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3. Plant Growth-Promoting Bacterial Endophytes

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Abstract:

Bacterial endophytes ubiquitously colonize the internal tissues of plants, being found in nearly every plant worldwide. Some endophytes are able to promote the growth of plants. For those strains the mechanisms of plant growth-promotion known to be employed by bacterial endophytes are similar to the mechanisms used by rhizosphere bacteria, e.g., the acquisition of resources needed for plant growth and modulation of plant growth and development. Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms; those that establish close associations with plants, such as the endophytes, could be more successful in plant growth promotion. Several important bacterial characteristics, such as biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and production of siderophores and phytohormones, can be assessed as plant growth promotion (PGP) traits. Endophytic bacteria survive in close association with their host plants. These bacteria become an integral part of the host tissue system.

Almost every plant species is found to be in association with these bacteria. These are the promising agents that promote their host growth even in stressed environments, like in phytoremediation. They flourish their host's growth either directly or indirectly. In this paper we will discuss Plant Growth-Promoting Bacterial Endophytes.

Keywords:

Plant Growth, Promoting, Bacterial Endophytes, Disease, Abiotic Stresses, Biological Nitrogen Fixation, Host Plants, Colonization, Associated-Microbial, Nutrient Depletion.

3.1 Introduction:

"Microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects" are one definition of endophytes.

The lack of symptoms associated with endophytic colonization in plant tissue makes it more difficult to emphasize their mutualistic and symbiotic relationship with plants; nonetheless, recent observations of variety have shown that they may also be harmful.

Living in plant tissues has been found to provide more opportunities for communication than living in the exospheric regions. More advantages and the capacity to communicate with the host in a variety of environmental settings are provided by bacterial endophytes. Through direct or indirect means, they promote plant growth.

One way to directly promote anything is to either increase the intake of vital nutrients like iron, phosphorus, and nitrogen, or adjust hormone levels to produce gibberellins, cytokines, or auxin. [1]

Furthermore, by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves the molecule ACC, the direct precursor of ethylene in all higher plants, some endophytes can reduce the amounts of the phytohormone ethylene. The synthesis of compounds that are hostile to bacterial or fungal pathogens inhibits indirect promotion.

Rhizobacteria and bacterial endophytes have been observed to interact with medicinal plants, such as Zingiberaceae. Rhizobacteria colonize the rhizosphere region, while bacterial endophytes live inside plant tissue without harming host plants.

Through both direct and indirect means, plant growth-promoting bacteria (PGPB), such as rhizobacteria and endophytic bacteria, promote the growth and development of plants. PGPB directly modifies plant hormone levels, which either enhances plant growth or makes it easier for plants to absorb nutrients from the environment. The indirect method has to do with using biocontrol to prevent plant diseases.

Plant tissues are home to plant growth-promoting endophytes (PGPE), and the tight connections between endophytes inside plant tissues promote the exchange of nutrients and the activity of enzymes. Plant growth is positively impacted by the growth-promoting hormones that endophytic bacteria make and distribute to plant tissues.

The ability of endophytes to release insoluble phosphate and supply nitrogen to their host plants is essential. Microbial endophytes compete with other microbial pathogens for the same ecological niches because they invade plant tissues without exhibiting symptoms.

As a result, the known plant-endophyte connection enhances plant health through various strategies that endophytes exhibit and may help shield the plant host from microbial disease.

Plant growth-promoting (PGP) chemicals are a class of bioactive molecules produced by PGPE that have many biological functions. [2]

3.2 Plant-Growth-Promoting Endophytes (PGPES):

Plant-growth-promoting rhizobacteria (PGPRs) and PGPEs work together to enhance the antioxidant system, improve nutrition uptake, control plant hormones, and produce siderophores. Based on their ecological interactions, bacterial endophytes linked to plants can be divided into three categories: helpful, harmful, and neutral.

Additionally present as PGPEs are several genera of generally regarded harmful bacteria, such as Pseudomonas, Enterobacter, Bacillus, Klebsiella, and Burkholderia, which support plant growth and development in both normal and stressful environments.

