



# Exploring Endophytes of Ginger for Sustainable Plant Growth Promotion and Secondary Metabolite Production

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**Abstract:** Endophytes, residing within plant tissues, have garnered attention for their ability to enhance plant health and contribute to secondary metabolite synthesis. Ginger, a rhizomatous and aromatic plant with medicinal properties, is renowned for its volatile oils and bioactive compounds such as gingerol, paradol, and shogaols. Widely utilized in Ayurvedic and Chinese medicine, ginger has demonstrated efficacy in treating various ailments. The bioactivity and quality of ginger extracts are intricately linked to the presence of endophytic fungi and bacteria residing within the plant's rhizomes. These endophytic microorganisms contribute significantly to the metabolite profile of ginger, producing compounds with diverse bioactivities, including antibacterial, antifungal, cytotoxic, anticancerous, antioxidant, and anti-inflammatory properties. The synthesis of these metabolites is often triggered by host-specific phytopathogens. Interestingly, many of these metabolites have been identified to possess characteristics that foster plant development. This review explores the potential of the endophytic community within ginger and delves into the role of their metabolites in enhancing plant growth, shedding light on the intricate interactions between plants and microbes. Understanding these dynamics not only enriches our knowledge of plant-microbe associations but also unveils the promising prospects of harnessing the endophytic potential for sustainable plant cultivation and medicinal applications.

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## 1. Introduction

*Zingiber officinale*, also known as ginger, a native plant to South Asia belonging to the Zingiberaceae family, is a monocotyledonous herb that is extensively

used as a spice, food, and medicine. The largest producer of Ginger is India, and majority of the production happens in Kerala. The name Ginger is suggested to have come from the regional Tamil word 'Injiver'. The Ginger rhizome is extensively used in the cuisine and research as it acts as a potential source for bioactive phenolics such as gingerols, paradols etc (Srinivasan *et al.* 2017). In addition to its flavor-enhancing abilities, ginger is renowned for its antioxidant, anti-inflammatory, antibacterial, and antipyretic activities.

Endophytes are endosymbiotic of bacteria and fungi that inhabit the internal parts of plant tissues (Singh and Dubey, 2015). Endophytes enhance the growth of the host plant and help to absorb nutrients from the soil and improve its resistance to abiotic or biotic stressors. Endophytes are colonized in any part of a plant like the stem, roots, petioles, leaves, inflorescences, fruits, seeds and also inside the dead and hollow hyaline cells of plants (Kobayashi and Palumbo, 2000). Endophytes can interact with the foreign invaders; thus, plays an important role in the development of plants. The population of endophytes in a plant varies and depends upon the type of host species, the developmental stage of the host, and environmental condition (Gouda *et al.* 2016). The presence of endophytic microbes from ginger plant and its role in direct and indirect plant growth promotion are reported (Rohini *et al.* 2018, Jasim *et al.* 2014, Sabu *et al.* 2019 and Chu *et al.* 2011)

The presence of essential oils and metabolites like shogaols, gingerols, and zingerone imparts the characteristic flavour of ginger. The rhizome is speculated to include glycolipids and diterpenes in addition to these chemicals. Apart from the taste, aroma and flavour, ginger is also known for its medicinal value, enhanced by the presence of polyphenolic antioxidants. Due to a variety of chemical components, ginger rhizomes have been found to have strong anti-inflammatory, antioxidant, and cancer-preventive properties. Although endophytes are residing in every part of the ginger, most of the endophytes reported from rhizome part (Jasim *et al.* 2014, Zhang *et al.* 2018, Rahayu *et al.* 2019). However, Ginting *et al.* (2013) isolated endophytic fungi from leaf, rhizome, root, and stem of red ginger plant.

## 2. Occurrence of Endophytic Bacteria in Ginger

Ninety-six numbers of endophytic bacteria isolated from rhizome of ginger (*Zingiber officinale*) (Rohini *et al.* 2018). A total of 57 strains were isolated from the rhizome of ginger and identified and were grouped into genera *Serratia*, *Enterobacter*, *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Agrobacterium*, *Ochrobactrum*, *Bacillus* and *Tetrathiobacter* (Zhang *et al.* 2018). The presence of

