

Plant Growth Promoting and Endophytes Bacteria: Mechanisms, Applications, and Future Perspectives for Sustainable Agriculture

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Abstract:

Background: Ensuring long-term food safety and security is a serious global issue, mainly in developed nations where the constancy of food supply is under threat from an uncertain agriculture climate change. Rapid population growth and decreasing soil fertility is unnecessary dependence on chemical fertilizers are the major global concerns in sustainable agriculture. The different environmental stresses such as waterlogging, drought, saline, ultraviolet (UV) radiation, pH (acidic/sodic soils) and temperature variations, which have a thoughtful impact on plant growth and quality. This review aims to highlights the role of Plant Growth Promoting Bacteria (PGPB) in sustainable agriculture, with a specific focus on nitrogen fixation and other plant growth promoting mechanisms that recover crop productivity and stress tolerance.

Key Findings: PGPB have emerged as promising biological agents that can improve plant growth, nutrient uptake and stress resistance against biotic and abiotic stresses as well as reduce the use of synthetic agrochemicals. PGPB has one of the most significant contributions in facilitating Biological Nitrogen Fixation (BNF). The nitrogen-fixing bacteria are composed of free-living, associative and endophytic species, which convert atmospheric N into plant available forms, thus improving the nitrogen use efficiency and reducing the needs of chemical N fertilizers. Moreover, PGPB improve the growth of plants by producing phytohormones, solubilizing phosphates, secreting siderophores, mobilizing nutrients and inhibiting phytopathogens. These positive interactions increase the productivity of the crops, soil fertility, ecosystem stability, and buffer against pollution in the environment.

Perspectives for the future: There is a need for additional research to identify top performing microbial strains, establish stable bioformulations, test the technologies on a large scale in the field, and combine PGPB technologies with precision agriculture and climate smart farming techniques. The efficacy and commercialization of PGPB-based products are anticipated to increase with the development of microbial biotechnology and formulation approaches

Conclusion: the combine application of plant growth promoting bacteria is based on biofertilizers and biopesticides offers a sustainable and environmentally responsible approach to enhancing agricultural productivity, maintaining soil health and ensuring long term food security. Adapting them can play a crucial role in role in reducing chemical inputs while supporting resilient and sustainable farming systems.

Keywords: Plant Growth-Promoting Bacteria (PGPB), Nitrogen fixing bacteria, Sustainable Agriculture, Biofertilizers, Biopesticides, Endophytic bacteria.

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I. Introduction

1.1 Importance of Nitrogen in Plant Growth and Health Hazards of Agrochemicals

The present-day challenges for agriculture and global food security are serious; climate change and rapid global population growth. Global warming, odd or irregular rainfall, drought, salinity and other abiotic stress factors have resulted in an ever-growing demand for agricultural production to meet the nutritional needs of humans. These constraints are negatively affecting the crop productivity and threatening sustainable agriculture [1,2].

In order to increase the food demand, the agriculture sector is mainly depending on chemical fertilisers and pesticides for good crop production and protection from pests and diseases. In addition to the other nutrients, the macronutrients (N, P, and K) exert significant influence in the plant growth, metabolism and productivity [3].

Nitrogen is important for the plants as it is a major component of chlorophyll, amino acids, proteins, nucleic acids and several metabolic compounds directly associated with crop growth and yield. Thus, the farmers need excessive amounts of nitrogenous fertilizers to obtain higher productivity and largely depend upon the chemical fertilizers contributing more agricultural production. But their continuous and excessive use has created serious environmental and health problems [4]. Chemical fertilizers and pesticides are the main warning alarm for all the research community for enhancing the yield and agricultural productivity ultimately declining of agriculture economy [5]. More pesticides nearly 76% were used in India and 44% globally chemical fertilizers and pesticides create the environmental pollution reduced soil fertility and negative impacts on human health [6,7].

1.2 Endophytes as Eco-Friendly Alternatives with Advantages over PGPB

Several plant pathogens also affected the plant productivity and estimated 20% to 30% crop loses globally, and some crops suffering up to 50% yield loss in regions heavily affected by diseases. The continuous loss of food crops significantly increases the food insecurity in many parts of the world mainly in developing countries where agriculture plays a central role in the economy [8]. A large number of edible crops like maize, wheat and legumes are affected by insecticides, pesticides and different pathogens worldwide and need to find out the serious alternative to overcome these limitations.

