

# Metagenomics: An Advanced Approach for Endophyte Study and Prospecting

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## ABSTRACT

Endophytes are beneficial microorganisms that reside in different plant tissues. The culturable endophytes are limited so that the knowledge about the microbial communities inside plant tissue is confined. In this study, we concentrated on the metagenomic studies of endophytes that opened a vast knowledge about them. The study of endophytes through the genetic level contributes detailed knowledge about microbial diversity, their composition, functions, and metabolic pathways. This will help to understand their role in plant growth and development. Through metagenomic studies, we can build more information about how endophytes promote plant growth promotion and the different aspects like biofertilization, plant growth hormone production, and stress tolerance. The use of metagenomics gives the importance of plant microbiota to the sustainable use of agriculture and ecological functions.

**Keywords:** Endophytes, Metagenomics, Biofertilization, Secondary metabolites, Stress tolerance.

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## INTRODUCTION

Plants are colonised by plenty of microbes in their tissues. Microbial endophytes are microorganisms that reside within plant tissue and complete their life cycle without causing visible harm or disease symptoms.<sup>[1]</sup> Endophytes encompass a variety of microorganisms, including bacteria, actinomycetes, and fungi.<sup>[2]</sup> They are indeed common plant colonizers and play a crucial role in the health and productivity of plants.<sup>[3]</sup> Endophytes can interact with plants in a mutualistic, neutral, pathogenic, or beneficial way.<sup>[4,5]</sup> The beneficial microbes, called plant growth-promoting endophytes, influence plant survival and functioning, thereby assisting the host plant's overall functioning. This mutualistic relationship exemplifies how plants and microbes work together to optimize survival and performance.<sup>[6,7]</sup> According to studies, barely 1% of the endophytes present in nature have

been cultivated and biotechnologically investigated.<sup>[8-10]</sup> Culture-dependent methods have been crucial for the isolation and identification of endophytic bacteria. Only a small percentage of the overall microbial community is culturable, but many other groups are not.<sup>[11]</sup> The use of molecular techniques established a tremendous knowledge about the unculturable endophytes and their interactions with the host plant.

The metagenomics approach has indeed revolutionized the study of microbial endophyte communities.<sup>[12]</sup> Metagenomics is the genomic analysis of microorganisms through the direct extraction and sequencing of DNA from an assemblage of microorganisms.<sup>[13]</sup> The complete taxonomic categorization, as well as the functional and metabolic pathways of the active endophytic microbes, are revealed by metagenomic research.<sup>[14]</sup> Discovering novel genetic features and processes of plant-microbe interactions is made easier by metagenomics. Metagenomics enables the study of microorganisms easier and more precise regardless of the kind of environmental samples used. Utilizing metagenomics techniques, researchers can analyse the genomes of microorganisms particularly those associated with plants, known as endophytes. It also

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enables the identification of potential genes involved in plant growth and physiological processes. This method has aided in the study of many genes that promote plant growth and have either known or unidentified roles.<sup>[15]</sup>

A potent sequencing tool for microbial research is Next-Generation Sequencing (NGS). This will help in better understanding and describing the microbiome. Next-Generation Sequencing (NGS) technology has been used in the analysis of soil, rhizospheres, and endophytic microbes by providing high-resolution insights into microbial diversity using 16S rRNA amplicon sequencing, 454 pyrosequencing, and ITS sequencing.<sup>[16-20]</sup> With the aid of this method, it is possible to examine the metagenome of bacterial endophytes and eliminate sequences with plant origin from the entire dataset.<sup>[21]</sup> These techniques are important valuable tools to explore entire microbial communities within plant tissues that possess unique properties that contribute to plant growth and health including phytohormone synthesis, cellular metabolism, and nitrogen fixation from plant root internal tissues.<sup>[15]</sup> Illumina sequencing, which produces large and error-free data sets, is currently the most used NGS technique.<sup>[22]</sup>

