

# Chapter 15

## Diversity, Ecology, and Conservation of Fungal and Bacterial Endophytes



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**Abstract** Every plant present on the earth is accompanied by internal association of microbes generally pronounced as endophytes. Bacterial and fungal endophytic microbes are among one of them. Their presence was noted in various niches ranging from mountain to sea, from forest to desert, and, moreover, in cold and in hot spring, copper mine wasteland, agronomic crops, prairie plants, deepwater rice, and grass ecosystem. These endophytic microbes are agriculturally important because of their various plant growth-promoting traits. They are found to inhabit the seeds, roots, stems, and leaves and even the periderm. These endophytic microbes generally enter the plant tissues via several “hotspots” like root system and mitigate with biotic and abiotic stresses, help to cure human diseases by producing several secondary metabolites, help in the induction and expression of plant immunity, exclude plant pathogens by niche competition, as well as actively participate in phenylpropanoid metabolism and antioxidant activities. The discoveries of novel bioactive compound and defense activator like antifungal, antibacterial, antiviral, and antitumor compounds, antibiotics, secondary metabolites, and volatile insecticides attributed to these endophytes are utilized as therapeutic agents in the field of pharmaceutical, medicine, agriculture, and industries. The conservation of endophytic microbes and their gene pools is an emerging and vital issue, even though the development is scary.

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## 15.1 Introduction

The plant's intercellular or intracellular spaces harbor a huge sort of unnoticed group of microorganisms known as endophytes. Fungal and bacterial microbes are among one of them. De Bary conceptualized the term "endophyte" in (1866). With the advancement of research in the field, definitions of endophytes have been modified several times. According to Tervet and Hollis (1948), endophytes are those microorganisms which live inside the plant without any disease symptoms. Petrini (1991) explained endophytes as an internal association of microorganism with plant at any stage of its life cycle without giving any disease symptoms which are further modified by Sikora et al. (2007) who termed this internal association of microorganism with plants may be beneficial, neutral, or detrimental to its host plant. The literature meaning of endophyte is "inside the plant," and the microorganisms which inhabit the inner tissue of the plant are known as endophytic microorganism (Fig. 15.1). It may be further extended to endophytic fungus, bacteria, or actinomycetes (Pandey et al. 2012, 2014, 2016). Almost all plants are the dwellers of these indigenous microbes which can reside intercellularly or intracellularly and responsible for nutrient assimilation, their processing, induction of the defense system, and synthesis of secondary metabolites (Pandey et al. 2017).

History of exploitation of fungal and bacterial endophytes for their plentiful novel bioactive compounds (Strobel et al. 2002; Harper et al. 2003) and their use in agriculture (James 2000), biotechnology (Berger 2009), and pharmaceutical (Strobel et al. 2004; Gangadevi and Muthumary 2008) is a century long. The fungal and bacterial endophytes are reported to produce novel bioactive compounds which are previously untapped such as insecticides (Ryan et al. 2008), antimicrobials (Liu et al. 2010a; De Melo et al. 2009; Wang et al. 2009; Bacilio-JimeÂnez et al. 2001), etc. responsible for induction of immune response and restriction of pathogen entry inside the plant tissues (Sturz et al. 2000; Lodewyckx et al. 2002). Production of non-food crops for biomass and biofuel production is also reported from bacterial endophytes (Rogers et al. 2012). Fungal and bacterial endophytes have been known to play a significant role in soil fertility, plant growth, and biocontrol activities through various mechanisms. Stable microbial endophytic communities with defense arsenals could able to control plant pathogens by producing antifungal metabolites and antibiotics, de novo synthesis of structural compounds, induction and expression of molecular-based plant immunity, exclusion of other organisms from niche competition, or induced systemic resistance (ISR). Fungal and bacterial endophytes are sometimes much more bioactive by inducing rapid and stronger responses against stress in the host than the rhizospheric microbes or any other microbes (Pandey et al. 2012). Understanding of the plant endophytes interaction should provide a range of application.

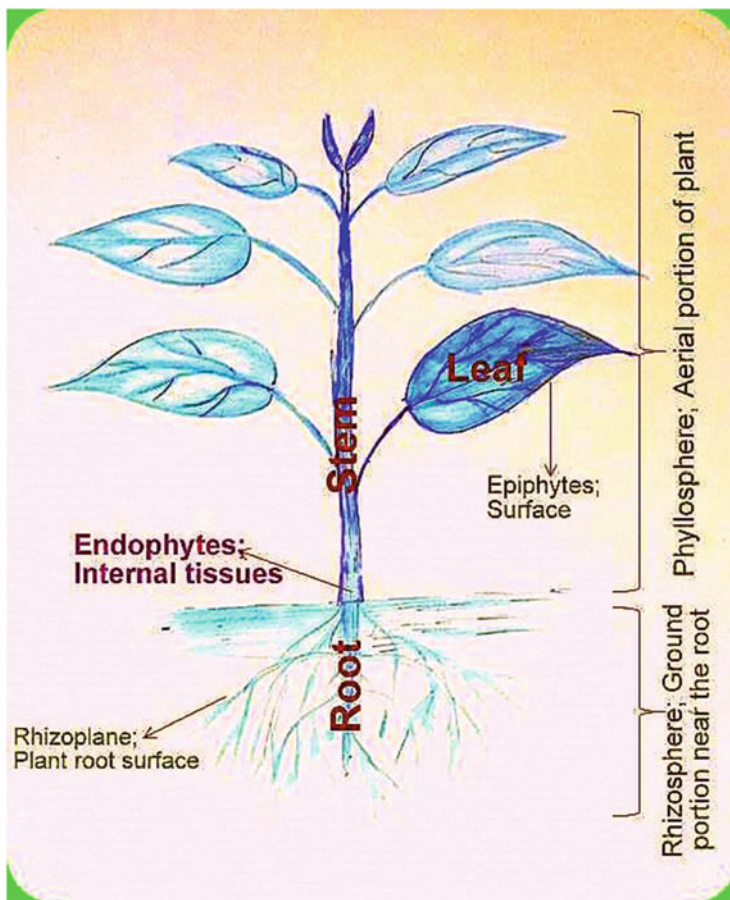


Fig. 15.1 Plant part showing localization of endophytic and epiphytic microbes

## 15.2 Evolutionary Origins and Ecological Consequences of Fungal Endophytes

Vogl in 1898 reported the presence of an endophytic mycelium inside the grass seed *Lolium temulentum*. Freeman in 1904 reported an endophytic fungus in annual grass *Persian darnel*. It is found that certain microorganisms able to penetrate the plant tissues, either with the help of cell wall-hydrolyzing enzymes like pectinase and cellulase or certain other mechanisms, and reside inside the plant tissue and coevolved. During the course of coevolution, they may be adapted toward the internal milieu of the host plant by the mechanisms of cross talk (Pathak 2011).

Colonization behavior and degree of colonization of fungal endophytes are influenced by numerous factors such as host tissue type, host genotype, the microbial taxon type, and strain type and several biotic and abiotic environmental factors

(Hardoim et al. 2015). Different colonization mechanisms for different classes of fungal endophytes have been identified:

1. *Clavicipitaceous* endophytes have narrow host range; colonize shoot and rhizome, are transmitted vertically and horizontally; and infect grasses.
2. Non-clavicipitaceous endophytes were recovered from symptomless tissues of nonvascular plants (Bryophytes), ferns, conifers, and angiosperms and have been distinguished based on phylogeny and life history traits. They have a wide host range and colonize root, shoot, and rhizome. They are transmitted vertically as well as horizontally.
3. *Balansiaceous* or grass endophytes grow within all aboveground plant organs of grasses. These types of endophytes are transmitted vertically through the seeds. They belong to the genera of clavicipitaceous which include *Epichloe* and *Balansia*.
4. Non-balansiaceous endophytes: Most of the non-balansiaceous endophytes belong to the *Ascomycota*, and their colonization pattern may be intracellular or intercellular. Most of them belong to the genera *Acremonium*, *Alternaria*, *Cladosporium*, *Fusarium*, *Geniculosporium*, *Phoma*, and so on (Rodriguez et al. 2009; Tenguria et al. 2011; Hardoim et al. 2015).

### 15.3 Host Variability and Biodiversity of Fungal Endophytes

Fungal endophytes form a variety of associations with their host which include (1) symbiotic, (2) mutualistic, (3) antagonistic, and (4) slightly pathogenic. Their associations with host plant influence ecology and evolution of both fungal endophytes and host plant. A single endophyte was found to be inhabitants of different hosts rather than in a specific host. Their distribution in the different plant tissues affected by their ability to utilize nutritional substances which could be synthesized in different plant part of the same host and their relationship in the different host varies with the nature of ecological and geographical conditions. The interaction between fungal endophytes and the host is controlled at the gene level, involving the genes of both the partners which are inflected by the environment. The encounter of endophyte by the host plant modulates the level and pattern of gene expression in the host plant. The diversity of fungal endophytes has multidisciplinary effects on the ecosystem such as the enhancement of primary productivity, retention, and flow of nutrient and along with the development of resistance to the pathogen entry. Fungal endophytes have been isolated from almost all plant groups ranging from palms, grasses, sea grasses, large trees, lichens, and medicinal plants. The diversity of fungal endophytes may vary in different plant parts with the location of host plant in different geographic sites such as temperate or tropical. Most of the fungal endophytes isolated belong to ascomycetes, and several may also belong to basidiomycetes. Endophytic fungi from aquatic, temperate, tropic, and xerophytic conditions are also reported (Pandey et al. 2014).

Endophytic fungi, *Alternaria alternata*, *Cladosporium cladosporioides*, *Chaetomium globosum*, *C. herbarum*, *Gliocladium roseum*, *Curvularia lunata*, *Nigrospora sphaerica*, and *Phyllosticta* spp. were reported as frequently occurring fungi in a leaf of medicinal climber and grasses (Shankar and Shashikala 2010). A semiarid region of Pakistan, where climatic conditions are exceedingly harsh with low rainfall, was helpful in understanding the frequency of colonization of endophytic mycoflora and species richness of medicinal plant *Withania somnifera*. Among the endophytic fungal isolates, 4 isolates belong to the class *Ascomycetes*, and 20 isolates belong to the class *Deuteromycetes*. The highest species richness was noted from the stem, and *Alternaria alternata* was found to be the most dominant endophyte. In the isolated class of fungi, *Deuteromycetes* were found to be the most prevalent (Khan et al. 2010). In a survey, it was found that sapwood of *Hevea brasiliensis* had greater endophytic fungal diversity in comparison to the leaves, while their colonization frequency was more in the leaves as compared to the sapwood (Gazis and Chaverri 2010). Endophytic fungal diversity has been investigated based on its relative frequency, isolation, and colonization rates in medicinal plants (*Adhatoda vasica*, *Ocimum sanctum*, *Withania somnifera*, *Cannabis sativa*, and *Viola odorata*) of Himachal Pradesh, India, which covers 15 fungal genera and 18 species (*A. clavatus*, *A. flavus*, *A. varicolor*, *Penicillium chrysogenum*, *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata*, *Haplosporidium* sp., *Phoma* sp., *Nigrospora* sp., *Colletotrichum* sp., *Cladosporium* sp., *Stemphylium* sp., *Fusarium* sp., *Geotrichum* sp., *Phomopsis* sp., *Trichoderma* sp., and *Rhizopus* sp.) (Gautam 2013). An effort was made to examine the diversity, distribution, and phylogeny among the endophytic fungi from the different plant sources on the basis of ITS1–5.8S-ITS2 sequence of Western Himalayas which showed diverse taxonomic affinities among isolated fungal endophytes. In the sum of 72 endophytic fungal strains isolated, only two belong to *Basidiomycetes*, whereas the rest belongs to *Ascomycetes*. *Alternaria* spp. and *Fusarium* spp. consist more than half (54.2%) of the strains isolated. Most of the genera of *Alternaria* strains were reported from *Artemisia annua* and *Rauwolfia serpentina*, whereas most of the *Fusarium* spp. were reported to be inhabitants of *Artemisia annua*, *Withania somnifera*, and *Platanus orientalis*. The fungal endophytes obtained from the conifers such as from *Pinus roxburghii*, *Cedrus deodara*, and *Abies pindrow* harbored the most diverse endophytic fungi of 13 different genera and offer significant taxonomic variants (Qadri et al. 2013).

## 15.4 Evolutionary Origins and Ecological Consequences of Bacterial Endophytes

Bacterial endophytes have been studied for more than 100 years (Anu Ranjan 2012). The first endophytic bacterial presence within the plant tissues was reported in 1926 (Hallman et al. 1997). Their relationship may have been intended to develop during the hundred million years ago along with the higher plants appeared on the Earth

(Anu Ranjan 2012). The evolution of endophytic bacteria is believed to be initiated from the symbiosis of rhizobia with leguminous plants. The interaction of rhizobia and leguminous plants is mediated by the signal exchange between them through the following steps: (1) curling of root hairs, (2) the appearance of infection threads, and, finally, (3) the appearance of nodule primordia in the inner root layers. During the course of evolution, initially the bacterial cell becomes surrounded by the plant membrane and forms the structure called symbiosome resembling to the mycorrhizal arbuscules, in which atmospheric nitrogen is fixed and transferred in exchange for the carbohydrates (Hardoim et al. 2015).

The entry of bacterial endophytes into the host involves recognition and chemotaxis for the attachment to the root surface of the host plant in the rhizosphere (Lugtenberg and Kamilova 2009). Type IV pili encoded by *pilAB* (Dorr et al. 1998), LPS (lipopolysaccharide) (Balsanelli et al. 2010), and EPS (exopolysaccharide) (Meneses et al. 2011) along with the various other components of bacterial surface are thought to be of essence for the endosphere colonization. It is believed that the endophytic bacteria in the form of several microcolonies may enter into the cells (1) through thin-walled apical root zone, (2) from the root hair zone, and (3) from the basal root zone of the emergence site of the lateral roots (Zachow et al. 2010) either as active or passive penetration and sometimes a combination of both. Active penetration takes place with the aid of cell wall-degrading enzymes (cellulolytic) like endoglucanase which hydrolyze the exodermal cell wall of plants (hydrolysis of  $\beta(1-4)$  linkage of the cellulose) (Reinhold-Hurek et al. 2006). The passive penetration takes place via cracks produced by the emergence of lateral roots in the basal root zone (Compant et al. 2005).

Bacterial endophytes provide a defense to the host plant by colonizing the tissues and the other parts earlier than the pathogenic microorganism. The colonization extent of the endophytic bacteria in the host's tissues makes them suitable for the adaptation in a specific location (Hallman et al. 1997). They have been reported to successively cross the endodermis and flourish in different plant parts as the endophytes such as in leaves, roots, stem, tubers, etc. (Yonebayashi et al. 2014; Upreti and Thomas 2015; Perez-Rosales et al. 2017; Compant et al. 2005). The strain-dependent mechanism of O-antigen chain of bacterial lipopolysaccharides (Dekkers et al. 1998; De Weger et al. 1989), vitamin B1, NADH dehydrogenases from the bacteria having a high growth rate (Dekkers et al. 1998; Simons et al. 1996), and type IV pili (Dorr et al. 1998) was found to be involved in the root colonization of the bacterial endophytes.

