Chapter 19 Bioprospecting of Endophytes for Agricultural and Environmental Sustainability

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19.1 Biodiversity of Endophytes

Endophytes are the most diverse group of microorganisms found in virtually every plant on earth. They are widely spread in various environment conditions. Although endophytes commonly refers to mostly fungi or bacteria it has already been recognized in marine algae (Smith et al. 1989; Stanley 1992) and mosses and ferns (Petrini et al. 1992; Raviraja et al. 1996). Endophytes were reported to be chiefly isolated from plants ranging from palm to large trees, marine grasses and lichens. The biodiversity of endophytes are mainly classified into three categories such as fungal, bacterial and algal endophytes.

19.1.1 Fungal Endophytes

Fungal endophytes are most commonly studied among other endophytes. Fungi are plant like organism that lack chlorophyll, true roots, stems and leaves. An endophytic fungus lives in inter or intra cellular spaces in stem, petiole and leaves of a plant for a part or whole of their life cycle. Most endophytes isolated belong to ascomycetes and their anamorph basidiomycetes. The total biodiversity of fungal endophytes may be classified into two major categories. These include the

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	Clavicipitacious	Nonclavicipita	ceous	
Criteria	Class-1	Class-2	Class-3	Classs-4
Host range	Narrow	Broad	Broad	Broad
Tissue colonized	Shoot and rhizome	Shoot, root and rhizome	Shoot	Root
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
In plantacolonization	Extensive	Extensive	Limited	Extensive
In planta biodiversity	Low	Low	High	Unknown

 Table 19.1
 Symbiotic criteria used to characterize fungal endophytic classes

 Table 19.2
 Fungal endophytes with their host range

Fungal				Host	Colonized
endophytes	Phyllum	Class	Order	range	part
Colletotrichum	Ascomycota	Sordariomycetes	Glomerellales	Wide	Shoots & roots
Curvularia	Ascomycota	Dothideomycetes	Pleosporales	Wide	Shoots & roots
Epichloë	Ascomycota	Sordariomycetes	Hypocreales	Grasses	Shoots
Fusarium	Ascomycota	Sordariomycetes	Hypocreales	Wide	Shoots & roots
Neotyphodium	Ascomycota	Sordariomycetes	Hypocreales	Grasses	Shoots
Piriformospora	Basidiomycota	Agaricomycetes	Sebacinales	Wide	Roots
Serendipita	Basidiomycota	Agaricomycetes	Sebacinales	Wide	Roots

clavicipitaceous (CE), which infect some grasses confined to cool regions and the non-clavicipitaceous endophytes (NCE), which are widely distributed and found in asymptomatic tissues of non vascular plants, conifers, ferns and angiosperms. However, NCE are reported to be restricted to Ascomycota and Basidicomycota groups (Jalgaonwala et al. 2011; Bhardwaj and Agrawal 2014). Symbiotic criteria used to characterize fungal endophytic classes are shown in Table 19.1.

Typically CE occurs on plant shoots where they form systemic intercellular infections. CE are fastidious in culture and are restricted to some grasses that grow in warm and cool season (Bischoff and White 2005). NCE are primarily ascomycetes fungi recovered from land plants, terrestrial eco systems ranging from agro biosystems to biomes, that are widespread from tropic to tundra (Arnold 2007). The examples of fungal and bacterial endophytes with their host range is shown in Tables 19.2 and 19.3 respectively.

19.1.2 Bacterial Endophytes

Bacterial endophytes are the second most studied endophytes after fungi. More than 16 phyla of bacterial endophytes belonging to about 200 genera were reported, most of which belong to the phyla Actinobacteria, Proteobacteria and Firmicutes

Bacterial			
endophytes	Examples	Host plant species	Colonizing area
Proteobacteria	Erwinia, Pseudomonas	Alfalfa (Medicago sativa L.)	Roots
γ-proteobacteria	Pseudomona, Serratia	Black pepper (Pipernigrum L.)	Roots
β-proteobacteria	β-proteobacteria	Grape (Vitis sp.)	Stems
Proteobacteria	Proteobacteria	Radish (Raphanus sativus L.)	Leaves and roots
Actinobacteria	Corynebacterium	Sugar beet (Beta vulgaris L.)	Roots
β-proteobacteria	Burkholderia cepacia	Wheat (Triticum aestivum L.)	Roots
α-proteobacteria	Erwinia.,	Soybean (Glycine max (L.)	Stems, leaves,
	Agrobacterium	Merr.	roots

Table 19.3 Bacterial endophytes with their host range

(Golinska et al. 2015). Gram positive and negative bacterial species majorly contribute to the diversity of bacterial endophytes viz., *Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevibacterium, Microbacterium, Pseudomonas, Xanthomonas* etc (Sun et al. 2013). These endophytes resides intracellularly in root and shoot cells of plants. In thisintracellular form bacteria loss cell wall but continue to divide and metabolize. These wall-less intracellular forms of bacteria are called L-forms (Allan et al. 2009). Actinomyces are prokaryotic organisms belonging to the phylum actinobacteria resembling mycelium like fungus and are spore producers (Chaudhary et al. 2013).

