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Endophytic microbial advancement through NGS technology: Unlocking the power of the genome

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Abstract

The application of endophytic microorganisms for the protection of plant against to several diseases represents a significant contribution in sustainable agriculture. These endophytes live within internal plant parts and interact to their surrounds without causing any harmful effect and establishment through symbiotic relationship that can induce plant defence system. Endophytic bacteria and fungi have shown not only inhibit a wide range of phytopathogens, viz., bacteria, fungi, and viruses, through the production of bioactive metabolites substances, competition for habitat, space and nutrients, and the activation of plant immune responses but also actively participated to plant growth promoting activities like secretion of phytohormone, enhancing nutrient uptake and influences plant biochemical pathway. This review represents a scenario of the different types of endophytes, their mechanism of action as biocontrol agents and biofertilizers successful case studies, and their role in integrated pest and disease management systems with plant growth promotion and additionally, discusses the recent advanced Next Generation Technology, *viz.*, next generation sequencing, multi-omics studies for endophytic microbial identification and research, challenges, limitations, regulation and future scopes for the commercialization and application of endophytes as a biocontrol agents and biofertilizers to support sustainable agriculture in crop improvement with ecological resilience.

Keywords: Endophytic microorganisms, phytopathogens, sustainable agriculture, advanced NGS technology, bioactive metabolites

Introduction

Plant health is critically interconnected with microbial community that both above and below soil (Trivedi *et al.*, 2020; Van Elsas *et al.*, 2019) [194, 198]. Among the mega diversity of plant-associated microorganisms form of endophytes (Ali *et al.*, 2021; Afzal *et al.*, 2019) [16, 7], these organisms that reside internally within plant tissues leaves, stem and root (Mishra *et al.*, 2021) [175], and represent an underexplored frontier in microbial ecology and agricultural biotechnology (Dubey *et al.*, 2020) [61]. Endophytes has grown exponentially in recent years (Rana *et al.*, 2023) [11], not only for their plant growth-promoting activities (Srivastava *et al.*, 2024) [187], but also specially for their ability to work as natural biocontrol agents (Bhardwaj *et al.*, 2023; Baron *et al.*, 2022) [32, 25] for inhibit harmful virulent phytopathogens (Bhagya *et al.*, 2019; Emitaro *et al.*, 2024) [31, 67].

Today's agricultural sector is facing several types of biotic challenges (Anand *et al.*, 2023; Chaudhary *et al.*, 2022) [17, 45], including the rapid evolution like novel strain are developed of pest and phytopathogen (Anand *et al.*, 2023; Anand *et al.*, 2019; Adeleke *et al.*, 2022; Kumar *et al.*, 2021) [17, 18, 4, 202], control to conventional agrochemicals (Adetunji *et al.*, 2022; Bamisile *et al.*, 2021; Dubey *et al.*, 2020) [5, 23, 61], results, loss of soil-crop biodiversity (El-Saadony *et al.*, 2022) [66], and exceeded vulnerability of crops due to monoculture strategies (Bharti *et al.*, 2025; Negi *et al.*, 2025) [33, 134]. Chemical control measures are widely adapted, while effective in the short term, often environmental contamination, health risks, and non-target effects, thereby necessitating more sustainable alternatives (Sharma *et al.*, 2025) [180]. Endophytic microorganisms especially endophytic bacteria and fungi (Akram *et al.*, 2023) [13] have emerged as potent in curing plant health (Wijesekara and Xu, 2023) [208], enhancing plant growth, and suppressing diseases (Basit *et al.*, 2021; Eid *et al.*, 2021) [27, 64] through enhance plant defence and eco-friendly mechanisms (Hardoim *et al.*, 2015) [81].

Endophytes are group of different types of microbes that colonize and establish, internal plant tissues (Kobayashi and Palumbo, 2000; Mengistu, 2020) [102, 125] without causing any apparent disease (Carroll, 1986; Petrini, 1991) [40, 155]. They are exhibiting numerous properties and play a crucial role in promoting plant growth, inducing stress tolerance (Shaffique *et al*., 2022; Ullah *et al*., 2019) [178, 196], and offering protection against harmful pathogens (Rabiae *et al*., 2025; Radouane *et al*., 2024) [163, 164], thereby contributing significantly and perform to enhance plant health and productivity (Tariq *et al*., 2025; Pandey *et al*., 2023) [191, 179]. Their internal colonization permits them to interact intimately with the plant's metabolic and defence responses, enabling both localized and systemic benefits (Riseh *et al*., 2025; Ryan *et al*., 2008) [167, 171], further enhances their appeal for inclusion in integrated pest and nutrient management practices (Sena *et al*., 2024) [177], especially under organic or resource-conserving agricultural models (Kandel, 2016; Santoyo *et al*., 2016) [93, 176].

From a biological control point of view, endophytes have antagonistic biotic effects on plant pathogens through several well-characterized mechanisms (Fite *et al*., 2023; Doty *et al*., 2022; Pandit *et al*., 2022) [68, 60, 145]. For example, the production of antimicrobial compounds *e.g.*, antibiotics, lipopeptides, volatile organic compounds (Nimbulkar *et al*., 2025; Prabhu *et al*., 2025; Narayanasamy *et al*., 2023; Panwar *et al*., 2021) [138, 158, 130, 147], induction of host defence responses through systemic resistance pathways, competition for ecological niches and nutrients (Gulyamova *et al*., 2025; Mamarasulov *et al*., 2025) [120], enzymatic degradation of phytopathogen cell walls (Thomas *et al*., 2024; Gagné-Bourque, 2015) [192, 70], and siderophores production (Chagas *et al*., 2018) [42] that sequester essential micronutrients like iron (Compant *et al*., 2005; Pieterse *et al*., 2014) [51, 156].

The earth's surface diversity of endophytes is vast yet widely underexplored (Sivalingam *et al*., 2024; Dos Reis *et al*., 2022; Aghdam and Brown, 2021; Harrison *et al*., 2020) [185, 59, 8, 84], as traditional culture-dependent methods often don't cover to their hidden complexity (Mametja *et al*., 2025) [121]. Chaurasia *et al.* (2018) [46] suggested that *Actinomycetes*: an unexplored microorganism for plant growth promotion and biocontrol in vegetable crop.

According to Kayode *et al.* (2025) [98] previous discoveries have denoted that nearly 300,000 species worldwide-harbour one or more endophytic microbial communities. Endophytes live multiple domains of life, including bacteria, fungi, actinomycetes, and even archaea. Among bacterial endophytes, diversity is commonly dominated by members of the phylum *Proteobacteria* (alpha, beta, and gamma classes) (Anand *et al*., 2023) [17], with the most frequently found genera like *Bacillus*, *Pseudomonas*, *Enterobacter*, *Streptomyces*, *Klebsiella*, *Azospirillum*, and *Rhizobium* (Ahmed *et al*., 2024). These organisms may be Gram-negative rods or Gram-positive spore-formers (Beskrovnaya *et al*., 2021) [30] and exhibit high metabolic mechanism (Soni and Keharia, 2021) [186] and can survive in the dynamic internal environments of plant tissues (Andryukov *et al.*, 2020; Van Elsas *et al*., 2019) [19, 198]. Fungal endophytes dominated by the genera such as *Penicillium*, *Trichoderma*, *Neotyphodium* *Fusarium* non-pathogenic strains, *Cladosporium*, and *Aspergillusm* (Grabka *et al.*, 2022; Fontana *et al.*, 2021; Al-Ani *et al.*, 2019) [75, 69, 15], these genera have shown the ability to confer stress tolerance and enhance pathogen resistance in host plants (Deepa *et al.*, 2024; Akram *et al*., 2023; Arnold *et al*., 2001) [56, 13, 21].