Bacterial endophytes from the endodermis and the root cortex of the plants have been employed to advocate that the bacteria from the rhizosphere are able to penetrate and colonize the root tissues (Sturz et al. 2000). During colonization process root cortex becomes the part of the soil-root microbial environment, which provides an apoplastic pathway of entry of microorganism (from the root epidermis to the shoot) (Algam et al. 2005). Secondary roots and the intercellular space colonization were observed after the disruption of the mucilage layer with the aid of scanning electron microscopy (Bacilio-Jimenez et al. 2001). Studies of the bacterial endophytic colonization patterns in the vegetative tissues and effects of

bacterial endophytes on the plant growth offer a better understanding of the biological interaction of the plant-bacterial endophytes (Algam et al. 2005). These studies have been performed by inoculating the endophytic bacteria into the host plants and comparing the inhibition of disease symptoms (Hastuti et al. 2012; Kumar et al. 2015; Zhao et al. 2017). Bacterial endophytes are able to penetrate deep within the plant and spread systemically to reach the aerial tissues and have been found to be an efficient biological nitrogen fixer (Verma et al. 2001). According to a report, the entry of the bacterial endophytes in the plant tissue may be accompanied (1) through the root zone, (2) by the aerial parts of the plant, (3) through the stems, or (4) by the cotyledons (Compant et al. 2010). They may either be localized at the point of entry or are able to spread throughout the plant, and such isolate can live (1) in the intercellular spaces, (2) inside cells, and up to some extent (3) in the vascular system (Sharma et al. 2005). Autofluorescent protein (AFP) and green fluorescent proteins (GFP) were employed to study the colonization and localization patterns of endophytic bacterial microbes and plant-endophytic bacterial interaction (Ryan et al. 2008; Akbari and Akbari 2017). GFP was used for the chromosomal integration and the expression of *gfp* gene in endophytic bacteria (Montalbán et al. 2017) and can be identified by the epifluorescence microscopy or confocal laser scanning microscopy (Villacieros et al. 2003; Germaine et al. 2004). The use of the  $\beta$ -glucuronidase (GUS) reporter system has been developed to monitor the bacterial colonization within the root intercellular spaces, stele, xylem vessels, stems, and other several parts of rice seedlings (James et al. 2002). Nitrogenase (*nif*) gene expression in the rice root and the responses of rice roots toward the endophytic bacterial colonization were reported (Miche et al. 2006). Investigation of the endophytic bacterial gene expression in the rhizosphere and phyllosphere by *in vivo* expression technology (IVET) and the recombination *in vivo* expression technology is very useful to study and understand the essential endophytic bacterial genes used to enter inside, to compete, to colonize, and to suppress the phytopathogens (Zhang et al. 2006; Ryan et al. 2008).

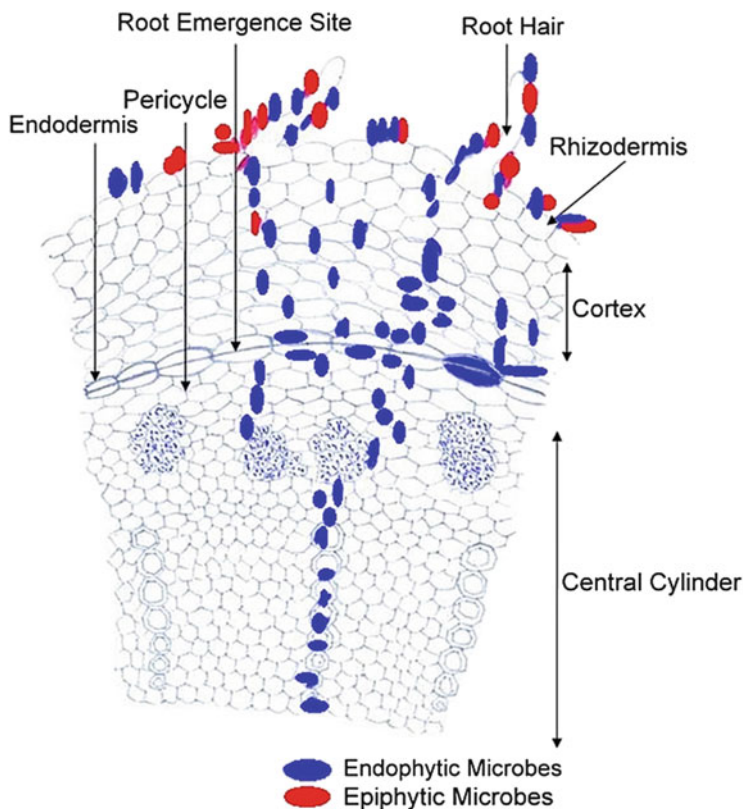
The bacterial endophytes may be beneficial, neutral, or detrimental to its host (Sikora et al. 2007). This association may be some time pronounced as endophytism, and probably all plants in their life cycle may harbor bacterial endophytes (Mercado-Blanco and Bakker 2007). They are reported to be mostly positive symbiotic to its host (Li et al. 2012; Egamberdieva et al. 2017), in which host plants provide nutrients for the growth and multiplication of bacterial endophytes and endophytes pay off in the form of releasing various active and novel metabolites which are used by the plants for its the development, regulation of physiology, and induction of defense mechanisms (Lodewyckx et al. 2002; Li et al. 2008). It is reported that the symbiotic relationship between the bacterial endophytes and plants can act as a switch between mutualistic and parasitic form, where a change in a particular gene segment may convert the endophytes into a detrimental parasitic form (Kogel et al. 2006). Hence, the bacterial endophytes from one host can obey different lifestyles in a different host. On the basis of life strategies, Hardoim et al. (2008) have described the endophytic bacteria in the following groups: (1) facultative bacterial endophytes, organisms that can (optionally or without restriction) live inside plants and in other

habitats also; (2) obligate bacterial endophytes, organisms that are (restricted) strictly bound to life inside a plant; (3) opportunistic bacterial endophytes, organisms that occasionally enter plants for their own needs; and (4) passenger bacterial endophytes, organisms that enter the plant by chance.

## 15.5 Host Variability and Biodiversity of the Bacterial Endophytes

The bacterial endophytes are found to inhabit the majority of healthy and symptomless plants. They are found to inhabit the seeds, roots, stems, and leaves and even the periderm also (Johri 2006; Mastretta et al. 2009). The presence of endophytic bacteria in the copper mine wasteland, agronomic crops and prairie plants, deepwater rice, grass ecosystem, pea cultivars in field condition, tropical grasses of the *Brachiaria* genus, and prairie plants has been reported (Zinniel et al. 2002; Verma et al. 2001; Ryan et al. 2008). These endophytic bacteria live in a variety of tissue types, inside many plant species, and thereby suggested their ubiquitous existence. Endophytic bacteria of a plant host are not confined to a single species, but they may go to several genera and species (Ryan et al. 2008). A number of endophytes from rice (Biswas et al. 2000; Ji et al. 2014; Sev et al. 2016), maize (Rosenblueth and Martinez Romero 2004; Mousa et al. 2015; de Abreu et al. 2017; Bodhankar et al. 2017), wheat (Zinniel et al. 2002; Pan et al. 2015; Robinson et al. 2015; Herrera et al. 2016), cotton (Reva et al. 2002), sorghum (Nascimento et al. 2014; Li et al. 2016), cacti (Puente et al. 2009), apple tree (Miliūtė and Buzaitė 2011; Miliute et al. 2016), bananas (*Musa* spp.) (Ngamau et al. 2012; Souza et al. 2014; Karthik et al. 2017), Antarctic moss (*Sanionia uncinata*) (Park et al. 2013), chili pepper (Paul et al. 2013), sphagnum (Shcherbakov et al. 2013), tomato (*Solanum lycopersicum* L.) (Romero et al. 2014), ginger (Chen et al. 2014), *Helianthus tuberosus* (Montalbán et al. 2017), potato, and *Arabidopsis* (Hallmann et al. 2001; Sessitsch et al. 2004; Hong et al. 2015) were reported. Beneficial bacterial endophytes have been isolated from the various plant species (Mastretta et al. 2009; Forchetti et al. 2007) along with the different plant parts like tubers, seeds, stems, buds, roots, seedlings, root bark, and plant tissues such as the intercellular space, the vascular tissue, and the periderm (Fig. 15.2), which make them suitable candidate for the plant growth promotion as they are less exposed to the external biotic and the abiotic stresses. According to an estimate, approximately 129 kinds of the bacterial endophyte consisting of both Gram-negative and Gram-positive species representing more than 54 genera were already identified from the various host crops (Zhenhua et al. 2012). Endophytic bacterial isolates from the leaves of neem (*Azadirachta indica*) have hydrocarbon degradation potential (Singh and Padmavathy 2015). Endophytic bacterial strains from the roots and crude seed extracts of a Cu-tolerant population of *Agrostis capillaris* were assessed for their influence on Cu tolerance and phytoextraction (Kolbas et al. 2015). A total of 536 bacterial and fungal endophytes from the root,





**Fig. 15.2** Sites of colonization by endophytic microbes in plant's root

stem, and leaves of *Amaranthus spinosus* were recovered successfully. It was found that the bacterial endophytes are more prominent in the root than in either stem or leaves, whereas, fungal endophytes are more prominent in stem than in either roots or leaves. Furthermore, Gram-negative endophytic bacteria were found to be more in comparison to Gram-positive endophytic bacteria in total isolate isolated (Sharma and Roy 2015). Among the 62 endophytic bacterial isolates, isolated from the leaves, roots, and stems of a healthy *Lepidium perfoliatum* L. plant, a sum of 34 isolates was isolated from the leaves, 27 were isolated from roots, and 1 isolate was isolated from stems. Among them, 66% were similar to *B. subtilis*, 14% were similar to *B. flexus*, 6% were similar to *B. licheniformis*, 5% were similar to *B. mojavensis*, 5% were similar to *B. sonorensis*, 2% were similar to *B. cereus*, and 2% were similar to *B. safensis* (Li et al. 2017). In a study, 125 strains of bacterial endophytes were identified based on 16S rRNA gene sequencing from the endangered traditional medicinal plants *Ferula* spp. in arid regions of Xinjiang belonging to 3 different phyla and 29 genera. Among the isolates, 3 strains isolated from the roots were

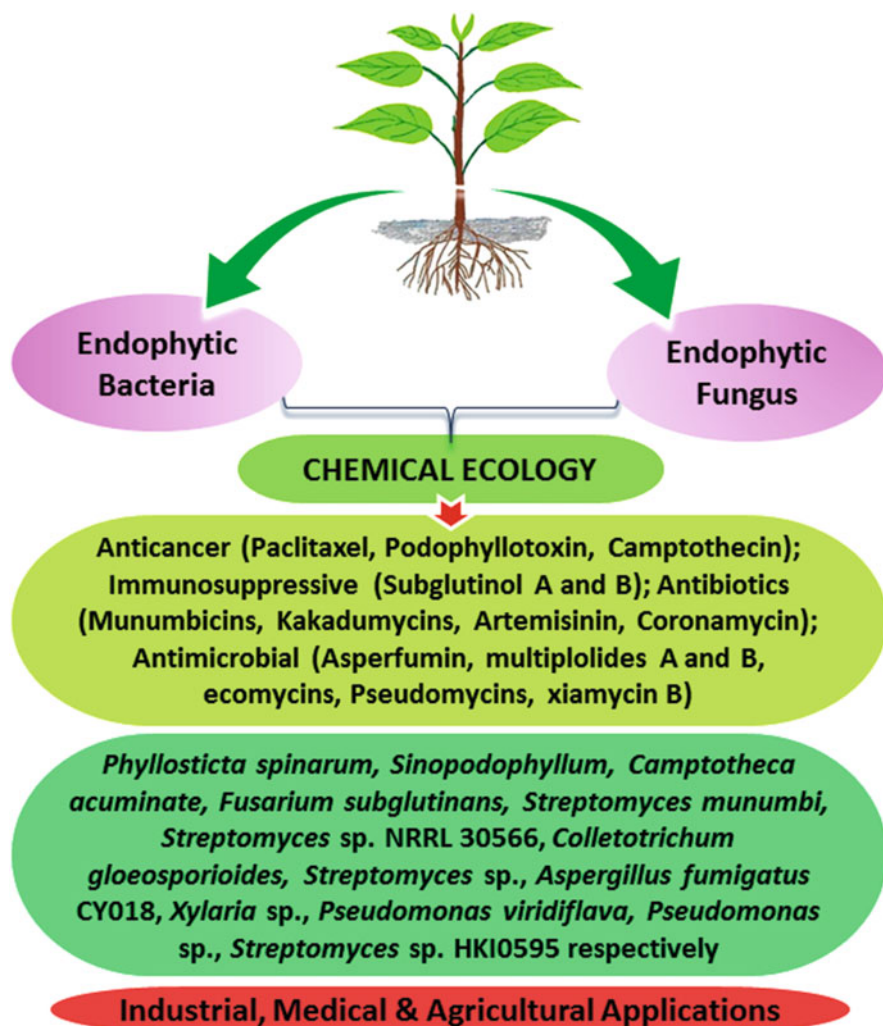
identified as a novel species of the genera *Porphyrobacter*, *Paracoccus*, and *Amycolatopsis* (Yonghong et al. 2017). A total of 133 bacterial endophytes were isolated from the root crown, stem, and leaf tissues of 34 plants consisting of 8 different species, collected from the Oregon coast growing under harsh conditions from 4 different sites. Among the 133 isolates, 94 are identified as unique and represent 36 different taxonomic groups. Among the total isolates, 20.3% of the isolates belong to *Pseudomonads*, 8.2% were *Curtobacterium*, 8.2% were *Microbacterium*, 7.5% were *Bacillus*, and 6% were *Xanthomonas* (Dombrowski et al. 2017).

## 15.6 Chemical Ecology of Fungal and Bacterial Endophytes

Nowadays world population is facing various health problems caused by bacteria, virus, fungus, protozoan, and other microorganisms which could be due to the drug resistance or parasitism, and this generates an alarming situation to the world population for their survival. Research based on the invention of medicinal and novel bioactive compounds from the endophytic microbes is a promising task. There is a substantial demand for the production of new drugs, particularly antibiotics, anticancer agents, immunomodulator compounds, and agrochemicals that are eco-friendly and cause less or no damage to the milieu and replace the synthetic fertilizers and pesticides (Pandey et al. 2013, 2014) (Fig. 15.3).