19.1.3 Algal Endophytes

Algae are simple chlorophyll bearing vascular plants. Algae are eukaryotic organisms that have no roots, stems or leaves. A number of endophytes are now known that grows within seaweeds and algae (Andrew et al. 2013). One such example is *Ulvella leptochaete*, which has recently been discovered from host algae including Cladophora and Laurentia from India. Recently a team led by Dr. Felix Bast, Assistant Professor, Central University of Punjab (2016), Bathinda able to discover the microscopic endophytic algae within its host seaweeds. The research involved analysis of green seaweeds, *Cladophora glomerata* collected from Calicut, Kerala and red seaweeds, *Laurentia obtusa* collected from Mandapam, Tamil Nadu.

19.2 Interaction Between the Endophytes and Their Host Plants

The endophytes, which are a major part of plant micro-ecosystems, found in healthier tissues are in an endosymbiotic relationship with the plants they invade. In a greater view it is found that these endophytes not only enhance the tolerance of the plant, in biotic or abiotic stress helps but also improves the quality as well as the quantity of the secondary phytocompounds that are used as bioactive compounds or drugs (Jia et al. 2016). Due the development of sophisticated new versatile pseudo branches of life-sciences such as proteomics, genomics and their subdivided branches such as metabolomics, trancriptomics, it became much easier to determine molecular interaction at the most nano level (Rout and Sahoo 2013; Pervez et al. 2016; Abdurakhmonov 2016). On the basis of these advanced modes of evaluations it is found that the endophytic population may greatly be affected by the age of the plant, genetic variations in the plant, or environmental background of the host plant that lies in it (Jia et al. 2016). Taking the benefits into count, further bioengineer the endophyte with desired efficiency can be designed and incorporated into the host plant to produce the required quantity of the primary, secondary phytocompounds or drugs. Apart from the growth of the host plant, proper uptake of the nutrients by the host plant the disease resistance of the host plant against pathogen can also be improved (Mei and Flinn 2010).

19.3 Transmission of Endophytes

Endophytes which lives inside plants for a part or whole of their life cycle can be transmitted from parents to offspring or between two individual plants in a community. There are basically two types of transmission.

19.3.1 Vertical Transmission

Vertical transmission is a method of direct transfer from parents to offspring. Vertically transmitted fungal endophytes are typically considered clonal and transmit via fungal hyphae penetrating the embryo within the host's seeds (e.g., seed transmitting forms of *Epichloe*) (White et al. 1993; David and Manish 2011).

19.3.2 Horizontal Transmission

Horizontal transmission is among individual plants in a community. Reproduction through asexual or sexual spores leads to horizontal transmission, where endophytes may spread between plants in a population or community (Mariusz et al. 2014). Most fungi are terrestial, growing as hyphae and producing thick walled non motile spores. Spores are single-celled propagules which separate from the organism and can get dispersed. In both sexual and asexual transmission fungi produce spores that disperse among plants via air or through animals.

19.3.3 Transmission of Fungal Endophytes

A fungal hyphae is a long branching filamentous structure required for its vegetative growth. In vertical transmission endophyte is found in the embryo of infected seed. As the seed germinates, the endophyte grows into emerging leaf and finally grows up the stem and into the seed head of the reproductive plant.

19.3.4 Transmission of Bacterial Endophytes

Mostly bacterial endophytes are originated from rhizosphere or phyllosphere, however some are transmitted through seeds Rhizosphere is the narrow region of the soil that is directly influenced by root secretions and associated soil microorganisms. Endophytic bacteria have been isolated from monocotyledaneous [*Miscanthus* giganteus, Iris pseudacorus, Phragmites australis, Lythrum salicaria and Cladium mariscus], dicotyledonous [Gosspium hirsutum, Cucumis sativis and Beta vulgaris] to herbaceous plants. The root system secrets chemical exudates (flavinoids, amino acids, carbohydrates, organic acids). Bacterial endophytes attracted towards these chemicals and by chemotactic movement it gets attached to the root. Various molecules like cellulose, pectinase, superoxide dismutase etc. secreted by bacteria to colonize plants. Finally bacteria enter through roots and spread to other parts. Plant roots exude many organic compounds that stimulates microbial growth and can have major impact on the composition of rhizosphere microbiome (Grayston et al. 1998; Miethling et al. 2000).