Tariq *et al.* (2025) [191] strongly suggested that nowadays advances in Next-Generation Sequencing technologies have revolutionized the identify of these symbiotic endophytic microbes by overcoming the limitations and challenges of traditional culture-dependent techniques. NGS offers a powerful base for exploring the genomic, transcriptomic, and functional potential of endophytic microbes (Shishodia *et al*., 2025; Verma *et al*., 2024; Bielecka *et al*., 2022; Chen *et al*., 2022; Wani *et al*., 2022; Bosamia *et al*., 2020) [18, 203, 34, 48, 207, 38] at unprecedented depth. It has become a powerful platform in modern life sciences, investigating innovations in consumer genomics, translational research, and diagnostics at molecular level (Kaur *et al*., 2024; Sagita *et al*., 2021; Kaul *et al*., 2016) [133, 173, 96]. Platforms such as Illumina NGS enable high-throughput sequencing (Prasanna *et al*., 2022) [161] that reveals genetic variations, taxonomic diversity, and metabolic pathways within microbial communities (Patel *et al*., 2025; Deepa *et al*., 2024) [149, 56]. These tools have analysed that each plant species harbours a unique core microbiome that can be altered by genotype (Maestro-Gaitán *et al*., 2025; Tabassum *et al*., 2024; Latz *et al*., 2021; Mina *et al*., 2020) [118, 190, 109, 126], developmental stage (Bintarti *et al*., 2022) [35], environmental conditions (Sahu and Mishra, 2021), and agricultural practices (Berg *et al*., 2014) [29].

According to Deepa *et al*. (2024) [56] through metagenomic, meta-transcriptomic, and functional genomic approaches, NGS enables the discovery of novel genes, biosynthetic pathways, and secondary metabolites with potential biotechnological applications. Additionally, the integration of Next Generation Sequencing technology with microbial biotechnology opens new doors for crop improvement in sustainable agriculture by developing the biofertilizers, bio-control, and stress-resilient crop varieties presented in Figure1.

This review therefore focuses the endophytic microbial advancement through NGS technology: unlocking the power of the genome", highlighting on scenario of endophytes, the interconnected with plants surrounds, advantages, their limitations and challenges, how to identified their potential genes or traits or endophytes through Next Generation Sequencing technologies-driven discoveries are shaping their application through case studies in agricultural microbial biotechnology and beyond.

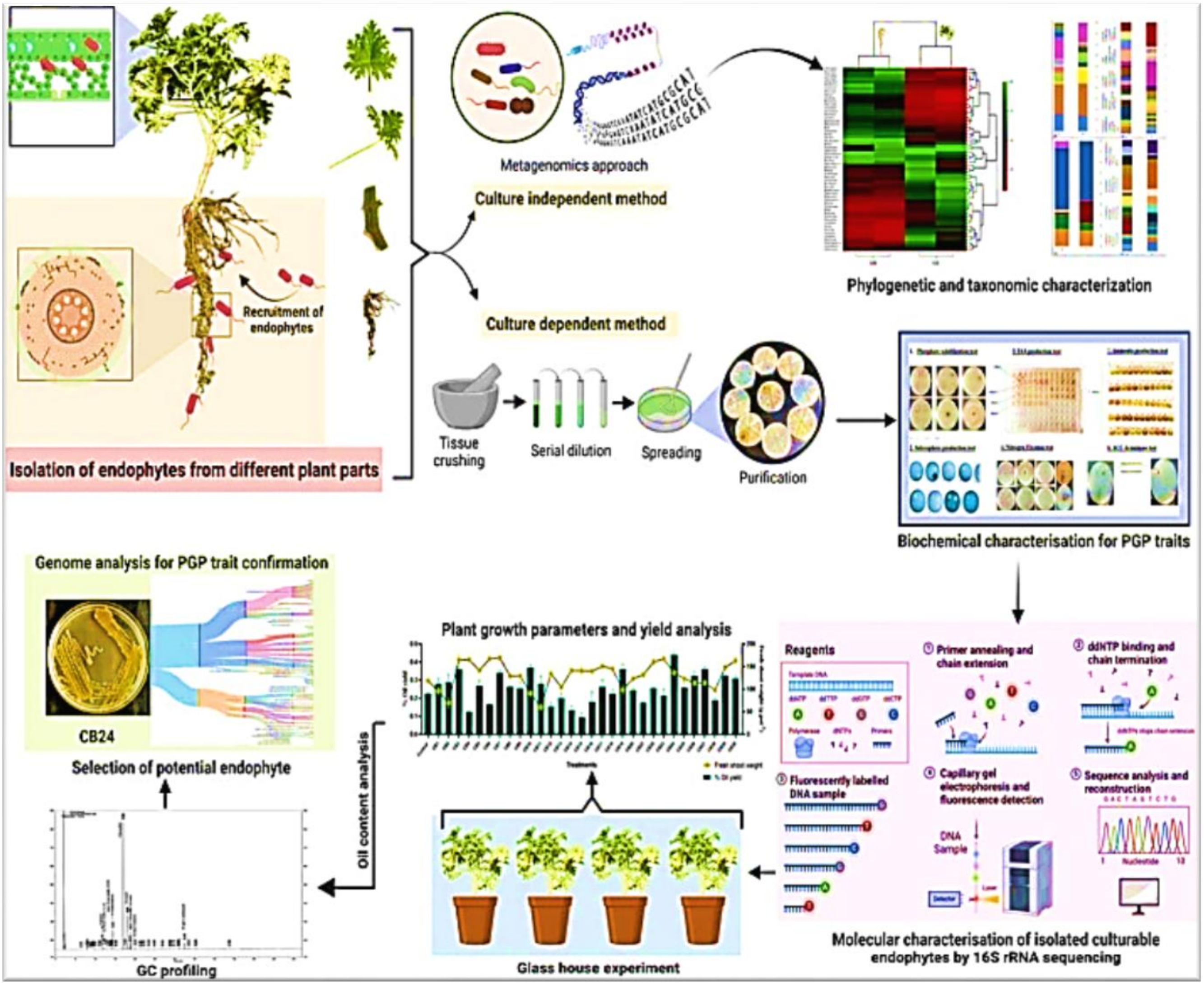


Fig 1: Endophytic Microbial Analysis through Traditional Culture-Dependent Methods and NGS Technology (Adapted from Deepa *et al*.,2024)

Endophytic Microbes

Endophytic microorganisms are having a taxonomically wide range and ecologically diverse group that colonize (Nair *et al*., 2014) [129] and establish the internal plant tissues without causing any disease symptoms or damages (Harrison and Griffin, 2020) [83]. These endophytes occupy a crucial ecological niche, symbiotic relationship (Kuźniar *et al*., 2025; Zou *et al*., 2025) [108, 218] that can wide range from mutualistic to commensal (Bard *et al*., 2024; El-Metwally *et al*., 2023) [24, 65] and sometimes opportunistic (Mishra *et al*., 2021), based on the host physiology, biochemical and environmental conditions (Hajji-Hedfi *et al*., 2025; Hakansson *et al*., 2018) [78, 79]. Endophytes are not only widespread across terrestrial and aquatic plant (Lazar *et al*., 2022) [110] but also are recognized as integral form of the plant holobiont systems (Pramanic *et al*., 2023; Durand *et al*., 2021; L'Hoir *et al*., 2021; Lyu *et al*., 2021) [160, 62, 111, 116] a functional unit forming the host and its relation with microbial communities (Grzyb *et al*., 2024; Anand *et al*., 2023; Dittami *et al*., 2021; Khare *et al*., 2018) [76, 17, 57, 101].

According to Vasileva *et al*. (2019) [199] some common endophytic bacterial genera isolated from agronomic plants reported in literature and found that diversity of endophytes in leguminous plants are more as comparison to non-leguminous plants.

Verma *et al*. (2021) [202] revealed that potential application of endophytes is stress controller to manangement of abiotic stress from drought and salinity in crop plants, through different mechanism.

Plant-Microbial relationships at phyllo-spheric and rhizospheric zone

Zhao *et al*. (2024) [217] reported that endophytic microbes and rhizospheric microbes are efficient to promote growth of the plants directly as well as by indirectly through increasing macronutrient and mineral uptake, plant protection against pathogens respectively presented in Figure 2. Naturally synthesized bioactive compounds with antimicrobial activities can be exploited in various sectors, especially in the Agricultural Biotechnology sectors.