four different endophytic bacterial strains were identified from ginger rhizome including *Pseudomonas* sp. (Jasimetal.2014). Distribution and population diversity of endophytic bacteria at different growth stages of ginger plants was studied by Chen *et al.* (2014). Seven endophytic actinobacterial isolates were isolated from the ginger rhizome (Rahayu *et al.* 2019) and had pancreatic lipase inhibitor activity. Endophytic *Serratia* sp. ZoB14 isolated from ginger rhizome was found to have inhibitory effect towards *Pythium myriotylum* and also against other pathogens (Sabu *et al.* 2019 ). Chu *et al.* (2011) isolated *Pseudomonas* sp., *Bacillus* sp., *Brachybacterium* sp., *Stenotrophomonas* sp. and *Rahnella* sp. from ginger plants. Studies of Huang *et al.* (2022) indicated that the composition of the endophytic microbiota underwent a shift during the progression of rhizome rot disease. *Enterobacteriaceae*, *Lachnospiraceae* and the bacterial genera *Clostridium*, *Bacteroides*, *Acrobacter*, *Dysgonomonas*, *Anaerosinus*, *Pectobacterium* and *Lactococcus* were relatively abundant in the bacterial community of rhizomes exhibiting bacterial decay symptoms but were also present in asymptomatic rhizomes. Bacterial flora from *Z. zerumbet* endosphere and rhizosphere yielded a total of 109 isolates from different tissue sets *viz.*, leaves, rhizomes, axenically grown *in vitro* plantlets and rhizosphere (Peter *et al.* 2021).

### 3. Occurrence of Endophytic Fungi in Ginger

A total of 563 endophytic fungal isolates were isolated from the leaves and rhizomes of ginger which were grouped into 12 species belonging to 8 genera based on morphological characters and Internal Transcribed Spacers (ITS) sequence analysis (Gupta *et al.* 2022). Endophytic fungi included *Acremonium macroclavatum*, *Cochliobolus geniculatus*, *Colletotrichum gloeosporioides*, *Curvularia affinis*, *Glomerella cingulata*, *Lecanicillium kalimantanense*, *Myrothecium verrucaria*, *Rhizopycnis vagum* etc. were successfully isolated from different parts of ginger plant (Ginting *et al.* 2013). *C. gloeosporioides* was found in stem and leaf, whereas *G. cingulate* and teleomorph of *C. gloeosporioides* was found in rhizome of red ginger plant (Ginting *et al.* 2013). Bussaban *et al.* (2001) found that *C. gloeosporioides* and its teleomorph stage *Glomerella* spp. and *Phomopsis* spp. were dominant endophytes on wild ginger *Amomum siamense*. Effect of ginger endophyte *Rhizopycnis vagum* on rhizome bud formation and protection from phytopathogens was studied (Anisha *et al.* 2018). Six fungal morphotypes were isolated from the rhizome fragment of torch ginger (*Etilingera elatior* (Jack) RM Smith) and five of them related to *Trichoderma* spp. while one isolate was identified as *Pestalotiopsis* sp. (Lutfia *et al.* 2020). Fungi were observed to

predominate bacterial species when isolated from rhizomes of wild ginger congener, *Zingiber zerumbet* Smith with colonization frequency values ranging from 12.5 to 50% (Keerthi *et al.* 2016). Arbuscular mycorrhizal and dark septate endophyte fungal associations in two dominant ginger species (*Zingiber montanum* and *Z. officinale*) of northeast India was studied (Pandey *et al.* 2020). Both the gingers had dual colonization of AMF and DSEF structures in different cortical cells of the same examined root segments and revealed the Intermediate type of AM morphology.

#### 4. Production of Plant Growth Metabolites by Ginger Endophytes

A type of auxin hormone produced by plants, indole-3-acetic acid (IAA) modifies cell orientation, organ development, fertility and cell elongation among other cellular processes to affect plant development. The endophytic bacteria isolated from the ginger plants were reported for the IAA production (Jabborova *et al.* 2020, Rohini *et al.* 2018, Zhang *et al.* 2018). In order to control the growth and development of their host, bacterial pathogens and symbionts of plants and algae also create IAA (Labeeuw *et al.* 2016). Endophytic bacterial species growing in ginger rhizome roots accounts for the increased Indole Acetic Acid (IAA) amount while compared with that of the control plant rhizomes grown in similar conditions. This aids in the plant growth enhancement (Rohini *et al.* 2018). According to the recent reports, *Pseudomonas*, *Pantoea agglomerans*, *Aeromonas*, *Serratia*, *Enterobacter asburiae* and *Rhizobium* were among the organisms in the samples with high levels of IAA production (Chen *et al.* 2014).

As with the cultivation of any other plant, growing ginger is likely to have deleterious plant pathogens. The main ailments that impact the production of ginger crops include soft rots, bacterial wilts, leaf spots, etc. The most prevalent of these is soft rots, which is a fungus caused by *Pythium* sp., followed by bacterial wilt infection caused by *Ralstonia* sp. *Phyllostic* sp. And *Pyricularia* sp. are responsible for leaf spots.

