid (IPyA) pathway utilized in IAA synthesis may potentially be characterized by the use of *Rhizobium* and *Bradyrhizobium* species as well as the indole-3-acetamide (IAM) pathway identified in IAA producing *Agrobacterium tumefaciens*. Other similar IAA producing microorganisms such as *Bacillus subtilis* produce IAA via the tryptamine pathway, demonstrating that there are multiple ways, through microbial interactions, to positively influence the growth and development of plants.

2.3.2 Indirect mechanism of PGPR

Siderophore production

Iron plays a critical role in the healthy growth of plants because it is needed as a co-factor by all the enzymes involved in respiration, photosynthesis, and nitrogen fixation. Although iron is a common element found throughout most soils, it is frequently unavailable to plants because of the predominance of oxidized forms (Fe+3), which combine with various elements to form an insoluble mineral compound. Plants have two primary methods of extracting iron from their environment: (1) they excrete organic compounds into their rhizosphere that chelate iron to increase the solubility of the iron-containing compound or (2) they take up the iron-containing compound through various complexation reactions formed between their chelating organic compounds and the Fe+3 iron-containing compound. Some rhizosphere bacteria secrete iron-chelating organic compounds to increase iron availability. For instance, siderophores, which are low-molecular-weight organic compounds produced by the two species of Pseudomonas bacteria, Pseudomonas fluorescens and Pseudomonas aeruginosa, possess functional groups such as hydroxamate and catechol that provide them with remarkable binding capacity for iron. In addition to enhancing the uptake of iron, the iron-chelating compounds secreted by these bacteria possess significant antibiotic properties that enable iron-chelating bacteria to possess greater competitive potential than those that are unable to produce these compounds, thereby supporting plant nutrition, limiting the ability of pathogens to grow, and restricting the availability of iron to pathogenic fungi. Therefore, siderophore-producing rhizobacteria foster greater plant health by improving nutrient uptake and suppressing pathogens.

Antibiotic production by PGPR

Microbial antagonists (e.g., *Bacillus* and *Pseudomonas spp*.) are being explored as possible natural substitutes for chemical pesticide use in agriculture because they promote plant development. By producing extracellular metabolites that inhibit fungi and bacteria (including plant diseases), these bacterial antagonists can reduce disease incidence caused by low concentrations of their extracellular metabolites. Many active metabolites of Bacillus species (e.g., *B. subtilis* and *B. amyloliquefaciens*) exist and include a variety of antibiotics, some of which have been characterized for over 50 years (e.g., *subtilin* and *bacilysin*). Two biosynthetic mechanisms (NRPS and PKS) are responsible for producing these antimicrobial metabolites. *B. amyloliquefaciens* contains at least nine distinct antibiotic biosynthetic gene clusters.

Pseudomonas spp. (*P. fluorescens* and *P. aeruginosa*) have also been extensively investigated for their antimicrobial abilities. The array of bioactive compounds produced by *P. fluorescens* and *P. aeruginosa* includes their well-known antibiotics (e.g., phenazines and 2,4-diacetylphloroglucinol). All of these bioactive compounds have a range of biological effects (antimicrobial and anti-cancer). Other than producing antibiotics, PGPR also play important roles in improving soil health and enhancing plant growth in multiple ways as shown in fig-3, such as improving soil pH through the addition of nutrients; promoting root colonization by altering the composition of root exudates; and aiding in the proliferation of beneficial microorganisms, such as arbuscular mycorrhizal fungi; and breaking down toxins produced

by plant pathogens. In summary, PGPR contribute to the promotion of plant health as well as to the protection of plants from soil-borne pathogens through many different mechanisms.