Endophyte distribution within plants depends on a combination of ability to colonize and allocation of plant resources. Root endophytes often colonize and penetrate the epidermis at sites of lateral root emergence, below the root hair zone, and in root cracks (Dong et al. 2003; Compant et al. 2005; Zakria et al. 2007). These colonizers are capable of establishing populations both inter- and intracellularly (Hurek et al. 1994; Zakria et al. 2007). After initial colonization, some endophytes can move to other areas of the plant by entering the vascular tissues and spreading systemically (Compant et al. 2005; Zakria et al. 2007; David and Manish 2011). Using endophytes labeled with green-fluorescent-protein (GFP), David and Manish (2011) demonstrated the transport of the endophytes from seeds into plant roots and tissues, and endophytes injected into stems moved into the roots and rhizosphere, suggesting that there may be a continuing movement of organisms throughout the root microbiome.

19.4 Endophytes for Environment and Agriculture Sustainability

Endopytes which live a part or full of their life cycle inside the host plant and secrete wide variety of compounds essential for the growth of plants and protection from environmental conditions. Bioactive compounds are of enormous importance to plants as well as human are produced by endophytes. The phytocompounds secreted by the endophytes acts as biocontrol agent by exerting protective action of the plants from repeated grazing of herbivores on same plant. The compounds produced by endophytes are of immense importance as antibiotics, drugs or medicinal compounds useful for food industry and the compounds of high relevance in research. Endohytes are involved in phytoremediation, biodegradation, and nutrient cycling and thus reduces use of pesticides in agriculture and protect our environment from hazardioaus chemicals. Summarising the profound applications of endophytes and its impact on plants, human and environment, they have been proved to be a boon and not a ban and have potential to sustain the agriculture in a better way. Various application of endophytes by means of which it promote plant growth as well as sustain the environment and agriculture are given below.

19.5 Applications of Endophytes

The efficiency of endophytes to produce novel bioactive compounds with unique structures and bioactivities have proven to be helpful in agricultural sustainability, environmental conservation and ecotoxicological importance is currently being explored to their maximum extent. In lieu of a huge reservoir, these vast potentialities of secondary products, as well as their exploitation for agricultural and environmental benefaction, are scanty. The current development in endophytic research is mainly focused on evaluating endophytic microbial populations inhabiting plants, which enhances plant growth, disease resistance and the ability to tolerate or withstand the external environment. It can be assumed that these humble researches will emerge as a boon and would certainly leave a wide impact on agricultural science, environmental sustainability as well as for the welfare of mankind through their personification in technological advancement in phytological technologies. The impact of endophytes that enhances plant growth, disease resistance, agricultural and environmental sustainability as well as its ecotoxicological importance is shown in Table 19.4.

19.5.1 Nutrient Cycling

Nutrient cycling is an important process of balancing of the nutrients and make it available for each ecosystem components. The nutrients are made available by the degradation of biomass by saprophytes and recycled into the environment thus

Table 19.4 Impact of en	dophytes in agricultural	Table 19.4 Impact of endophytes in agricultural and environmental sustainability			
		Endophytic organism	Bioactive compounds/		
Function	Plants	Bacteria	Phytocompounds	Function	References
Phytostimulation	Phragmites communis, Potamogeton crispus, Nymphaea crispus, Majas marina	Aeromonas caviae, A. salmonicida, Bacillus niacin, B. aryabhattai, B. sphaericus, B. simplex, Delttia tsuruhatensis, Paenibacillus lautus, Enterobacter hormaechei, Floxobacterium oceanosedimentum	Aromatıc phytocompounds	Biodegradation (Chlorpyrifos Fenpropathrin Naphthalene Bifenthrin) and P-soluhilization	Chen et al. (2012)
		Klebsiella terrigena, Lactococcus lactis, Microbacrium flavescens, Paenibacillus barcinonensis, Pantoea agglomerans, Pseudomonas jessenii, P. taiwanensis, P. xanthomarina, Staphylococcus epidermidis			
	Arabidopsis thaliana, Ptatycladus orientalis	Phyllobacterium brassicacearum, B. subtilis	Abscisic acid (ABA)	Decrement of leaf transpiration (A. <i>thaliana</i>), stomatal conductance (<i>P.</i> <i>orientalis</i>)	Bresson et al. (2013); Liu et al. (2013)
	Glycine max Lavandula dentate	P. putida, B. thuringiensis	Gibberellin Indole-3-acetic acid (IAA)	Growth promotion	Armada et al. (2014)
	Vitis vinifera L cv.	Bacillus, Mirococcus and Pantoa genera	Ammonia, IAA, IAA-like molecules, siderophores and lytic enzyme	Phosphate solubilization and growth promotion	Baldan et al. (2015)

(continued)

