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RESEARCH ARTICLE

BENEFICIAL MICROBIOMES AND THEIR PLANT GROWTH PROMOTING ATTRIBUTES.

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ABSTRACT

The plant microbiome (epiphytic, endophytic, and rhizospheric) is crucial for plant growth, development, and soil health. Plants and rhizospheric soil are rich natural resources that harbor microbial hotspots and play important roles in global nutrient balance and ecosystem function. The varied assemblage of microorganisms are important components of soil-plant systems, where they interact intensely in the rhizosphere/phyllosphere or as endophytes. Plant growth promoting (PGP) microorganisms have emerged as a valuable and promising tool for achieving long-term agricultural sustainability. Plant growth promoting (PGP) microorganisms are bacteria that promote plant growth, they have emerged as a vital and promising instrument for agriculture's long-term viability. PGP microbes promote plant growth and development directly or indirectly, either by releasing plant growth regulators/phytohormones, phosphorus, potassium, and zinc solubilization, biological nitrogen fixation, or by producing siderophore, ammonia, HCN, and other secondary metabolites that are antagonistic to pathogenic microbes. The PGP microbes belonged to different phylum of archaea (Euryarchaeota); bacteria (Acidobacteria, Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes and Proteobacteria) and fungi (Ascomycota and Basidiomycota), which include different genera namely *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Brevibacterium*, *Burkholderia*, *Collimonas*, *Curtobacterium*, *Diplococcus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Flexibacterium*, *Gluconoacetobacter*, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Herbaspirillum*, *Klebsiella*, *Methylobacterium*, *Microbiospora*, *Micrococcus*, *Micromonospora*, *Nocardiodides*, *PaeniBacillus*, *Pantoea*, *Penicillium*, *Piriformospora*, *Planomonospora*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, *Thermomonospora* and *Xanthomonas*. For sustainable agriculture, these PGP microorganisms might be utilized as biofertilizers/bioinoculants instead of chemical fertilizers.

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INTRODUCTION

Plant-microbe interactions are critical for plant development, growth, and soil health. Understanding the plant microbiome and its beneficial properties might have a number of implications for sustainable agriculture. Decoding microbial diversity linked with plants from various ecosystems has recently received a lot of attention. Microbial diversity is thought to be vital for the long-term viability of agricultural production systems. Microbe-plant interaction is regarded as a complicated mechanism in which distinct types of microorganisms effectively communicate without sharing the same cellular regions (Ajar *et al.*, 2017). Plant-microbe interactions may be classified into three categories: epiphytic, endophytic, and rhizospheric. The rhizosphere is the zone of soil where roots stimulate microbial activity by releasing substrates.

It is characterized by increased microbiological activity as the distance from the plant roots increases, and it is an ideal method for acquiring culturable beneficial bacteria. The capacity of rhizospheric bacteria to bind to root surfaces allows them to gain the most advantage from root exudates. The varieties of rhizospheric bacteria are known to be influenced by a variety of parameters including soil type, moisture, pH, and temperature, as well as plant age and circumstances. Microbial species belonging to different genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Aspergillus*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Haloarcula*, *Halobacterium*, *Halococcus*, *Haloferax*, *Methylobacterium*, *PaeniBacillus*, *Penicillium*, *Piriformospora*, *Pseudomonas*, *Rhizobium* and *Serratia* were revealed from rhizosphere of different crop plants (Barea *et al.*, 2005, Lavania *et al.*, 2006, Tilak *et al.*, 2005, Yang *et al.*, 2009, Kumar *et al.*, 2016, Verma *et al.*, 2016, Yadav *et al.*, 2017, Verma *et al.*, 2015).