The association of endophytes may serve as potential eco-friendly alternatives for improving nutrient uptake, stress tolerance and overall plant productivity. The ability of establishment of stable endophytes within plant tissues makes them promising approach for sustainable crop improvement under changing climatic conditions. These endophytes live inside plant tissues and form a more stable, intimate association allowing them to directly regulate plant metabolism, improve nutrient uptake and increase stress tolerance. Endophytes generally provide more reliable and sustained yield enhancement compared to PGPB because of their internal colonization and consistent interaction with the host [9,10,11,12].

II. Endophytic Microorganisms and Their Biological Importance

2.1 Ecological Adaptation of Endophytic Bacteria

Plants are surrounded by and live with a very diverse community of microbes, both external and internal. Endophytes are one of these microorganisms that is a unique ecological group as it colonizes the inner tissues of plants without producing visible pathogenic symptoms. The definition of endophytes has developed a lot in the last decades, from a pathogen-centered view of plant microbiology towards a larger view of plant beneficial–microbe interactions. Endophytes can be broadly described as any microorganism that colonise plant tissues without causing apparent disease symptoms in the host plant [13,14].

The study of endophytic microorganisms, especially bacteria, has gained great interest due to the various functional roles in plant growth, nutrient utilization, stress response, and ecological adaptation. Intimate, sometimes mutualistic associations are formed with their host plants by these microorganisms which colonize different plant tissues, such as roots, stems, leaves, flowers, vascular tissues and seeds [15]. The endophytes occupy a protected niche in the plant tissue, which protects them from the stressful conditions of the external environment like UV radiation, temperature fluctuation, nutrient deficiencies, competition from other microorganisms, etc [16].

Endophytic bacteria have more than just a tissue colonization function, it is biological. They play an active role in plant growth and development through various mechanisms such as production of phytohormones, nutrient availability improvement, biological nitrogen fixation, phosphate solubilization, production of siderophores and inhibition of phytopathogens. Moreover, a large number of endophytes elicit systemic resistance and regulate plant defense mechanisms, enhancing plant health and resilience to unfavourable environmental conditions [17].

2.2 Endophytes in Sustainable Agriculture

The increasing environmental issues raised by overuse of chemical fertilizers and pesticides, scientists have been interested in microbial alternatives as a sustainable option in agriculture. Traditional farming methods that are highly dependent on synthetic agrochemicals lead to soil degradation, contamination of ground water, eutrophication, biodiversity loss and greenhouse gas emissions [7]. As a result, microorganisms associated with the plants, such as endophytic bacteria, have become potential biological agents for reducing the use of chemical inputs in maintaining the productivity and environmental considerations of the crop [18].

In recent years, advances in the study of the plant microbiome have reinforced the notion that plants and their associated microbial communities constitute a functional and ecological unit, the “plant holobiont”. In this context, microbial communities have vital roles in the nutrition, metabolism, immunity, growth regulation and adaptation of the plant to changes in the environment. Consequently, microorganisms are now known to play critical roles in plant survival, in the functioning of ecosystems and in agricultural sustainability [19,20].

2.3 Plant Microbe Interaction are Influence by the Environment

Environmental conditions play a crucial role in plant–microbe interaction. Plants tend to be selective in recruiting and stress-tolerant microorganisms under conditions of drought, salinity, nutrient deficiency, and temperature. This adaptive association is a co-evolutionary association where both plants and microorganisms benefit from each other's interactions that enhance their survival and fitness in tough environments [21].

Endophytic bacteria have been shown to be a valuable source for sustainable agriculture due to their metabolic versatility, ecological adaptability and multiple plant growth promoting properties. They are promising options to develop next generation biofertilizers, biostimulants and biocontrol agents as they can improve nutrient-use efficiency, boost plant productivity, reduce reliance on synthetic agro-chemicals and increase the tolerance of plants to various stress factors [22].

III. Diversity and Distribution of Endophytic Bacteria

3.1 Community Structure of Endophytic Bacteria

The endophytic bacteria are a very heterogeneous group of microorganisms that reside in plants without expressions of plant disease symptoms. They differ a great deal between plant species, tissue type, development and environment. The bacteria associated with host plants inside of their tissues are referred to as endophytic bacteria, and most of them are included within four bacterial taxa: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. *Bacillus*, *Pseudomonas*, *Azospirillum*, *Enterobacter*, *Rhizobium*, *Serratia*, *Burkholderia*, *Micrococcus* and *Streptomyces* are endophytic genera commonly reported [23,24]. They have different metabolic pathways and are equally important for plant growth promotion, nutrient cycling, stress tolerance, and disease suppression.