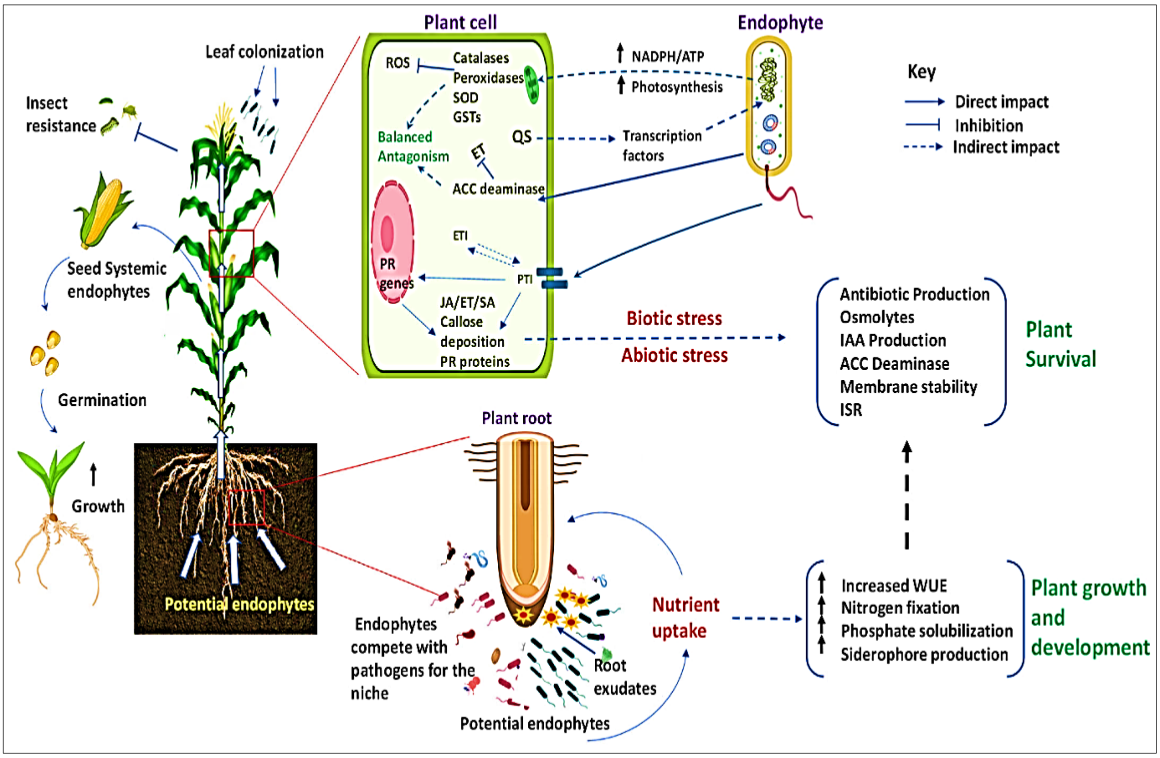
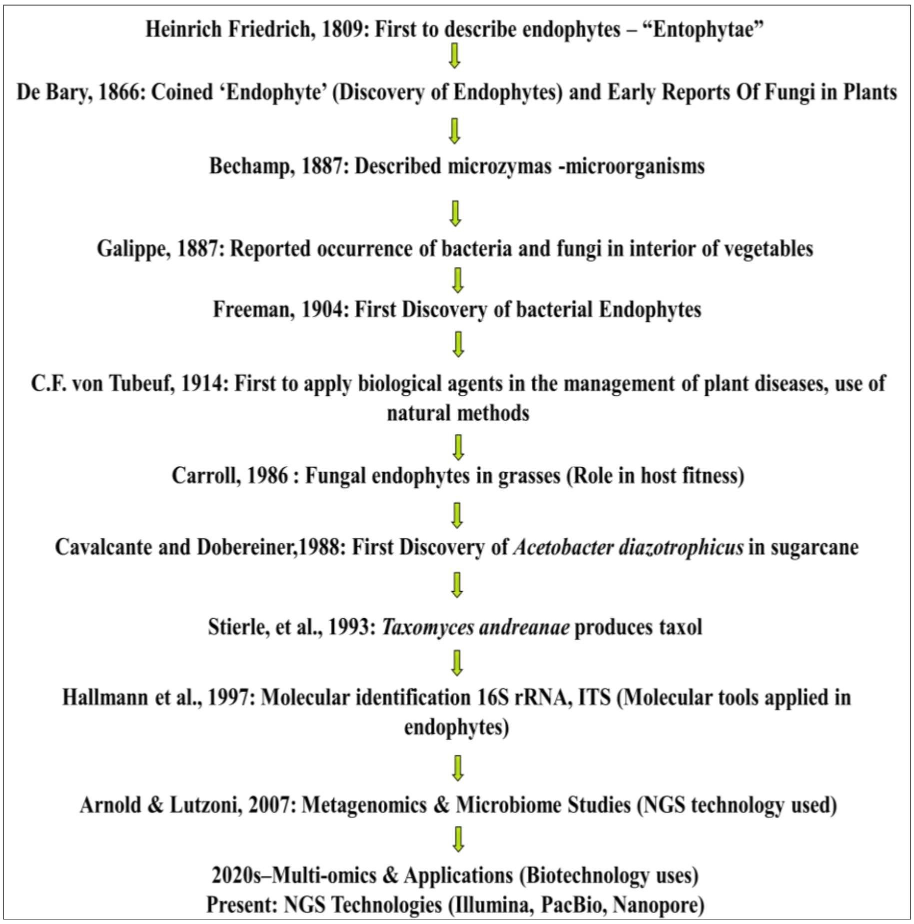


Fig 2: Mechanisms of Plant-Endophytic Microbial-Interaction (Adapted from Zhao *et al*.,2024)

Timeline of Endophyte Research and identification

Endophytes were first described by J. H. Friedrich Link, the German botanist, in 1809. They were about plant parasitic fungi. the French scientist Béchamp were later coined the term as "microzymas". There was a strongly belief that plants were healthy under sterile conditions and it was not until 1887 that Victor Galippe identified bacteria normally occurring inside plant tissues (Hardoim *et al*., 2015) [81], history of endophytic microorganism shown in Figure 3.



Heinrich Friedrich, 1809: First to describe endophytes – "Entophytae"

De Bary, 1866: Coined 'Endophyte' (Discovery of Endophytes) and Early Reports Of Fungi in Plants

Bechamp, 1887: Described microzymas -microorganisms

Galippe, 1887: Reported occurrence of bacteria and fungi in interior of vegetables

Freeman, 1904: First Discovery of bacterial Endophytes

C.F. von Tubeuf, 1914: First to apply biological agents in the management of plant diseases, use of natural methods

Carroll, 1986 : Fungal endophytes in grasses (Role in host fitness)

Cavalcante and Dobereiner,1988: First Discovery of *Acetobacter diazotrophicus* in sugarcane

Stierle, et al., 1993: *Taxomyces andreanae* produces taxol

Hallmann et al., 1997: Molecular identification 16S rRNA, ITS (Molecular tools applied in endophytes)

Arnold & Lutzoni, 2007: Metagenomics & Microbiome Studies (NGS technology used)

2020s–Multi-omics & Applications (Biotechnology uses)
Present: NGS Technologies (Illumina, PacBio, Nanopore)

Fig 3: A schematic representation, timeline of endophytic microbial research and identification in 1809 up to present year

Classification of Endophytic Microorganisms

According to Das *et al*. (2025) [159] obligate endophytes rely redefined as entirely on the host plant for growth, development, establishments, and reproduction without host can't survive. These are often associated in long-time, co-evolved relationships with specific plant lineages. In case of facultative endophytes are colonizing plant tissues under favourable conditions and free-living in the soil or rhizosphere under an unfavourable condition. Most bacterial endophytic species belong to this category (Compant *et al*., 2005; Compant and Vacher, 2019) [51, 52], presented in Table 1.