Soft rots also referred to as rhizome rots are caused by *Pythium* and *Fusarium* sp, which are fungi whereas it is also caused by bacterial species like *Ralstonia*. Although, the soft rot disease is commonly occurring, it doesn't imply that the disease is of not grave nature. The rhizome rot disease is a highly infectious, destructive disease that accounts for the destruction of plant. The pathogen infects the roots, collar, and succulent sections of the rhizome to cause the disease. Various physical, biological and chemical measures have been considered and practiced to ward off the infections of soft rot that have actively reduced the disease (Rai *et al.* 2018). The symptoms of

soft rot are lessened by a number of bacterial species that exhibit antagonistic activity, according to earlier reports. *Bacillus* species are one of the biocontrol agents that live in the rhizosphere of plants and are thought to be a source of resistance to unfavourable environments. They support plant growth and health and show promising anti-microbial properties (Gerayeli *et al.* 2018). The endophytic bacterial strains isolated from ginger rhizome found to have the ability to produce IAA, ACC deaminase and siderophore (Jasim *et al.* 2014). The endophytic *Serratia* sp. isolated from ginger, the rhizome was observed to have the presence of an array of plant growth promoting traits with in vivo growth enhancement effect on *Vigna unguiculata* seedlings (Sabu *et al.* 2019).

## 5. Antagonistic Activity of Endophytes from Ginger

The antagonistic activity of isolated endophytic fungi against *F. oxysporum* varied with the inhibition value range from 1.4 to 68.8%. *C. affinis* (JMbt7), *F. solani* (JMd14), and *G. cingulata* (JMr2) had significantly high antagonistic activity with the value above 65%; (Ginting *et al.* 2013). Endophytic microorganisms from *Zingiber officinale* were screened for *in vitro* inhibition against *Pythium myriotylum*. From this, *Burkholderia vietnamiensis* ZoB74 was selected as an organism with remarkable antifungal effect (Rohini *et al.* 2018a). Biocontrol potential of fungal endophyte *Aspergillus terreus* isolated from leaves of ginger and its bioactive metabolite terrein, against ginger leaf spot phytopathogen, *C. gloeosporioides* was reported (Gupta *et al.* 2022). The ginger endophyte *Rhizopycnisvagum* was found to prevent *P. myriotylum* infection in ginger with associated enhancement in germination and bud development (Anisha *et al.* 2018). Ginger endophytic fungi including *Trichoderma* and *Pestalotiopsis* sp. displayed potential antifungal activities against *Fusarium oxysporum*, *Ganoderma boninense*, and *Rigidoporus lignosus* as representative phytopathogens in a dual culture plate assay (Lutfia *et al.* 2020). The growth inhibition of *Pythium myriotylum*, an economically significant phytopathogen of cultivated ginger by the ginger endophytes *Fusarium Solani* and *F. oxysporum* isolates were reported (Keerthi *et al.* 2016). *Pseudomonas aeruginosa*, a ginger endophyte, has a very potent inhibitory effect on *Pythium myriotylum* (Jasim *et al.* 2014). Endophytic *Paraconiothyrium* sp. from *Zingiber officinale* Rosc displayed broad-spectrum antimicrobial activity by production of danthronan anthraquinone derivative (Anisha *et al.* 2018). *Bacillus pumilus*, *B. amyloliquefaciens* and *B. subtilis* exhibited antagonism to *P. myriotylum* (Peter *et al.* 2021).

Rhizomes of *Zingiber zerumbet* collected from their natural habitat and reported earlier to have high zerumbone content were selected for isolation

of endophytes. Isolate identified as *Klebsiella aerogenes* yielded maximal antagonistic activities against *P. myriotylum*. Volatile metabolites were extracted and GC-MS metabolite profiling detected alkanes and fatty acid methyl esters as the predominant constituents in the solvent extracts. (Harsa and Nair, 2020). Antimicrobial peptides extraction from rhizome of *Zingiber Zerumbet* Showed the inhibitory potential of necrotrophic *Pythium myriotylum* revealed maximal inhibition of proteases (75.8%) compared to other hydrolytic enzymes (Raj *et al.* 2020).

## 6. Secondary Metabolite Production

Endophytic microbes residing in ginger rhizomes and the metabolites produced by these fungi may have a possible role in the bioactivity of the ginger extracts, quality of the spice, as well can have effect on human health, as these are not removed by routine washing. These metabolites are often produced against host specific phytopathogens and may show a broad range of bioactivity which includes antibacterial, antifungal, cytotoxic, anti cancerous, antioxidant, and anti-inflammatory activity. Many metabolites have plant growth promoting properties. Thus, these endophytes play an important role in the plant yield and disease resistance shown by improved varieties. Metabolites produced by endophytic microbes are also important in the context of raw consumption of ginger as medicine and spice. Among the four varieties studied, fifteen isolates were identified. The various genera identified were *Acremonium* sp., *Gliocladiopsis* sp., *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp., *Phlebia* sp., *Earliella* sp., and *Pseudo Aureobasidium* sp. The endophytes are unique, due to varying host genotypes. The bioactive metabolites from fungal extracts include tyrosol, benzene acetic acid, ergone, dehydromevalonic lactone, N-aminopyrrolidine, and bioactive fatty acids and their derivatives such as linoleic acid, oleic acid, myristic acid, n hexadecanoic acid, palmitic acid methyl ester, and methyl linoleate. The presence of bioactive endophytic fungi is the reason for the differences in the performance of the different ginger varieties (Anisha and Radhakrishnan, 2017).