The endophytic bacteria are not evenly distributed in plants and this is host and environmental dependent. Endophytes tend to be most abundant and diverse in plant roots, which are the points of contact between the soil and the plant. Many studies have shown that endophytic bacteria can enter and spread in the vascular tissues, allowing them to be distributed in stems, leaves, flowers, fruits and seeds [17]. This systemic colonization allows for continued endophyte-host plant interaction and helps to maintain plant health and productivity [25].

The endophytic bacterial community composition and structure is dependent on plant genotype. Root exudates and secondary metabolites, such as sugars, amino acids and organic acids, flavonoids and phenolic compounds are different types and amounts released by various plant species and cultivars. Such compounds can serve as chemotropic signals which promote and attract certain microorganisms. Genotype dependent recruitment patterns imply a close evolutionary relationship between plants and the associated microorganisms and thus highly specialized plant–microbe interactions [23].

Endophytic bacteria are influenced by environment conditions and they are diversified in their distribution. Soil pH, temperature, moisture, nutrient availability, salinity, and climatic conditions as well as field practices of cultivation have significant influences on endophytic bacterial communities. Environmental stress response is often associated with the acquisition of microorganisms that have heightened stress tolerance features, including drought, salinity, nutrient shortages and extreme temperatures. These beneficial organisms bring about enhanced plant adaptation and survival to stressful situations [26].

3.2 Advances in the Study of Endophytic Diversity

Endophytes bacteria have recently been shown to be ecological value in enhancing plant fitness and helping in acquisitions of nutrients, especially during environmental stress [27]. However, much of the microbial diversity has not been cultivated under laboratory conditions due to an inability of artificial media to duplicate the nutritional complexity of plant tissues, Culture-independent methods like metagenomics and next-generation sequencing have since shown a far greater diversity of microorganisms than can be achieved by standard culture methods [28].

The endophytic bacteria are remarkable divers and play a significant role in the growth and nutrition uptake, stress tolerance and stability of the plant ecosystem. To design the efficient microbial inoculants and sustainable agricultural management, the factors controlling the distribution and composition of endophytic community needs to be understood. Continued research combining microbiome profiling, functional genomic approaches, and investigations of plant–microbiome interactions will facilitate the further development of endophytic bacteria as tools to further enhance plant improvement efforts and environmental sustainability [23,24].

IV. Importance of PGPB (plant growth-promoting Bacteria) in plants

4.1 Mechanism of Plant Growth promotion

Plant Growth Promoting Rhizobacteria (PGPR) is also known as Plant Growth Bacteria (PGPB) are the beneficial microorganisms that colonizing the rhizosphere and plant tissues which increase the plant growth through a variety of direct and indirect mechanism. Due to their ability to improve nutrient acquisition, suppress plant pathogens and stimulate plant development, PGPB have emerged as promising bio alternatives to chemical fertilizers and pesticides in sustainable farming. Numerous studies have demonstrated that PGPB are successfully applied in a wide range of economically important crops, resulting in increase biomass production improved nutrient efficiency and higher crop yields [29,30].

PGPB have several direct growth promoting activities like biological nitrogen fixation, phosphate solubilization, mobilization of potassium, production of siderophores, synthesis of phytohormones like Indole-3-acetic acid (IAA), gibberellins and cytokinin's. They also indirectly in the process of suppressing phytopathogens by producing antimicrobial products competing for nutrients, interactions enhance plant health biomass and resistance to normal and stressful environments [9,10,11,12].

4.2 The Influence of Environmental factors on PGPB Performance

Soil properties and environmental conditions are very important factors in the effectiveness of PGPB. Soil texture is a key factor in microbial colonization, activity and growth, and soils that lack a large amount of clay generally offer better conditions for establishment and growth of PGPB than do compacted soils that have a high clay content [26]. It also determines the performance of the bacteria, with soil pH affecting the performance of many PGPB strains, which display optimal activities under neutral pH. For instance, *Bacillus amyloliquefaciens* has lower growth promoting efficiency in acidic soils than in neutral soils [31]. The effect of temperature on bacterial survival, metabolism, and efficiency of biofertilisation is very significant. Some beneficial strains have an optimum temperature for growth: *Bacillus subtilis* XZ18-3 is optimum at 25 - 30 °C [32]. In the same way, proper soil moisture is also vital for good colonization and microbial activity, while drought stresses may limit the establishment of bacteria and decrease their potential to suppress plant pathogens [33]. (Table. 1)