Table 1: Classification of Endophytic Microorganisms in different Categories with examples (Das *et al*., 2025)

Basis of Classification	Category	Examples
On the Basis of Microbial Type	Bacterial Endophytes	*Pseudomonas, Bacillus, Enterobacter, Klebsiella, Streptomyces*
	Fungal Endophytes	*Epichloë, Trichoderma, Fusarium, Aspergillus, Penicillium*
On the Basis of Colonization Pattern	Obligate Endophytes	*Epichloë coenophiala, Arbuscular mycorrhizal fungi*
	Facultative Endophytes	*Pseudomonas fluorescens, Trichoderma harzianum*
Transmission Mode	Vertical Transmission	*Epichloë spp. in grasses*
	Horizontal Transmission	*Fusarium, Alternaria, Colletotrichum*
Symbiotic Relationship	Mutualistic Endophytes	*Trichoderma spp., Azospirillum brasilense*
	Commensal Endophytes	*Some Penicillium spp.*
	Latent/Opportunistic Endophytes	*Fusarium oxysporum (non-pathogenic strains)*
On the Basis of Fungal Ecological Classes	Class1 (Clavicipitaceous)	*Epichloë coenophiala* (seed transmitted in tall fescue)
	Class 2 (non-clavicipitaceous)	*Trichoderma, Alternaria, Fusarium* (broad host range, colonize roots/shoots)
	Class 3 (non-clavicipitaceous)	*Colletotrichum, Ustilago* (above-ground limited colonization)
	Class 4 (non-clavicipitaceous)	*Phialocephala, Chloridium* (root-restricted, melanized)

Transmission Mode of Endophytic microorganism

Endophytes can enter plants tissue or cell through natural openings like hydathodes or stomata and lenticels, as well as through wounds on the root surface or tips (Mattoo and Nonzom *et al*., 2020; Kumar *et al*., 2020) [124, 92]. Once inside, they colonize intercellular spaces, vascular tissues and sometimes even intracellular compartments, depending on host-microbe mutualistic relation (Khan *et al*., 2025; Stotz *et al*., 2022) [100, 195], presented in Figure 4. According to Ahlawat *et al.* (2022) [9] colonization patterns are affected by both microbial traits (e.g., motility, adhesion, quorum sensing) and host factors (*e.g*., immune responses, secondary metabolite production).

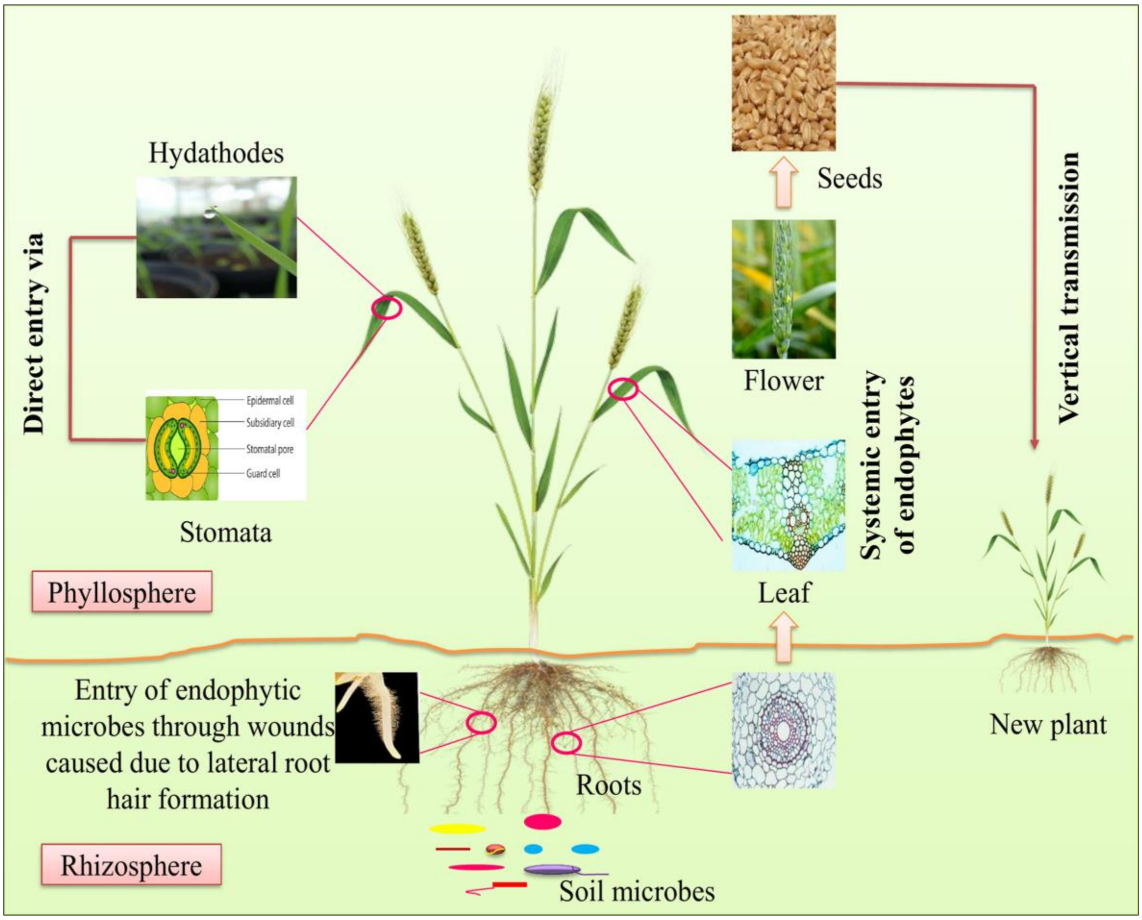


Fig 4: Entry of Endophytes through different Mode (Adapted from Ahlawat *et al.*, 2022)

Diversity Drivers

Anand *et al*. (2023) [17] reported the cluster data analysis was performed by "VOS viewer version 1.6.16," 2020 represented in Figure 5. The map highlights the most frequently used bibliographic terms to understand the most active research areas and results can be grouped into five clusters. The first cluster is represented by green balls exhibit 241 items, and devoted to stress factors and adaptation. Keywords are related to abiotic stress (*e.g.*, salinity and drought) and biotic (pathogen). Cluster 2 is represented by blue balls exhibit 224 items, keywords highlight the reactivity of endophytes, the endophytic production of metabolites, and the antibacterial activity of the obtained bioactive compounds. The third cluster is highlighted by yellow balls (192 items), and mainly concerns colonization mechanisms, with several keywords devoted to culture, and bacterial and fungi growth. Cluster 4 is represented by violet balls, 75 items, and devoted to remediation, with keywords related to contamination and detoxification. The keywords contaminants denote to heavy metals and organic pollutants. Finally, cluster 5 is exhibit 168 items, represented by red balls, mainly devoted to genome and genetic expressions.