Better understanding of plant endophytes through metagenomic analysis will lead to develop solutions to environmental problems caused by the use of chemical fertilizers and creating biological control alternatives for treating plant diseases.<sup>[23]</sup> Because the plant associated beneficial endophytes can directly affect plant growth and health through the production of phytohormones, enhancing nutrient uptake and phosphate solubilization, and tolerating biotic and abiotic stresses, these bacteria can provide advantages to the host plant. Additionally, they have the capacity to exert indirect action by eliciting a variety of physiological and immunological responses from the host, which may lead to the expansion of a particular microbiome to combat the pathogen infection. This advantageously aroused microbiome can compete for plant resources and niche spaces or restrict the establishment of plant diseases by producing antibiotics, enzymes that break down fungal cell walls, or siderophores, among other things.<sup>[24-26]</sup> They generate biologically active metabolites having enormous potential for use in pharmaceuticals, medicine, and agriculture.

### Metagenomics and Biofertilization

Chemical fertilizers are crucial in ensuring that the world's population's increasing food needs are met. To produce crops with the highest yields possible, the three main commercial fertilizer types, Nitrogen (N), Phosphate (P), and Potassium (K) are employed.<sup>[27]</sup>

However, the increasing utilisation of chemical fertilizers in agriculture has a negative effect on ecosystems. Chemical fertilizers are not properly absorbed by plants, causing them to eventually reach water bodies through leaching and causing eutrophication. Additionally, they may affect soils in several ways, diminishing their ability to store water and disrupting the balance of soil fertility.<sup>[28]</sup> In addition to being expensive, they produce more greenhouse gases due to fossil fuel use in their production. It has been necessary to create alternative fertilizers for a very long time that are affordable, efficient, and environmentally benign and that function without harming the environment.<sup>[29]</sup>

Biofertilization is the process of enhancing plant growth by improving the availability or supply of essential nutrients.<sup>[30]</sup> Biofertilizers are typically bacteria or fungi that can fix nitrogen, solubilize phosphorus, potash, and perform other tasks.<sup>[31]</sup> Numerous Plant Growth-Promoting Bacteria (PGPB) aid in nitrogen fixation and phosphate solubilization. Metagenomic study of microbiomes facilitates more understanding about the genetic basis of these activities and how much they are contributed to plant health and productivity. Several studies proved that the knowledge of endophytes through metagenomic analysis helps for the eco-friendly use of biofertilizers instead of chemical fertilizers and sustainable growth in agriculture.

Metagenomic analysis of sugarcane revealed the presence of uncovering novel *nifH* gene-producing microbes colonizing the different tissues that facilitate the growth through nitrogen fixation.<sup>[32]</sup> Their study reports more than 20 of the most abundant N<sub>2</sub>-fixing microbes and among them some are renowned examples of N<sub>2</sub>-fixing bacteria like *Rhizobium*, *Azospirillum*, *Enterobacter*, etc. More than 23 bacterial species that contribute plant growth promotion were found in rice from West Bengal's coastal saline zone using 16S rRNA gene-based metagenomic analysis. Among them, an isolate of *Kosakonia* was shown to have both the capacity to fix nitrogen and to promote plant growth properties.<sup>[33]</sup> Metagenomic analysis of bacterial endophytes of *Emilia sonchifolia* reported genes *nifD*, *nifK*, and *nifH* for nitrogen fixation.<sup>[34]</sup> Metagenomic data of bacterial endophytes of *Panax ginseng* reported the presence of nitrogen fixation genes. The partial amplification of the *nifH* gene revealed two bacterial endophytes *Stenotrophomonas maltophilia* and *Agrobacterium tumefaciens*, having the *nifH* gene.<sup>[35]</sup>