The phyllosphere is a frequent habitat for microorganisms and plants to collaborate. Phyllosphere refers to the leaf surface, whereas phyllo sphere refers to the zone of leaves inhabited by microorganisms. This plant portion, particularly the leaves, is exposed to dust and air currents, leading to the creation of characteristic flora on their surface, which is helped by cuticles, waxes, and appendages, which aid in the attachment of microorganisms. The level of material impacts in leaf diffuseness or exudates determines whether phyllospheric bacteria survive or multiply on leaves. The main nutritional components (amino acids, glucose, fructose, and sucrose) are found in the leaf exudates, and such specialized habitats may create a niche for nitrogen fixation and secretions of chemicals that promote plant development. The phyllospheric microorganisms may play an important role in suppressing airborne infections that cause plant disease. Microbes found on leaf surfaces are extremophiles and can endure extremes of temperature (5-55°C) and UV light. Microbes such as *Achromobacter*, *Agrobacterium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Brevibacterium*, *Burkholderia*, *Diplococcus*, *Flexibacterium*, *Methylobacterium*, *Microbiospora*, *Micrococcus*, *Micromonospora*, *Nocardioidea*, *Pantoea*, *Penicillium*, *Planomonospora*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, *Thermomonospora* and *Xanthomonas* have been reported in the phyllosphere of different crop plants (Hornschuh *et al.*, 2002, Verma *et al.*, 2014, Holland *et al.*, 2000, Verma *et al.*, 2103, Tancos *et al.*, 2017, Dobrovolskaya *et al.*, 2017, Verma *et al.*, 2015).

Endophytic bacteria are microorganisms that colonize the inside of plant components such as the root, stem, or seeds without harming the host plant. The word endophyte comes from the Greek words endon (inside) and python, which means "in the plant" (plant). Endophytic microorganisms penetrate host plants mostly through wounds that arise naturally as a result of plant growth, as well as through root hairs and epidermal junctions. Endophytes can be passed down either vertically (from parent to child) or horizontally (from offspring to parent) (among individuals). Influences such as the physicochemical structure of the soil, plant development phase, and physiological condition, as well as a variety of external factors, can all affect the endophytic microbiome (Lian *et al.*, 2008, Mitter *et al.*, 2017). Most plant species have endophytic microorganisms that range from symbiotic to harmful. Some endophytic microbial species *Achromobacter*, *Azoarcus*, *Burkholderia*, *Collimonas*, *Curtobacterium*, *Enterobacter*, *Flavobacterium*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella*, *Microbiospora*, *Micromonospora*, *Nocardioidea*, *Pantoea*, *Planomonospora*, *Pseudomonas*, *Serratia*, *Streptomyces* and *Thermomonospora* have been identified from different host plants (Verma *et al.*, 2014, Verma *et al.*, 2015, Suman *et al.*, 2016, Hallmann *et al.*, 1997, Quadt-Hallmann *et al.*, 1997, Ryan *et al.*, 2008).

Biodiversity of beneficial microbiomes: Plant associated microbes mainly includemembers from archaea (Euryarchaeota), bacteria (Acidobacteria, Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes, and Proteobacteria), and fungi (Acidobacteria, Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes, and Proteobacteria (Ascomycota and Basidiomycota). According to a literature analysis, the distribution of microorganisms varied across all bacterial phyla, although Proteobacteria were the most prevalent and widespread, followed by Actinobacteria.

The phylum Deinococcus-Thermus and Acid bacteria had the fewest microorganisms, followed by Bacteroidetes (Yadav, 2015, Saxena *et al.*, 2016, Shukla *et al.*, 2016, Yadav *et al.*, 2015, Suman *et al.*, 2015). Acidimicrobiia, Actinobacteria, Coriobacteriia, Nitriliruptoria, Rubrobacteria, and Thermolephilia are the five groups of gram-positive bacteria of the Actinobacteria phylum. Actinobacteria are the most common and have been shown to interact with plants growing in a variety of settings as well as severe climates. It also contains *Streptomyces*, one of the biggest bacterial genera (Singh *et al.*, 2016, Yadav *et al.*, 2017, Sahay *et al.*, 2017). The rhizospheric Actinobacteria are the most common in nature, and their contributions to soil systems are critical to agriculture and forest management. *Bacillus* and *Bacillus* related genera are members of the phylum Firmicutes, which are the most culturable and colonize a variety of plants, including wheat, rice, maize, soybean, and chickpea (Hung and Annapurna, 2004, Hungria *et al.*, 2015, Kämpfer *et al.*, 2016, Liu *et al.*, 2015, Sun *et al.*, 2008). Among different phylum the Proteobacteria one of the predominant phylum including many dominant genera including *Brevundimonas terrae*, *Bosea sp.* and *Methylobacterium sp.* from α -proteobacteria; *Burkholderia sp.*, *Burkholderia cepacia*, *Variovorax ginsengisoli*, *Janthinobacterium lividum* and *Janthinobacterium sp.* from β -proteobacteria and *Aeromonas*, *Pantoea*, *Providencia*, *Pseudomonas*, *Psychrobacter* and *Yersinia* from γ proteobacteria class (Verma *et al.*, 2016, Yadav *et al.*, 2017, Verma *et al.*, 2015, Suman *et al.*, 2016, Verma *et al.*, 2016).