These are usually indirect processes, like antibiosis, induction of systemic resistance (ISR), and competitive exclusion, which work together to limit the harmful effects of other phytopathogenic microbes.

PGPEs stimulate plant development through a variety of pathways, including nitrogen fixing; generation of siderophores, phosphate solubilization, auxin synthesis, and 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, the majority of which have extensive documentation.

Endophytic bacteria belonging to the genera Pantoea, Bacillus, Burkholderia, Klebsiella, and others were recovered from native or hybrid genotypes of maize. [3]

3.3 Review of Literature:

A functional definition of endophytic behavior has been proposed by some authors, who classify any bacterium as an endophyte if it is able to be isolated from plant tissue that has been infected on the surface or retrieved from inside the plant and does not cause visible damage to the plant.

Regarding the community, it is unclear if it is advantageous for bacterial endophytes to live inside plant tissues as opposed to rhizosphere bacteria, which are bacteria that live outside of plant roots.

It is evident that bacterial endophytes can benefit the host plant in a number of ways, most notably by promoting growth and protecting it from pathogens. Additionally, bacterial endophytes are more effective at communicating and interacting with the plant than rhizosphere bacteria in a variety of environmental settings (Ali et al., 2012). [4]

Understanding plant-associated bacteria's ecological role and interactions with plants is crucial for understanding their potential to benefit plants, as well as for biotechnological applications.

According to recent research, the release of secondary metabolites by the related bacteria may be responsible for the encouragement of plant growth (Hamayun et al. 2010).

Therefore, to sustain the production of medicinal plants for their novel natural products, research on plant-microbe interaction and evaluation of the role of indigenous pre-dominant culturable endophytic and epiphytic bacteria, associated with ethnomedicinal plants, for plant growth promotion and antagonistic activity are needed. [5]

The selection of capable rhizobacteria with PGP properties is necessary for the use of beneficial bacteria as agricultural inputs to increase crop productivity. In this nutrient-rich, protected environment of the root interior, nature chooses endophytes that are competitively suited to occupy suitable niches without putting the host plant under pathological stress. It is preferable to screen these bacteria for the most promising isolates with appropriate colonization and PGP characteristics rather than screening them for additional PGP agents.

The majority of studies have shown that, after incubation, bacterial flora is randomly removed from plates or morphological representatives are chosen for additional examination.

Nevertheless, certain superior bacteria with high colonization capacity and PGP properties may be eliminated by this kind of selection.

As a result, it's critical to conduct a thorough analysis of every isolated bacterium and identify the strains with the highest colonization rates and PGP properties. However, screening every strain for every PGP characteristic will be an expensive and time-consuming procedure.

We would just need to determine the PGP characteristics of the isolates that are able to establish themselves in rice roots, as the ability to colonize roots is a prerequisite for a rhizobacteria to be deemed a real PGPR (Silva et al., 2003).

In order to aid in the selection of the isolates with colonization and plant growth promoting rhizobacteria (PGPR) potential, we were therefore interested in locating a significant auxiliary tool. [6]

There is usually no one strategy that bacteria can use to promote plant growth because the processes that these organisms can use to impact plant growth vary among species and strains.

Research has been done on the potential of certain bacteria, including endophytic bacteria, to encourage the growth of plants.

According to Gauero et al. (2013), endophytes are bacteria or fungi that inhabit interior plant tissues, can be separated from the plant following surface cleaning, and have no detrimental effects on plant growth. [7]

3.4 Objectives:

- The function of endophytes in promoting plant growth.
- Endophytes increase plant growth by fixing nitrogen, generating phytohormones, absorbing nutrients, and providing resistance against biotic and abiotic stressors.
- The most well-researched methods of bacterial plant growth promotion involve giving plants nutrients and resources that they don't already have, like fixed nitrogen, iron, and phosphorus.

3.5 Research Methodology:

This study's overall design was exploratory. The research paper is an endeavor that is founded on secondary data that was obtained from reliable online resources, newspapers, textbooks, journals, and publications. The research design of the study is mostly descriptive in nature.

3.6 Result and Discussion:

Numerous stressful environments are present for plants all the time, and they need a variety of response mechanisms to cope. One such mechanism is the interaction between soil bacteria and roots, which facilitates nutrition availability, growth stimulation, and disease suppression.