Both monobasic (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) and dibasic (HPO<sub>4</sub><sup>2-</sup>) phosphate are absorbed by plants from the soil. However, 95-99% of the phosphorus in soil is unavailable to plants because it exists in precipitated,

immobilized, and insoluble forms.<sup>[36]</sup> Phosphorus Solubilizing Microorganisms (PSM) are microorganisms that are capable of converting insoluble phosphate molecules into phosphate that is readily available to plants.<sup>[37]</sup> This increases plant growth, productivity, and quality. *Azotobacter*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Micrococcus luteus* have all been discovered as P-fertilizers.<sup>[38]</sup> Phosphate solubilization activities in an endemic legume *Humboldtia brunonis* Wall. revealed the presence of *Brevibacillus brevis* in culture-based studies and metagenomic analysis identified a broader range of phosphate solubilizers including, *Bradyrhizobium*, *Enterobacter*, *Fusirrhizobium*, *Methylobacterium*, *Salmonella*, *Rhizobium*, *Acidobacterium*, etc. but are not responsive to culture-based techniques.<sup>[39]</sup> Role of Plant Growth Promoting Rhizobacteria in suppressive soils against *Fusarium oxysporum* f. sp. *cubense* based on metagenomic analysis showed the presence of *Pseudomonas*, *Burkholderia*, and *Bacillus* are known as PGPR that can solubilize phosphate.<sup>[40]</sup> The study of rice seedling establishment of rice seedling showed plant growth promotion activities and nine endophytic bacteria showed phosphate solubilization activity.<sup>[41]</sup>

### Metagenomics and Phytohormone Production

Microorganisms that enhance plant development are known as plant growth-promoting microorganisms, which produce various plant hormones that play a significant role in maintaining plant health and vitality. Plant phytohormones stimulate plant development and serve as growth regulators. Five key phytohormones that serve as Plant Growth Regulators (PGRs) are auxin, gibberellins, cytokinin, ethylene, and abscisic acid.<sup>[42]</sup> *Acetobacter*, *Azotobacter*, *Herbaspirillum*, and *Rhizobium* have been reported to synthesize auxin, cytokinin, and gibberellins.<sup>[43]</sup> Indole-3-Acetic Acid (IAA), is a important plant hormone secreted by endophytes, that supports the growth and development of plants.<sup>[44,45]</sup> It plays a crucial role in promoting plant cell elongation, differentiation, and division. It enhances seeds and tubers germination, stimulates roots and xylem formation, improves lateral root initiation, and regulates vegetative growth and adventitious root formation.<sup>[46]</sup> Beyond the primary growth functions it also contributes to pigment production and the biosynthesis of metabolites, and regulates plant responses to gravity, light, and fluorescence. It also aids in photosynthetic processes and increases plant tolerance to adverse environmental conditions.<sup>[47]</sup>

Using the shotgun approach the potential functional genes present in the endophytic microbiomes of maize

roots were studied.<sup>[48]</sup> Analysis of the metagenome revealed the presence of genes *ipdC* and *iaaM*, which encode indole-3-pyruvate decarboxylase and tryptophan 2-monooxygenase respectively. IAA production capacity of the endophytic microbiome of *Emilia sonchifolia* is indicated by enzyme coding gene annotations like *ipdC* and *tnaA*.<sup>[34]</sup> Bacterial endophytes isolated from maize through metagenomic analysis have been identified as producers of indole acetic acid including species such as *Sphingobium*, *Burkholderia*, *Chitinophaga*, *Staphylococcus*, and *Bacillus*.<sup>[49]</sup>

### Metagenomics and Stress Tolerance

Various environmental factors negatively affect plant growth, development, and yield. These include salinity,<sup>[50]</sup> temperature,<sup>[51]</sup> drought,<sup>[52]</sup> the presence of harmful metals or organic contaminants, and other stresses.<sup>[53]</sup> Among these drought has emerged as one of the most significant and increasingly common challenges. It has a profound effect on various physiological and metabolic processes in crop plants and severely affects their development and productivity.<sup>[54]</sup> Under drought stress crop plants show slower growth and development due to its profound effects on root physiology, nutrient intake, leaf structure, photosynthetic activity, and seedling germination.<sup>[55,56]</sup>