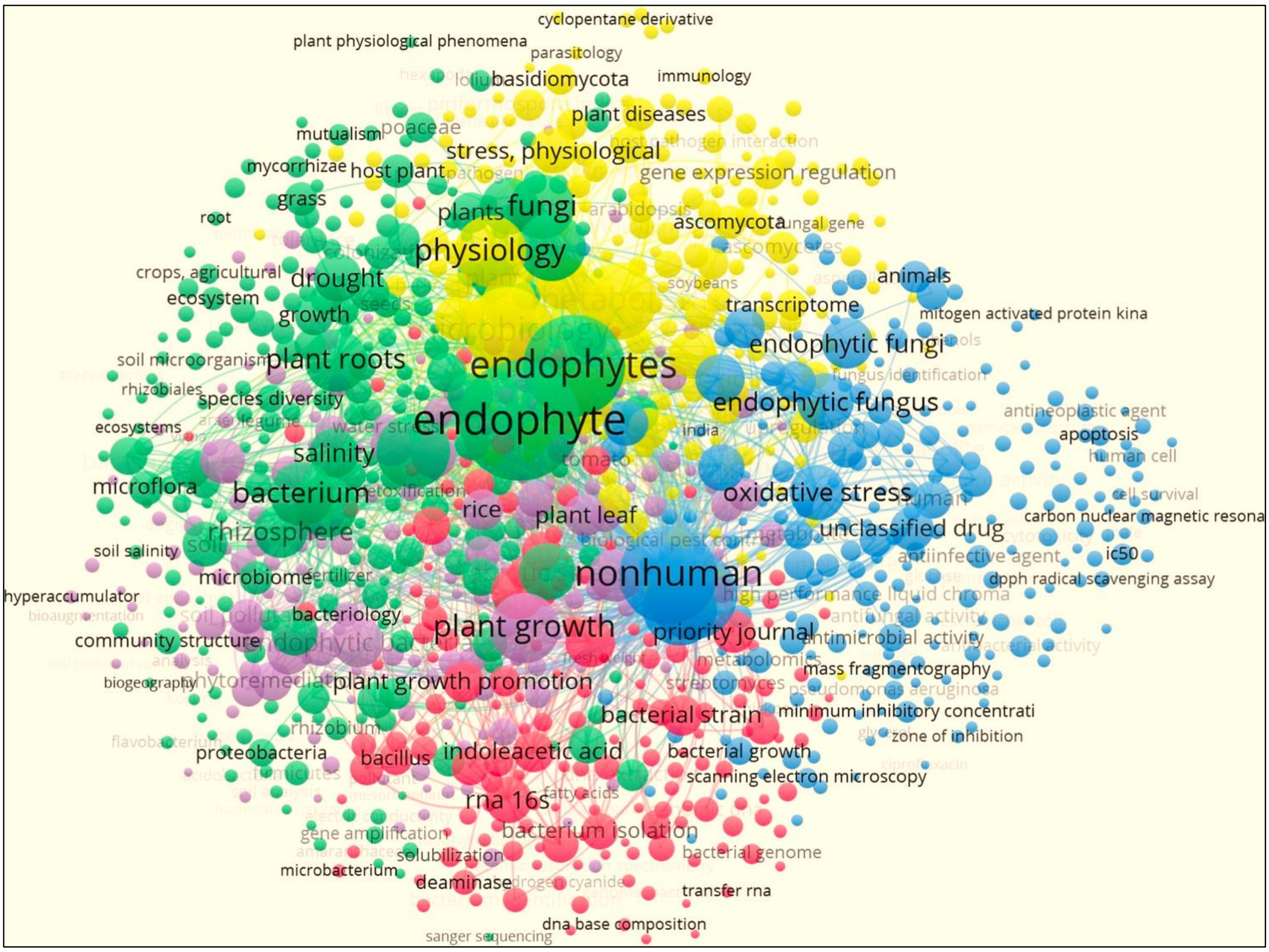


Fig 5: Reports the cluster analysis provided from the co-occurrence network of keywords of the papers extracted from the SCOPUS platform (Adapted from Anand *et al*., 2023)

Endophytic community consisted by a range of biotic and abiotic factors (Kandel, 2016) [93].

Plant genotype and species: The host plant genome makeup affects its root exudates substance and physicochemical process, which in turn modulate according to microbial needs.

Tissue specificity: Endophytic populations vary with its living plant parts like roots, stems, leaves, flowers, and seeds, each offering unique microhabitats.

Developmental stage: The successional dynamics of plant growth affect microbial turnover and taxonomical functional.

Environmental variables: Soil types, pH, EC, temperature, moisture level, and agricultural management practices *e.g.*, chemical pesticide and fertilizer use, crop rotation and patterns significantly influence endophytic functions.

Advantages of using Endophytic microorganism

Endophytic microorganisms exhibit advantages through a complicated and multifaceted array of mechanisms, dealing with direct antagonism, boost of plant immunity, and competitive ecological interactions (Lu *et al*., 2021) [189]. These mechanisms are the result of millions of years of co-evolution between plants and their associated microbiota and reflect both microbial lifestyle and ecological resilience. The studying of these association at a molecular, cellular and physiological level is important for making effective biological control strategies in modern agriculture, presented in Figure 6 (Tariq *et al*., 2025) [191]. Chaudhary *et al*. (2022) [45], and Kumar *et al.* (2022) [45] suggested that endophytes are promising biofertilizers in crop improvement for agriculture production. Field-level applications of endophytic biocontrol agents have observed promising results under natural disease pressures. Aarthi *et al*. (2025) [1] conducted an experiment in both greenhouse and field condition, application of a consortium of *Bacillus subtilis* and *Trichoderma viride* significantly minimized the incidence of disease like damping-off, root rot, and leaf spot in vegetable crops. There results strongly suggested that consortium of *Bacillus subtilis* and *Trichoderma viride* acted synergistically, providing broad-spectrum control and promoting plant growth.

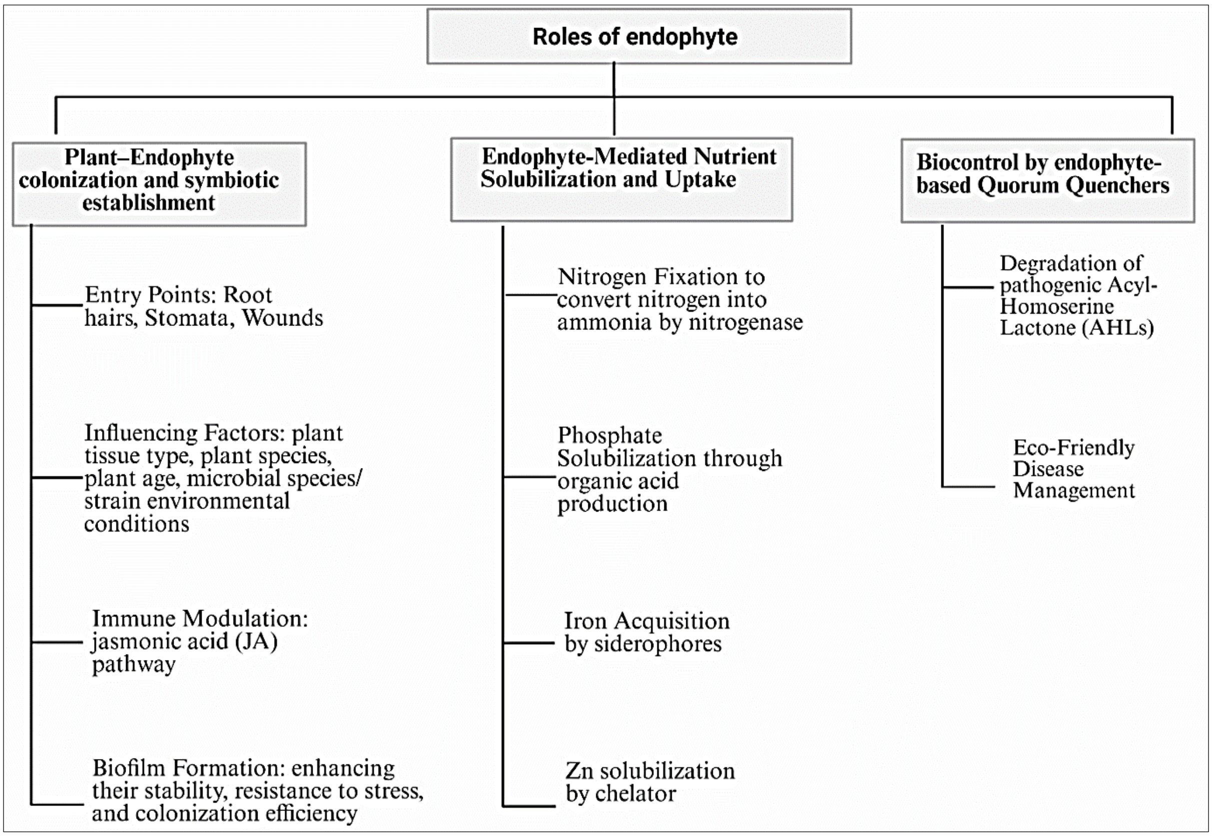


Fig 6: Advantages of Endophytic Microorganism through different Strategies (Adapted from Tariq *et al*., 2025)

Antibiosis and Secondary Metabolite Production: One of the most effective mechanisms of the plant for the production of bioactive secondary metabolites with antimicrobial compounds (Basit *et al*., 2021; Jain *et al*., 2019; Zaynab *et al*., 2018) [27, 89, 215]. These include antibiotics, cyclic lipopeptides, phenazines, siderophores, terpenoids, and volatile organic compounds VOCs, (Heredia-Bátiz *et al*., 2025; Navarro *et al*., 2019) [85, 131] and these compounds directly inhibit the growth or reproduction of pathogenic fungi, bacteria, or nematodes (Jha *et al*., 2022) [90] by disrupting cell wall integrity (Gogoi *et al*., 2024) [73], inhibiting essential enzymes, or generating reactive oxygen species (Oyebamiji *et al*., 2024) [173]. For example, *Bacillus subtilis* produces lipopeptides degrading substances such as surfactin, fengycin, and iturin, which form pores in fungal membranes, leading to cytoplasmic shrinkage finally death of the cell (Ongena and Jacques, 2008) [141]. Similarly, *Pseudomonas fluorescens* synthesizes phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol (DAPG), both of which are efficient antifungal agents (Raaijmakers *et al*., 2002; Njuguna, 2025) [162, 139]. Endophytic *Streptomyces* spp. produces a broad spectrum of antibiotics that exhibit antagonism activity toward various phytopathogenic species *e.g*., *Fusarium*, *Ralstonia*, and *Pythium* spp.