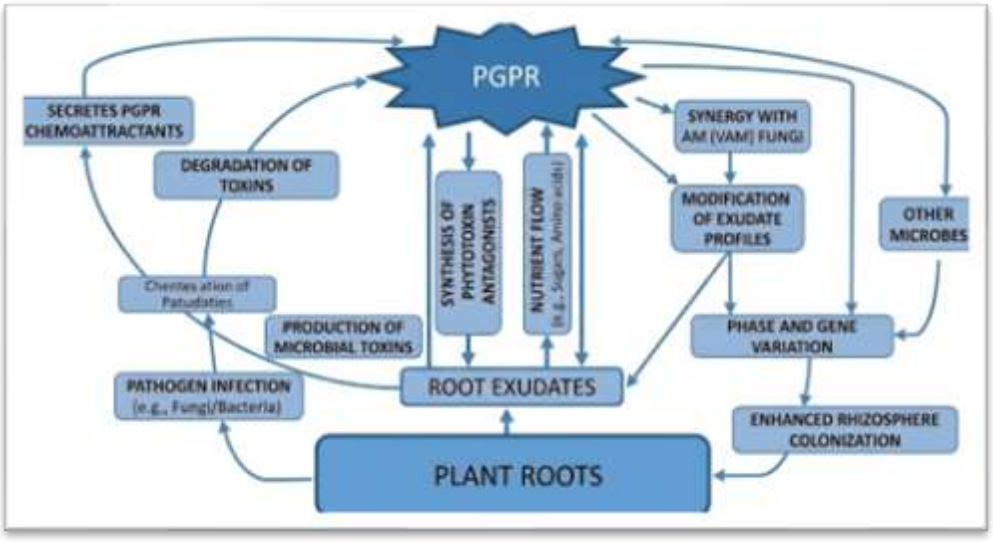


Fig-2.3: Interaction of plant root exudates, pathogen, PGPR and other beneficial microbes in the rhizosphere.

2.4 Antioxidant potential of Rhizobacteria

2.4.1 Free Radicals and Oxidative stress

Free radicals are highly reactive chemicals with at least one unpaired electron, meaning they can easily bond with other molecules, creating new ones. In plants, many forms of ROS(Reactive oxygen species) (including O_2-, H_2O_2, •OH, and $^1O_2$) are generated as a metabolic product during normal cellular processes such as photosynthesis, respiration, and photorespiration. However, exposure to extreme biotic and/or abiotic stress (e.g., increased salinity, drought, heavy metal toxicity, or disease) will disrupt the dynamic state that exists between the production of ROS and the plant's antioxidant defense mechanisms. This disrupted balance creates an increase in the cellular concentration of ROS, resulting in "oxidative stress."

The excessive buildup of ROS within cells can have a number of serious impacts on the cellular structure and its function including:

- Lipid Peroxidation: Attack of polyunsaturated fatty acids found in cell membranes results in malondialdehyde (MDA) formation and compromise of membrane fluidity and importance to the structural integrity of the cell.

- Protein Carbonylation: Induction of irreversible structural damage to proteins results in denaturation of the protein and loss of enzymatic function.

- DNA Degradation: Creation of single- and double-strand breaks in the DNA as well as base modifications and cross-linking could result in mutations or cell death.

In order to protect cells from the oxidative damage caused by excessive ROS in their metabolic processes, plants employ either internal or external regulatory systems to neutralize excess radicals and maintain homeostatic balance.

2.4.2 Microbial Antioxidant compounds

The rhizosphere has microorganisms associated with it. The major groups of these organisms include PGPR (plant growth-promoting rhizobacteria) and make a broad range of Non-enzymatic and Enzymatic secondary metabolites, both of which play a role in controlling the oxidative stress experienced by plants through microbial synthesis of antioxidants. Many of the antioxidants produced by microorganisms are used for scavenging, as donors of hydrogen atoms, or as chelators to safely neutralize free radicals before the free radicals can damage important biomolecules.

Non-Enzymatic Antioxidants

Many of the protective metabolites made by Rhizobacteria are low-molecular weight Non-Enzymatic Antioxidants, which include the following:

• Phenolic Compounds and Flavonoids: These act as excellent hydrogen donors to terminate radical chain reactions.

• Carotenoids: Carotenoids are composed of highly conjugated units of isoprene that can effectively quench $^1O_2$ (singlet oxygen) while dissipating excess excitation energy.
• Exopolysaccharides: Exopolysaccharides (EPS) consist of extracellular polymeric matrices that contain hydroxyl and carboxylic groups to stabilize free radicals and bind toxic pro-oxidant heavy metals.
• Volatile Organic Compounds(VOCs): Microbial VOCs (such as alcohols, ketones, terpenoids, etc.) induce increased synthesis of secondary metabolites, which can enhance the overall antioxidant capability of host plants.