Endophytes help crop plants to adapt environmental challenges, by enhancing the plants to withstand harsh conditions, supporting plant development, suppressing phytopathogens, and activating stress-induced genes in plants that are not typically activated in response to stress.<sup>[57]</sup> Studies on *Ananas comosus* identified several endophytic bacteria including species from the genera *Staphylococcus*, *Bacillus*, and *Providentia*. They can boost drought tolerance, encourage growth, and increase disease resistance.<sup>[58]</sup> Similar to this, Sandhya et al<sup>[59]</sup> showed that many endophytic bacterial strains obtained from different crops, helped plants to withstand drought conditions up to a matric potential of 1.02, and also had the ability to promote growth. Inoculating wheat seedlings with the endophytic strain *Pantoea albagi*, which was isolated from the plant *Albagi sparsifolia*, significantly improved their growth under drought conditions.<sup>[60]</sup> The endophyte-treated plants also showed increased accumulation of soluble sugar and reduced levels of malondialdehyde indicating enhanced stress resistance and reduced cellular damage. The endophytic bacterium *Bacillus subtilis* B26 helped to mitigate drought stress in the grass *Brachypodium distachyon* by enhancing the expression of stress-induced genes.<sup>[61]</sup> Additionally, endophytic fungal strains *Ampelomyces* sp. and *Penicillium* sp. isolated from soil subjected to drought and high



salinity were found to improve tomato plants tolerance to both salinity and drought.<sup>[62]</sup>

Endophytic bacteria help plants combat drought stress by producing volatile compounds, abscisic acid, ACC-deaminase, and IAA.<sup>[63]</sup> The endophytic bacterial strain *Bacillus amyloliquefaciens* has been shown to effectively alleviate salinity stress in plants by significantly increasing the production of abscisic acid after inoculation. Additionally, rice subjected to stressful conditions inoculated with *B. amyloliquefaciens* also led to notable improvements in the levels of some key antioxidant amino acids, including cysteine, aspartic acid, glutamic acid, phenylalanine, and proline.<sup>[64]</sup> Similarly, research on the effects of pre-treating maize seed with the endophytic bacteria *Corynebacterium hansenii* and *Bacillus subtilis*, demonstrated enhanced growth and physiological responses in maize under drought stress.<sup>[65]</sup> Pre-treatment with *C. hansenii* under drought conditions resulted in increased relative water content, leaf proline, and chlorophyll levels. Additionally, under stressful conditions, ACC deaminase (1-aminocyclopropane-1-carboxylate) enzymes produced by endophytic microbial strains reduce the amounts of ethylene in plants thereby mitigating stress-related damage.<sup>[66]</sup> According to Jaemaeng<sup>[67]</sup> the endophytic bacterial strain *Streptomyces sp.* enhanced rice's ability to withstand salt stress by producing 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD), which works by converting an ethylene precursor into ammonia and -ketobutyrate, lowering the level of ethylene in plants.

Metagenomic studies aim to delineate the metabolic pathways of genes responsible for both known and novel secondary metabolites allowing bacterial adaptation to harsh salinity.<sup>[68]</sup> The metagenomic study of two fingermillets cultivars revealed the presence of genes encoding stress response.<sup>[69]</sup> Shotgun metagenome analysis of uncultured microbial communities within endophytic bacteria has identified populations of Proteobacteria and Actinobacteria that are potentially involved in plant growth promotion and abiotic stress tolerance.<sup>[70]</sup> Additionally, changes in the endophytic bacterial communities of wheat, assessed by 16S rRNA sequencing, have been correlated with variations in drought stress conditions, highlighting the dynamic role of these microbial communities in responding to environmental stress.<sup>[71]</sup> The metagenomic studies of the desert plant *Senna ilatlica* revealed the presence of bacterial endophytes.