### 15.6.1 Chemical Ecology of Fungal Endophytes

A fungal endophyte produces a large number of diverse bioactive molecules which are previously unknown. A number of bioactive compounds from the endophytic fungus have been isolated which provides protection by producing antimicrobial compounds like antifungal, antibacterial, and antiviral (Fig. 15.3). Researches are mostly concentrated on the investigation and discovery of natural bioactive compounds and improving the productivity by taking advantage of genetic engineering and other measures (Pandey et al. 2014). The endophytic fungus is also reported to produce the bioactive compound that acts as an anticancer agent (Strobel et al. 1996, 1997). Paclitaxel (Taxol) is the world's first billion dollar anticancer drug mainly obtained from the bark of the Pacific yew tree which is a tetracyclic diterpenoid (Strobel et al. 1996, 1997). The *Taxus* trees are very rare and unable to meet the market need. Hence, an alternative strategy should be needed to develop. The production of Taxol (paclitaxel) by endophytic fungus, such as *Phyllosticta spinarum* (Kumaran et al. 2008), *Bartalinia robillardoides* (Gangadevi and Muthumary 2008), *Pestalotiopsis terminaliae* (Gangadevi and Muthumary 2009), and *Botryodiplodia theobromae* (Pandi et al. 2010), is an attractive invention and generated more attention in the treatment of various cancers because of its unique



**Fig. 15.3** Overview of chemical ecology of fungal and bacterial endophytic microbes

mode of action as compared to the other anticancer agents. Podophyllotoxin has been used as a precursor for synthesis of anticancer drug and having the antimicrobial and antioxidative properties which are mainly produced by the genera of an endangered fungal species *Sinopodophyllum* (or *Podophyllum*) and *Aspergillus fumigatus*, *Fusarium oxysporum*, and *Phialocephala fortinii* (Eyberger et al. 2006; Kusari et al. 2008, 2009; Kour et al. 2008). Similarly, camptothecin, a pentacyclic quinoline alkaloid, was found to be working as an antineoplastic agent and important precursor of anticancer drugs topotecan and irinotecan (Shaanker et al. 2008), which are reported to inhibit the intranuclear enzyme topoisomerase-I required for the DNA

replication and transcription (Pandey et al. 2014), and is predominantly isolated from the *Camptotheca acuminata* (Liu et al. 2010b). The anticancer terpenoid indole alkaloids known as vinblastine and vincristine produced from an endophytic fungus interfere with microtubule and mitotic spindle (Zhao et al. 2010). Pestacin (1,3-dihydroisobenzofuran) and isopestacin (isobenzofuranone) having antioxidant activity are obtained from an endophytic fungus *P. microspora* and able to scavenge superoxide and hydroxyl free radicals (Strobel et al. 2002; Harper et al. 2003). Immunosuppressive nontoxic bioactive compound subglutinols A and B are identified from the endophytic fungus *Fusarium subglutinans* and propose to have an active role in allograft rejection in transplant (Lee et al. 1995). A number of endophytic fungi such as *Phyllosticta* spp., *Nodulisporium* spp., and *Xylaria* sp. are isolated from *Dipterocarpus* trees and were described to possess antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* (Sutjaritvorakul et al. 2011). The antimicrobial compound asperfumin produced by an endophytic fungus *Aspergillus fumigatus* CY018 was found to inhibit the *Candida albicans* (Liu et al. 2004). The antifungal compounds sordaricin (Pongcharoen et al. 2008) and multiplolides A and B (Boonphong et al. 2001) produced from *Xylaria* sp. were also found to be active against *Candida albicans*. Besides these bioactive compounds, endophytic fungus was reported to produce various sorts of antibiotic, which is briefly dealt under the head biological control by fungal and bacterial endophytes in the section functional ecology.

### 15.6.2 Chemical Ecology of Bacterial Endophytes

Likewise, the bacterial endophytes may also be utilized for the production of novel pharmaceutical agents and agrochemical compounds. There is tremendous scope for the isolation of novel bioactive medicinal compounds from endophytic bacteria. Various categories of natural products have been reported to be produced by the bacterial endophytes. The endophytic bacterium produces some harmful compounds to keep out the predators from the host plants (Suto et al. 2002). An ample range of low molecular weight compounds has been isolated from the bacterial endophytes which are active at their low concentrations against a range of animal and plant pathogens. Many bacterial endophytes such as *Pseudomonas* and *Burkholderia* are well recognized for producing secondary metabolites that include antibiotics, anti-cancer compounds, and insecticidal, antifungal, antiviral, and immunosuppressant agents (Lodewyckx et al. 2002). The grass endophytic bacteria *Pseudomonas viridiflava* are reported to produce ecomycins, a family of novel lipopeptides, which was found to be active against the pathogenic fungi *Cryptococcus neoformans* and *Candida albicans*. The antifungal compound pseudomycins was also described to be produced by plant-associated pseudomonads (Strobel and Daisy 2003). The previously unknown novel compounds xiamycin B, indosespene, and sespenine together with the previously known compound xiamycin A are also reported from the culture broth of endophytic *Streptomyces* sp. HKI0595. These compounds are

used as a biocontrol agent and found to have strong antimicrobial activities against different pathogenic bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* (Ding et al. 2011). Several compounds and antibiotics, namely, ammonia, butyrolactones, 2,4-diacetylphloroglucinol, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin, and zwittermicin A, were reported to be produced by antagonistic endophytic bacteria (Whipps 2001). Several endophytic bacteria were known to produce hydrolytic enzymes that cause cell wall lysis and utilized to control fungal pathogens (Backman and Sikora 2008). The endophytic bacteria were found to produce biosurfactants and used as an antimicrobial compound. The endophytic bacteria *Pseudomonas fluorescens* was found to produce cyclic lipopeptide surfactants such as viscosinamide and tensin and act as antifungal against *Rhizoctonia solani* and *Pythium ultimum* (Nielson et al. 1999, 2000). The protein fractions 42 and 75 from the *Bacillus amyloliquefaciens* and *Bacillus pumilus* act as elicitor molecules in the induced resistance against the pathogen *Xanthomonas vesicatoria* in the tomato plant with increasing peroxidase (POX) and polyphenol oxidase (PPO) activities (Lanna-Filho et al. 2013). The antimicrobial compounds surfactin and fengycin A homologs were identified from the endophytic *Bacillus amyloliquefaciens* and are found to be active against the peanut bacterial wilt (BW) caused by *Ralstonia solanacearum* (Wang and Liang 2014). The presence of tannins, alkaloids, saponins, glycosides, amino acids, proteins, and phenolic compounds was described from the endophytic bacteria *Lactobacillus* sp. isolated from the leaf tissues of *Adhatoda beddomei* (Swarnalatha et al. 2015).

## 15.7 Functional Ecology of Fungal and Bacterial Endophytes

### 15.7.1 Biofertilization

The plant growth promotion by increasing the availability or supply of major and minor nutrients is designated as biofertilization (Gaiero et al. 2013) (Fig. 15.4).

#### 15.7.1.1 Production of Phytohormones

It is well known that the plant hormones are organic compounds effective in very minute concentration and subsequently on their synthesis transported to the other location where they can interact with specific target tissue and regulate the physiological function of the host plant and hence referred to as plant growth regulators or phytohormones (auxins, gibberellins, ethylene, cytokinins, and abscisic acid) (Pandey et al. 2017). Phytohormones producing endophytic microbes under extreme conditions also involve the output of numerous other secondary metabolites such as

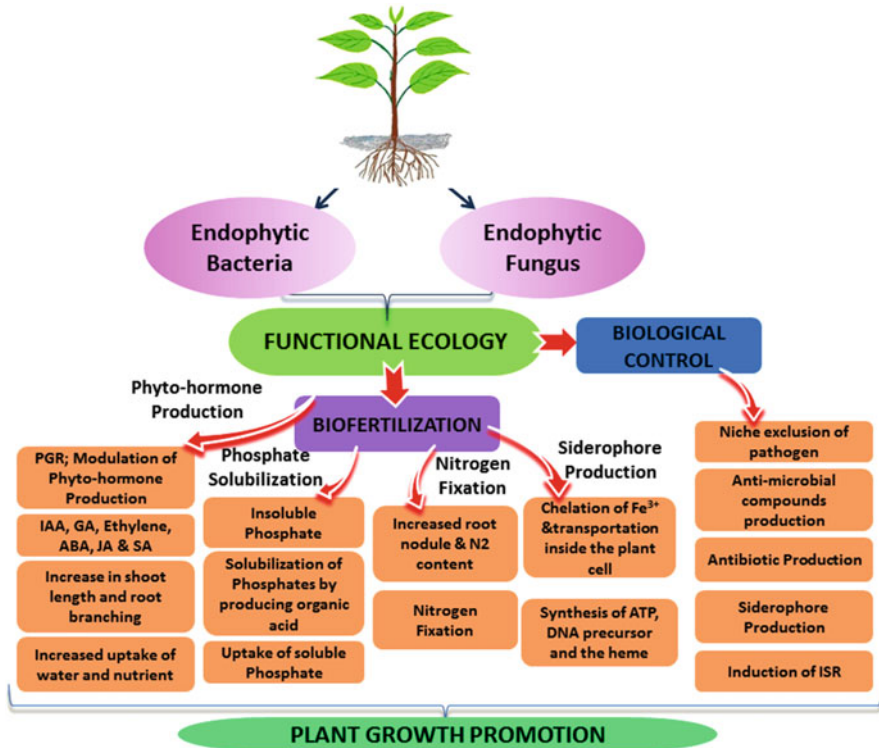


Fig. 15.4 Overview of functional ecology of fungal and bacterial endophytic microbes

flavonoids to avoid the unfavorable conditions (Khan et al. 2011). Different microbial endophytic groups are reported to produce the IAA (indole-3-acetic acid), the most important auxin which regulates plant development such as the cell expansion, division, and differentiation, gene regulation, and the other tropic response (Ratul et al. 2013; Pandey et al. 2017). The endophytic bacterial isolates from the root of *Hyptis marruboides* Epling, a *Lamiaceae*, were studied to synthesize IAA in DYGS medium supplemented with tryptophan. In the total endophytic bacterial isolates, 52% isolates were able to synthesize IAA, and the isolates RF18 (95.13 µg/ml), RG9 (39.28 µg/ml), RF13 (16.21 µg/ml), and RG24 (11.96 µg/ml) were identified as to produce significant amounts of IAA under test conditions (Vitorino et al. 2012). Endophytic bacterial isolates from the duckweed (*Landoltia punctata*) were examined for their plant growth promotion ability by the production of phytohormone indole-3-acetic acid (IAA). Out of 71 isolates, 27 were identified as positive for IAA production in which *Deinococcus* sp. L2-88 was reported to produce the highest quantity of IAA, i.e., 713.2 + 11.6 µg/ml (Kittiwongwattana 2015). Endophytic bacterial isolates from sugar beet roots were reported to produce indole-3-acetic acid (IAA) in vitro and are reported to positively affect the plant height, their fresh and dry weights, and leaves per plant, along with levels of phytohormones when

compared to the control plant (Shi et al. 2009). The fungal endophytes such as *Fusarium oxysporum* CSE15, *Trichoderma harzianum* CSE10, *Cadophora malorum* CSE14, and *Phialophora mustea* CS7E2 recovered from the *Crocus sativus* Linn were reported to produce 109.5, 95.12, 92.94, and 73.52 mg L<sup>-1</sup> of IAA, respectively (Wani et al. 2016). Fungal endophytes like *Gibberella fujikuroi* (Bomke et al. 2008), *Penicillium citrinum* (Khan et al. 2008), *Chrysosporium pseudomerdarium* (Hamayun et al. 2009), and *Aspergillus fumigatus* (Khan et al. 2011) were reported to produce GAs in their culture medium and have an active GA biosynthesis pathway. The culture filtrates of endophytic fungi *Paecilomyces formosus* LHL10 isolated from the roots of cucumber plant showed the presence of different forms of GAs such as GA1, GA3, GA4, GA8, GA9, GA12, GA20, and GA24. When the endophytic *Paecilomyces formosus* LHL10 is evaluated under salinity stress by inoculation, it was found that the endophyte significantly enhanced vegetative parameters of cucumber as compared to uninoculated control plant and elevated production of GA3, GA4, GA12, and GA20 contents modulates the salinity stress (Khan et al. 2012). Another most highly studied example of production of plant growth regulators is the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which brings down the level of plant hormone ethylene and reduces the abiotic stresses. It is reported that the elevated ethylene levels inhibit cell division, DNA synthesis, and root/shoot growth. A number of microbial endophytes have the ability to produce ACC deaminase, and thereby increased plant growth was reported (Gaiero et al. 2013). Besides IAA, GA, and ethylene, there are several other phytohormones such as abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) which are also responding in several abiotic stresses as a stimulus and hence act as a defense signaling substances (Shinozaki and Yamaguchi-Shinozaki 2007). Jasmonic acid (JA) induces the biosynthesis of defense proteins and defense-related secondary metabolites along with modulation of various physiological events associated with the biotic and abiotic stresses (Brodersen et al. 2006; Balbi and Devoto 2008; Lorenzo et al. 2004). Abscisic acid (ABA) modulates the stomatal closure and regulates the plant growth and development during stress conditions (Wasilewska et al. 2008; Shinozaki and Yamaguchi-Shinozaki 2007). Salicylic acid (SA) was likewise found to modulate the induction of flowering, biosynthesis of ethylene, the behavior of stomata, and respiration in several plants (Wildermuth et al. 2001; Rivas-San Vicente and Plasencia 2011; Rabe et al. 2013; Ambrose et al. 2015). Role of salicylic acid in defense is well studied; its application induces pathogenesis-related (PR) genes (Rabe et al. 2013). A novel gibberellin (GA)-producing basidiomycetous endophytic fungus *Porostereum spadiceum* AGH786 was found to be capable of producing six types of GAs besides modulating the JA and ABA production in salt-stressed soybean seedlings. It was found that endophytic fungus *Porostereum spadiceum* AGH786 inoculated seedlings were able to synthesize high levels of GAs and low ABA and JA. The finding indicates that the endophytic fungus ameliorates the effect of salinity by modulating endogenous phytohormones of the seedlings. Interestingly, in GA production when compared with the most efficient GA-producing fungus *Gibberella fujikuroi*, the endophytic fungus *Porostereum spadiceum* AGH786 was

found to produce higher amounts of bioactive GA3 significantly (Hamayun et al. 2017).

### 15.7.1.2 Nitrogen Fixation

Nitrogen fixation by the conversion of atmospheric nitrogen to ammonia is a well-studied form of biofertilization employed by endophytic microorganism in plant growth promotion (Gaiero et al. 2013). Nitrogen (N) is the most common nutrient required for the optimal growth of the plant, and in spite of its 78% of presence in the atmosphere, its availability is limited to the plants. The availability of nitrogen to the plant could be enhanced by the application of endophytic microbes. Endophytic microbes have the capacity to convert free nitrogen into the ammonia by biological nitrogen fixation using a complex enzyme system of nitrogenase. A full 60% of the earth's total nitrogen fixation is escorted by biological nitrogen fixation (BNF) (Pandey et al. 2017). In 1986, the discovery of nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* in the sugarcane stem by Brazilian scientists draws attention of several scientists for investigations of more nitrogen-fixing endophytes such as *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azospirillum lipoferum*, *Klebsiella pneumoniae*, *Azorhizobium caulinodans*, *Novosphingobium sediminicola*, and *Ochrobactrum intermedium* (Cavalcante and Dobreiner 1988; Schloter et al. 1994; Muangthong et al. 2015; Moyes et al. 2016). Further, the presence of *Gluconacetobacter diazotrophicus* was also noticed in sugarcane, sweet potato, and pineapple (Silva-Froufe et al. 2009). The study of DNA sequence of nitrogenase reductase gene (*nifH*) from the endophytic bacterial strain isolated from the Brachiaria forage grasses was demonstrated that the *nifH* gene sequences were highly similar to the N<sub>2</sub>-fixing organisms (Kelemu et al. 2011). Although the sufficient work was held by the researcher to investigate the nitrogen-fixing endophytes from the sugarcane, the mechanism dealing with the proportion of fixed nitrogen contributed to plant and the level of nitrogen fixed by endophytes still remains unclear (Giller and Merckx 2003). The cocultivation of *Arabidopsis* and tobacco seedlings with endophytic fungus *Piriformospora indica* demonstrates a considerable transfer of nitrogen from the agar plates into the aerial part of the seedlings and helps in growth promotion of *Arabidopsis* and tobacco seedlings by stimulation of the NADH-dependent nitrate reductase enzyme and expression of a nitrate reductase gene (Sherameti et al. 2005).