Enzymatic defense mechanism

Rhizobacteria not only use chemical scavengers, but also use their own internal enzymes to neutralize potentially harmful reactive intermediates (before the raw chemicals leave the bacteria's cell or in its system):
Superoxide Dismutase (SOD): converts the very dangerous superoxide radical into H_2 (hydrogen peroxide) via dismutation of superoxide radicals and molecular oxygen.
Catalase (CAT): rapidly breaks down toxic hydrogen peroxide (H_2O_2) and converts it to harmless water (H_2O) and molecular oxygen (O_2).
Peroxidase (POD): utilizes organic substrate to accept hydrogen peroxide molecules and reduces the number of hydrogen bonds on that hydrogen peroxide plasma and puts them, back together again, to form molecules of (electrically neutral) peroxides.

DISCUSSION

Himachal Pradesh has many different types of agro-climatic zones with different types of plants and soil types that allow for the growth of rhizobacteria(H. Kumar et al., 2025). The assessment of rhizobacteria in relationship to organic farming and horticulture has caused an increase in research studies looking into isolating indigenous, stress-tolerant strains from the soils of Himachal Pradesh. The goal of these studies is to increase crop yields and reduce the impact of environmental stress(Meena et al., 2024).
Bacterial communities are highly diverse in the rhizosphere soils of Himachal Pradesh. Researchers routinely isolate indigenous strains from major genera, such as *Bacillus, Pseudomonas* and *Azotobacter*. The predominant, multi-functional PGPR in the temperate and subalpine soils of regions such as Shimla, Kullu and Solan, are *Bacillus* and *Pseudomonas*; these bacteria are studied for their high degree of cold-temperature function and biocontrol over a broad range of pathogens(Joshi et al., 2024). Free-living nitrogen-fixing Azotobacter strains also occur in abundance in productive agricultural valleys (i.e., Kangra and Mandi), and are considered to be excellent natural soil fertility enhancers.
According to recent scientific research, native strains found in Himachal Pradesh efficiently display a variety of traits that contribute to plant growth and adaptation to the mountainous terrain.
The organic acids produced by an array of isolates (i.e., *Bacillus* and *Pseudomonas*) are responsible for releasing insoluble phosphorus in mountain soils; these organic acids release inorganic phosphates which free insoluble or bound forms of tricalcium-phosphate into soluble forms available for plant uptake (bioavailability)(Wang et al., 2023).
In addition, free-living diazotrophs such as azotobacter found on crops from Himachal Pradesh fix atmospheric nitrogen (N_2) directly into ammonia (NH_3) through the nitrogenase process, using the pathogenic nitrogenase pathway that does not require oxygen to create an abundance of available nitrogen in between plants at higher elevations(Jehani et al., 2023). Moreover, the production of indole-3-acetic acid (IAA), which stimulates root branching and elongation, allows plants to keep soil stable on sloped surfaces; the production of high-affinity iron-chelating compounds (siderophores) limits iron to fungal pathogens in the close vicinity(*The Interaction between Plants and Rhizosphere Microbes - EPrints Soton*, n.d.).
An important component of apple-dominated horticulture in Himachal Pradesh is managing the rhizosphere around apple trees; it is especially important to consider rhizosphere management when managing apple replant disease (ard), a difficult-to-manage disease that occurs when young apple trees are planted in the same soil as older apple trees(Ma et al., 2025). Recent field studies have shown that while single-strain PGPR inoculations/fungicides (like *Bacillus subtilis)* will provide some benefit to controls, they will perform better in the field when used with natural arbuscular mycorrhizal fungi or with biocontrol agents considered specialized biofumigants (i.e., brassica seed meals).
Soil collected from the area surrounding plant roots is the basis for isolating the beneficial microorganisms rhizobacteria that naturally establish themselves on plant roots and contribute to the long-term productivity and health of a plant(Dlamini et al., 2022). This particular portion of soil surrounding plant roots is referred to as rhizosphere soil and the organisms inhabiting it are referred to as rhizobacteria. It is considered to be a biologically active area because it

harbours many types of microbes that have interactions with the plant. Microbes in the rhizosphere use the exudates produced from the root for their own nutrient source. When researchers isolate rhizobacteria from the rhizosphere they have the opportunity to recover pure cultures of bacteria from this diverse community and then study them in the laboratory for their morphological, biochemical, physiological, and plant growth-promoting qualities and characteristics in a controlled environment(Chauhan et al., 2023).