### Metagenomics and production of secondary metabolites

Endophytes has the ability to produce diverse kinds of bioactive secondary metabolites for their own

establishment and also to boost the plant immune system. Production of metabolite is an effective method for biocontrol.<sup>[72]</sup> Various metabolites produced by endophytes include hydrolytic enzymes, cyanides, antibiotics, etc. and they are potential sources of different biological activities like antimicrobial, anti-inflammatory, antifungal, anticancerous, etc. Metagenomic techniques play an important role in elucidating the bioactive compounds produced by the endophytes. Unculturable Actinobacteria from the *Streptomyces* species, which are known to produce a variety of bioactive compounds and antibiotics, have been found in several studies on medicinal plants.<sup>[73,74]</sup> The genome of the bacterial endophytes, *Streptomyces* species, are rich source of bioactive compounds and contains genes that encode the organic substances ecomycins, munumbicins, xiamycins, kakudumycins, and pseudomycins.<sup>[7]</sup> The possibility of bacterial endophytes serving as a source of new metabolites has therefore been confirmed.<sup>[75,76]</sup> Additionally, in order to defend themselves against diseases, unculturable endophytes also produce enzymes like pectinase.<sup>[77]</sup> Like xiamycin from *Streptomyces* GT2002/1503, the bioactive substances generated by microbial endophytes have antibacterial and antiviral activities. The endophytic fungus *Taxomyces andreanae* isolated from the bark of Pacific yew produces the novel anticancer and antitumour Paclitaxel (Taxol).<sup>[78]</sup> The gene *bcnB*, which encodes hydrogen cyanide synthase, was identified.<sup>[48]</sup> This gene enhances the production of Hydrogen Cyanide (HCN) and HCN has been reported to be useful in the encouragement of the host plant's growth. They also discovered the genes *ubiC* and *phzF*, which encode for chorismate lyase and phenazine biosynthesis, respectively. These genes produce the compounds phenazine and 4-hydroxybenzoate, which aid the host's defense mechanisms and biocontrol processes.<sup>[79]</sup> Terpenoid is a crucial secondary metabolite involved in defense mechanisms, stress tolerance, and other processes. Metagenome of *E. sonchifolia* contains genes involved in the mevalonate pathway of terpenoid production.<sup>[34]</sup> The use of the metagenomic technique in studying complex microbial communities associated with lignocellulosic energy food crops such as *Populus nigra*, *Eucalyptus camaldealensis*, and *Arundo donax* has been studied.<sup>[80]</sup> They revealed that the plant microorganisms produced cellulase-coding genes, indicating that they could eventually be employed to hydrolyze cellulose and lignocellulose found in the environment. The results of a metagenomic analysis of various watermelon cultivars showed that the microbiome associated with the fruit is involved in the metabolism of carbohydrates and fruit ripening. All cultivars contained numerous

genes related to various cellular processes including signal transduction, post-translational modification, carbohydrate metabolism, transcription, intracellular trafficking, and amino acid and energy metabolism. In this study, different families of structurally related glycoside hydrolases, glycosyl transferases, carbohydrate esterases, auxiliary activities, and carbohydrate-binding modules of enzymes that degrade, modify, or create glycosidic bonds were identified.<sup>[81]</sup>