Harsh environmental conditions like salinity, drought stress, extreme temperatures and UV radiation may have a negative impact on PGPB survival and activity. Microbial colonization and metabolic activity is typically inhibited by high salinity; however, some microbes like *Pseudomonas putida* have been found to be tolerant of moderate salinity and promote plant growth in such environments [34]. Similarly, drought stress may affect bacterial proliferation and rhizosphere colonization, which will affect their beneficial effect on plants. Another influence of the organic matter content is that it increases nutrient availability and creates favourable habitat for microbial growth, which in turn influences PGPB activity. Low organic matter soils tend to have low microbial diversity and low plant growth promoting potential [35]. In addition, UV radiation can harm microbial cells and affect the viability of the inoculant. To address these constraints, protective formulations of microbials, including microbial encapsulation and UV-protective carriers, have been developed to enhance the survival of microbials in the field and their field performance [36] and are also shown in table. 1.

4.3 Significance of PGPB in Sustainable Agriculture

Pollution of the environment, erosion of soil and overuse of chemical fertilizers and pesticides have raised awareness of the use of PGPB-related technologies in sustainable agriculture. PGPB also increase availability of nutrients, tolerance to stress and suppress plant pathogens to improve crop productivity and decrease the use of synthetic agrochemicals. The incorporation of their role in modern agriculture system provides a sustainable and cost-effective solution for the production of safe food the enhancement of soil fertility and health and the sustainability of crop production [31,32,33,34,36].

Table.1: Abiotic Stress Factors Affecting Performance of PGPB and Endophytic Bacteria plant Performance.

Stress Factor	Effect on Microorganisms	Representative Example	References
Salinity	Decreases microbial colonization and metabolic activity	<i>Pseudomonas putida</i> is Moderately tolerant to salinity	37
Low pH	Slow down bacterial growth and activity	<i>Bacillus amyloliquefaciens</i> is less efficient at low pH levels	31
High Temperature	Affects survival, metabolism, and biofertilizer performance	<i>Bacillus subtilis</i> XZ18-3 is Optimally active at 25–30°C	32
Drought	Restricts colonization and pathogen suppression	Reduced activity of <i>Bacillus licheniformis</i> under water deficit conditions	33
UV Radiation	Causes cellular damage and reduces viability	Encapsulation improves microbial stability and survival	36

V. Endophytes as Internal Plant Growth Promoters

5.1 Endophytes Colonization and Plant Association

Endophytes are the microorganism that resides in the plants internal tissues that does not produce any visible disease symptoms The rhizosphere microorganism that primarily interact with the surface of root, endophytes establish intimate and long-lasting relations within the plant tissues, allowing direct interaction with plant metabolic and physiological processes. These microorganisms colonize various plant organs, including roots, stems, leaves, flowers, vascular tissues, and seeds, where they contribute to plant growth, development, and adaptation to environmental stresses [17,27].