		Endonhytic organism	Dissetius someondal		
Function	Plants	Bacteria	Phytocompounds	Function	References
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	Z. mays L., Solanum lycopersicum L., Citrullus lanatus	B. cereus, B. licheniformis, B. pumilus, B. simplex, Bacillus sp., B. thuringiensis, Burkholderia	Growth hormones	Growth promotion	Xia et al. (2015)
	Thunb.	gladioli, B. gladioli pv. Alliicola, Paracoccus halophilus, Stenotrophomonas maltophilia, and Stenotrophomonas so			
	Phaseolus vulgaris L.	Trichoderma polysporum, Trichoderma atroviridae and	Proteolytic enzymes and phosphate	Phytoactivation and/or	Pierre et al. (2016
		Trichoderma harzianum	solubilization factors	phytoinhibition	
	A. thaliana	Kosakonia radicincitans	20S proteasome alpha-3 subunit	Growth promotion	Witzel et al. (2017)
	Simmondsia chinensis	Bacillus sp., Streptomyces sp., Methylobacterium aminovorans, Rhodococcus pyridinivorans and Oceanobacillus kimchi	Growth hormones	Growth promotion	Perez-Rosales et al. (2017)
		Fungus			
	Suaeda japonica	Penicillium sp.	Bioactive gibberellins (GAs) and other inactive GAs	Enhanced seed germination	You et al. (2012)
	Panax ginseng	Aspergillus sp., Cladosporium sp., Engyodontium sp., Fusarium sp., Penicillium sp., Plectosphaerella sp., Verticillium sp. and Ascomycete sp.	Triterpenoid saponins and ginsenosides	Root growth and protection	Wu et al. (2013)
	Tinospora cordifolia Phoma sp. and Calotropis procera	Phoma sp.	Growth hormones	Growth promotion	Kedar et al. (2014)

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Table 19.4 (continued)

Proteolytic enzymes. Enhanced seed Pierre et al. (2016)	germination	Enhanced IAA, ACCPlant growthZahoor et al. (2017)deaminase and solubilize phosphatepromotionsecretion		Plant growth de Araujo et al. promotion and (2000) protection	Enhanced IAA, Plant growth Gangwar et al. siderophore promotion, (2014) production solubilization
sp., Trichoderma sp., Penicillium metabolites citrinum, P. nigricans	ie, T. zianum	Mucor sp.	Actinomycetes	Microbispora, Streptomyces and Streptosporangium	Streptomyces albosporus, S. aureus, Enha S. cinereus, S. globisporus, S. siderc griseofuscus, S. roseosporus, S. produ viridis, S. griseorubruviolaceus, Actinopolyspora sp., Micromonospora sp., Saccharopolysporasp.
Enantia chlorantha, Kigelia africana	Phaseolus vulgaris	Brassica campestris		Z. mays L.,	Aloe vera, Mentha arvensis and Ocimum sanctum

Table 19.4 (continued)					
		Endophytic organism	Bioactive compounds/		
Function	Plants	Bacteria	Phytocompounds	Function	References
	Saccharum officinarum	Streptomyces sp. and Micromonospora sp.	Enhanced IAA, siderophore production	Plant growth promotion, and biosolubilizing activities of insoluble phosphate and leonardite,	Sinma et al. (2015)
	Triticumae stivum	Streptomyces nobilis, S. kunmingensis, S. mutabilis,	Enhanced IAA production,	Plant growth promotion,	Anwar et al. (2016)
	Solanum lycopersicum	S. enissocaesilis, S. djakartensis	siderophore, and HCN and ACC deaminase production	phosphate solubilization	
	Dracaena cochinchinensis Lour.	Streptomyces sp., Nocardiopsis sp., Brevibacterium sp., Microbacterium sp., Tsukamurella sp., Arthrobacter sp., Brachybacterium sp., Nocardia sp., Rhodococcus sp., Kocuria sp., Nocardioides sp. and Pseudonocardia sp.	1	Plant growth promotion and protection	Salam et al. (2017)
Phyto-immobilization		Bacteria			
	Albizia lebbeck	Bacillus sp., Rhizobium sp., Pseudomonas sp., Xanthomonas sp., Salinococcus sp. and Marinomonas sp.	1	Phytoaccumulation of cromium	Manikandan et al. (2015)

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Sea plui	Sedum plumbizincicola	Achromobacter piechaudii	1	Resistance to cadmium, zinc and lead and phosphate solubilization	Ma et al. (2016)
Lep	Leptochloa fusca	Pantoea stewartii, Microbacterium arborescens and Enterobacter sp.	1	Bioaugmentation, plant growth promotion, removal of both organic and inorganic pollutants	Ashraf et al. (2017)
		Fungus			
Fes	Festuca	Neotyphodium	1	Enhanced	Soleimani et al.
aru pra	arundinacea and F. pratensis			cadmium tolerance and partial immobilization	(2010a)
 G.	G. max L.	Exophiala, Metarhizium, Promicromonospora and P. funiculosum	1	Enhanced cupper tolerance	Khan and Lee (2013)
Trii	Triticum	Arbuscular Mycorrhizal Fungus (AMF)	1	Enhanced cupper zinc, iron and manganese tolerance	Khan et al. (2014)
Ζ. Υ	Z. mays	Exophiala pisciphila	I	Enhanced cadmium tolerance	Wang et al. (2016)
0	Oryza sativa	Piriformospora indica	1	Modulation of the anti-oxidative enzyme system, enhanced arsenic tolerance	Mohd et al. (2017)