### Metagenomics and Phytoremediation

Adverse environmental conditions are due to the high quantity of pollutants generated through various activities and their removal is economically challenging. Biological agents can be an alternative method to remediate this problem. The breakdown of contaminants using microorganisms is known as phytoremediation or bioremediation. Endophytes contribute important to bioremediation. To remove metals from the soil and encourage plant development and production, endophyte-based bioremediation is applied.<sup>[62]</sup> Some bacterial endophytes have the ability to withstand high levels of pollutants while exhibiting Plant growth promotion.<sup>[82]</sup> Endophytic bacteria were isolated from *Sedum plumbizincicola*, a Zn/Cd hyper-accumulator plant. Five of the isolates helped the plant to grow better such as the production of IAA, phosphate and phosphorus solubilization, siderophores, and ACC deaminase activity. Additionally, these bacteria showed high tolerance to metals like Pb, Zn, and Cd.<sup>[83]</sup> *Bacillus*, *Pseudomonas*, and *Arthrobacter* are regarded as the three most common genera of bacteria in metal-contaminated environments.<sup>[84]</sup> Zn-tolerant rhizobia isolated from Zn mining soil has been reported to promote the growth of *Leucaena leucocephala* in contaminated environments.<sup>[85]</sup> In the study by,<sup>[86]</sup> Cu-resistant *Kocuria sp.* was a potential Plant Growth-Promoting Rhizobacterium (PGPR), which was isolated from the dry tailing of a copper mine.

The bioremediation process of hydrocarbon-contaminated soil by microbial communities was revealed by metagenomic analysis through Illumina sequencing.<sup>[87]</sup> The structural and functional properties of microbial communities are identified via shotgun metagenomics. Using shotgun metagenomics, identified the 'HM resistome'- a comprehensive collection of all the heavy metal resistance genes- present in agricultural soil with and without cadmium pollution was identified. The study functionally annotated genes involved in the translocation of heavy metals, with P-type ATPases functioning in detoxification and the efflux of cadmium being *czcA*, *czcD*, *czrA*, etc. The analysis also revealed

multiple genes associated with resistance to Copper (Cu), Nickel (Ni), Iron (Fe), and Cobalt (Co).<sup>[88]</sup> The metagenomic analysis is employed to study the microbial adaptation mechanism in contaminated sediments under natural conditions. The results of the study revealed functional gene annotations that include a significant number of genes related to DNA recombination, DNA damage repair, and heavy-metal resistance. In addition, the study also identified genes involved in the exportation, resistance, and reduction pathways of Mercury (Hg), Lead (Pb), Copper (Cu), Arsenic (As), Zinc (Zn), Cadmium (Cd), and Chromium (Cr).<sup>[89]</sup>

Metagenomic analysis was used to study the microbial community and their function involved in cd-contaminated and non-contaminated soil.<sup>[90]</sup> The study revealed the presence of more metabolic genes in cd-contaminated soil including those encoding for ABC transporters and, genes involved in the detoxification process. Comparative metatranscriptomic and metagenomic analysis has been used to study the Cr (VI) remediation genes of unculturable microbes leading to the identification of six novel genes including *gsr* and *mcr* that confer Cr tolerance.<sup>[91]</sup> These genes demonstrated high Cr (VI) removal efficiencies and good stability in the treatment of Cr (VI)-contaminated industrial wastewater. Enhanced lead phytoextraction by endophytes from indigenous plants is studied.<sup>[92]</sup> The combined use of EDTA and isolated endophytes from indigenous plants was studied. Treatment with microbial inoculum alone and combined use treatment revealed the most effective lead phytoextraction in *Brassica juncea* and *Helianthus annuus* respectively. They also validated these results through metagenomic analysis and confirmed that plant-microbe interaction is important for phytoremediation.

### Metagenomics and Biocontrol

Pests and pathogens destroy plants thereby reducing productivity. This brings out the use of chemicals that contaminate the surroundings. The control of plant diseases and phytopathogens using microorganisms is biocontrol. Bacteria and fungi are used as biocontrol agents. By facilitating physical touch or by using particular pathogen-specific defense mechanisms (hyperparasitism, predation, etc.), they can directly suppress infections. They may also produce lytic enzymes, antibiotics, or other substances that have an indirect effect via enhancing plant defenses. Endophytic microbiota act as biocontrol agents through various mechanisms, such as competition for a niche or substrate, engaging hyperparasitism and predation,

synthesizing allelochemicals like antibiotics, lytic enzymes, and siderophores, and including systemic resistance in plants.<sup>[93]</sup>