Microbiota, which are classified as (1) endosphere (inter or intracellular tissues), (2) rhizoplane microbial (on the root surface and perhaps attached to root hairs), and rhizosphere microbial (soil adjacent to the root surface), can be found associated with plants to varying degrees and locations.

The host genotype, soil type, production methods, and other factors affect each of these communities' composition (Figure 3.1).

Recently, the possibility of using endospheric bacteria as acclimatization agents for abiotic stress response has been raised.

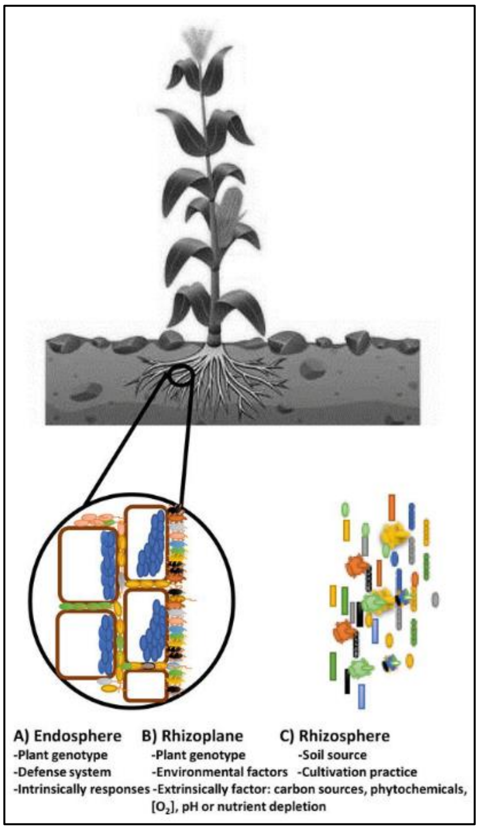

Figure 3.1: Localization of maize associated-microbial communities. (A) Endosphere; here, communities are affected by plant genotype, its defenses system, and intrinsic responses; (B) in the rhizoplane, extrinsic factors like carbon sources or phytochemicals, [O2], pH, or nutrient depletion, affect microbial community composition; (C) in the rhizosphere, communities are strongly affected by soil source, cultivation practice, and others. [8]

Plant Associated Bacteria (PAB) can be divided into two categories based on the area in which they have colonized: associated bacteria, which include rhizospheric (near the root) and rhizoplanic (on the root surface) bacteria, as well as endophytic bacteria. The term "endophytic bacteria" describes bacteria that proliferate inside plant parts such as roots, stems, or seeds without endangering the host plant. Increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, hydraulic activity, roots and shoot length, yield, and resistance to abiotic stresses like drought, flood, salinity, etc. are all possible outcomes of these bacteria's promotion of plant growth.

Biological nitrogen fixation (BNF), phytohormone synthesis, phosphate solubilization, suppression of ethylene biosynthesis in response to biotic or abiotic stress (induced systemic tolerance), and other processes are some of the direct and indirect ways that PAB might boost plant growth.

Indirect methods include creating resistance to pathogens. The current review intends to concentrate on the molecular aspects of rhizospheric and endophytic bacteria's capacity to promote plant growth. Based on the fundamental processes by which it promotes plant growth, PAB has been categorized as a plant growth promoting bacterium (PGPB), which directly induces plant growth, and a bio-controller, which protects plants by preventing the growth of pathogens and/or insects (Figure 2). The PGPB discussion in this review has left out rhizobia related to leguminous plants.