(continued)

		Endophytic organism	Bioactive compounds/		
Function	Plants	Bacteria	Phytocompounds	Function	References
Phytotransformation		Bacteria			
	Achillea millefolium, Dactylis glomerata, Solidag ocanadensis and Trifolium	Microbacterium foliorum and Plantibacter flavus	1	Petroleum tolerance and degredation	Lumactud et al. (2016)
Bioremediation	aureum Lolium multiflorum	Psudomonas	Alkanes	Diesel degradation	Andria et al. (2009)
	Zea maize	Burkholderia cepacia	Phenol, toluene	Petroleum tolerance and degredation	Wang et al. (2010)
	Poplar	Psudomonas putida	Trichloroethylene	Co-contamination of nickel and trichloroethylene tolerance and degredation	Weyens et al. (2015)
	Alium macrostemon	Enterobacter	Pyrenes	Pyrenes tolerance and degredation	Sheng et al. (2008)

become accessible to living system. Endophytes have been reported to have important role in biodegradation of the litter of the inhabitating host plant (Muller et al. 2001; Kumaresan and Suryanarayanan 2002; Osono 2003, 2006; Korkama-Rajala et al. 2008; Fukasawa et al. 2009; Osono and Hirose 2009; Promputha et al. 2010). The endophytes colonize with in the plants initially, during biodegradation of litter, and is facilitated through antagonistic interaction of the saprophytes (Thormann et al. 2003; Fryar et al. 2001; Terekhova and Semenova 2005).

19.5.2 Plant Growth Promotion by Endophytes

Endophytes orchestrate many ubiquitous roles in the plant growth by colonizing the internal tissue in almost every part of the plant (Santoyo et al. 2016). Moreover, these endophytes could efficiently execute this spectacular attribution of plant growth enhancement via certain important interrelated mechanisms like phytostimulation, phytoimmobilization, phytostabilization, phytotransformation, phytovolatilization, phytofilterastion, biofertilization and biocontrol (Conesa et al. 2012). Phytostimulation is a direct manifestation of plant growth promotion through the up-regulation of phytohormones directly or indirectly (Bloemberg and Lugtenberg 2001). Likewise, phytoimmobilization is another remediation technology where contaminants are effectually removed by the means of plants, the intake and sequential release into the soil is further followed by immobilization in either a mineralemended soil or a geomate commonly known as mineral-containingmat (Arthur et al. 2005). Removal of the contaminants from the location is quite sophisticated therefore stabilization provides another important and effective mode of technical amelioration strategy adapted by nature for self-sustenance (Perez-de-Mora et al. 2011). Together phytoimmobilization and phytostabilization leads to phytotransformation or otherwise known as phytodegradation (sequestration and/or compartmentalization) phytocompounds involve the transformation or deterioration of intricate organic molecules into unadorned or simple molecular form that could be further absorbed into plant tissue in necessary (Etim 2012). The efficient degradation of contaminants or the xenobiotics, the subsequent release is sometimes regarded as phytoextraction/phytovolatilization where they are efficiently removed out of the plant system into the atmosphere (Limmer and Burken 2016). Phytofiltration is a fascinating scheme tailored by the plants where, with the help of certain surface anchorage phytocompounds, contaminants or nutrients are either absorbed/bound to the nonliving part of the plant (biosorptation), or to the root (Rhizofiltration), or to the seedlings (Blastofiltration) (Conesa et al. 2012). Though there is tremendous advantage in the usage of this phenomenon for the agricultural remediation but till date the advancement of research in the field is scanty. Biofertilazation on the other hand is one of the most growing, demanding, advanced and widely researched area of agriculture and environmental science. It is a technique where living microorganisms effectively aggrandize the nutrients and mineral uptake of the plants by means of establishing a symbiotic relationship with the plant and simultaneously sustains the dynamic nature of the soil (Roychowdhury et al. 2017). And finally, the protection of plant from pathogens before or after the harvest by the help of endophytic microbes is another growing sector of agricultural research where the endophytes produce a bioactive compound or induces the host plant to produce a specific and/ or a group of phytoactive compound that inhibits the growth and survivability of the pathogens (Eljounaidi et al. 2016; Shahzad et al. 2017).