### 15.7.1.3 Phosphate Solubilization

Phosphorus (P) is the second most important major nutrient after nitrogen required for the optimal growth of plants. Their role in the metabolic processes, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration is well established (Khan et al. 2009). The immobilization limits the availability of phosphorous to the plants as the phosphorous rapidly forms complex with the other



elements in the field (Miller et al. 2010; Gurikar et al. 2016; Edwards et al. 2016). Hence, phosphate fertilizers are needed to meet the demands of phosphorus to the developing plants (Yazdani et al. 2009; Oliveira et al. 2009). But, the addition of phosphatic fertilizers is very costly and ecologically unfair, and this problem is overcome by the application of endophytic microorganisms in environmentally friendly manner. The endophytic microorganisms provide the available phosphorous to the plants by various mechanisms (Pandey et al. 2017; Senthilkumar et al. 2009). These endophytic microorganisms are capable to secrete different types of organic acids which help in the lowering of pH in the soil delimited by the plant's root and consequently release the bound forms of phosphate in the soils for uptake of plant (Pandey et al. 2017). Among the organic acids, the gluconic acid (GA) and to a lesser extent  $\alpha$ -ketogluconic acid are found to be the most frequent agents of inorganic phosphate solubilization (Oteino et al. 2015). The endophytic bacterial strains when inoculated into *P. sativum* L. are able to produce 14–169 mM gluconic acid (GA) which helps in the solubilization phosphate in the range of 400–1300 mg L<sup>-1</sup> (Oteino et al. 2015). The capacity of endophytes from the root of *Hyptis maruboides* Epling, a *Lamiaceae*, to solubilize calcium phosphate in GELP medium and iron phosphate in the modified Reyes basal medium indicates that of the 42 endophytic isolates, 20% isolates were able to solubilize the inorganic phosphate. It is observed that endophytic strains of root zone make available the soil phosphorus to the plant, thereby increasing vegetation and improving plant growth (Vitorino et al. 2012). The phosphate-solubilizing bacterial endophytes from the meristematic tissues of strawberry promote plant growth, which is signified by the biomass accumulation and improvement in the development of other plant parts (Armando et al. 2009). The endophytic isolates from the ginseng plants were reported to solubilize phosphate, detected by extracellular solubilization of precipitated tricalcium phosphate with glucose as a sole source of carbon (Thamizhvendan et al. 2010). The *Parthenium hysterophorus* L. root endophytic fungus *Curvularia geniculata* (Tracy & Earle) Boedijn was investigated for their ability to solubilize different sources of phosphorus (P) [ $\text{AlPO}_4$ ,  $\text{FePO}_4$ , and  $\text{Ca}_3(\text{PO}_4)_2$ ] by inoculation into pigeon pea (*Cajanus cajan*) plants. It is found that the plants exhibited superior growth over uninoculated control plants and the fungus solubilized different sources of P in the order of  $\text{FePO}_4 > \text{AlPO}_4 > \text{Ca}_3(\text{PO}_4)_2$  (Priyadharsini and Muthukumar 2017).

#### 15.7.1.4 Siderophore Production

Siderophore is an iron-chelating molecule of about 1000 molecular weight and reported to be produced by many microorganisms (Logeshwaran et al. 2009). Their biosynthesis is influenced by the presence of iron, and their synthesis is suppressed if the iron is abundant in the environment (Verma et al. 2011; Loaces et al. 2011; Rungin et al. 2012). The production of siderophore is considered as very important due to the leading role of iron in the nitrogen fixation and assimilation processes (Sauv tre and Schr der 2015; Mukherjee et al. 2017). Many endophytic

microbes were reported to secrete siderophores under iron-limiting conditions (Senthilkumar et al. 2009; Logeshwaran et al. 2009). The siderophore-secreting endophytic microbes inside the plant tissues help in the transport of  $\text{Fe}^{3+}$  inside the plant cell and intensify the plant growth and productivity through the synthesis of ATP, DNA precursor, and the heme (Stintzi et al. 2000; Chincholkar et al. 2000; Logeshwaran et al. 2009; Loaces et al. 2011; Kumar et al. 2016). Its deficiency can alter the DNA synthesis and growth of cell and hamper with the several metabolic processes such as photosynthesis and the mitochondrial reactions (Chincholkar et al. 2000). The functions of siderophore in the inhibition of plant pathogens were identified (Compant et al. 2005). Siderophore production ability of endophytic bacteria *Methylobacterium* spp. was tested by chromeazuroil agar assay test (CAS), Csáky test (hydroxamate type), and Arnow test (catechol type), and it was found that a total of 37 strains of *Methylobacterium* spp. were positive for chromeazuroil agar assay test. *Methylobacterium* spp. was found to be producing a hydroxamate type of siderophore in the Csáky assay (Lacava et al. 2008). The production of siderophore provides competitive advantages to endophytic microbes for their successful colonization into the plant tissues and exclusion of other harmful microorganisms from the same ecological niche (Loaces et al. 2011). The endophytic bacterial isolates HKA-72 and HKA-113 were reported to synthesize Fe (III)-chelating siderophores, indicated by the formation of a yellowish-gold halo around the endophytic bacterial colonies grown on CAS agar plates. It was proposed that the siderophore production was very important for the iron supply to the growing plant as well as associated endophytes as endophytic bacteria have to also compete with the plant cell for its growth (Senthilkumar et al. 2009).

### 15.7.2 Biological Control

The use of chemical pesticides may be pessimistic because it develops pathogen resistance (Vinale et al. 2008), and thus, scientist focuses on the alternative means by utilizing nonchemical agents such as microorganisms in the agricultural fields to control phytopathogen (Barakat and Al-Masri 2005; Pandey et al. 2012). The promotion of plant growth and protection from phytopathogens by taking advantage of biological agents is considered as biological control or biocontrol (Fig. 15.4). Among these biological agents, the scientist gave special attention toward endophytes by taking account of their beneficial role on host plant (Firáková et al. 2007) because they are systemically distributed inside the plant and more competent to colonize the plant tissues than to phytopathogens (Rai et al. 2007). Numerous endophytes have been reported with biocontrol potential against various plant pathogens such as fungus, bacterial, viral, insect, and nematodes (Hallman et al. 1997; Ryan et al. 2008; Monteiro et al. 2017). The endophytes have been proven to prevent disease development through endophyte-mediated de novo synthesis of novel compounds (Ryan et al. 2008). Studies of the endophytic strains of novel metabolites should identify new drugs for effective treatment of disease in humans,

plants, and animals (Strobel et al. 2004). The endophytic inoculant trades for the biological control in the past few decades witnessed an approximately annual growth rate of 10% worldwide (Lahlali and Hijri 2010). Host specificity and pattern of host colonization, population dynamics, potentiality to enter within host tissues, and the ability to induce systemic resistance are certain factors which accompanied the effectiveness of endophytes as biocontrol agents (Gonzalez et al. 2016; Yadav and Yadav 2017; Hu et al. 2017). A number of different mechanisms that are used by the endophytic microbes to enhance the plant growth by suppression of phytopathogens are explained by researchers (Backman and Sikora 2008; Bacilio-JimeÁnez et al. 2001; Weyens et al. 2009) which include (1) ability to synthesize chelators that effectively chelate elements necessary for growth and making it unavailable to phytopathogens; (2) capacity to synthesize antimicrobial compounds, antibiotics, cell wall-lysing enzymes, and hydrogen cyanide, which effectively suppresses the growth of phytopathogens; (3) potentiality to enhance growth of plant by effectively competing with phytopathogens for nutrition and space; (4) synthesis of phytohormones to colonize specific niches inside the plant; and (5) ability to induce systemic resistance.

### 15.7.2.1 Biological Control Through Fungal Endophytes

The endophytic fungi were isolated from an aromatic and medicinal plant *Monarda citriodora* Cerv. ex Lag. which showed variable degrees of antagonism in different assays against plant pathogenic fungi *Sclerotinia* sp., *Colletotrichum capsici*, *Aspergillus flavus*, and *Aspergillus fumigatus*, and this suggests that the endophytes from the plants with medicinal values could be explored as promising biocontrol agents and employed as a defensive metabolite resource (Katoch and Pull 2017). The volatile organic compounds (VOCs) of endophytic fungi *Muscodor* spp. and *Simplicillium* sp. from the *Coffea arabica* were found to be effective against the phytopathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Phoma* sp., *Botrytis cinerea*, *Fusarium solani*, *Fusarium verticillioides*, *Cercospora coffeicola*, and *Pestalotia longisetula* (Monteiro et al. 2017). Several mechanisms may be involved by endophytic fungi in biological control, in which antibiotic production is one of the chief mechanisms (Lu et al. 2000; Pandey et al. 2014). An endophytic fungus *Colletotrichum gloeosporioides* isolated from Chinese traditional herb *Artemisia Annu*a was found to produce artemisinin with their fungistatic nature to the plant pathogen (Lu et al. 2000). *Phomopsis* sp. isolated from *Erythrina crista*, a medicinal plant, was reported to produce phomal, a polyketide lactone identified as anti-inflammatory metabolite (Weber et al. 2004). Endophytic fungus was also reported to have anti-insect property to the host plant against herbivorous insects through the production of toxic repellent (Pandey et al. 2014). The antimicrobial activity of endophytic fungus isolates isolated from the leaves and branches of *Ocimum* sp. (Tulsi) was assessed and found effective against pathogenic microorganisms such as *Candida albicans*, *Penicillium chrysogenum*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Mycobacterium smegmatis* (Pavithra et al. 2012).

A diverse number of endophytic fungus isolates belong to the species of *Phyllosticta* spp., *Nodulisporium* spp., and *Xylaria* sp.1 isolated from *Dipterocarpus* trees which were reported to produce antimicrobial compounds with antimicrobial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* (Sutjaritvorakul et al. 2011). The endophytic fungi *Aspergillus fumigatus* CY018 and *Xylaria* were reported to inhibit *Candida albicans* (Liu et al. 2004; Boonphong et al. 2001; Pongcharoen et al. 2008). *Xylaria* sp.1 was found to have broad spectrum antimicrobial activity (Liu et al. 2008). Entomopathogenic endophytic fungi *B. bassiana* and *Clonostachys rosea* were active against the coffee berry borer (Vega et al. 2008).

### 15.7.2.2 Biological Control Through Bacterial Endophytes

Bacterial endophytes attribute to antagonistic activity due to their faster colonization than phytopathogen, induction of defense mechanisms, and synthesis of antimicrobial secondary metabolites. To understand the interaction and colonization of bacterial endophytes with plants and their proposed role in plant growth promotion and biological control, many studies were performed (Algam et al. 2005; Bacilio-JimeÁnez et al. 2001). The production and secretion of antibiotic, a molecule that either kills the target pathogens or inhibits their growth, is a well-known mechanism by which bacterial endophytes control the plant diseases (Compant et al. 2005). Bacterial endophytes were reported to synthesize a wide variety of antibiotics having antagonistic activity against several pathogens and commercially utilized in pharmaceutical, medical, and agricultural field (Castillo et al. 2002; Castillo et al. 2003; Ezra et al. 2004; Park et al. 2005; Wang et al. 2007). A wide spectrum antibiotic munumbicins by an endophytic *Streptomyces munumbi* isolated from the *Kennedia nigricans* and a novel antibiotic kakadamycins from the endophytic *Streptomyces* sp. NRRL 30566 of *Grevillea pteridifolia* were reported from the same author (Castillo et al. 2002; Castillo et al. 2003). The endophytic *Streptomyces* sp. isolated from *Monstera* sp. was found to produce a novel antibiotic coronamycin active against the pathogenic fungus *Cryptococcus neoformans* (Ezra et al. 2004). Endophytic bacterial strains *Corynebacterium flavescens* and *Bacillus pumilus* from rice were evaluated for their ability to enhance plant growth by successfully excluding rice pathogen *Azospirillum brasilense* (Bacilio-JimeÁnez et al. 2001). Endophytic bacterial strains of *Pseudomonas* sp., *Bacillus* sp., and *Methylobacterium* sp. isolated from different agroecosystems were isolated and screened for their nematocidal action against *Meloidogyne incognita* in host *Abelmoschus esculentus* L. (Vetrivelkalai et al. 2010). Possible mechanism of nematode control by bacterial endophytes might be due to production of secondary metabolites, e.g., 2,4-diacetylphloroglucinol and other lytic enzymes, antibiotics, hydrogen cyanide (Ahl et al. 1986; Vetrivelkalai et al. 2010), toxic metabolites such as bacillopeptidase, subtilin E, and a lactamase (Vetrivelkalai et al. 2010). Plant growth-promoting endophytic bacteria isolated from the corm and roots of banana were tested for their ability to induce systemic resistance against BBTV (Harish et al.

2008). The endophytic bacterial strains of genus *Phyllobacterium* isolated from *Epimedium brevicornum* Maxim displayed a wide spectrum antimicrobial activity against fungal and bacterial phytopathogens such as *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Botrytis cinerea*, and *Botrytis fabae* (He et al. 2009). Endophytic bacteria trigger a phenomenon known as induced systemic resistance (ISR) which is phenotypically similar to systemic-acquired resistance (SAR), but ISR differs from SAR in that the inducing bacterium does not have any visible symptoms on the host plant (Van Loon et al. 1998; Ryan et al. 2008).

## 15.8 Conservation of Fungal and Bacterial Endophytic Microbial Diversity

As discussed in the previous section, the importance of endophytic microorganisms has been ever realized from the ancient period of time in the dairy industry, fermentation industry, biocontrol, production of antibiotics, and novel secondary metabolites. Each individual plant on earth is host to one or several endophytes (Strobel et al. 2004; Ryan et al. 2008). Endophytes have been isolated from surface-sterilized plant tissues or extracted from internal plant tissues (Pandey et al. 2012). Of the total plant species reported (approximately 300,000 present on the earth), each plant species carry one or more endophytes in their lifetime, and nearly all vascular plant species investigated to date were reported to harbor endophytic microorganisms (Pathak 2011). The presence of endophyte in the copper mine wasteland, agronomic crops and prairie plants, deepwater rice, grass ecosystem, pea cultivars in field condition, tropical grasses of the *Brachiaria* genus, and prairie plants has been reported earlier (Zinniel et al. 2002; Verma et al. 2001; Ryan et al. 2008). Endophytes have been reported to benefit the human welfare directly and serve as a major resource in the development of biotechnology. The conservation of endophytic microbial gene pools is an emerging and vital issue, even though development is scary. In the previous studies, conservation dealt with endophytic bacteria almost entirely addressed in context of ex situ conservation. However, obviously this strategy is quite inadequate for ensuring conservation of huge endophytic microbial diversity.