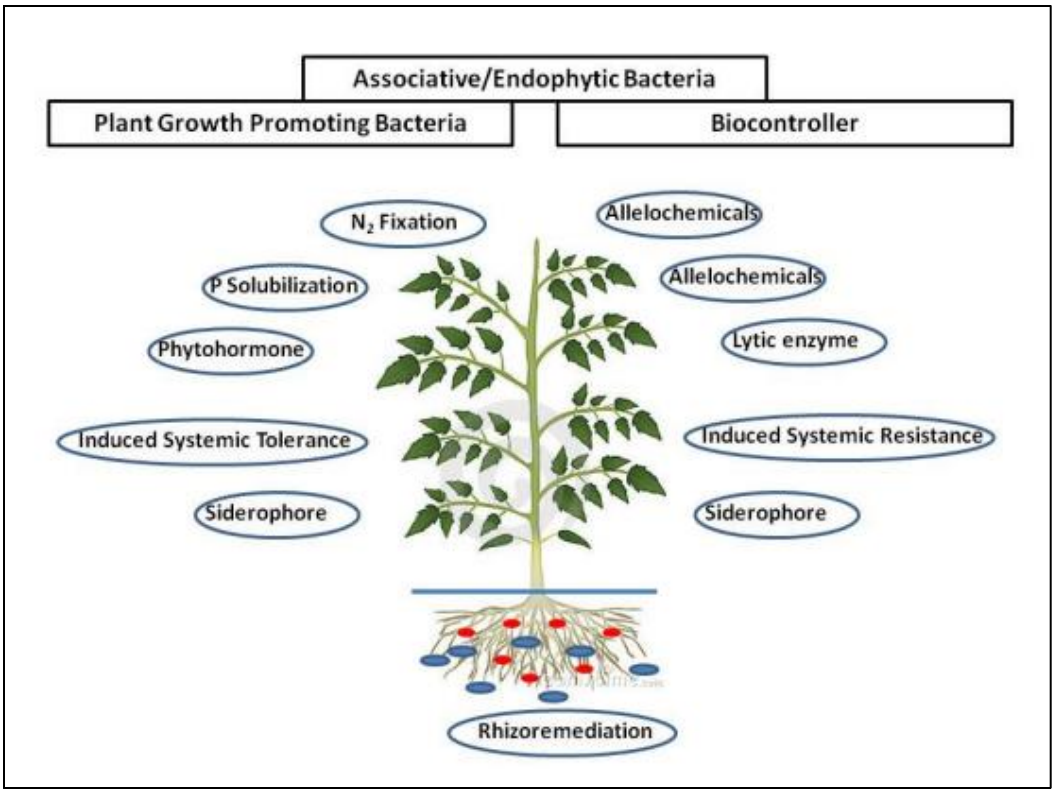

Figure 3.2: Properties of associative/endophytic bacteria for plant growth improvement. [9]

Associative/endophytic bacteria have been categorized as biocontrol and Plant Growth Promoting Bacteria (PGPB) based on their characteristics.

By supplying nutrients (nitrogen, phosphorus, and iron), producing plant hormones, and maybe enabling plants to withstand abiotic stresses, PGPB may be beneficial to related plants.

Through hostility and/or generated systemic resistance, biocontrol bacteria (right panel in figure) shield plants from the invasion of pathogenic microbes.

3.6.1 Plant Growth Promotion Mechanisms:

Figure 3.3 provides an overview of the various ways in which bacteria aid in plant growth. It has been demonstrated that both endophytic and rhizospheric bacteria support plant growth and work through comparable processes.

Compared to rhizospheric colonizers, endophytes have the benefit of not being affected by shifting soil conditions once they have become established within the host plant's tissues.

Variations in temperature, soil pH and water content, and the presence of soil bacteria that may compete for binding sites on host plant root surfaces are some of these shifting variables that may prevent rhizospheric bacteria from growing and functioning.

The microorganisms' stimulation of growth can result from nitrogen fixation, phytohormone production, biocontrol of phytopathogens in the root zone (by producing siderophores, antifungal or antibacterial agents, nutrient competition, and the induction of systemic acquired host resistance or immunity), or by improving the availability of minerals.