Competition for Nutrients and Ecological Niches: Endophytic microorganisms exhibit most powerful strategy, suppress the pathogens through various way like niche exclusion (Wallis, 2021) [205], effectively living internal plant parts tissues (Omomowo and Babalola, 2019) [140] and competing with potential pathogens for space and limited nutrients (Liu *et al*., 2017) [113] such as iron, nitrogen, and carbon sources (Blumenstein *et al*., 2015) [37]. The ability of endophytes to colonize, stabilize the plant environment before pathogen (Bamisile *et al*., 2021) [23] and confers a competitive edge, minimizing the chances of pathogen establishment (Gómez-Lama *et al*., 2025; Khan *et al*., 2025) [74, 100]. This mechanism is important in root systems where nutrient fluxes are high and microbial interactions are intense (Dutilloy *et al*., 2022) [63].

According to Neilands, (1995) [136] iron is a critical micronutrient, and many endophytes produces siderophores high-affinity iron-chelating compounds to sequester iron from the rhizosphere to apoplast. By low iron availability, they create a hostile environment for iron-dependent pathogens.

Induced Systemic Resistance (ISR): ISR defines to the activation of plant defence responses triggered by beneficial microorganisms, including endophytes (Oukala *et al*., 2021; Tonelli *et al*., 2020; Rashid and Chung, 2017) [142, 193, 166] Unlike systemic acquired resistance (SAR), which is mediated by salicylic acid (SA)-dependent pathway (Sun *et al*., 2021; Islam *et al*., 2019) [189, 88] and triggered by pathogenic infection (Kamle *et al*., 2020; Roychowdhury *et al.*, 2024) [92, 170], ISR is often mediated by jasmonic acid (JA) and ethylene (ET) pathways (Yanti, 2019; Yang *et al.*, 2015) [212, 211] and does not necessary the presence of a pathogen (Cho *et al*., 2008) [49].

Endophytes such as *Pseudomonas fluorescens* and *Trichoderma asperellum* have been observed to prime host plants for a quick and stronger defence response against pathogen attack (Aarthi *et al*., 2025) [1]. This priming involves enhanced expression of pathogenesis-related (PR) genes or protein, production of reactive oxidative enzymes (e.g., peroxidases, polyphenol oxidases), and accumulation of secondary defence metabolites (Adeleke *et al.*, 2022) [3].

Production of Lytic Enzymes: Many endophytic microorganism's production of extracellular hydrolytic enzymes for capable of degrading pathogen cell walls (Sahoo *et al*., 2025; Riseh *et al*., 2024; Panicker and Sayyed, 2022) [174, 167, 146] and include chitinases, β-1,3-glucanases, proteases, cellulases, and lipases (Chandoliya *et al*., 2023; Admassie *et al*., 2022; Lopes *et al*., 2021) [43, 6, 114]. This enzymatic degradation and ruptured the structural integrity of fungal and bacterial pathogens, leading to lysis finally death (Kandi *et al*., 2022) [94]. For example, *Trichoderma* spp. has these enzymes in a coordinated manner to parasitize and degrade fungal hyphae a process called as mycoparasitism (Harman *et al.*, 2004) [82]. Similarly, *Streptomyces* and *Bacillus* spp. secretes proteases and chitinases enzymes that lysis pathogen cell walls in situ.

Productions of Volatile Organic Compounds (VOCs): Endophytes produce a wide range of low molecular weight compounds Volatile Organic Compounds (VOCs) with biocontrol activities (Ling *et al.*, 2024) [112] such as antibacterial, nematocidal and antifungal, activities (Karslı and Şahin, 2021; Roy *et al*., 2019) [95, 169], *viz.*, 2,3-butanediol, hydrogen cyanide (HCN) and acetoin, have been identified and reported to suppress pathogen growth, development and spore germination, and other activities (Naz *et al*., 2022) [132]. These compounds also have provided beneficial effects on plant physiology, enhancing plant health growth, biotic and abiotic stress tolerance.

Activation of Phytohormone Levels: Most of these beneficial endophytes are produce phytohormones (Younas *et al*., 2025; Cosoveanu *et al*., 2021; Xu *et al*., 2018) [213, 53, 210] such as indole-3-acetic acid (IAA), ethylene, gibberellins, cytokinin, and abscisic acid (ABA). These compounds not only promote plant growth, development and other activities but also mediate stress responses and fortify plant defences (Sharma *et al*., 2023) [184]. Additionally, hormone mimics or hormone-activating enzymes e.g., ACC deaminase help avoid abiotic stresses and stop pathogen-induced senescence, and enhancing plant vigour (Sadaf *et al*., 2016) [172].

Biofilm Formation and Colonization Resistance: Biofilm formation defence characteristics by endophytic bacteria creates a physical and biochemical barrier that suppresses pathogen colonization (Ajijah *et al*., 2023; Pinski *et al.*, 2019) [11, 157]. Biofilms are complex extracellular polymeric substances (EPS) produced by endophytic microbial communities (Velmourougane *et al*., 2017) [200] and promote adherence to plant tissues, protect against environmental stresses, and provide nutrient exchange (Gogoi *et al*., 2021) [72]. Endophytic strains of *Bacillus velezensis* and *Pseudomonas putida* have resulted strong biofilm-forming capabilities, contributing to persistent colonization and pathogen exclusion characters (Patil *et al*., 2022) [150].

Quorum Quenching and Signal Interference: Some endophytes have quorum quenching activity, disrupting the communication systems of phytopathogens (Kusari *et al*., 2015) [107] that rely on quorum sensing (QS) to coordinate virulence (Venkatesh *et al*., 2019) [201], biofilm formation, and toxin secretion (Joo *et al*., 2021; Alagarasan *et al*., 2017) [91, 14]. Endophytes serve as N-acyl homoserine lactone (AHL) lactonases (Paul *et al*., 2023) [152] and acylases enzymes degrade signalling molecules like N-acyl homoserine lactones (AHLs) (Pellissier *et al*., 2021; Shastry and Rai, 2017) [153, 181], hindering the pathogens incapable of searching coordinated attacks (Anandan *et al*., 2019) [18].

These mechanisms enable endophytic microorganisms to function as more effective and multifaceted biocontrol agents (Abid Mehmood *et al*., 2025) [2]. Their internal stabilization, capacity for symbiotic relationship, and breadth of antagonism activity make them evenly equipped for deployment in sustainable crop protection and management strategies (Negi *et al*., 2024) [133].

A broad-level understanding of these interactions provided by multi-omics technologies, synthetic biology, and microbial ecology will be key to utilizing endophyte-based formulations for real-world agricultural.

Microbial Biotechnology Advancement through NGS

Recent era has supported advancements in Next-Generation Sequencing (NGS) technologies, revolutionizing of endophytic microbiomes and our understanding their functions in plant growth promotion and biocontrol. Culture-dependent methods, still valuable but have some limitations in failing to cover the full spectrum of microbial diversity within plant tissues. NGS technologies enables high-throughput, culture-independent profiling to identify of endophyte communities at an unprecedented resolution, allowing researchers to characterize and analyse microbial composition, function, and interaction, compatibility with high accuracy (Tariq *et al.*, 2025) [191].

Meta transcriptomics and Meta-Proteomics: These advanced omics technologies offer snapshots of endophytic gene expression (RNA-seq) and protein study under *in vivo* plant colonization surrounding. By detecting real-time interactions between endophytes and host plants, scientists and researchers can identify genes involved in immune priming, oxidative stress response mechanism, and signal response pathways. Meta transcriptomic studies offer in *Pseudomonas* and *Streptomyces* endophytic genera have linked expression of defence-mechanism proteins with phytopathogen suppression (Mabaso *et al*., 2025) [117]

Metagenomics and Functional Annotation: Whole-genome shotgun metagenomics techniques surpass amplicon sequencing by providing dept study into the functional potentialities or capabilities of endophytes (Huang *et al*., 2023) [86]. Researchers to reconstruct metabolic pathways, detect biosynthetic gene clusters their expression responsible for antibiotics, siderophores, and phytohormones, and study horizontal gene transfer that transfer to biocontrol traits. For instance, metagenomic analysis has performed that certain *Bacillus* endophytes possess non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) gene clusters responsible for lipopeptide production (Patil *et al*., 2025) [151].