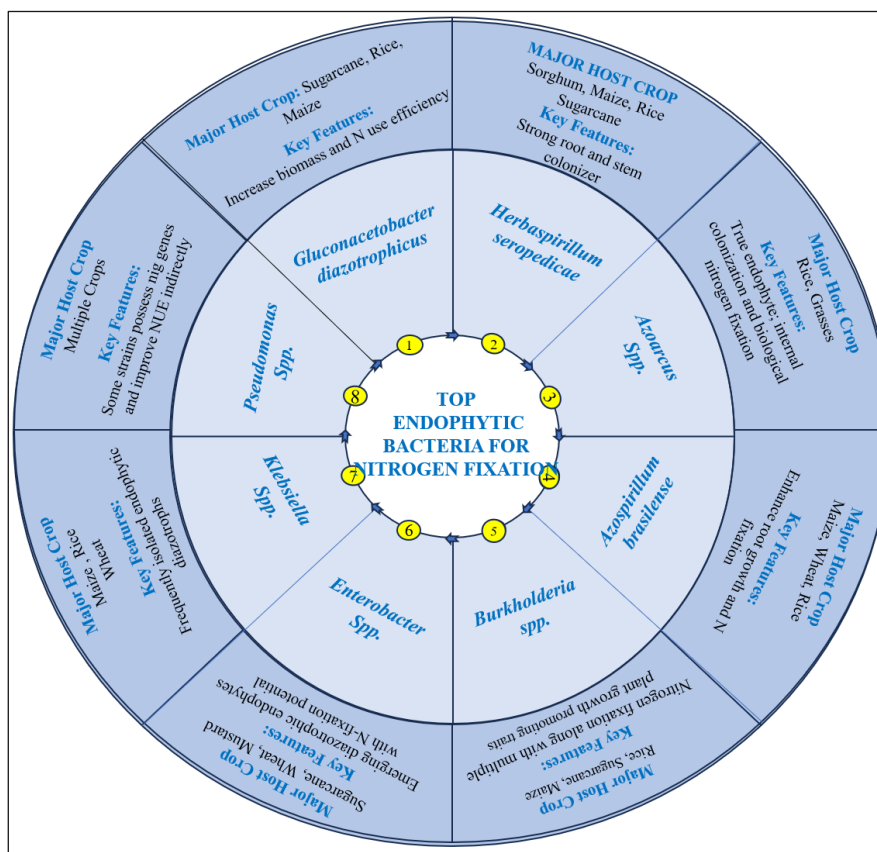
Endophytic bacteria can directly or indirectly stimulate plants to grow. These enhance the uptake and use of nutrients, through the provision of biological nitrogen fixation, phosphate solubilization and nutrient translocation in plant tissues. Endophytes play a role in improving nitrogen-use efficiency by regulation nitrogen assimilation pathways of N assimilation and ensure efficient N allocation across the plant. Many endophytes also synthesize plant growth regulators like cytokinin, gibberellins and indole-3-acetic acid (IAA) which excite root development, cell division, and overall plant growth, in addition they produce siderophores and other bioactive metabolites that enhance micronutrient availability, suppress phytopathogens, and improve plant tolerance to abiotic stresses such as drought, salinity, and temperature extremes [27].

5.2 Endophytes in the major crops of agriculture.

The use of endophytic bacteria in economically important crops has been shown to provide beneficial effects in many studies. In *Oryza sativa*, endophytes have been reported to improve the levels of nitrogen absorption, drought tolerance, and yield [37]. In wheat (*Triticum aestivum*), the endophytic microorganisms play a role for nutrient uptake, resistance against diseases and better crop productivity [17]. (Figure. 1)

On the same lines, endophytes enhance root growth, uptake of nutrients and resistance against stress in maize, *Zea mays*, leading to higher biomass production and grain yield of the plant [24]. Endophytic bacteria have been shown to enhance biological nitrogen fixation and growth of soybean, *Glycine max*, which helps with higher yield [27]. The endophytic diazotrophs are also important in the process of N fixation in sugarcane (*Saccharum officinarum*) and hence in improving cane yield and sugar content [38]. (Figure.1)

Figure. 1: Multi-ring circular representation endophytic diazotrophic bacteria, their host crops, plant growth-promoting traits, and contribution to biological nitrogen fixation and nitrogen use efficiency in agricultural crops. Adapted from published literature including [17,18,19,24,27,37,39,40,41].



5.3 Contribution to the sustainable agriculture

Endophytic bacteria, known for their soil biology capabilities to increase nutrient-use efficiency, stress tolerance, plant growth and decreased need for chemical fertilizers and pesticides, have benefits to sustainable agriculture. They are closely linked to their host plants and thus can provide benefits consistently and over time, regardless of environmental conditions. This has led to an increasing interest in the use of endophytes as biofertilizers, biostimulants and biocontrol agents for enhancing crop productivity and reducing environmental impacts [23,42].

As the role of endophytes in plant–microbe interactions is now better understood, their contribution to crop improvement programmes in the future is becoming a possibility. New technologies in microbial biotechnology, genomics and microbiome research will enable the identification and development of "elite" endophytic strains with improved endophytic plant growth promotional properties. Endophyte-based technologies could play a crucial role in enhancing the productivity of the crops, soil health and global food security within the framework of climate-smart and sustainable agriculture [43].