Endophytes helps in the protection and survival of host plants with production of a repertoire of chemically diverse and structurally unprecedented secondary metabolites reported to exhibit an incredible array of biological activities including antimicrobial, anticancer, antiviral, antioxidants, antiparasitics, immunosuppressants, immunomodulatory, antithrombotic, and biocontrol ability against plants pathogens and nematodes (Toghueo *et al.* 2019). Endophytic actinomycetes isolated from surface-sterilized plant tissue strongly

inhibit *Colletotrichum musae* and *Fusarium oxysporum* (Taechowisan *et al.* 2003). *Streptomyces* sp. isolated from the root tissues of *Alpinia Galanga* Antagonist of phytopathogenic fungi; *Alternaria porri*, *Colletotrichum gloeosporioides*, *Colletotrichum musae*, *Curvularia* Sp., *Drechslera* sp., *Exserohilum* Sp., *Fusarium oxysporum*, *Verticillium* sp. and *Sclerotium rolfsii*. 3-methylcarbazole and 1-methoxy-3-methylcarbazole are the major active ingredients purified from the culture filtrate and the crude extract from *Streptomyces* sp. (Taechowisan *et al.* 2012).

Anisha *et al.* (2022) characterized metabolite(s) of non-pathogenic, endophytic *Fusarium oxysporum* from *Zingiber zerumbet* rhizome to display antagonism towards *Pythium myriotylum*. The same isolate also shows the cytotoxic effect in HeLa cell lines with IC<sub>50</sub> value of 57.9 lg/ml. An endophytic fungus isolated from rhizomes of *Zingiber zerumbet*, shows radical scavenging activity and COX II inhibition (Nongalleima *et al.* 2013). Fungal endophytes from *Z. nimmonii*, as potential sources of antioxidative and DNA protective compounds. *Bipolaris specifera*, *Alternaria tenuissima*, *Aspergillus terreus*, *Nectria haematococca* and *Fusarium chlamydosporum* extracts exhibited a potentially high antioxidant capacity. Characterization of the extracts revealed an array of phenolic acids and flavonoids. *N. haematococca* and *F. chlamydosporum* extracts contained quercetin and showed DNA protection ability (Das *et al.* 2017). *Streptomyces zerumbet* W14, a novel species of the endophyte genus *Streptomyces*, was isolated from the rhizome tissue of *Zingiber zerumbet*. The isolated compounds were identified to be methyl 5-(hydroxymethyl) furan-2-carboxylate and geldanamycin. Bioassay studies showed that compound 1 had antibacterial activity against *Staphylococcus aureus* ATCC 25923 and methicillin resistant *S. aureus* strain Sp6 (clinical isolate) (Taechowisan *et al.* 2019).

## 7. Future Perspectives

Due to the upsurge of disease burden in the world, there is a high demand for newer drugs. From the studies conducted on endophytes in the last two decades have proved that they are capable of producing new drugs (Saxena *et al.* 2021). Medicinal plants and the endophytes which are associated with it have many important bioactive compounds and secondary metabolites that contribute to most of the natural drugs available in the pharmaceutical industry (Singh and Dubey, 2015). Endophytes are rich in novel secondary metabolites that are effective for antiarthritic, antimicrobial, anticancer, antidiabetic, anti-insect, and immunosuppressant activities (Jalgaonwala *et al.* 2011). The bioactive compounds, such as camptothecin, diosgenin, hypericin,

paclitaxel, podophyllotoxin, and vinblastine, commercially produced by endophytic microbes in plants have important roles in both agricultural as well pharmaceutical industries (Joseph and Priya, 2011).

Bacterial endophytes are a source of bioactive compounds that helps enhance the growth and developments of plants through various mechanisms. Gathering further knowledge on the symbiotic relationship of hosts and endophytic pathogens through experimental models might contribute much more to the agricultural–scientific field. The advancement in scientific technology has in fact enhanced our ability to gain more knowledge on the topic yet, there are several areas that remain dormant. Considering the importance of endophytes which are associated with wild and domestic plants, studies should be conducted to explore them for both agricultural and medicinal applications. To understand and utilize the benefits offered by endophytic microbes we are to learn and grasp on the various species in the community and how well they interact with each other and with that of the plants.

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