Rhizobacteria provide a number of mechanisms to enhance plant growth. Directly, they provide nitrogen to the plant through biological fixation, solubilize phosphorus, mobilize potassium, produce phytohormones (e.g., IAA, gibberellins, cytokinins) and ammonia, which improve nutrient availability for the plant and stimulate root growth and development(Hasan et al., 2024). Indirectly, rhizobacteria protect the plant from disease-causing organisms by inhibiting their growth through the production of antibiotics, siderophores, hydrogen cyanide (HCN), and lytic enzymes, as well as by inducing systemic resistance (ISR) to disease-causing organisms, all of which collectively contribute to sustainable agriculture and agricultural production. The importance of isolating rhizobacteria from the rhizosphere of *Pogostemon cablin* (patchouli), a valuable aromatic and medicinal crop whose yield and growth are highly influenced by soil microbial communities(A. Kumar et al., 2024). Indigenous rhizobacteria will have a better connection with the local environmental features of the site and, therefore, have a higher likelihood of establishing successfully on the plant's root system (compared with introduced bacteria). The application of these local native PGPR can enhance nutrient acquisition and alleviate the impact of various environmental conditions on the growth of plants while reducing reliance on chemical fertilizers in the sustainable production of patchouli.

In addition to having well established microbial communities of native PGPR, the process of isolating rhizobacteria creates an opportunity to screen the PGPR characteristics of these microorganisms (PGPR) (Guardiola-Márquez et al., 2023). Some critical PGPR characteristics include ammonia production, phosphate solubilization, siderophore production, catalase production, antioxidant activity, ACC deaminase activity, and enzyme production. Identifying the best PGPR can then be done using greenhouse/field trials for use as a biofertilizers or as a biocontrol agent. The screening of PGPR is essential as PGPRs can exhibit different levels of activity and identifying the best PGPR will increase the chances of producing a successful microbial inoculum(Nagrale et al., 2023).

The isolation of rhizobacteria is also a means to better understand the diversity of microorganisms found with plants at their roots. Through morphological, biochemical, and molecular characterisation of these microorganisms we can obtain valuable information about the taxonomy of these microorganisms, and their ecological and agricultural functions(Dabban et al., 2024). Such information can be used to develop microbial formulations that will increase the fertility of soil, enhance nutrient cycling, reduce pollution to the environment from too much fertiliser, and promote environmentally sustainable agriculture.

In this study, rhizobacteria isolated from the rhizosphere soil of *Pogostemon cablin* yielded distinct different bacterial isolates with different colony morphologies and biochemical characteristics. When evaluated for catalase activity, ammonia production, and antioxidant potential it was determined that some of the isolates had potential plant growth-promoting properties. This suggests that the isolated rhizobacteria have the potential to be used as biofertilisers to improve the growth, health, and productivity of patchouli and other economically important crops, as well as support environmentally sustainable agriculture.

CONCLUSION

Rhizobacteria associated with *Pogostemon cablin* have potential to be a good biological asset for improving plant growth and sustainable cultivation of patchouli. These beneficial microorganisms enhance plant growth due to lots of mechanisms like nutrient solubilization, nitrogen fixation, production of siderophores, generation of phytohormones and inhibiting soil borne pathogens. These microorganisms can improve plant growth through stimulating root development and nutrient uptake which may lead to increased biomass, and also synthesis of essential oils produced by *Pogostemon cablin*. Rhizobacteria also help plants cope with dry, salty, heavy metal contaminated or otherwise stressed conditions and less use of chemical fertilizer and pesticides. Even though quite promising prospect of using rhizobacteria in patchouli growing is due to its role, nothing is known about the diversity and functionality of *Pogostemon cablin* rhizobacteria.

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