VI. Functional Trait of Beneficial Endophytes

6.1 The Production of Siderophores and their Acquisition of Iron.

The diverse useful characteristics of the endophytic bacteria play an important role in plant growth and yield. The beneficial microorganisms carry out biochemical and physiological processes such as biological nitrogen fixation, solubilization of phosphates, production of siderophores, production of phytohormones, and secretion of hydrolytic enzymes that are beneficial to the plants. Each of these activities will improve the nutrient availability, stimulate plant growth, boost stress resistance and improve the general health of plants [29]. (Table.2)

Iron is an essential micronutrient for plants and microorganisms, but not available in soil due to the creation of insoluble complexes. Low-molecular weight (LMW) iron-chelating compounds called siderophores are produced by many endophytic bacteria that are involved in mobilizing iron and its uptake by plants. Siderophores also aid in biological disease control by competing for available iron and in improving the iron nutrition and contribute to plant health [27]. (Table.2)

6.2 Nutrient Mobilization and Phosphates solubilization

One of the most essential macronutrients for plant growth is phosphorus, but a large percentage of soil phosphorus is unapproachable to plants. Phosphate-solubilizing endophytic bacteria (PSAB) secrete organic acids and phosphatases that break down insoluble phosphate compounds into soluble phosphates that are accessible to the plants. This process helps to uptake of phosphorus, facilitate root growth and plant productivity in wide-ranging [44]. (Table.2)

The endophytic bacteria are known to produce plant growth regulators like IAA (indole-3-acetic acid), gibberellins and cytokinins. These phytohormones regulate the growth of the cells, extension of the roots, diversity of tissues and uptake of nutrients. IAA-producing endophytes are of special importance because they enhance the volume of plant root system and therefore the plant uptake of water and nutrients in agriculture and under stress conditions resulting in better plant growth [9]. (Table.2)

6.3 Enzymatic Activities and Plant Adaptation.

The endophytic bacteria are known to excrete a variety of extracellular enzymes such as proteases, cellulases, amylases, pectinases, catalases, etc. The enzymes facilitate recycling of nutrients, help microbes penetrate within the plants and enhance adaptation to variable environments. Enzymatic activities also help to decompose complex organic compounds and facilitate enhanced nutrient availabilities and survival of microbes in the host plant [45]. (table.2)

The endophytic bacteria possess numerous plant growth promoting properties and its benefits are often due to several of these properties. The synergistic effect of nitrogen fixation, nutrient solubilization, phytonutrient production, siderophore production, and antioxidant induction as well as enzymatic activities play major role in plant growth, NUE, stress tolerance and disease resistance. The multifunctional interactions indicate the endophytic bacteria as an interesting group for biofertilizer and sustainable agricultural technologies development. [4,29]. (Table.2)

Table. 2. Plant Growth-Promoting Activities of Beneficial Endophytic Bacteria

PGP Activity	Mechanism	Representative Bacterial Species	Supporting References
Biological Nitrogen Fixation	Conversion of atmospheric N ₂ into plant-available ammonia	<i>Azotobacter chroococcum</i> , <i>Azospirillum brasilense</i> , <i>Herbaspirillum seropedicae</i> , <i>Gluconacetobacter diazotrophicus</i> , <i>Burkholderia vietnamiensis</i>	[26,27,46].
Phosphate Solubilization	Solubilization of insoluble phosphates through organic acid production	<i>Bacillus megaterium</i> , <i>Bacillus subtilis</i> , <i>Pseudomonas fluorescens</i> , <i>Rhizobium leguminosarum</i> , <i>Enterobacter cloacae</i>	[44,45]
Siderophore Production	Chelation and mobilization of iron for plant uptake	<i>Pseudomonas fluorescens</i> , <i>Pseudomonas putida</i> , <i>Bacillus subtilis</i> , <i>Azotobacter vinelandii</i> , <i>Burkholderia cepacia</i>	[26,27,46].
IAA Production	Synthesis of indole-3-acetic acid to stimulate root growth and nutrient uptake	<i>Azospirillum brasilense</i> , <i>Pseudomonas fluorescens</i> , <i>Bacillus amyloliquefaciens</i> , <i>Enterobacter cloacae</i> , <i>Serratia marcescens</i>	[26,27]
Antioxidant Induction	Increased plant antioxidant defense mechanisms under stress conditions	<i>Bacillus subtilis</i> , <i>Pseudomonas fluorescens</i> , <i>Enterobacter spp.</i> , <i>Paenibacillus polymyxa</i>	[26,46]

VII. Abiotic Stress Tolerance in Beneficial Endophytes

7.1 Endophytic bacteria have a significant contribution in stress

The abiotic stresses that affect global agriculture productivity are salinity, drought, high and low temperatures, pH values and nutrient deficiency. Under these environmental constrictions, plants growth, physiology, nutrient uptake and crop production are adversely affected, which makes great challenges for sustainable agriculture [26]. With the rising frequency and severity of such stresses as climate change continues the need for environmentally friendly strategies to improve crop resilience and productivity is growing.