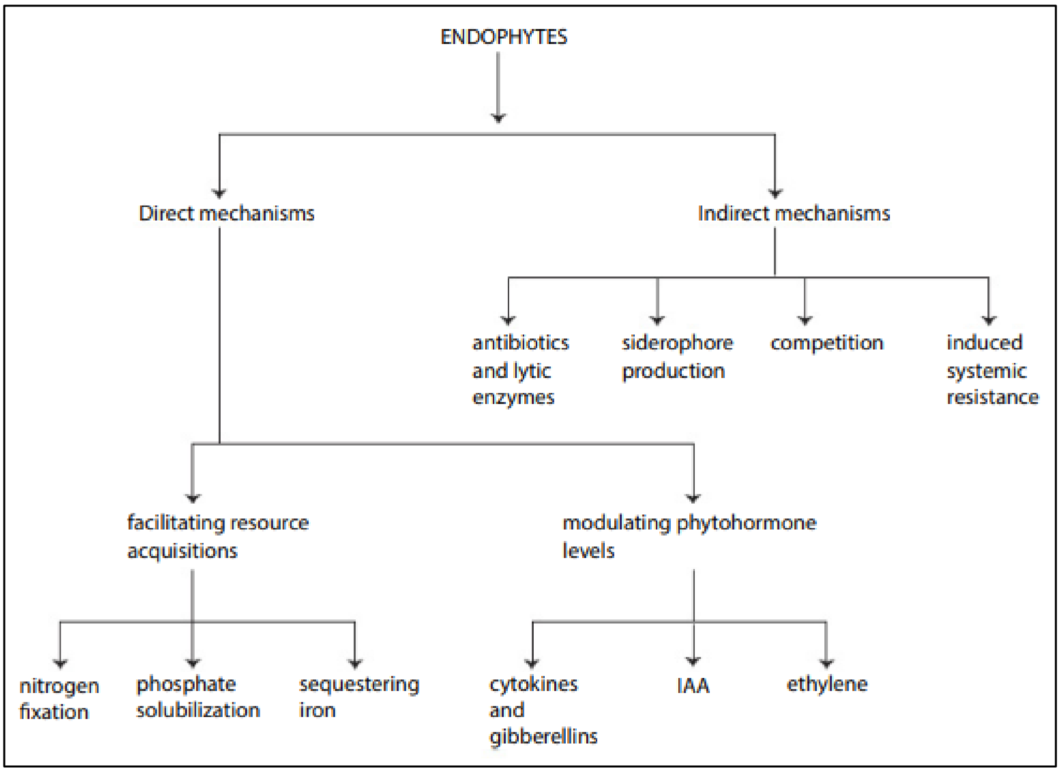

Figure 3.3: Outline of various mechanisms adapted by endophytes to promote plant growth.

Characteristics that encourage plant development IAA was produced by 55 different bacterial strains, with varying quantities ranging from 0.6 to 79.243 mg L-1. Through the production of enzymes that mineralize organic phosphorus, phosphate solubilizing bacteria allow plants to absorb accessible phosphate from insoluble phosphate.

Out of the 57 bacterial isolates evaluated in this study, 29 of them were able to solubilize phosphate, with a phosphate solubilizing index ranging from 1.06 to 5.59. 98% of the bacterial isolates showed evidence of catalase activity. For every bacterial isolate, there were differences in the bubble formation. ROS detoxification by bacteria is aided by catalase. [10] 51 different bacterial isolates were capable of producing ammonia. Nessler's reagent addition resulted in a deep yellow to brownish tint, which suggested more ammonia accumulation. Bacteria create ammonia, which serves as a source of nitrogen for plant growth.

Our findings showed that nitrogen could be fixed by both endophytic and rhizobacteria. This activity was observed in 32 bacterial isolates in all. Nitrogen-fixing bacteria raised the pH of the semisolid NFb medium, causing the medium's hue to shift from green to blue and the bacteria to form pellicles on it. 27 bacterial isolates may generate exopolysaccharides. The bacteria produced a mucoid substance on the medium and precipitated after being mixed with absolute ethanol. A total of 21 bacterial isolates were able to produce ACC deaminase.

By splitting the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), into α-ketobutyrate and ammonia, the enzyme ACC deaminase was able to lower the ethylene level. In this investigation, no bacterial isolate exhibiting properties related to HCN synthesis was found. HCN was created by bacterial antagonists as a kind of biological control. A total of 16, 35, 29, and 11 bacterial isolates were able to produce the enzymes cellulase, pectinase, protease, and chitinase in this investigation. Both bacterial endophytes and rhizobacteria have the ability to manufacture all of the hydrolytic enzymes. Microorganisms can enter plant tissue thanks to the activities of the enzymes cellulase and pectinase. Enzymes like protease, cellulase, and chitinase were used in biological control to prevent plant diseases.