Beneficial microbiomes: Plant-associated microbes have been shown to benefit plant growth by fixing atmospheric nitrogen, solubilizing minerals like phosphorus, potassium, and zinc, and producing siderophores and plant growth hormones like cytokinins, auxins, and gibberellins, or indirectly by producing antagonistic substances and inducing resistance against plant pathogens (Tilak *et al.*, 2005, Verma *et al.*, 2016, Compant *et al.*, 2005, Glick, 1995, Kour *et al.*, 2017). Biological nitrogen fixation (BNF) is one of the biological alternatives to nitrogen fertilizers that might lead to more productive and environmentally friendly agriculture. Many associative microorganisms have been discovered that fix atmospheric nitrogen and provide it to their linked host plants. A variety of nitrogen fixing microbes like *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gluconoacetobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, and *Serratia* have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants (Niste *et al.*, 2013, Yadav *et al.*, 2016, Olivares *et al.*, 2013). Plant-associated bacteria generate auxins and gibberellins, which are plant growth hormones. Gibberellins are more commonly produced by root-associated bacteria, while auxins are produced by all plant-associated microorganisms. Auxins can speed up (by boosting cell elongation) or slow down the development of roots and stems (through cell division and differentiation). The synthesis of such growth regulators by microorganisms benefits the host plant in a variety of ways, including facilitating root system development, which increases water and nutrient absorption and plant survival. Plant-associated microorganisms have a wide range of abilities to produce these phytohormones (Yadav *et al.*, 2015, Chaiarn and Lumyong, 2011, Xie *et al.*, 1996). A wide range of microbial organisms can generate the auxin phytohormone indole acetic acid (IAA). As part of their colonization strategy, bacteria employ this phytohormone to interact with plants, including phyto-stimulation and

circumvention of basal plant defensive systems. It has also been recommended that IAA has been used to stimulate plant growth or reduce weed development. Phosphorus (P) is an important macronutrient for biological development and growth. Microbes provide a biological rescue mechanism, allowing insoluble inorganic P in soil to be dissolved and made accessible to plants. The capacity of certain microorganisms to convert insoluble P to an accessible form, such as orthophosphate, is a critical characteristic for improving plant yields in PGP microbes. In agriculture, rhizospheric P-utilizing microorganisms might be a valuable source of plant growth promoters. P-solubilization is a characteristic shared by microorganisms associated with several crops. Members of the Burkholderia, Enterobacter, Halolamina, Pantoea, Pseudomonas, Citrobacter, and Azotobacter families have been identified as PGP microorganisms with P solubilizing properties (Kumar *et al.*, 2017, Yadav *et al.*, 2015, Illmer and Schinner, 1992, Kaur *et al.*, 2017, Wakelin *et al.*, 2004, Pradhan and Sukla, 2006, Yadav *et al.*, 2016). Organic acids such as gluconate, ketogluconate, acetate, lactate, oxalate, tartarate, succinate, citrate, and glycolate are produced by bacteria to solubilize P. Depending on the carbon source used as a substrate, the kind of organic acid generated for P solubilization may vary.