19.5.3 Bioremediation/Biodegradation

The method of removal of wastes and hazardous pollutants from the environment employing micro-organisms is known as bioremediation. Endophytes have the potential to breakdown complex compounds. The role of endophytes in bioremediation (Mastretta et al. 2009) resulted in improved biomass production under conditions of stress due to cadmium, thus can withstand higher cadmium concentration when compared to uninoculated plants. The impact of endophytes that enhances plant growth by bioremediation is shown in Table 19.4.

19.5.4 Phytostimulation

Up-regulation or down-regulation of phytocompounds specifically growth hormones and other bioactive compounds plays an indispensable role in plant growth and development. These phytohormones and bioactive compounds are generally self-induced within the plants in coordination with the time and certain abiotic factors which influences plant sustenance within that particular biosphere. But in some case the modulation of these phytohormones or other bioactive phytocompounds is induced by biotic factors living within the plant in a symbiotic relation as endophytes. These endophytes or endo-rhizosphers are currently been exploited for their capacity to produce exopolysaccharides, growth hormones (Indole-3-acetic acid), phyto-enzymes (1-Aminocyclopropane-1-carboxylic acid [ACC] deaminase), volatile compounds, osmoregulatory hormone and antioxidants (Table 19.4) (Vurukonda et al. 2016).

Phytohormones such as auxins, cytokinins and gibberellic acids are produced by bacterial endophytes (Bloemberg and Lugtenberg 2001). The most highly studied example of phytostimulation involves lowering plant hormone ethylene levels by 1-aminocyclopropane-1- carboxylate deaminase (ACC). Several endophytesthat release ACC deaminase have been shown to increase plant growth including *Arthrobacter* spp. and *Bacillus* spp. in pepper plants (*Capsicum annuum*) as well as *Pseudomonas putida* and *Rhodococcus* spp. in peas (*Pisum sativum*) (Sziderics et al. 2007; Belimov et al. 2001). Higher amounts of bioactive GA3. GA4 and GA7 that induced maximum plant growth in rice and soyabean varieties by *Cladosporium sphaerosperrmum*, a fungal endophyte isolated from the roots of *Glycine max* (L)

Merr (Humayun et al. 2009). Endophytes also facilitate uptake of important nutrients from soil, water and organic matter for growth and development of plants. Phyllobacterium brassicacearum and Bacillus subtilis have been evaluated for enhancing the abscisic acid (ABA) in Arabidopsis thaliana and Platycladus orientalis respectively. The up-regulation ABA hormone resulted in decrement of leaf transpiration in A. thaliana while increment stomatal conductance in P. orientalis (Bresson et al. 2013; Liu et al. 2013). P. putida embellished the growth of Glycine max by inducing the secretion of the hormone gibberellins. In Lavandula dentate the hormone indole-3-acetic acid (IAA) was enhanced by B. thuringiensis, which further improved K-contents and proline with simultaneously decrease glutathione reductase and ascorbate peroxidase (Armada et al. 2014). The production of IAA was also enhanced in Triticum by a group of microbes namely Rhizobium leguminosarum, R. phaseoli and Mesorhizobium ciceri (Hussain et al. 2014). In a different study a total of 377 bacterial endophytes were isolated form Vitis vinifera L cv. and were tested for plant growth promoting (PGP) abilities along with their effect of A. thaliana was also evaluated. It was found that the endophytes could able to promote ammonia production, phosphate solubilization, IAA and IAA-like molecules biosynthesis, siderophores and lytic enzyme secretion. Further twelve effective endophytes mainly belonging to Bacillus, Mirococcus and Pantoa genera were specifically selected for further studies (Baldan et al. 2015). Again 12 root endophytes from different strains of Solanum lycopersicum mostly belonging to species of Pseudomonas, Rhizobium, Rhodococcus and Agrobacterium were isolated and analyzed for their PGP properties. Improvement in the production of organic acids, IAA, ACC deaminase and siderophores was observed. The impact was further verified on A. thaliana root growth using vertical agar plate assay (Abbamondi et al. 2016). In a most recent study, 101 endophytic bacteria were isolated from Simmondsia chinensis root of which 8 endophytic bacteria belonging to Bacillus sp., Streptomyces sp., Methylobacterium aminovorans, Rhodococcus pyridinivorans and Oceanobacillus kimchi were the partial sequencing of 16S rDNA gene. Later it was found that of these 8 endophytic bacterial species only two endophytes namely R. pyridinivorans and O. kimchi showed efficient PGP properties (Perez-Rosales et al. 2017).