Through metagenomic analysis, microbial populations and their effect on controlling diseases are studied. Comparative metagenomics has been used to uncover the microbial population and biocontrol organisms that act against the plant pathogenic fungus *Magnaporthe oryzae*.<sup>[94]</sup> and they identified several microbes with antagonistic potential against *M. oryzae*, suggesting they could serve as potential biocontrol agents for management of rice blast disease. The microorganisms living in and around banana fields affected by *Fusarium* wilt disease are studied.<sup>[95]</sup> They looked at both plants showing disease symptoms and those without symptoms and investigated the presence of microbiota as biocontrol agents and the production of antagonist compounds against phytopathogens. The results showed the presence of two main potentially beneficial microbes, *pseudomonadales* and *streptomycetaceae*. Manipulating the population of *pseudomonads* and *streptomycetes* in rhizosphere soils could help us to reduce or diminish the disease development in plants, even in the presence of the pathogen. Endophytic strain *Bacillus amyloliquefaciens* suppresses *Fusarium oxysporum* infection of Chinese wolfberry by modifying its rhizosphere bacterial community.<sup>[96]</sup> The biocontrol potential of *B. amyloliquefaciens* HSB1 and FZB42 was tested against five fungal pathogens that commonly cause root rot in Chinese wolfberries. Metagenomic sequencing assessed bacterial community shifts in the wolfberry rhizosphere following the introduction of each biocontrol strain. The results indicated that HSB1 and FZB42 differentially altered the abundances of various taxa and positively influenced several functions of the inherent wolfberry rhizosphere bacteria.

## CONCLUSION

Scientists are interested in studying endophytes and their interactions with the host plant. The invention of molecular techniques like metagenomics opened a vast area to study microbial organisms. The concept of exploring the whole microbiome of a plant provides various knowledge about them and it also helps to understand the diversity, structure, and functions of these microorganisms. Through metagenomic studies, the contributions of endophytes to the growth and development of plants and the genetic level relatedness can be understood. Metagenomics research can focus on specific genes with known functions. However, recent advancements in Next-Generation Sequencing (NGS) technology, particularly shotgun metagenomics

have greatly expanded our ability to study microbial communities. This high-throughput sequencing method allows scientists to analyze the entire genetic makeup of endophytic communities. Nowadays comprehensive views of the genes present in these microbial communities are obtained through metagenomic analysis rather than looking at a few specific genes. Detailed study of endophytes and their functions can lead to future studies in economic and environment-friendly ways.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**NGS:** Next-Generation Sequencing; **rRNA:** Ribosomal Ribo Nucleic Acid; **ITS:** Internal Transcribed Space; **PGPB:** Plant growth Promoting Bacteria; **PGPM:** Plant Growth-Promoting Microorganisms; **PGRs:** Plant Growth Regulators; **IAA:** Indole-3-acetic acid; **ACC:** 1-aminocyclopropane-1-carboxylate; **HCN:** Hydrogen Cyanide; **DNA:** Deoxy Ribo Nucleic Acid; **EDTA:** Ethylene Diamine Tetra Acetic acid.

## SUMMARY

- Endophytes are organisms that reside within plant tissues in a beneficial way to promote plant growth and productivity.
- Less than one percentage of microbial endophytes will be available for research and study in culture conditions. Metagenomic analysis unveils this hidden microbial world in a broad scenario
- Metagenomic analysis provides more insights into the functional and metabolic pathways, essential for plant-microbe interactive development.
- Multifaceted roles of endophytes in plant growth including the production of plant growth regulators, and assimilation of nutrients will be analyzed in greater detail through metagenomic sequencing



- Metabolic profiling is an advancing area of phytochemical research. Genome-based analysis of endophytes will bring some genes to the limelight which has a greater role in plant metabolic pathways. So, bioactivity studies in cognition of these interactive pathways will be a future perspective in endophyte research.
- Other allied aspects like bioremediation, stress tolerance, and biocontrol will be disclosed in greater detail through metagenome sequencing and analysis.

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