Ethylene is a plant hormone that is produced in response to stress and can hinder plant development. By cleaving the plant-produced ethylene precursor 1-aminocyclopropane-1-carboxylate, certain bacteria can reduce the amount of ethylene in the plant (ACC). By supporting plant development in the presence of ethylene, such bacteria can alleviate the effects of numerous stresses. After such spurts, ACC-deaminase producing bacteria may play a role in controlling ethylene levels, ensuring that ethylene levels remain below the point where development is hampered. In general, ethylene is a necessary metabolite for plant growth and development (Suman *et al.*, 2016, Penrose and Glick, 2003, Glick *et al.*, 2007, Siddikee *et al.*, 2010). Ethylene has been identified as a stress hormone in addition to being a plant growth regulator. The endogenous level of ethylene is greatly raised under stress circumstances such as those caused by salt, drought, water logging, heavy metals, and pathogenicity, which has a detrimental impact on total plant development. Plant growth and development are aided by PGP microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces ethylene levels, induces salt tolerance, and reduces dehydration stress in plants. *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, and *Rhizobium* have all been found to have ACC deaminase activity (Siddikee *et al.*, 2010, Khalid *et al.*, 2006, Srivastava *et al.*, 2014, Sun *et al.*, 2009, Xu *et al.*, 2014).

The indirect process of plant development occurs when bacteria produce inhibitory chemicals or increase the host's inherent resistance to diseases, reducing or preventing the harmful effects of infections on plants (Gulati *et al.*, 2009, Selvakumar *et al.*, 2008, Mishra *et al.*, 2011, Selvakumar *et al.*, 2011, Mishra *et al.*, 2009, Mishra *et al.*, 2008, Vyas *et al.*, 2010). Production of siderophore, chitinases, antibiotics, fluorescent pigment, and cyanide generation can all be used to suppress phytopathogenic microorganisms (Lottmann *et al.*, 2000, Yadav *et al.*, 2016). Biocontrol systems are environmentally benign, cost-effective, and aid in the

improvement of soil consistency and the preservation of native soil flora (Jha *et al.*, 2013, Uppal *et al.*, 2008, Verma *et al.*, 2017). To be effective, the Biocontrol agent must be able to function in a wide range of circumstances, including pH, temperature, and ion concentrations. Pathogens, nematodes, and insects are all slowed by biocontrol chemicals. Pathogens can be limited directly by biocontrol microbes producing antagonistic substances, competition for iron, detoxification or degradation of virulence factors, or indirectly by inducing systemic resistance (ISR) in plants against certain diseases, signal interference, competition for nutrients and niches, and interference with pathogen activity, survival, germination, and speculation. Iron is an important nutrient for practically all species and is required as a cofactor in many enzyme activities. To obtain enough iron, bacteria produce a siderophore that can bind Fe³⁺ with a high affinity, thus solubilizing the metal for effective absorption. Bacterial siderophores are low-molecular-weight molecules with strong Fe³⁺ chelating affinities that help to solubilize and transport this element into bacterial cells. In the presence of iron, siderophore-producing bacteria may bind and transport the iron-siderophore complex through the production of particular proteins. Microorganisms that produce siderophores are advantageous to plants because they can limit the growth of plant diseases.

Application of Individual Microbes for Improvement of Crop Performance: Various helpful microbes have been commercialized (Berg, 2009), but their efficiency in terms of helping agricultural plants in fields has not always been constant. Nonetheless, it has been demonstrated that combining beneficial microorganisms for application to agricultural plants improves plant performance more effectively. Under restricted water circumstances, the alkaloid content of two medicinal plants, *Withania somnifera* and *Hyocyanus niger*, was enhanced by using PGPBs such as *Azospirillum*, *Azotobacter*, *Pseudomonas*, and *Bacillus* species (Rajasekar and Elango, 2011, Ghorbanpour *et al.*, 2013). In terms of endosymbiotic PGPBs, Chandrakala *et al.* screened and identified one *Rhizobium* species from the rice rhizosphere that could solubilize silicate and boost rice plant growth potential. *Bradyrhizobium japonicum* has been demonstrated to aid soybean resistance to high As levels in the soil, and *Sinorhizobium fredii* has been shown to successfully nodulate a variety of legumes, hence encouraging host plant development (Lopez-Baena *et al.*, 2016). Vemulapally *et al.* screened and identified *Frankia spp.* from *Casuarina spp.* nodules, one of which (*F. casuarinae* Cc13 strain) could form nodules in the roots of *C. equisetifolia* seedlings and enhance plant development following inoculation. Certain bacterial species can synthesize Jasmonic Acid and Abscissic Acid, according to Forchetti *et al.* (Forchetti *et al.*, 2007). However, it is uncertain if the amounts of these hormones in plants may be directly altered by the related microorganisms.