Table 3.1 Plant growth-promoting traits of selected bacteria

Isolate	Characteristics[a]											
	N_2-Fix	Cellu	IAA (mg/L)	PSI	Catal	Prot	NH_3	EPS	Pec	ACC	HCN	Chit
4RH22	+	+	10.8	1.1	-	-	++	-	-	+	-	-
4EA10.1	+	+	9.2	1.2	+	+	+++	+	+	+	-	-
TAT2	-	-	10.2	5.6	+	-	+	-	-	-	-	-
TA2	+	-	3.8	1.3	+	-	++	-	+	-	-	-
YAT19	+	-	5.5	1.4	+	+	+	+	+	-	-	+
YAT9	-	-	9.0	1.1	+	+	+	-	+	-	-	-
TAT19	+	-	7.6	1.2	+	+	+	+	-	-	-	+
YAT12	-	-	6.5	1.2	+	+	+	+	+	-	-	-
TAT17	+	+	10.0	1.3	+	+	+	+	+	-	-	-
4RH8	+	+	71.1	-	+	-	+	+	+	+	-	-

[a]N_2-Fix= nitrogen fixation; Cellu= cellulase production; IAA= indol acetic acid production; PSI= phosphate solubilizing index= ratio clear zone and colony diameter; Catal= catalase production; Prot= protease production; NH_3= ammonia production; EPS= exopolysaccharide production; Pec= pectinase production; ACC= ACC deaminase production; HCN= HCN production; Chit= chitinase production.

High potential microorganisms were chosen as biostimulants based on their PGP properties. Ten bacterial isolates—eight rhizobacteria (RH22, TAT2, YAT19, YAT9, TAT19, YAT12, TAT17, and 4RH8) and two endophytes (EA10.1 and TA2)—were chosen for the plant growth assay in the current investigation.

The PGP properties exhibited by the chosen bacteria ranged from four to ten. With the exception of HCN synthesis, we chose bacterial isolates that reflected every PGP characteristic.

According to a prior study, bacteria have a variety of ways of acting that can both stimulate plant growth and reduce illness.

A. Effect of Selected Bacteria and Application Method on Rice Seedling Growth:

The outcomes demonstrated that specific bacterial isolates could improve the growth of rice seedlings. Three, five, six, and three bacterial isolates in total considerably boosted the rice seedlings' root length, leaf count, shoot count, and fresh weight at the root. When compared to the control plant, the bacterial isolates had no discernible impact on the height of the rice seedling shoots (Figure 3.4). [11]

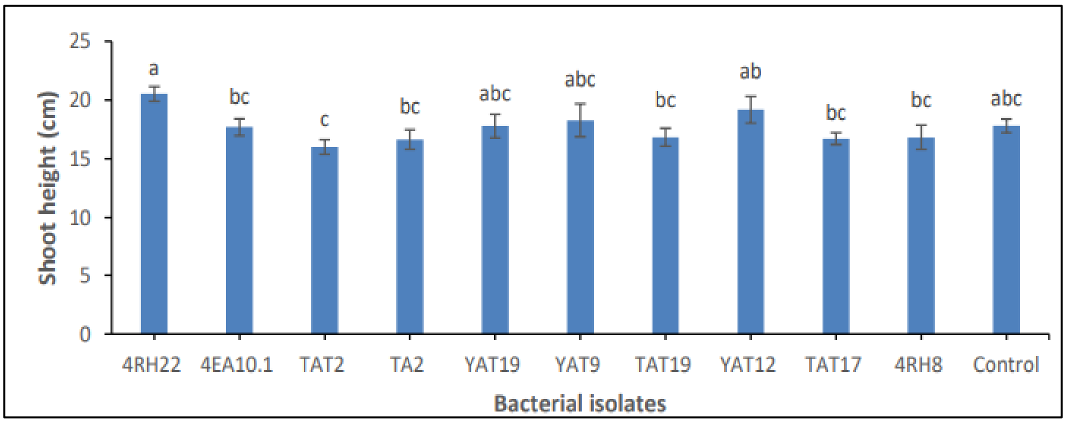

Figure 3.4: Effect of bacterial treatments on shoot height of rice seedling. Bars with same letter are not significantly different according to DMRT (p<0.05).