### 15.8.1 *In Situ Conservation of Fungal and Bacterial Endophytic Microbes*

In situ conservation, conservation of endophytic microbial diversity assured in their natural habitats, i.e., in different geographical locations notable by their exceptional levels of biodiversity and endemism and such locations are defined as hotspots. The

copper mine wasteland, agronomic crops and prairie plants, deepwater rice, grass ecosystem, pea cultivars in field condition, tropical grasses, terrestrial and marine geothermal sites, deep ocean trenches, and polar regions are considered as one of them. Conservation of the areas with natural populations of high-valued endophytic microbes is a fundamental condition for the conservation of endophytic microbial diversity. Conservation strategies of endophytic species in agriculturally important plant genus *Zea* were studied as it became domesticated from its wild ancestors (teosinte) to modern maize (corn) and moved from Mexico to Canada. Kernels from populations of four different teosintes and ten different maize varieties were screened for endophytic bacteria by culturing, cloning, and DNA fingerprinting using terminal restriction fragment length polymorphism (TRFLP) of 16S rDNA. Analysis of data showed that there was a core microbiota of endophytes that was conserved in *Zea* seeds across boundaries of evolution, ethnography, and ecology (Johnston-Monje and Raizada 2011). *Silene paradoxa*, a well-adapted plant to extreme environments, were evaluated for their seeds, roots, and aerial plant parts associated with their endophytic bacterial communities. Molecular analysis of high-throughput sequencing of the 16S rRNA genes (microbiota) of bacterial communities isolated from seeds demonstrates that the endophytic bacterial communities were transferred to the next generation of plants as seed endophytes. Their study proves that the plants with special characters and from specific location could provide an in situ conservation of endophytic microbes (Mocali et al. 2017). In situ conservation is seen as the most appropriate means of conserving endophytic biodiversity.

An all-taxa biodiversity inventory (ATBI) has been proposed for systematic investigation and makes a record of the full diversity of living organisms, including species, number, and value of organisms from the selected habitats. The microbial ATBIs have been proposed to search new species utilized for resolving the biotechnological exploration and discovery leading to expose the exact relationship between various environments and different genotypes; to investigate the occurrence and richness of un-culturable microbes, cataloging the infrequent microbes; and to collect the information related to genotypic, phenotypic, and ecology of microbial distribution (Cannon 1995; Bull et al. 2000).

### ***15.8.2 Ex Situ Conservation of Fungal and Bacterial Endophytic Microbes***

Ex situ conservation of endophytic microbial diversity is the preservation of endophytes and its components outside their natural habitats. Here, the components refer to gene banks of endophytes, man-made wild field bank of plants with potential endophytes, in vitro plant tissues containing endophytes, artificial propagation of plants containing high-valued endophytes, maintaining botanical gardens, and most importantly endophytic microbial culture collections. Ex situ conservation involves

conservation of genetic resources of endophytic species and provides insurance against extinction.

The idea of the microbial culture collection was first set up in the late nineteenth century, when the pure culture techniques for the isolation of pure cultures of microbes such as solid culture media (potato, gelatin, and agar) were introduced to the target collection, maintenance, and distribution of microbial strains among researchers and scientist, and it was considered to be a means to preserve microbial diversity *ex situ* (Smith 2003). The idea of the establishment of the microbial culture collection is important in the context of availability of microbial resources for fundamental research and studies. The repository of culture collection helps in understanding the mechanisms of metabolic and evolutionary patterns of valuable microorganisms as they play a fundamental role in the development and advancement of agriculture, industry, and health sectors (Sharma et al. 2017). Culture collections have provided repositories for the safe storage of live germplasm of all the microbial species along with the threatened and endangered species. It includes nucleic acid sequences and gene banks too (Sly et al. 1990). Preservation of microbes often requires different preservation methods to ensure optimal viability, storage, purity, and stability of individual microbial strains. However, application of molecular methods such as use of polymerase chain reaction (PCR) and sequence analysis of culturable microbes from the sample and analysis of biodiversity of nonculturable microbes by direct extraction of nucleic acids from the environment is a more robust method. The identity and diversity of a microbial species can be evaluated by analysis of its genetic material (16S rRNA gene).

Several repositories at international level like the World Federation for Culture Collections (WFCC, 2011) ([www.wfcc.info](http://www.wfcc.info)) and European Culture Collection Organisation (ECCO, 1981) ([www.eccosite.org](http://www.eccosite.org)) and at national level like Korean Agriculture Culture Collection (KACC, 1995) ([genebank.rda.go.kr/eng/mic/itr/GeneInfo.do](http://genebank.rda.go.kr/eng/mic/itr/GeneInfo.do)), Korean Collection for Type Cultures (KCTC, 1985) ([kctc.kribb.re.kr/English/ekctc.aspx](http://kctc.kribb.re.kr/English/ekctc.aspx)), Biological Resource Centre (BRC, 2016) ([www.brc.a-star.edu.sg](http://www.brc.a-star.edu.sg)) at Republic of Korea, Belgium Coordinated Collection of Microorganism (BCCM, 2017) ([bccm.belspo.be](http://bccm.belspo.be)) at Belgium, Czech Collection of Microorganism (CCM, 2017) ([www.sci.muni.cz/ccm](http://www.sci.muni.cz/ccm)) at Republic of Czech, Pasteur Culture Collection of Cyanobacteria (PCC, 2015) ([cyanobacteria.web.pasteur.fr](http://cyanobacteria.web.pasteur.fr)) at France, Microbial Type Culture Collection (MTCC, 2017) and Gene Bank ([www.mtccindia.res.in](http://www.mtccindia.res.in)) at IMTECH, Chandigarh, Indian Type Culture Collection (ITCC, 2010) ([www.iari.res.in](http://www.iari.res.in)) at IARI, New Delhi, and National Agriculturally Important Microbial Culture Collection (NAIMCC, 2017) ([nbaim.org.in](http://nbaim.org.in)) at ICAR-NBAIM Mau India were recognized by National Biodiversity Authority (NBA), Government of India, being functional for microbial culture collection. In spite of these recognized repositories, few repositories are being also reported which exclusively dealt with endophytic microbes. The North Carolina Arboretum Germplasm Repository (TNCAGR, 2017) founded in 2008 makes an effort to conserve the endophytic microbes from the native plants. It is noted that they were collected and conserved, more than 2000 germplasm samples of seeds and endophytes from the diverse region with the purpose of boosting their use in collaborative projects related with food and

health in native region. By the use of advance technology and regional natural resources, TNCAGR identifies and produces endophytic microbial samples and extracts for researchers to discover, develop, and validate natural medicines for human health and wellness. Collected endophytic strains are found to have antibiotic, antiviral, anticancer, antioxidant, antidiabetic, immunosuppressive, and anti-insecticidal activity ([ncarboretum.org](http://ncarboretum.org)). Similarly, the personnel collection repository by GRBio, 2017 (Global Registry of Biodiversity Repositories) group collected and maintained the fungal endophytes isolated from different crop and pasture species ([grbio.org](http://grbio.org)).

### 15.8.2.1 Preservation of Fungal and Bacterial Endophytic Microbes

The preservation and maintenance of endophytic microbial cultures are very essential for the study of biodiversity and systematics. Endophytic microbes have huge diversity; therefore various methods of culturing and preservation are needed to secure their morphology, physiology, viability, and genetic constituents over the time. The laborious work, cost, and time for each method must be effective. Preservation of endophytes is highly required for further studies involving their interaction with plants, their mode of action, and their utilization. However, there is scarcity of literature on the endophytic preservation methods, and there is no well-accepted specific preservation strategy for endophytes. Thus, the traditional methods or the preservation strategies generally used for common microbes can be used for endophytes preservation.

The fundamental methods of culturing for preservation are continuous growth, drying, and freezing. In continuous growth methods, the endophytic cultures are inoculated on agar for growth, and this method is used for short-term storage. These cultures either can be stored from 5 to 20 °C or can be frozen to enhance the time interval between the subculturing. The other most useful method of endophytic culture preservation is drying that is helpful to produce spores and resting structures. The materials frequently required for the drying method are silica gel, soil, and glass beads. Certain fungi have been stored up to 11 years on silica gel by drying method (Smith and Onions 1983). The drying method is technically very simple and cost-effective with less labor work. Freezing methods containing cryopreservation are also really useful in broad ranges. Certain less costly methods such as storage in distilled water are also used for preservation. These low-cost techniques are very good, but not considered as permanent preservation (Nakasone et al. 2004).

Microorganisms have the property of genetic instability. The development of new techniques for the preservation of genotype should be the highest priority. However, none of the methods ensure the complete preservation of genotype; long-term storage can be possible by lyophilization, freezing, and L-drying (Kidby 1977). The spores of AM endophytes are viable after L-drying, and the capacity of spores to survive was affected by several factors (Tommerup and Kidby 1979). Glycerol is used for freezing endophytic microbes, very hardly passes into the cell, and acts as a cryoprotectant to reduce the formation of ice crystals both intracellularly and extracellularly (Ghera 1981). The glycerol concentrations between 5 and 50% at



freezing from  $-10$  to  $-80$  °C have been tested for various microbes, including endophytes (Kirsop and Snell 1984; Fontaine et al. 1986).

The short-term preservation by serial transfer technique is used for the maintenance of microbial and endophytic culture up to 1 year. The method is very simple, cost-effective, and widely applicable. However, it is very time taking and laborious, but the periodic transfer of culture is a good option for small collections with continuous use of short time (less than 1 year). The microbial culture must be checked time to time for contamination either by mites or by other microbes and for moisture content. The replacement of nutrient-rich media with nutrient-poor one during the transfer helps to preserve healthy cultures. Certain endophytic fungi require specific media for their preservation (Bacon 1990). The cultures are stored either at room temperature or at 4 °C after the establishment. The preserved culture must be assured that no contamination and dehydration over the time (Nakasone et al. 2004). The oil overlay over the agar slants growing endophytic cultures is a low-cost and less maintenance method. The preservation of endophytic cultures from this method is up to several years at room temperature. This method is very effective for non-sporulating endophytic microbes that are not liable to freeze-drying. One advantage of this method is that the overlaid oil reduces the mite infestations (Burdshall and Dorworth 1994). The microbial cultures grown on agar slants are covered up to 10 mm layer of autoclaved high quality of mineral oil or liquid paraffin. The test tubes are kept at room temperature in an upright position. The level of oil in culture tubes should be checked over the time (Nakasone et al. 2004). Most endophytic microbial cultures which are frozen at  $-20^{\circ}$  to  $-80$  °C in freezer stay viable. The microbial endophytic cultures grown on their respective agar slants or test tubes with screw cap bottles can be directly kept in freezer. The repeated freezing and thawing is not recommended because it reduces the viability of cultures significantly. In freezing technique the storage in liquid nitrogen is a very powerful technique of preservation for all those microbes that are not able to lyophilize. The main benefits of liquid nitrogen storage are reduction in the genetic variability of stocks, time-saving, less labor intensive to handle, reduction in repeated pathogenicity tests, prevention of contamination, and enhanced long-term viability in cultures.

Lyophilization, or freeze-drying, is a less expensive and permanent preservation method, but it does not cover all microbes like some fungi. This technique is very effective for the microbes that form numerous and small propagules. This process is effective for many spore-forming fungi that produce more numbers of spores about 10  $\mu\text{m}$  or less in diameter. The dry skimmed milk powder (sterile 5 or 10% solution) and filter sterilized bovine serum are the two most common materials used during the lyophilization; however the other proteinaceous materials also can be used in place of these two. Lyophilization and freezing in glycerol are the most acceptable technique for long-term storage of endophytic microbes. Certain species of bacteria that are preserved by this method are remaining viable up to 30 years (Ghera 1981; Fontaine et al. 1986).

## 15.9 Conclusion

Endophytes are naturally occurring microorganisms and are an excellent source of bioactive novel compounds. They are widely exploited in pharmaceutical, medical, and agricultural industries. The use of endophytes makes higher and quicker yields of bioactive compounds in contrast to the large number of mature plants processed to achieve low yields of final products. Endophytes minimized threat of extinction of plants as compounds from endophytes are produced in vitro and in a controlled manner. They are also helpful in minimizing the labor cost and time in order to achieve valuable and large quantity of biological compounds. Fungal and bacterial endophytes have been basically ignored for their conservation. Despite of their ignorance for conservation, their role in production of natural novel compounds, secondary metabolites and industrially important natural products such as insecticides, antimicrobials, therapeutics, etc., is well studied. Secondary metabolites are as well responsible for adaptation, immune induction, and interaction with the environment. Plants associated with endophytes resist the intrusion of pathogens in their part or tissues by producing antimicrobial compounds. Fungal endophytes are the well-understood and well-investigated group of microorganisms as compared to bacterial endophytes. The discovery of novel bioactive compounds from the fungal and bacterial endophytes provides possibilities to overcome the problems associated with several medical problems such as cancer, tuberculosis, etc. Environmental problems like pollution control, biodiversity conservation, etc. are being dealt with endophytic microbes which are helpful in detoxification of industrial effluents, treatment of oil spills, and sewage treatments. With all the above concern contents, there is an increasing demand of research to study ecology, to identify and develop new competent inocula, and to investigate and explore new industrial products from endophytes with its commercial potential in life sciences.