Plant microbiomes and microbiomes from various extreme ecosystems contain the most molecular and chemical diversity in the world. The relevance of microbes in sustainable agriculture (microbes may be utilized as biofertilizers/bioinoculants) and industry has sparked research into microbial diversity (microbes may be used for the production of different compounds of pharmaceutical importance). Beneficial microorganisms may have a significant impact on nutrient cycle and environmental detoxification.

Table 1. Bacterial strain that forms biofilm and their contribution to agriculture

Phylum	Class	Bacterial strain	Host plant	Isolated from	Function	Reference
Proteobacteria	AlphaProteobacteria	<i>Azospirillum brasilense</i>	Wheat	rhizosphere	Nitrogen fixation	Souza <i>et al.</i> , (2014)
Proteobacteria	AlphaProteobacteria	<i>Azospirillum brasilense</i>	Sorghum	rhizosphere of sorghum	enhanced NO and indole-3-acetic-acid	Koul <i>et al.</i> , (2015)
Firmicutes	Bacilli	<i>Bacillus amyloliquefaciens</i>	Tomato		Biocontrol	Nihorimbere <i>et al.</i> , 2012
Firmicutes	Bacilli	<i>Bacillus cereus</i>	Arabidopsis	rhizoplane	Biocontrol	Beauregard <i>et al.</i> , (2013)
Firmicutes	Bacilli	<i>Bacillus cereus</i>	Arabidopsis	commercial strain	Biocontrol	Bais <i>et al.</i> , (2006)
Firmicutes	Bacilli	<i>Bacillus cereus</i>	Arabidopsis	commercial strain	Biocontrol	Bais <i>et al.</i> , (2006)
Firmicutes	Bacilli	<i>Bacillus amyloliquefaciens</i>	Chickpea	alkaline soil	enhanced plant growth	Kumar <i>et al.</i> , (2016)
Firmicutes	Bacilli	<i>Bacillus cereus</i>	Arabidopsis	Rhizosphere	Biocontrol	Beauregard <i>et al.</i> , (2013)
Proteobacteria	Alpha proteobacteria	<i>Ensifer meliloti</i>	Alfalfa	metal polluted soil	remediation of metal contaminated soils	Raklami <i>et al.</i> , (2019)
Actinobacteria	Actinobacteria	<i>Microbacterium sp.</i>	Sugarcane	sugarcane stem	Nitrogen fixation	Lin <i>et al.</i> , (2012)
Proteobacteria	Gammaproteobacteria	<i>Pseudomonas sp.</i>	Rice	sewage sludge	bioremediation of phenanthrene	Zhou and Gao, (2019)
Proteobacteria	Gammaproteobacteria	<i>Pseudomonas entomophila</i>	Wheat	rhizoplane of wheat	tolerance to abiotic stress	Ansari and Ahmad, (2018)
Proteobacteria	Gamma proteobacteria	<i>Pseudomonas putida</i>	Chickpea	roots of chickpea	synergistically enhances plant growth	Kumar <i>et al.</i> , (2016)
Proteobacteria	Gamma proteobacteria	<i>Pseudomonas putida</i>	Plant source unknown	rhizosphere of chickpea	plant growth promoting activities	Srivastava <i>et al.</i> , (2008)
Firmicutes	Bacilli	<i>Paenibacillus polymyxa</i>	Peanut	peanut rhizosphere	biocontrol against crown rot disease	Haggag and Timmusk (2008)
Proteobacteria	Gamma proteobacteria	<i>Pseudomonas aeruginosa</i>	Arabidopsis and sweet basil	and human pathogenic strains	antimicrobial activity	Walker <i>et al.</i> , (2004)

Microbiomes abound in all kinds of habitats, including those with extremes of pH, temperature, salinity, and water stress, and they're said to be beneficial for agriculture because they can be used as PGP agents (biofertilizers) for sustainable agriculture and biofortification of micronutrients like Fe and Zn.

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