Beneficial endophytic bacteria are one powerful factor playing an important role on plant resistance to adverse environmental conditions. They possess several mechanisms of adaptation which help them survive stressful conditions while at the same time building up the plant's resistance or tolerance to these conditions. The stress-tolerant endophytes produce metabolites such as osmoregulators, antioxidants and exopolysaccharides

which help protect the cells of plants from the stress of osmotic imbalance, oxidative damage and physiological disruption. Moreover, endophytic bacteria have been reported to be able to regulate plant hormone levels and to trigger stress-related stress signalling pathways in the plants that help them withstand environmental stress. These processes lead to better growth, nutrient uptake, water use efficiency and plant responses to stress [46].

Recently, it has been revealed that beneficial endophytes can include bacteria that alter the plant's genes that regulate its responses to stress. These microorganisms activate genes that increase the antioxidant defence, osmotic adjustment, ion homeostasis and stress signaling pathways, which consequently increases plant resistance and tolerance to abiotic stress such as drought, salinity, extreme temperatures and other abiotic stresses. All such plant–microbe interactions are crucial in boosting the resilience of the crop in altered environment [47].

7.2 Importance of Biofertilizer Development

However environmental factors, such as pH, salinity, temperature and water availability, are also important to take into consideration when choosing biofertilizer candidates as organisms able to survive under such conditions. In the field, the microbial strains with a higher tolerance to stress are more likely to be able to survive, to colonize plant tissues and to continue their plant growth-promoting properties. Hence, the identification and development of stress resilient endophytic bacteria are crucial for successful use of microbial inoculants under Sustainable and Climate-smart Agriculture [26,48,47].

Stress-tolerant endophytic bacteria are a good approach in improving the sustainability of farming in the context of climate change. Future studies should focus on the molecular mechanisms of stress adaptation, identification of superior strains of microbes and development of consistent microbial formulations which will perform consistently in different environmental situations. These improvements will help promote the broader use of endophyte-based technologies to help boost crop productivity, soil health, and food security. [48].

VIII. The isolation, characterization and cultivation of endophytic bacteria.

8.1 Cultivation and Morphological characterization.

The successful isolation of the endophytic bacteria demands that epiphytic microorganisms on plant surface must be removed and viable microbial communities must remain inside the plant. Therefore, one of the vital steps in the endophyte isolation procedures is the surface sterilisation. Standard disinfection techniques used are sequential treatment with ethanol and hypochlorite solutions and then several rinses with sterile distilled water. Ethanol disrupts cell membranes and sodium hypochlorite is a strong oxidizing disinfectant that will aid in the removal of surface deposits [49]. Final rinse water is normally plated on nutrient agar media to check the effectiveness of the surface sterilization. There was no microbial growth, which suggests that epiphytic microorganisms were eliminated and that bacteria subsequently isolated were from the plant's internal tissues [14].

The plant tissues are surface sterilized and the interior bacteria are released by macerating or homogenizing the tissues under aseptically controlled conditions. A series of dilutions are made and the homogenate is then spread or poured onto an appropriate culture media. The techniques are employed to aid in the growth and isolation of a wide range of bacteria including aerobic and facultative anaerobes. Initial identification of bacterial isolates is done using their colonial characteristics, which include shape, size, colour, texture, margin, elevation and opacity. While morphological characteristics are not adequate for species identification, they are helpful in differentiating bacterial isolates. These are then streaked repeatedly on nutrient agar plates for obtaining pure culture and then stored long term in nutrient agar slant or in glycerol stocks for further research [14].

The nutritional and physiological complexity of plant tissue is not reproducible using artificial media - some microorganisms living inside plants cannot be cultured in the laboratory. However, extraction of viable endophytes is still necessary for the functional characterization, development of biofertilizers and agricultural application studies. Hence, combining culture-dependent and culture-independent methods is essential to gaining a full knowledge of the endophytic microbial community [14,50].