Endophytic fungus also imparts phytostimulation by modulating the production of bioactive phytocompounds within their host plant species. This phenomenon was shown in a study where 35 fungal endophytes were isolated from a halophyte Suaeda japonica and were identified by internal transcribed spacer (ITS). Their PGP ability was verified by their treatment with Waito-c rice seedling and moreover, their secondary metabolites such as bioactive gibberellins (GAs) and other inactive GAs were detected by HPLC and GC-MS SIM analysis (You et al. 2012). In Panax ginseng 38 strains of endophitic fungus belong to Aspergillus, Cladosporium, Engyodontium, Fusarium, Penicillium, Plectosphaerella, Verticillium and Ascomycete species were isolated and investigated for their capacity to produce saponins and ginsenosides which were detected by HPLC (Wu et al. 2013). Phoma species isolated from two medicinal plants namely Tinospora cordifolia and Calotropis procera was evaluated for its PGP activity on Zea mays, where it was observed that the fungus indeed showed the ability to promote the plant growth (Kedar et al. 2014). *Trichoderma* endophytes specifically *T. atroviridae*, *T. polysporum* and *T. harzianum* isolated from the roots of *Phaseolus vulgaris* could able to synthesize and release proteolytic enzymes and phosphate solubilization factors. Furthermore most of the active volatile and non-volatile metabolites that had certain stimulatory or inhibitory impact on *P. vulgaris* seed germination were perticularly released by *T. polysporum* and *T. harzianum* (Pierre et al. 2016). A recent study proves that IAA, ACC deaminase and solubilize phosphate secretion enhanced in the plant *Brassica campestris* by *Mucor* species which was identified by 18S and 28S rRNA ITS 1 and 4 sequence homology (Zahoor et al. 2017).

Apart from endophytic bacteria and fungus other microorganisms such as algae, acticomycetes, protozoa and cyanobacteria also mediate phytostimulation and contribute in host plant growth and development (Table 19.4) (Vejan et al. 2016). A study on *Nostoc* in crop plants like *Oryza sativa* and *Triticum* under axenic conditions improves the growth and development by modulating the hormones such as IAA (Hussain et al. 2015). Overall it can be stated that endophytic microbes are an effective means of phytostimulants.

19.5.5 Phytoimmobilization

In the remediation process where in the use of phytocomponents are at the verge of being highly exploited and could be further accelerated in conjugation with the symbiotic endophytes like bacteria, fungus and many other related microorganisms (Ma et al. 2011). Previously substantiation convey, bacterial endophytes as an effective tool that could be implemented in regulation of physiological changes including immobilization of osmolytes, certain micronutrients along with osmotic acclimatization, stabilization of membrane ion conductivity which is directly or indirectly linked with changes in the membrane phospholipid composition (Compant et al. 2005). Phytoimmobilization and transformation ultimately leads to increased ability to sustain or tolerate the stress induced by the abiotic component or factor. Plants like Elsholtzia splendens and Commelina communis which could tolerate or withstand copper concentration, have been reported to encounter an increase in dry weight of the root as well as the shoot when inoculated with endophytic bacteria in comparison to the control (Sun et al. 2010). On the other hand endophytes that are genetically modified convey an additional channel for phyto-associated neutralization of the contaminants and there by ameliorating the stress induced by these contaminants in the site (Divya and Kumar 2011). On the basis 16S rRNA gene sequencing phylogenic analysis reveled of about 118 isolates comprising of 17 proteobacterial genera in the chromium treated plant sample of Albizia lebbeck. The proteobacterial genera commonly comprised of Bacillus, Rhizobium, Pseudomonas, Xanthomonas, Salinococcus and Marinomonas (Manikandan et al. 2015). There are certain multi-metal resistance endophytes, one of such endophyte is Achromobacter *piechaudii* a bacterium isolated from *Sedum plumbizincicola* and characterized by morphological features, biochemical and 16S rDNA sequencing and phylogenic analysis. The bacterial strain portrayed increased level of resistance to cadmium, zinc and lead along with other salutary properties such as solubilization of phosphors and production of IAA and lastly presence of the strain significantly increased the availability of cadmium, zinc and lead in soil (Ma et al. 2016). Effective bioremediation can be generally achieved by constructed wetland vegetated with *Leptochloa fusca*. It was found that, when a combination of three endophytic bacteria namely *Pantoea stewartii*, *Microbacterium arborescens* and *Enterobacter* species used for bioaugmentation, the consortium of the bacteria could enhance the growth of *L. fusca* and simultaneously contributed to the removal of both organic and inorganic pollutants and also ameliorated the toxicity in the constructed wetland (Ashraf et al. 2017).