Genome-Wide Association Studies (GWAS): Integrating NGS with GWAS enables researchers analyse to link between plant genetic traits and endophytic microbiome structure and function (Kumar *et al*., 2025) [106]. These interactions provide information, how to boast plant immunity, metabolic signalling, and receptor expression affect microbial colonization, establishment, and biocontrol potential (Mani and Kushwaha, 2023) [122].

High-Throughput 16S rRNA and Internal Transcribed Spacer (ITS) Amplicon Sequencing: PCR-based sequencing of conserved genomic regions, such as 16S rRNA for endophytic bacteria (Zhang *et al*., 2022**)** [216] and Internal Transcribed Spacer for fungi, facilitates taxonomic analysis into complex endophytic communities (Singh *et al*., 2023; Błaszczyk *et al*., 2021) [184, 36]. Through this technology has covered numerous unexplorable and unidentified endophytic microbes in cereals, leguminous and non-leguminous crop plants, such as wheat, maize, rice, and soybean. For their genome sequencing studies using Illumina Mi Seq and Hi Seq platforms have demonstrated (Dong *et al*., 2023) [58] that each plant species hosts a unique reservoir of microbiome, affected by genotype, development stage, and surrounding (Bulgarelli *et al*., 2012) [39].

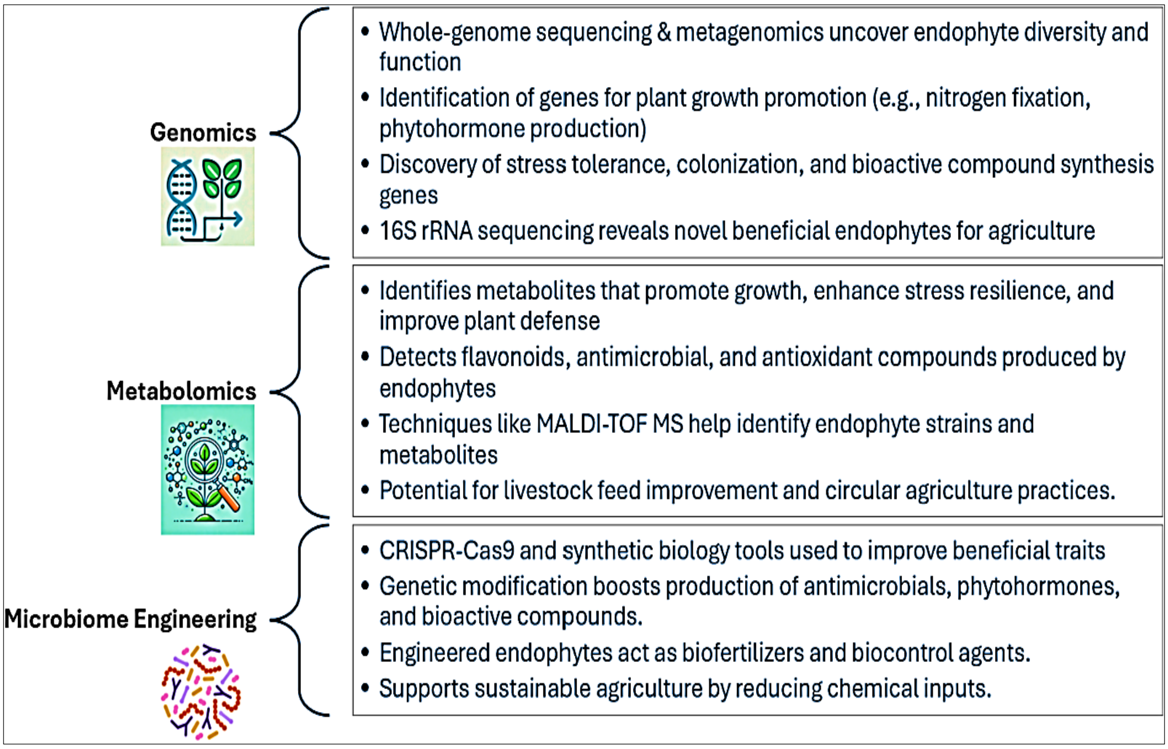
Comparative Genomics and **Pan-Genomics:** These technologies permit for the comparison of various endophytic microbial strains from the same or different species or genera knowing conserved and unique genomic sequences (Wang *et al*., 2025; Mahmoud *et al*., 2024; Peng *et al*., 2024; Chen *et al*., 2007) [214, 119, 156, 47]. Comparative genomic studies provide difference between pathogenic and non-pathogenic endophytic microbial genera, like *Fusarium* (Neik *et al*., 2020) [135] or *Xanthomonas* strains (Passarelli-Araujo *et al*., 2020) [148].

Single-Cell Genomics: This emerging technique enables the genomic characterization of individual unculturable and unidentified endophytes isolated from internal plant part tissues (Pradhan *et al*., 2025; Shishodia *et al*., 2025; Utturkar *et al*., 2016) [159, 182, 197]. It holds promise for resolving intragenic variability, strain-specific traits, and efficient endophyte-plant specificity. It has been mainly useful in studying low-diversity microbial taxonomy that could be allows for host defence (Cole *et al*., 2024) [50].

NGS-Enabled Bioprospecting: NGS technology offers to researchers can be identify microbial genomes for novel antimicrobial peptides, biosynthetic gene clusters, differential gene expression, and enzymes with potential agricultural applications. These studies are vital for developing next generation endophytic bioinoculants with multi-functional efficacy (Xu *et al*., 2022) [209].

Moreover, the integration of transcriptomics, proteomics, and metabolomics are leveraged to study and offers a framework into real-time microbial metabolism and cross-kingdom signaling, and apply endophytes for improved crop yield and stress resilience.

Manna *et al.* (2025) [123] Strongly suggested that omics-based strategies to enhance agricultural productivity through endophyte research. A schematic representation of how genomics, transcriptomics, proteomics, and metabolomics are leveraged to study and apply endophytes for improved crop yield and stress resilience presented in Figure 7.



- Whole-genome sequencing & metagenomics uncover endophyte diversity and function
- Identification of genes for plant growth promotion (e.g., nitrogen fixation, phytohormone production)
- Discovery of stress tolerance, colonization, and bioactive compound synthesis genes
- 16S rRNA sequencing reveals novel beneficial endophytes for agriculture

- Identifies metabolites that promote growth, enhance stress resilience, and improve plant defense
- Detects flavonoids, antimicrobial, and antioxidant compounds produced by endophytes
- Techniques like MALDI-TOF MS help identify endophyte strains and metabolites
- Potential for livestock feed improvement and circular agriculture practices.

- CRISPR-Cas9 and synthetic biology tools used to improve beneficial traits
- Genetic modification boosts production of antimicrobials, phytohormones, and bioactive compounds.
- Engineered endophytes act as biofertilizers and biocontrol agents.
- Supports sustainable agriculture by reducing chemical inputs.

Fig 7: Microbial Biotechnology Advancement through NGS **(**Adapted from Tariq *et al.,* 2025)

Several types of case studies have been reported and explored how Next Generation Sequencing has accelerated the identification, research and application of endophytes in crop achievement, health management, and microbial biotechnology.

Patel *et al.* (2025) [149] reported an endophyte of *Brucella* sp. PM1, from pomegranate, and analysis through whole-genome NGS, technology revealing ~3000 plant growth-promoting trait genes mapped to both direct mechanisms - hormone secretion, nutrient uptake and indirect mechanisms stress tolerance, colonization, and biocontrol. Their evidence showing capability for secondary metabolite production (ochrobactin), heavy-metal detoxification, and phytohormone biosynthesis pathways beyond IAA. There results concluded that *Brucella* genus is a good PGP candidates and identifying multifaceted beneficial traits through whole genome sequencing (WGS).

Yu *et al*. (2025) [214] identified *Klebsiella pneumoniae* strain YMK25, from maize, and demonstrated multiple in-vitro Plant Growth Promoting traits *viz.*, high IAA, N-fixation, inorganic/organic P solubilization, and siderophore production, sequenced and annotated through whole genome sequencing 5.12 Mb, 4,746 CDS, predicted PGP traits or related genes- nif/ure/gln, trp/ipdC, pho/phn, ent/fep, etc., and showed that YMK25 enhances maize seedling growth and soil nutrients in pot trials. Their results strongly suggested that YMK25 is a promising biofertilizer candidate.