B. Plant Growth Promotion:

To take use of bacteria for plant development and yield enhancement, inoculations with a target microorganism at a concentration significantly greater than that found in the soil are required.

The types and varieties of plants, the growth environment, and the bacterial strains all affect the benefits of applying bacteria.

Because there are more limiting nutrients available, plants perform better overall and are better able to protect themselves when diseased.

The application of endophytic bacteria, such as Azospirillum, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Rhizobium, and Serratia, which are chosen for different plant-growth-promoting activities, has been shown to increase yields (Table 3.2).

Oilseed rape and tomato were bacterized with endophytes to significantly improve seed germination and growth.

This also helped to lessen disease symptoms produced by Fusarium oxysporum f. sp. lycopersici (Sacc.) and Verticillium dahliae Kleb.

Likewise, the inoculation of dry beans with bacterial endophytes resulted in a 34% increase in shoot biomass and a 25% increase in overall biomass.

The isolated bacteria exhibited nitrogen xation, IAA generation, and antagonistic behavior towards the potato diseases Clavibacter michiganensis ssp. epedonicus, Fusarium sambucinum, and Pectobacterium atrosepticum.

It has recently been shown that the endophytic bacterium Pseudomonas sp. from the medicinal plant Tinospora stimulates pea plant growth.

Table 3.2: The bacterium showed phosphate solubilization, and production of IAA, siderophores, HCN, and ammonia. [12]

Bacterial Endophyte	Plant	Attribute
Pseudomonas spp. and *Serratia* spp.	*Brassica napus* and *Lycopersicon lycopersicum* L.	Antagonism against *Verticillium dahliae* Kleb and *Fusarium oxysporum* f. sp. *lycopersici* and plant growth promotion
Aureobacterium saperdae, *Bacillus pumilus*, *Burkholderia solanacearum* *Phyllobacterium rubiacearum*, *Pseudomonas putida*, and *P. putida*	Cotton	Antagonism against *Fusarium oxysporum* f. sp. *vasinfectum*
Herbaspirillum	Rice	Nitrogen fixation
Pantoea agglomerans	Rice	IAA, P-solubilization, nitrogen fixation, plant growth promotion
Herbaspirillum seropedicae	Rice	Nitrogen fixation
Acinetobacter, *Enterobacter*, *Pantoea*, *Pseudomonas*, and *Ralstonia*	Soybean	IAA, P-solubilization, nitrogen fixation
Methylobacterium extorquens, and *Pseudomonas synxantha*	Soybean	Adenine derivatives
Burkholderia sp.	*Vitis vinifera* L. cv. Chardonnay	Plant growth promotion
Bacillus, *Burkholderia*, *Erwinia*, and *Pseudomonas*	*Paphiopedilum*	IAA
Achromobacter xiloxidans, *Alcaligenes* sp. and *Bacillus pumilus*	Sunflower	Jasmonic acid, ABA
Azospirillum spp.	Maize	IAA, nitrogen fixation
Burkholderia	Sugarcane	pyrrolnitrin
Bacteria	*Solanum nigrum*	ACC deaminase, IAA, phosphate solubilization
Acinetobacter, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Pantoea*, and *Serratia*	Soybean	IAA, phosphate solubilization, nitrogen fixation
Serratia	Banana	Biocontrol against *Fusarium oxysporum*

3.7 Conclusion:

It appears that endophytes are crucial for safeguarding plants from biotic and abiotic stresses and for fostering plant growth. In several underdeveloped nations, these microorganisms are being successfully employed. This can be applied to lessen the quantity of chemicals used in agriculture, which is now one of the main sources of pollution. Important topics for additional research include the ability of some bacteria to modify plant metabolism and the promotion of bacterial colonization induced by particular carbonaceous exudates produced by plant roots. These topics may provide light on potentially mutualistic plant–endophyte connections.

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