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## References

- Ahl P, Voisarad C, Defago G (1986) Iron siderophores, cyanic acid and antibiotics involved in suppression of *Thielaviopsis basicola* by *Pseudomonas fluorescens* strain. J Phytopathol 116:121–134
- Akbari DL, Akbari LF (2017) Detection of GFP expression and colonization of wheat by two endophytic bacteria tagged with GFP gene. Int J Pure App Biosci 5:844–848
- Algam SA, Guan-lin X, Coosemans J (2005) Delivery methods for introducing endophytic *Bacillus* into tomato and their effect on growth promotion and suppression of tomato wilt. Plant Pathol J 4:69–74

- Ambrose KV, Tian Z, Wang Y, Smith J, Zylstra G, Huang B, Belanger FC (2015) Functional characterization of salicylate hydroxylase from the fungal endophyte *Epichloë festucae*. *Sci Rep* 5:10939. <https://doi.org/10.1038/srep10939>
- Anu Ranjan S (2012) Microbial endophytes of crop plants and their role in plant growth promotion. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, India
- Armando CFD, Francisco ECC, Fernando DA, Paulo TL, Manoel AT, Laura CA, Welington LA, Joao LA, Itamar SM (2009) Isolation of micro propagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. *World J Microbiol Biotechnol* 25:189–195
- Bacilio-Jimeñez M, Aguilar-Flores S, del Valle MV, Péreze A, Zepedae A, Zenteno E (2001) Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. *Soil Biol Biochem* 33:167–172
- Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. *Biol Control* 46:1–3
- Bacon CW (1990) Isolation, culture and maintenance of endophytic fungi of grasses. In: Labeda DP (ed) *Isolation of biotechnological organisms from nature*. McGraw-Hill, New York, pp 259–282
- Balbi V, Devoto A (2008) Jasmonate signaling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios. *New Phytol* 177:301–318
- Balsanelli E, Serrato RV, de Baura V, Sasaki G, Yates MG, Rigo LU, Pedrosa FO, de Souza EM, Monteiro RA (2010) *Herbaspirillum seropedicae* rfbB and rfbC genes are required for maize colonization. *Environ Microbiol* 12:2233–2244
- Barakat RM, Al-Masri MI (2005) Biological control of gray mold disease (*Botrytis cinerea*) on tomato and bean plants by using local isolates of *Trichoderma harzianum*. *Dirasat Agric Sci* 32:145–156
- BCCM (2017) Belgian Coordinated Collections of Microorganisms. <http://bccm.belspo.be/>. Accessed 27 May 2017
- Berger RG (2009) Biotechnology of flavours—the next generation. *Biotechnol Lett* 31:1651–1659
- Biswas JC, Ladha JK, Dazzo FB, Gianni YG, Rolfe BG (2000) Rhizobial inoculation influences seedlings vigor and yield of rice. *Agron J* 92:880–886
- Bodhankar S, Grover M, Hemanth S, Reddy G, Rasul S, Yadav SK, Desai S, Mallappa M, Mandapaka M, Srinivasarao C (2017) Maize seed endophytic bacteria: dominance of antagonistic, lytic enzyme-producing *Bacillus* spp. *3 Biotech* 7:1–13
- Bomke C, Rojas MC, Gong F, Hedden P, Tudzynski B (2008) Isolation and characterization of the gibberellin biosynthetic gene cluster in *Sphaceloma manihoticola*. *Appl Environ Microbiol* 74:5325–5339
- Boonphong S, Kittakoop P, Isaka M, Pittayakhajonwut D, Tanticharoen M, Thebtaranonth Y (2001) Multiplolides A and B, new antifungal 10-membered lactones from *Xylaria multiplex*. *J Nat Prod* 64:965–967
- BRC (2016) Biological Resource Centre. <https://www.brc.a-star.edu.sg/>. Accessed 27 May 2017
- Brodersen P, Petersen M, Nielsen HB, Zhu S, Newman MA, Shokat KM, Rietz S, Parker J, Mundy J (2006) *Arabidopsis* MAP kinase 4 regulates salicylic acid and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *Plant J* 47:532–546
- Bull AT, Ward AC, Goodfellow M (2000) Search and discovery strategies for biotechnology: the Paradigm shift. *Microbiol Mol Biol Rev* 64:573–606
- Burdshall HH Jr, Dorworth EB (1994) Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water in cryovials. *Mycologia* 86:275–280
- Cannon PF (1995) An ATBI—how to find one and what to do with it. *Inoculum* 46:1–4
- Castillo U, Harper JK, Strobel GA, Sears J, Alesi K, Ford E, Lin J, Hunter M, Maranta M, Ge H, Yaver D, Jensen JB, Porter H, Robinson R, Millar D, Hess WM, Condrum M, Teplow D (2003) Kakadumycins, novel antibiotics from *Streptomyces* sp. NRRL 30566, an endophyte of *Grevillea pteridifolia*. *FEMS Microbiol Lett* 224:183–190
- Castillo U, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robison R, Condrum MA, Teplow DB, Stevens D, Yaver D (2002) Munumbicins, wide spectrum antibiotics

- produced by *Streptomyces munumbi*, endophytic on *Kennedia nigricans*. *Microbiology* 148:2675–2685
- Cavalcante VA, Döbereiner J (1988) A new acid tolerant nitrogen-fixing bacterium associated with sugar cane. *Plant Soil* 108:23–31
- CCM (2017) Czech Collection of Microorganism. <http://www.sci.muni.cz/ccm/>. Accessed 27 May 2017
- Chen T, Chen Z, Ma GH, Du BH, Shen B, Ding YQ, Xu K (2014) Diversity and potential application of endophytic bacteria in ginger. *Genet Mol Res* 13:4918–4931
- Chincholkar SB, Choudhari BL, Talegaenkar SK, Kothari RM (2000) Microbial chelators, a sustainable tool for the biocontrol of plant diseases. In: Upadhyay RK, Mukerji KG, Chamola BP (eds) *Biocontrol potential and its exploitation in sustainable agriculture, Crop disease, weeds and nematodes*, vol I. Kluwen Academic/Plenum, New York
- Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem* 42:669–678
- Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl Environ Microbiol* 71:4951–4959
- de Abreu CS, Figueiredo JEF, Oliveira CA, dos Santos VL, Gomes EA, Ribeiro VP, Barros BA, Lana UGP, Marriel IE (2017) Maize endophytic bacteria as mineral phosphate solubilizers. *Genet Mol Res* 16:gmr16019294
- De Bary A (1866) *Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten*, Hofmeister's handbook of physiological botany, vol II. W. Engelmann, Leipzig, Germany
- De Melo FMP, Fiore MF, de Moraes LAB, Silva-Stenico ME, Scramin S, de Araújo Teixeira M, de Melo IS (2009) Antifungal compound produced by the cassava endophyte *Bacillus pumilus* MAHIM4A. *Sci Agric* 66:583–592
- De Weger LA, Bakker PAHM, Schippers B, van Loosdrecht MCM, Lugtenberg B (1989) *Pseudomonas* spp. with mutational changes in the O-antigenic side chain of their lipopolysaccharides are affected in their ability to colonize potato roots. In: Lugtenberg BJJ (ed) *Signal molecules in plant-microbe interactions*. Springer, Berlin, Germany, pp 197–202
- Dekkers LC, Phoelich CC, Fits LV, Lugtenberg BJJ (1998) A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. *Proc Natl Acad Sci U S A* 95:7051–7056
- Ding L, Maier A, Fiebig H-H, Lin W-H, Hertweck C (2011) A family of multicyclic indolosesquiterpenes from a bacterial endophyte. *Org Biomol Chem* 9:4029–4031
- Dombrowski JE, Hollenbeck VG, Martin RC (2017) Isolation and identification of bacterial endophytes from grasses along the Oregon coast. *Am J Plant Sci* 8:574–601
- Dorr J, Hurek T, Reinhold-Hurek B (1998) Type IV pili are involved in plant-microbe and fungus-microbe interactions. *Mol Microbiol* 30:7–17
- ECCO (1981) European Culture Collections' Organisation. <https://www.eccosite.org/>. Accessed 27 May 2017
- Edwards CL, Maguire RO, Alley MM, Thomason WE, Whitehurst GB (2016) Plant-available phosphorus after application of synthetic chelating agents. *Commun Soil Sci Plant Anal* 47:433–446
- Egamberdieva D, Wirth SJ, Shurigin VV, Hashem A, Abd Allah EF (2017) Endophytic bacteria improve plant growth, symbiotic performance of chickpea (*Cicer arietinum* L.) and induce suppression of root rot caused by *Fusarium solani* under salt stress. *Front Microbiol* 8:1887. <https://doi.org/10.3389/fmicb.2017.01887>
- Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. *J Nat Prod* 69:1121–1124
- Ezra D, Castillo UF, Strobel GA, Hess W, Porter H, Jensen J, Condrón M (2004) Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp. *Microbiology* 150:785–793

- Firáková S, Šturdíková M, Múčková M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. *Biologia* 62:251–257
- Fontaine MS, Young PH, Torrey JG (1986) Effects of long-term preservation of *Frankia* strains on infectivity, effectiveness, and in vitro nitrogenase activity. *Appl Environ Microbiol* 51:694–698
- Forchetti G, Masciarelli O, Alemanno S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Appl Microbiol Biotechnol* 76:1145–1152
- Freeman EM (1904) The seed fungus of *Lolium temulentum* L. *Phil Trans R Soc Lond (Biol)* 196:1–27
- Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. *Am J Bot* 100:1738–1750
- Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi, isolated from a medicinal plant, *Aegle marmelos* Correa ex Roxb. *World J Microbiol Biotechnol* 24:717–724
- Gangadevi V, Muthumary J (2009) Taxol production by *Pestalotiopsis terminaliae*, an endophytic fungus of *Terminalia arjuna* (arjun tree). *Biotechnol Appl Biochem* 52:9–15
- Gautam AK (2013) Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. *Arch Phytopathol Plant Protect* 47:537–544. <https://doi.org/10.1080/03235408.2013.813678>
- Gazir R, Chaverri P (2010) Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. *Fungal Ecol* 3:240–254
- Germaine K, Keogh E, Garcia-cabellos G (2004) Colonisation of poplar trees by gfp expressing bacterial endophytes. *FEMS Microbiol Ecol* 48:109–118
- Gherna RL (1981) Preservation. In: Gerhardt P, Murray RGE, Costilow RN et al (eds) *Manual of methods for general bacteriology*. American Society for Microbiology, Washington DC, pp 208–217
- Giller KE, Merckx R (2003) Exploring the boundaries of N<sub>2</sub>-fixation in cereals and grasses: a hypothetical and experimental framework. *Symbiosis* 35:3–17
- Gonzalez F, Tkaczuk C, Dinu MM, Fiedler Z, Vidal S, Zchori-Fein E, Messelink GJ (2016) New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. *J Pest Sci* 89:295–311
- GRBio (2017) The global registry of biodiversity repositories. <http://grbio.org/non-institutional-collection/fungal-endophytes-collection-research-group-agronomy>. Accessed 27 May 2017
- Gurikar C, Naik MK, Sreenivasa MY (2016) Azotobacter: PGPR activities with special reference to effect of pesticides and biodegradation. In: Singh DP, Singh HB, Prabha R (eds) *Microbial inoculants in sustainable agricultural productivity: research perspectives*. Springer, New Delhi
- Hallman J, Quadt-Hallman A, Mahafee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. *Can J Microbiol* 43:895–914
- Hallmann A, Quandt-Hallmann A, Miller WG, Sikora RA, Lindow SE (2001) Endophytic colonization of plants by the biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. *Phytopathology* 91:415–421
- Hamayun M, Hussain A, Khan SA, Kim HY, Khan AL, Waqas M, Irshad M, Iqbal A, Rehman G, Jan S, Lee IJ (2017) Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. *Front Microbiol* 8:686. <https://doi.org/10.3389/fmicb.2017.00686>
- Hamayun M, Khan SA, Iqbal I, Na CI, Khan AL, Hwang YH, Lee BH, Lee IJ (2009) *Chrysosporium pseudomerdarium* produces gibberellins and promotes plant growth. *J Microbiol* 47:425–430
- Hardoim PR, Overbeek LSV, Elsas JDV (2008) Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol* 16:463–471
- Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* 79:293–320

- Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. *Appl Soil Ecol* 39:187–200
- Harper JK, Arif AM, Ford EJ, Strobel GA, Tomer DP, Grant DM, Porco J, Oneill K (2003) Pestacin: a 1,3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron* 59:2471–2476
- Hastuti RD, Lestari Y, Suwanto A, Saraswati R (2012) Endophytic *Streptomyces* spp. as biocontrol agents of rice bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). *HAYATI J Biosci* 19:155–162
- He R-L, Wang G-P, Liu X-H, Zhang C-L, Lin F (2009) Antagonistic bioactivity of an endophytic bacterium isolated from *Epimedium brevicornu* Maxim. *Afr J Biotechnol* 8:191–195
- Herrera SD, Grossi C, Zawoznik M, Groppa MD (2016) Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiol Res* 186–187:37–43
- Hong CE, Jo SH, Moon JY, Lee J-S, Kwon S-Y, Park JM (2015) Isolation of novel leaf-inhabiting endophytic bacteria in *Arabidopsis thaliana* and their antagonistic effects on phytopathogens. *Plant Biotechnol Rep* 9:451–458
- Hu H-J, Chen Y-L, Wang Y-F, Tang Y-Y, Chen S-L, Yan S-Z (2017) Endophytic *Bacillus cereus* effectively controls *Meloidogyne incognita* on tomato plants through rapid rhizosphere occupation and repellent action. *Plant Dis* 101:448–455
- ITCC (2010) Indian Type Culture Collection. [http://www.iari.res.in/?option=com\\_content&view=article&id=1251&Itemid=1809](http://www.iari.res.in/?option=com_content&view=article&id=1251&Itemid=1809). Accessed 27 May 2017
- James EK (2000) Nitrogen fixation in endophytic and associative symbiosis. *Field Crop Res* 65:197–209
- James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PP, Olivares FL, Ladha JK (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Mol Plant-Microbe Interact* 15:894–906
- Ji SH, Gururani MA, Chun S-C (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars? *Microbiol Res* 169:83–98
- Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One* 6:1–22
- Johri BN (2006) Endophytic to the rescue of plant. *Curr Sci* 90:1315–1316
- KACC (1995) Korean Agricultural Culture Collection – Rural Development Administration. <http://genebank.rda.go.kr/eng/mic/itr/GeneInfo.do>. Accessed 27 May 2017
- Karthik M, Pushpakanth P, Krishnamoorthy R, Senthilkumar M (2017) Endophytic bacteria associated with banana cultivars and their inoculation effect on plant growth. *J Hortic Sci Biotechnol* 92:1–9. <https://doi.org/10.1080/14620316.2017.1310600>
- Katoch M, Pull S (2017) Endophytic fungi associated with *Monarda citriodora*, an aromatic and medicinal plant and their biocontrol potential. *Pharm Biol* 55:1528–1535
- KCTC (1985) Korean Collection for Type Cultures. <http://kctc.kribb.re.kr/English/ekctc.aspx>. Accessed 27 May 2017
- Kelemu S, Fory P, Zuleta C, Ricaurte J, Rao I, Lascano C (2011) Detecting bacterial endophytes in tropical grasses of the *Brachiaria* genus and determining their role in improving plant growth. *Afr J Biotechnol* 10:965–976
- Khan AL, Hamayun M, Kang S-M, Kim YH, Jung HY, Lee JH, Lee IJ (2012) Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *BMC Microbiol* 12:3. <https://doi.org/10.1186/1471-2180-12-3>
- Khan AL, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ (2011) Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, plant growth and isoflavone biosynthesis in soybean under salt stress. *Process Biochem* 46:440–447

- Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M (2009) Functional diversity among plant growth-promoting rhizobacteria. In: Khan MS, Zaidi A, Musarrat J (eds) *Microbial strategies for crop improvement*. Springer, Berlin, Heidelberg, pp 105–132
- Khan R, Shahzad S, Choudhary MI, Khan SA, Ahmad A (2010) Communities of endophytic fungi in medicinal plant *Withania somnifera*. *Pak J Bot* 42:1281–1287
- Khan SA, Hamayun M, Yoon HJ, Kim HY, Suh SJ, Hwang SK, Kim JM, Lee IJ, Choo YS, Yoon UH, Kong WS, Lee BM, Kim JG (2008) Plant growth promotion and *Penicillium citrinum*. *BMC Microbiol* 8:231–239
- Kidby DK (1977) Culture maintenance and productivity. In: Sandford PA, Laskin A (eds) *Extracellular microbial polysaccharides*. American Chemical Society, Washington DC, pp 1–13
- Kirsop BE, Snell JJS (eds) (1984) *Maintenance of microorganisms*. Academic, London
- Kittiwongwattana C (2015) Biodiversity of endophytic bacteria isolated from duckweed (*Landoltia punctata*) and their IAA production. *Thammasat Int J Sci Tech* 20:1–11
- Kogel K-H, Franken P, Huckelhoven R (2006) Endophyte or parasite- what decides? *Curr Opin Plant Biol* 9:358–363
- Kolbas A, Kidd P, Guinberteau J, Jaunatre R, Herzig R, Mench M (2015) Endophytic bacteria take the challenge to improve Cu phytoextraction by sunflower. *Environ Sci Pollut Res* 22:5370–5382
- Kour A, Shawl AS, Rehman S, Sultan P, Qazi PH, Suden P, Khajuria RK, Verma V (2008) Isolation and identification of an endophytic strain of *Fusarium oxysporum* producing podophyllotoxin from *Juniperus recurva*. *World J Microbiol Biotechnol* 24:1115–1121
- Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD (2016) Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech* 6:60. <https://doi.org/10.1007/s13205-016-0393-y>
- Kumar PMP, Ramesh S, Thipeswamy T, Sivaprasad V (2015) Antagonistic and growth promotion potential of endophytic bacteria of mulberry (*Morus* spp.). *Int J Ind Entomol* 31:107–114
- Kumaran RS, Muthumary J, Hur BK (2008) Production of taxol from *Phyllosticta spinarum*, an endophytic fungus of *Cupressus* sp. *Eng Life Sci* 8:438–446
- Kusari S, Lamshoft M, Spiteller M (2009) *Aspergillus fumigates* Fresenius, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. *J Appl Microbiol* 107:1019–1030
- Kusari S, Lamshoft M, Zuhlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J Nat Prod* 71:159–162
- Lacava PT, Silva-Stenico ME, Araújo WL, Simionato AVC, Carrilho E, Tsai SM, Azevedo JL (2008) Detection of siderophores in endophytic bacteria *Methylobacterium* spp. associated with *Xylella fastidiosa* sub sp. Pauca. *Pesq agropec bras, Brasília* 43:521–528
- Lahlali R, Hijri M (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS Microbiol Lett* 311:152–159
- Lanna-Filho R, Souza RM, Magalhães MM, Villela L, Zanotto E, Ribeiro-Júnior PM, Resende MLV (2013) Induced defense responses in tomato against bacterial spot by proteins synthesized by endophytic bacteria. *Trop Plant Pathol* 38:295–302
- Lee J, Lobkovsky E, Pliam NB, Strobel G, Clardy J (1995) Subglutinols A & B: immunosuppressive compounds from the endophytic fungus- *Fusarium subglutinans*. *J Org Chem* 60:7076–7077
- Li E, Jiang L, Guo L, Zhang H, Che Y (2008) Pestalochlorides A-C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. *Bioorg Med Chem* 16:7894–7899
- Li L, Sinkko H, Montonen L, Wei G, Lindström K, Räsänen LA (2012) Biogeography of symbiotic and other endophytic bacteria isolated from medicinal *Glycyrrhiza* species in China. *FEMS Microbiol Ecol* 79:46–68
- Li Y, Wang Q, Wang L, He LY, Sheng XF (2016) Increased growth and root Cu accumulation of *Sorghum sudanense* by endophytic *Enterobacter* sp. K3-2: implications for *Sorghum sudanense* biomass production and phytostabilization. *Ecotoxicol Environ Saf* 124:163–168

- Li Y, Cheng C, An D (2017) Characterisation of endophytic bacteria from a desert plant *Lepidium perfoliatum* L. *Plant Prot Sci* 53:32–43. <https://doi.org/10.17221/14/2016-PPS>
- Liu B, Huang L, Buchenauer H, Kang Z (2010a) Isolation and partial characterization of an antifungal protein from the endophytic *Bacillus subtilis* strain EDR4. *Pestic Biochem Physiol* 98:305–311
- Liu JY, Song YC, Zhang Z, Wang L, Guo ZJ, Zou WX, Tan RX (2004) *Aspergillus fumigatus* CY018, an endophytic fungus in *Cynodon dactylon* as a versatile producer of new and bioactive metabolites. *J Biotechnol* 114:279–287
- Liu K, Ding X, Deng B, Chen W (2010b) 10-Hydroxycamptothecin produced by a new endophytic *Xylaria* sp., M20, from *Camptotheca acuminata*. *Biotechnol Lett* 32:689–693
- Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou J (2008) Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. *Appl Microbiol Biotechnol* 78:241–247
- Loaces I, Ferrando L, Scavino AF (2011) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microb Ecol* 61:606–618
- Lodewyckx C, Vangronsveld J, Porteous F (2002) Endophytic bacteria and their potential applications. *Crit Rev. Plant Sci* 21:583–606
- Logeshwaran P, Thangaraju M, Rajasundari K (2009) Hydroxamate siderophores of endophytic bacteria *Gluconacetobacter Diazotrophicus* isolated from sugarcane roots. *Aust J Basic App Sci* 3:3564–3567
- Lorenzo O, Chico JM, Sánchez-Serrano JJ, Solano R (2004) Jasmonate-insensitive encodes a MYC transcription factor essential to discriminate between different jasmonate regulated defence responses in *Arabidopsis*. *Plant Cell* 16:1938–1950
- Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. *Plant Sci* 151:67–73
- Lugtenberg B, Kamilova F (2009) Plant-growth-promoting-rhizobacteria. *Annu Rev. Microbiol* 63:541–556
- Mastretta C, Taghavi S, van der Lelie D, Mengoni A, Galardi F, Gonnelli C, Barac T, Boulet J, Weyens N, Vangronsveld J (2009) Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. *Int J Phytoremediation* 11:251–267
- Meneses CHSG, Rouws LFM, Simoes-Araujo JL, Vidal MS, Baldani JJ (2011) Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus*. *Mol Plant-Microbe Interact* 24:1448–1458
- Mercado-Blanco J, Bakker PAHM (2007) Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. *Antonie Van Leeuwenhoek* 92:367–389. <https://doi.org/10.1007/s10482-007-9167-1>
- Miche L, Battistoni F, Gemmer S (2006) Up regulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. *Mol Plant-Microbe Interact* 19:502–511
- Miliūtė I, Buzaitė O (2011) IAA production and other plant growth promoting traits of endophytic bacteria from apple tree. *Biologija* 57:98–102
- Miliute I, Buzaitė O, Gelvonauskienė D, Sasnauskas A, Stanys V, Baniulis D (2016) Plant growth promoting and antagonistic properties of endophytic bacteria isolated from domestic apple. *Zemdirbyste-Agriculture* 103:77–82
- Miller SH, Brown P, Prigent-Cambaret C, Combes-Meynet E, Morrissey JP, O’Gara F (2010) Biochemical and genomic comparison of inorganic phosphate solubilisation in *Pseudomonas* species. *Environ Microbiol Rep* 2:403–411
- Mocali S, Fabiani A, Chiellini C, Gori G, Gonnelli C (2017) Endophytic and rhizospheric bacteria associated with *Silene paradoxa* grown on metal-contaminated soils are selected and transferred to the next generation of plants as seed endophytes. *GRA* 19:EGU2017–EGU9137
- Montalbán B, Thijs S, Lobo MC, Weyens N, Ameloot M, Vangronsveld J, Pérez-Sanz A (2017) Cultivar and metal-specific effects of endophytic bacteria in *Helianthus tuberosus* exposed to Cd and Zn. *Int J Mol Sci* 18:2026. <https://doi.org/10.3390/ijms18102026>



- Monteiro MCP, Alves NM, de Queiroz MV, Pinho DB, Pereira OL, de Souza SMC, Cardoso PG (2017) Antimicrobial activity of endophytic fungi from coffee plants. *Biosci J* 33:381–389
- Mousa WK, Shearer CR, Limay-Rios V, Zhou T, Raizada MN (2015) Bacterial endophytes from wild maize suppress *Fusarium graminearum* in modern maize and inhibit mycotoxin accumulation. *Front Plant Sci* 6:805. <https://doi.org/10.3389/fpls.2015.00805>
- Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O’Neil J, Frank AC (2016) Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytol* 210:657–668
- MTCC (2017) The Microbial Type Culture Collection and Gene Bank. <https://www.mtccindia.res.in/>. Accessed 27 May 2017
- Muangthong A, Youpensuk S, Rerkasem B (2015) Isolation and characterisation of endophytic nitrogen fixing bacteria in sugarcane. *Trop Life Sci Res* 26:41–51
- Mukherjee A, Bhattacharjee P, Das R, Pal A, Paul AK (2017) Endophytic bacteria with plant growth promoting abilities from *Ophioglossum reticulatum* L. *AIMS Microbiol* 3:596–612. <https://doi.org/10.3934/microbiol.2017.3.596>
- NAIMCC (2017) National Agriculturally Important Microbial Culture Collection. <http://nbaim.org.in/pages/services-culture-collectionnaimccculture-collectionnaimcc>. Accessed 27 May 2017
- Nakasone KK, Peterson SW, Jong SC (2004) Preservation and distribution of fungal cultures. *Biodiversity of fungi: inventory and monitoring methods*. Elsevier, Amsterdam, pp 37–47
- Nascimento MA, Nascimento CV, Antunes JE, Figueiredo MV, Tabosa JN, Martínez CR (2014) Selection of plant growth promoting bacteria in sweet sorghum (*Sorghum bicolor* (L.) Moench) under the effects of salinity. *BMC Proc* 8:P113
- Ngamau CN, Matiru VN, Tani A, Muthuri CW (2012) Isolation and identification of endophytic bacteria of bananas (*Musa spp.*) in Kenya and their potential as biofertilizers for sustainable banana production. *Afr J Microbiol Res* 6:6414–6422
- Nielson TH, Christophersen C, Anthoni U, Sorensen J (1999) Viscosinamide, a new cyclic depsipeptide with surfactant and antifungal properties produced by *Pseudomonas fluorescens* DR54. *J Appl Microbiol* 87:80–90
- Nielson TH, Tharne C, Christophersen C, Anthoni U, Sorensen J (2000) Structure, production characteristics and fungal antagonism of tensin-a new antifungal cyclic lipopeptide from *Pseudomonas fluorescens* strain 96.578. *J Appl Microbiol* 89:992–1001
- Oliveira CA, Alves VM, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimaraes CT, Schaffert RE, Sa NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado biome. *Soil Biol Biochem* 41:1782–1787
- Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol* 6:745. <https://doi.org/10.3389/fmicb.2015.00745>
- Pan D, Mionetto A, Tiscornia S, Bettucci L (2015) Endophytic bacteria from wheat grain as biocontrol agents of *Fusarium graminearum* and deoxynivalenol production in wheat. *Mycotoxin Res* 31:137–143
- Pandey PK, Yadav SK, Singh A, Sarma BK, Mishra A, Singh HB (2012) Cross-species alleviation of biotic and abiotic stresses by the endophyte *Pseudomonas aeruginosa* PW09. *J Phytopathol* 160:532–539
- Pandey PK, Singh AK, Singh S, Chandrakumar Singh M (2013) Bacterial Endophytes: current status and future prospects. In: Tiwari SP, Sharma R, Singh RK (eds) *Recent advances in microbiology*, vol 1. Nova Science, New York, pp 495–518
- Pandey PK, Singh S, RNS Y, Singh AK, MCK S (2014) Fungal endophytes: promising tools for pharmaceutical science. *Inter J Pharm Sci Rev Res* 25:128–138
- Pandey PK, MCK S, Singh AK, Singh S, Pandey AK, Pathak M, Kumar M, Shakywar RC, Patidar RK, Devi MB (2016) Arsenal of endophytic actinobacterial microbes. *Int J Curr Microbiol App Sci* 5:62–66

- Pandey PK, MCK S, Singh S, Singh AK, Kumar M, Pathak M, Shakywar RC, Pandey AK (2017) Inside the plants: endophytic bacteria and their functional attributes for plant growth promotion. *Int J Curr Microbiol App Sci* 6:11–21
- Pandi M, Manikandan R, Muthumary J (2010) Anticancer activity of fungal taxol derived from *Botryodiplodia theobromae* Pat., An endophytic fungus, against 7, 12 dimethyl benz(a)anthracene (DMBA)-induced mammary gland carcinogenesis in Sprague dawley rats. *Biomed Pharmacother* 64:48–53
- Park J-H, Choi GJ, Lee HB, Kim KM, Jung HS, Lee SW, Jang KS, Cho KY, Kim J-C (2005) Griseofulvin from *Xylaria* sp. strain F0010, an endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. *J Microbiol Biotechnol* 15:112–117
- Park M, Lee H, Hong SG, Kim O-S (2013) Endophytic bacterial diversity of an Antarctic moss, *Sanionia uncinata*. *Antarct Sci* 25:51–54
- PCC (2015) Pastuer culture collection of cyanobacteria. Web site – Institut Pasteur. <http://cyanobacteria.web.pasteur.fr/>. Accessed 27 May 2017
- Pathak KV (2011) Purification and characterization of antifungal compounds produced by banyan endophytic *Bacilli*. PhD Thesis, Sardar Patel University, Gujarat
- Paul NC, Ji SH, Deng JX, Yu SH (2013) Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens in vitro. *Plant Omics J* 6:441–448
- Pavithra N, Sathish L, Ananda K (2012) Antimicrobial and enzyme activity of endophytic fungi isolated from Tulsi. *J Pharm Biomed Sci* 16:1–6
- Perez-Rosales E, Alcaraz-Meléndez L, Puente ME, Vázquez-Juárez R, Quiroz-Guzmán E, Zenteno-Savín T, Morales-Bojórquez E (2017) Isolation and characterization of endophytic bacteria associated with roots of jojoba (*Simmondsia chinensis* (Link) Schneid). *Curr Sci* 112:396–401
- Petrini O (1991) Fungal endophytes in the tree leaves. In: Andrews JH, Hirano SS (eds) *Microbial ecology of leaves*. Springer, New York, pp 179–197
- Pongcharoen W, Rukachaisirikul V, Phongpaichit S, Kühn T, Pelzing M, Sakayaro J, Taylor WC (2008) Metabolites from the endophytic fungus *Xylaria* sp. PSU-D14. *Phytochemistry* 69:1900–1902
- Priyadharsini P, Muthukumar T (2017) The root endophytic fungus *Curvularia geniculata* from *Parthenium hysterophorus* roots improves plant growth through phosphate solubilization and phytohormone production. *Fungal Ecol* 27(A):69–77
- Puente ME, Li CY, Bashan Y (2009) Rock-degrading endophytic bacteria in cacti. *Environ Exp Bot* 66:389–401
- Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ, Riyaz-Ul-Hassan S (2013) Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. *Springerplus* 2:8. <https://doi.org/10.1186/2193-1801-2-8>
- Rabe F, Ajami-Rashidi Z, Doehlemann G, Kahmann R, Djamei A (2013) Degradation of the plant defence hormone salicylic acid by the biotrophic fungus *Ustilago maydis*. *Mol Microbiol* 89:179–188
- Rai R, Dash PK, Prasanna BM, Singh A (2007) Endophytic bacterial flora in the stem tissue of a tropical maize (*Zea mays* L.) genotype: isolation, identification and enumeration. *World J Microbiol Biotechnol* 23:853–858
- Ratul N, Sharma GD, Barooah M (2013) Screening of endophytic bacterial isolates of tea (*Camellia sinensis* L.) roots for their multiple plant growth promoting activities. *IJAEB* 6:211–215
- Reinhold-Hurek B, Maes T, Gemmer S (2006) An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* sp. strain BH72. *Mol Plant-Microbe Interact* 19:181–188
- Reva ON, Smirnov VV, Pattersson B, Priest FG (2002) *Bacillus* endophyticus sp. Nov isolated from the inner tissues of cotton plants (*Gossypium* sp.). *Int J Syst Bacteriol* 52:101–107
- Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. *J Exp Bot* 62:3321–3338