IX. Conclusions and suggested lines of further investigation

Despite the advances in elucidating the plant growth promoting, nutrient acquisition and stress tolerance effects of endophytic bacteria, there are still some gaps in understanding. There are only limited validations performed in large-scale arenas in different agroecological conditions, most of the studies have been conducted under controlled laboratory or greenhouse settings. To enable effective commercialization and implementation of endophyte technologies in sustainable agriculture, these gaps need to be addressed.

9.1 Major Research Priorities

9.1.1 Smart Nano-Biofertilizers: Stimuli-Responsive Delivery Systems

Biofertilizer technology holds promise for further innovations such as the incorporation of beneficial microorganisms with advanced nanomaterials. Present nano-bio-fertilizers are based on the principle of continuous release of microbial inoculants but future “Smart” delivery systems are based on the principle of responding to the specific environmental or biological signal. These carrier materials are responsive to stimuli, and release the microbial cargo upon exposure to root exudates, nutrient deficiency or drought stress. An example of such a "smart capsule" could be one that releases beneficial bacteria when the soil dries out, but does not when it is sufficiently wet. These systems of precision delivery can enhance the survival of microorganisms, ability to colonize possibly, economic viability and decrease resource wastage [51,52]

9.1.2 uses of CRISPR/Cas technologies and nanotechnology

In recent years, new technologies for gene editing have developed that could lead to better performance of desirable microorganisms. As the non-viral carriers for transferring CRISPR/Cas components into bacterial and plant cells, the two can be the carbon nanodots (CNDs) and gold nanoparticles (AuNPs). This method allows for specific modification of genes related to nitrogen fixation, siderophore production, stress resistance and nutrient solubilization. Combine nanotechnology with gene editing could help pave the way for "designer microorganisms" that can be customized for particular crops, soil conditions, and environmental conditions, helping to increase agricultural productivity and sustainability [51,52].

9.1.3 Digital Rhizosphere: Nano-Sensors and Smart Agriculture

Nanotechnology, biosensors and the Internet of Things (IoT) are coming together to provide opportunities to monitor soil and microbial health in real-time. Advanced nanosensors can detect the metabolites of the microbes, nutrients and changes in the environment in the rhizosphere. These systems enable the transmission of data via wireless network to the farmers to make more precise and timely interventions in agriculture. The new paradigm, 'digital rhizosphere' is poised to transform the way agriculture was being managed from a blanket and traditional approach of using inputs towards more data-driven approach which would enhance resource use efficiency and sustainability [53].

9.1.4 Omics-Based Approaches for Future Research

Endophytic bacteria and their interactions with their host plants are gaining insight into their functional roles through advanced omics technologies such as genomics, transcriptomics, proteomics, metabolomics and metagenomics. The combination of these strategies will aid the discovery of critical microbial genes, pathways and signaling networks that promote plant growth and adapt to plant stress. The knowledge generated will help in the development of future generation biofertilizers and microbial technologies in agriculture in a swift pace [54].

9.2 Further studies are needed on:

- Identification and screening of high efficiency endophytic strains fit to different agro climatic regions.
- Understanding of the molecular and genetic regulation of plant–microbe interaction.
- Scalable, stable and cost-effective microbial formulations for field.
- Linkage of microbial biotechnology to precision and climate-smart agriculture.
- Long-term ecological impacts on soil microbes from microbial products

X. Conclusion

The increasing global food demands, sustainable agriculture practices are needed due to overuses of chemical fertilizers and pesticides, in recent year beneficial microorganisms, particularly (PGPB) plant growth promoting bacteria and endophytic bacteria have been identified as environment friendly alternative which is capable of enhancing crop productivity while reducing the reliance on synthetic agrochemicals.

The endophytic bacteria inhibit host plants and contribute to plant growth through various mechanism including biological nitrogen fixation, phytohormone production, nutrient mobilization, enzymatic activities, enhanced photosynthetic efficiency, and improved tolerance to abiotic stresses. Their ability to improve nitrogen-use efficiency and nutrient acquisition highlights their potential to decrease fertilizer inputs and support sustainable crop production.

Despite considerable advances, further research is required to explain the molecular mechanisms underlying plant–endophyte interactions, identify elite microbial strains, and validate their efficiency under

various field conditions. The integration of microbial biotechnology with emerging technologies such as nanotechnology, genome editing, precision agriculture, and multi-omics approaches offers exciting opportunities for developing new and innovative biofertilizers and sustainable crop management practice.

In the future the potential of endophytic bacteria to improve soil health, boost security and ensure environmental sustainability will be vital.

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