Dong *et al*. (2023) [58] identified root endophytic microbial communities change across the growth stages of ratooning rice (cv. Jiafuzhan). NGS-based sequencing (16S + ITS) revealed clear shifts in root endophytic diversity across rice life stages. Their finding revealed that bacterial diversity highest at tillering stage, decreased at heading and ratooning and fungal diversity peaked at flowering and filling stages. Dominant bacterial endophytes Phyla: *Proteobacteria, Actinobacteria, Firmicutes.* Genera: *Pseudomonas, Burkholderia, Bacillus* and Dominant Fungal Endophytes Phyla: *Ascomycota, Basidiomycota.* Genera: *Fusarium, Trichoderma, Aspergillus.* Identify dominant endophytes (bacteria: genes for nitrogen fixation, organic matter decomposition, stress adaptation and fungi: functional guilds for saprotrophs, symbiosis, and plant defence) linked with plant growth, nutrient cycling, and stress tolerance. Their results demonstrated that NGS technology advances biotechnology by decoding endophytic diversity and functions, offering potential for biofertilizer and biocontrol applications in ratooning rice.

Huany *et al.* (2024) used to Next Generation Sequencing technology (Illumina high-throughput sequencing) for identification of the diversity of endophytic fungi in *Camellia reticulata* pedicels. In these studied revealed that *C. reticulata* pedicels host rich and diverse endophytic fungal communities, dominated by Ascomycota. All the isolates have broad antagonism activities against to nectar yeasts. *Alternaria alternata* D23 results showed that highest antimicrobial potential and producing multiple antibiotics. NGS technology strongly revealed endophytic fungal diversity and reported 1,034 OTUs (4 phyla, 24 classes, 77 orders, 161 families, 267 genera), while 4,036 metabolites (including amino acids, fatty acids, vitamins, nucleotides) uncovered novel bioactive compounds. Findings suggest that endophytic fungi contribute to nectar stability, plant defence, and reproductive fitness in cross-pollinated plants.

Endophytes can synthesize a larger number of secondary metabolites, including antibiotics, and plant growth promotion and disease suppression compound like siderophores, phytohormones, and VOCs etc. The redundancy and multifunctionality of these metabolites underscore the ecological importance of endophytes as keystone members of the plant microbiome. The endophytic lifestyle is spending within plant thought to be an evolutionary prospectus for microbial longevity, enabling colonization of a nutrient-rich and relatively protected environment. In return, plants benefit from enhanced nutrient acquisition, disease resistance, and stress tolerance. Some endophytes even exhibit vertical flow through seeds, indicating long-term host association and better co-evolution (Hardoim *et al*., 2015) [81].

The advancement of NGS technologies has overview expanded our capacity to find out the complexity of endophytic microbiomes and their ecological and functional roles in plant disease suppression. Future integration of multi-omics technologies data with AI-based analytics and systems. Researchers will further investigate, to optimization, and deployment of endophytic biocontrol agents in sustainable agriculture.

Overall, at functional characterization and taxonomic level diversity of endophytic microorganisms affects their ecology and evolutionary relationship. Identifying the complex interaction between host plant and endophytic microorganism at cellular, molecular, and ecological levels is important for their efficient characterization in biocontrol in sustainable agriculture.

Limitations and Challenges of Next Generation Sequencing Technology

Despite its power, Next Generation Sequencing technology faces numerous challenges and limitations like cost effective, incomplete reference databases, expertise for advanced bioinformatics studies, lack of standardized protocols causes variability across studies and difficulty in distinguishing true endophytic microbes from contaminants. Need of high-level functional predictions for experimental validation presented in Figure 8.

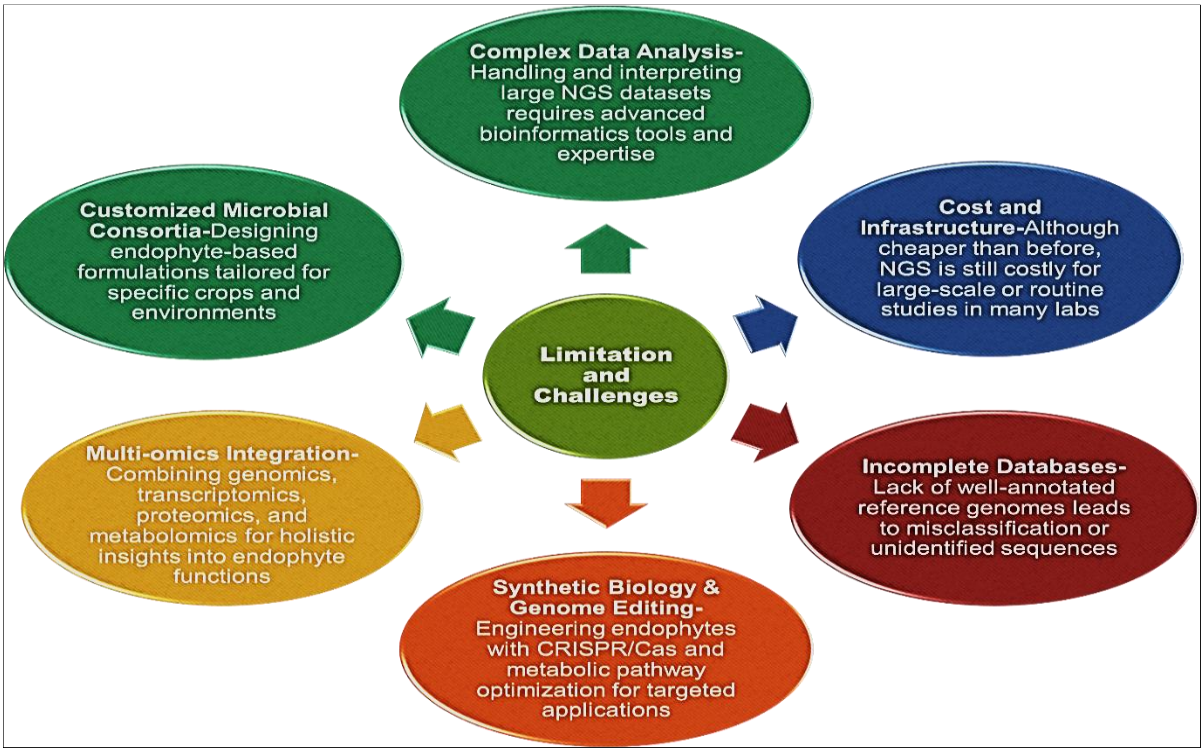


Fig 8: Limitations and Challenges of NGS Technology

Conclusion

Endophytic microorganisms represent a powerful antagonistic activity against disease or phytopathogens through defense mechanism pathway and their modes of action, ability to colonize internal plant tissues, and potential to enhance plant growth make them ideal candidates for sustainable agriculture. Future discovery and development should focus on overcoming the current drawback and exploring their full potential for commercial applications in agricultural sector. Endophytic microorganisms hold great potential in agriculture. In advancement of Next-generation sequencing (NGS) technologies has revealing their hidden diversity, functional traits genes, and bio-physio-chemical metabolic pathways beyond conventional methods. These technologies accelerate the discovery of efficient endophytes as biofertilizers, novel metabolites and biocontrol agents, in conclusion Next-generation sequencing (NGS) technologies making endophytes valuable potential tools for sustainable biotechnology. Integrating Next-generation sequencing (NGS) technologies with multi-omics and synthetic biology will further unlock their applications in sustainable agriculture for crop improvement, environmental sustainability.

Commercial Applications and Future Prospects

The commercialization of endophytic biocontrol agents is maintaining, with several products already available or under process. Advances in molecular biology, genomics, and bioinformatics and nanotechnology are facilitating the identification and characterization of promising potent endophytes. The future holds potential for engineering endophytes with enhanced biocontrol potent traits catch several diseases. Formulation of microbial consortia to facilitate broad level disease resistance, integration into integrated pest management programs. Further using to CRISPR-Cas based gene editing technology to investigate and manipulate plant-microbial interactions.

Conflict of Interest

The Authors declare no conflict of interest

Acknowledgement

The authors have no acknowledgements to make in this review article

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