- Robinson RJ, Fraaije BA, Clark IM, Jackson RW, Hirsch PR, Mauchline TH (2015) Endophytic bacterial community composition in wheat (*Triticum aestivum*) is determined by plant tissue type, developmental stage and soil nutrient availability. *Plant Soil* 4:1–6. <https://doi.org/10.1007/s11104-015-2495-4>
- Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Tansley review; fungal endophytes: diversity and functional roles. *New Phytol* 182:314–330. <https://doi.org/10.1111/j.1469-8137.2009.02773.x>
- Rogers A, McDonald K, Muehlbauer MF (2012) Inoculation of hybrid poplar with the endophytic bacterium *Enterobacter* sp. 638 increases biomass but does not impact leaf level physiology. *Glob Change Biol Bioenergy* 4:364–370
- Romero FM, Marina M, Pieckenstein FL (2014) The communities of tomato (*Solanum lycopersicum* L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing. *FEMS Microbiol Lett* 351:187–194
- Rosenblueth M, Martinez Romero E (2004) *Rhizobium etli* maize populations and their competitiveness for root colonization. *Arch Microbiol* 181:337–344
- Rungin S, Indananda C, Suttiviriya P, Kruasuwan W, Jaemsang R (2012) Plant growth enhancing effects by a siderophore producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). *Antonie Van Leeuwenhoek* 102:463–472
- Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett* 278:1–9
- Sauv tre A, Schr der P (2015) Uptake of carbamazepine by rhizomes and endophytic bacteria of *Phragmites australis*. *Front Plant Sci* 6:83. <https://doi.org/10.3389/fpls.2015.00083>
- Schloter M, Kirchof G, Heinzmann U, D bereiner J, Hartmann A (1994) Immunological studies of the wheat root colonization by the *Azospirillum brasilense* strains Sp7 and Sp 245 using strain specific monoclonal antibodies. In: Hegazi N, Fayez M, Monib M (eds) Nitrogen fixation with non-legumes. The American University in Cairo Press, Cairo, Egypt, pp 290–295
- Senthilkumar M, Swarnalakshmi K, Govindasamy V (2009) Biocontrol potential of soybean bacterial endophytes against charcoal rot fungus, *Rhizoctonia bataticola*. *Curr Microbiol* 58:288–293. <https://doi.org/10.1007/s00284008-9329-z>
- Sessitsch A, Reiter B, Berg G (2004) Endophytic bacterial communities of field grown potato plants and their plant–growth–promoting and antagonistic abilities. *Can J Microbiol* 50:239–249
- Sev TM, Khai AA, Aung A, Yu SS (2016) Evaluation of endophytic bacteria from some rice varieties for plant growth promoting activities. *JSIR* 5:144–148
- Shaanker RU, Ramesha BT, Ravikanth G, Gunaga R, Vasudeva R, Ganeshiah KN (2008) Chemical profiling of *N. nimmoniana* for camptothecin, an important anticancer alkaloid: towards the development of a sustainable production system. In: Ramawat KG, Merillion J (eds) Bioactive molecules and medicinal plants. Springer, Berlin, Germany, pp 198–210
- Shankar NB, Shashikala J (2010) Diversity and structure of fungal endophytes in some climbers and grass species of Malnad region, Western Ghats, Southern India. *Mycosphere* 1:265–274
- Sharma PK, Sarita S, Prell J (2005) Isolation and characterization of an endophytic bacterium related to *Rhizobium/Agrobacterium* from wheat (*Triticum aestivum* L) roots. *Curr Sci* 89:608–610
- Sharma S, Roy S (2015) Isolation and identification of a novel endophyte from a plant *Amaranthus spinosus*. *Int J Curr Microbiol App Sci* 4:785–798
- Sharma SK, Saini S, Verma A, Sharma PK, Lal R, Roy M, Singh UB, Saxena AK, Sharma AK (2017) National Agriculturally Important Microbial Culture Collection in the global context of microbial culture collection centres. *Proc Natl Acad Sci India, Sect B Biol Sci*. <https://doi.org/10.1007/s40011-017-0882-8>
- Shcherbakov AV, Bragina AV, Kuzmina EY, Berg C, Muntyan AN, Makarova NM, Malfanova NV, Cardinale M, Berg G, Chebota VK, Tikhonovich IA (2013) Endophytic bacteria of Sphagnum mosses as promising objects of agricultural microbiology. *Microbiology* 82:306–315

- Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmüller R (2005) The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water Dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J Biol Chem* 280:26241–26247
- Shi Y, Lou K, Li C (2009) Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biol Fertil Soils* 45:645–653
- Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. *J Exp Bot* 58:221–227
- Sikora RA, Schäfer K, Dababat AA (2007) Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. *Australas Plant Pathol* 36:124–134
- Silva-Froufe LG, Boddey RM, Reis VM (2009) Quantification of natural populations of *Gluconacetobacter diazotrophicus* and *Herbaspirillum* spp. in sugar cane (*Saccharum* spp.) using different polyclonal antibodies. *Braz J Microbiol* 40:866–878
- Simons M, van der Bij AJ, de Weger LA, Wijffelman CA, Lugtenberg BJ (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Mol Plant-Microbe Interact* 9:600–607
- Singh MJ, Padmavathy S (2015) Hydrocarbon biodegradation by endophytic bacteria from neem leaves. *LS: An Int J Life Sci* 4:33–36
- Sly LI, Lijima T, Kirsop B (eds) (1990) 100 years of culture collections proceedings of the Kral Symposium to celebrate the centenary of the first recorded service culture collection. International House, Osaka
- Smith D (2003) Culture collections over the world. *Int Microbiol* 6:95–100
- Smith D, Onions AH (1983) A comparison of some preservation techniques for fungi. *Trans Br Mycol Soc* 81:535–540
- Souza A, Cruz JC, Sousa NR, Procópio AR, Silva GF (2014) Endophytic bacteria from banana cultivars and their antifungal activity. *Genet Mol Res* 12:8661–8670
- Stintzi AC, Barnes JX, Raymond KN (2000) Microbial iron transport via a siderophore shuttle: a membrane ion transport paradigm. *PNAS* 97:10691–10696
- Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* 67:491–502
- Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. *J Nat Prod* 67:257–268
- Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, Fung PC, Ming Wah Chau R (2002) Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. *Phytochemistry* 60:179–183
- Strobel GA, Hess WM, Ford E, Sidhu RS, Yang X (1996) Taxol from fungal endophytes and issue of biodiversity. *J Ind Microbiol* 17:417–423
- Strobel GA, Hess WM, Li JY, Ford E, Sears J, Sidhu RS, Summerell B (1997) *Pestalotiopsis guepinii*, a taxol producing endophyte of the Wollemi Pine, *Wollemia nobilis*. *Aust J Bot* 45:1073–1082
- Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit Rev. Plant Sci* 19:1–30
- Sutjaritvorakul T, Whalley AJS, Sihanonth P, Roengsumran S (2011) Antimicrobial activity from endophytic fungi isolated from plant leaves in Dipterocarpous forest at Viengsa district Nan province, Thailand. *J Agric Tech* 7:115–121
- Suto M, Takebayashi M, Saito K, Tanaka M, Yokota A, Tomita F (2002) Endophytes as producers of xylanase. *J Biosci Bioeng* 93:88–90
- Swarnalatha Y, Saha B, Lokeswara Choudary Y (2015) Bioactive compound analysis and antioxidant activity of endophytic bacterial extract from *Adhatoda beddomei*. *Asian J Pharm Clin Res* 8:70–72
- Tenguria RK, Khan FN, Quereshi S (2011) Endophytes- mines of pharmacological therapeutics. *World J Sci Tech* 1:127–149

- Tervet IW, Holli JP (1948) Bacteria in the storage organs of healthy plants. *J Phytopathol* 38:960–967
- Thamizhvendan R, Yu YJ, Lee SH, Rhee YH (2010) Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. *J Microbiol* 48:559–565
- TNCAGR (2017) The North Carolina Arboretum Germplasm Repository. <http://www.ncarboretum.org/impact/germplasm-repository/>. Accessed 27 May 2017
- Tommerup IC, Kidby DK (1979) Preservation of spores of vesicular-arbuscular endophytes by L-drying. *Appl Environ Microbiol* 37:831–835
- Upreti R, Thomas P (2015) Root-associated bacterial endophytes from *Ralstonia solanacearum* resistant and susceptible tomato cultivars and their pathogen antagonistic effects. *Front Microbiol* 6:255. <https://doi.org/10.3389/fmicb.2015.00255>
- Van Loon LC, Bakker PA, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. *Annu Rev. Phytopathol* 36:453–483
- Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. *Biol Control* 46:72–82
- Verma SC, Ladha JK, Tripathi AK (2001) Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *J Biotechnol* 91:127–141
- Verma VC, Singh SK, Prakash S (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J Basic Microbiol* 51:550–556
- Vetrivelkai P, Sivakumar M, Jonathan EI (2010) Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in bhendi. *J Biopest* 3:452–457
- Villacieros M, Power B, S'anchez-Contreras M, Lloret J, Oraezabal RI, Martin M, Fernandez-pinas F, Bonilla I, Whelan C, Dowling DN, Rivilla R (2003) Colonization behaviour of *Pseudomonas fluorescens* and *Sinorhizobium meliloti* in the alfalfa (*Medicago sativa*) rhizosphere. *Plant Soil* 251:47–54
- Vinale FK, Sivasithamparam EL, Ghisalberti R, Marra R, Woo SL, Lorito M (2008) *Trichoderma* plant pathogens interactions. *Soil Biol Biochem* 40:1–10
- Vitorino LC, Silva FG, Soares MA, Souchie EL, Costa AC, Lima WC (2012) Solubilization of calcium and iron phosphate and in vitro production of Indole acetic acid by endophytic isolates of *Hyptis maritima* Epling (Lamiaceae). *Inter Res J Biotechnol* 3:47–54
- Vogl AE (1898) Mehl und die anderen mehlprodukte der cerealien und leguminosen. *Nahrungsm Unters Hyg Warenk* 12:25–29
- Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC (2007) Antimicrobial potentials of endophytic fungi residing in *Quercus variabilis* and *Brefeldia* A obtained from *Cladosporium* sp. *W J Microbiol Biotech* 23:79–83
- Wang H, Wen K, Zhao X, Wang X, Li A, Hong H (2009) The inhibitory activity of endophytic *Bacillus* sp. strain CHM1 against plant Pathogenic fungi and its plant growth-promoting effect. *Crop Prot* 28:634–639
- Wang X, Liang G (2014) Control efficacy of an endophytic *Bacillus amyloliquefaciens* strain BZ6-1 against peanut bacterial wilt, *Ralstonia solanacearum*. *Biomed Res Int* 465435:1–11
- Wani ZA, Mirza DN, Arora P, Riyaz-UI-Hassan S (2016) Molecular phylogeny, diversity, community structure, and plant growth promoting properties of fungal endophytes associated with the corms of saffron plant: an insight into the microbiome of *Crocus sativus* Linn. *Fungal Biol* 120:1509–1524
- Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frei dit Frey N, Leung J (2008) An update on abscisic acid signaling in plants and more. *Mol Plant* 1:198–217
- Weber D, Sterner O, Anke T, Gorzalczancy S, Martino V, Acevedo CJ (2004) Phomol, a new anti-inflammatory metabolite from an endophyte of the medicinal plant *Erythrina crista galli*. *J Antibiot* 57:559–563
- Weyens N, van'derLelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. *Trends Biotechnol* 27:591–598

- WFCC (2011) World Federation for Culture Collection. <http://www.wfcc.info/>. Accessed 27 May 2017
- Whipps J (2001) Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot* 52:487–511
- Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414:562–565
- Yadav A, Yadav K (2017) Exploring the potential of endophytes in agriculture: a minireview. *Adv Plants Agric Res* 6(4):00221. <https://doi.org/10.15406/apar.2017.06.00221>
- Yazdani M, Bahmanyar MA, Pirdashti H, Esmaili MA (2009) Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays* L.). *World Acad Sci Eng Technol* 49:90–92
- Yonebayashi K, Katsumi N, Nishi T, Okazaki M (2014) Activation of nitrogen-fixing endophytes is associated with the tuber growth of sweet potato. *Mass Spectrom (Tokyo)* 3(1):A0032. <https://doi.org/10.5702/massspectrometry.A0032>
- Yonghong L, Jianwei G, Li L, Asem MD, Yongguang Z, Mohamad OA, Salam N, Wenjun L (2017) Endophytic bacteria associated with endangered plant *Ferula sinkiangensis* K. M. Shen in an arid land: diversity and plant growth-promoting traits. *J Arid Land* 9:432–445
- Zachow C, Fatehi J, Cardinale M, Tilcher R, Berg G (2010) Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. *FEMS Microbiol Ecol* 74:124–135
- Zhang XX, George A, Bailey MJ, Rainey PB (2006) The histidine utilization (hut) genes of *Pseudomonas fluorescens* SBW25 are active on plant surfaces, but are not required for competitive colonization of sugar beet seedlings. *Microbiology* 152:1867–1875
- Zhao J, Zhou L, Wang J, Shan T, Zhong L, Liu X, Gao X (2010) Endophytic fungi for producing bioactive compounds originally from their host plants. In: Mendez-Vilas A (ed) Current research, technology and education topics in applied microbiology and microbial biotechnology. Formatex Research Center, Badajoz, Spain, pp 567–576
- Zhao LF, Xu YJ, Zheng A, Lai XH (2017) Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. *Braz J Microbiol* 49:269–278. <https://doi.org/10.1016/j.bjm.2017.06.007>
- Zhenhua X, Dongmei G, Xiuli S, Ying X (2012) A review of endophyte and its use and function. In: Proceedings of the International Conference on Environmental Engineering and Technology, Advances in Biomedical Engineering (ICEET'12), vol 8. Environmental Engineering and Technology, Zhangjiaji, China, pp 124–130
- Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmariski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl Environ Microbiol* 68:2198–2208