Fungus on the other side also plays an important role in phytoimmobilization, which is a direct representation of the capacity to tolerate and immobilize pollutants by concurrently increasing the amount of biomass (Sudha et al. 2016). Currently studies are focused on evaluating the capacity of endophytic fungus to induce metal tolerance and partial immobilization in plants. One such example can be cited for the endophytic fungus Neotyphodium that enhanced cadmium tolerance and its partial immobilization in infected plants named Festuca arundinacea and F. pratensis. Additionally it was observed that photochemical efficacy of photosystem II increased, indicating the reduction of cadmium stress (Soleimani et al. 2010a, b). Species of Exophiala, Metarhizium, Promicromonospora and Pencillium also showed increased tolerance to metal stress of copper and cadmium. But it was Pencillium funiculosum that highly ameliorated biomass yield, chlorophyll and total protein contents in its host plant Glycine max L. under Cu stress (Khan and Lee 2013). Arbuscular Mycorrhizal Fungus (AMF) is a group of mycorrhizal fungus that could penetrate roots through cortical cells of the vascular plants. In Triticum the grain and shoot yield was increased under different concentration of Zn, Cu, Fe and Mn when inoculated with AMF compared to un-inoculated Triticum (Khan et al. 2014). Dark Septate Endophytes (DSEs) are asexual chlamydospores ascomycetous fungi that inhabits within the living plant root in a symbiotic relationship. One of such endophyte is Exophiala pisciphila, which regulates physiological response in Z. mays under soil cadmium stress. The mechanism is not clear how such tolerance is achieved by Z. mays by the help DSE (Wang et al. 2016). The immobilization of soluble arsenic is another interesting feature displayed by these endophytic fungus. Piriformospora indica is one of such fungus the colony of which was isolated from Oryza sativa root and was evaluated for its impact on the plant. Primarily it was found that hyper-colonization of the fungus that ultimately alleviated biomass density, root amelioration, number of chlorophyll and stabilization of oxidoredox status by the modulation of the antioxidative enzyme system which finally protects the plants photo-system under stress induced due to hyperconcentration of arsenic (Mohd et al. 2017). The impact of endophytes that enhances plant growth by phytoimmobilization is shown in Table 19.4.

19.5.6 Phyto-Transformation

In nature, plants commonly perform this phenomenon to neutralize soil pollutants and furthermore this property could be enhanced by the help of endophytes. Mostly endophytic bacteria, fungus, actinomycites and up to some extent algae are widely being explored for their contribution to plant life, in phytotransformation (Shakoor et al. 2017). Presently the persistent organic pollutants like pesticides, explosives, industrial byproducts and other xenobiotics are the major source of abiotic stress.

The role of bacterial endophytes in the metabolism of toxic xenobiotics has already been described successfully in the phytotransformation of toluene and other organic pollutants into intermediate metabolites that could efficiently be used both by the plant and associated interacting micro-organisms (Aken et al. 2010). Burkholderia fungorum, a bacterial strain generally present in poplar root tissue, was isolated from oil refinery discharge that had the capability to transform dibenzothiophene, phenanthrene, naphthalene, fluorine and their removal (Andreolli et al. 2013). Prosopis juliflora was found to harborcertain endophytic bacterial species such as Aerococcus, Bacillus and Staphylococcus which was confirmed by 16S rRNA gene sequencing phylogenic analysis. The ability to ionize toxic heavy metals such as chromium, cadmium, copper, lead and zinc were also evaluated along with the capacity to promote plant growth was confirmed when inoculated in Lolium multiflorum L. (Khan et al. 2015). Four different plants, Achillea millefolium, Dactylis glomerata, Solidag ocanadensis and Trifolium aureum could able to grow bounteously in the soil contaminated with petroleum. It was later found that there were about 190 endophytic bacterial species that support these plants in phytotransformation of hydrocarbons. 16S rDNA sequencing showed the presence of Microbacterium foliorum and Plantibacter flavus in all the plants (Lumactud et al. 2016).

Endophytic fungus also contributes in phytotransformation of contaminants like bacterial endophytes and improves plant's fitness to withstand environmental stress. Alleviation of stress induced due to salt accumulation was achieved by a GAs producing basidiomycetous endophytic fungus *Porostereum spadiceum* (Hamayun et al. 2017). There are many other endophytic fungus that performs similar functions like *Penicillium minioluteum*, *P. funiculosum*, *Metarhizium anisopliae*, *Beauveria bassiana*, *Mucor* sp. etc. (Khan et al. 2011a, b; Greenfield et al. 2016; Zahoor et al. 2017). The impact of endophytes that enhances plant growth by phytotransformation is shown in Table 19.4.

19.5.7 Phytovolatilization

The phenomenon through which a plant can completely remove contaminants from the site and release them into atmosphere in a volatile form can be termed as phytovolatilization. This phenomenon is highly exploited in phytotechnology programs in standardization plant growth and survivability (Schiavon and Pilon-Smits 2017). Plants simultaneously interacts with diversified classes of chemical compounds including both organic and inorganic through either direct or indirect phytovolatilization (Limmer and Burken 2016; Schiavon and Pilon-Smits 2017). Volatilization of metals such as As, Se, Hg and organic compounds such as petroleum hydrocarbons are now archived by certain endophytic bacterium. Some of such endophytic bacterium includes Pseudomonas aeruginosa, P. putida, P. stutzeri, Rhodococcus wratislaviensis, Acinetobacter sp., Burkholderia sp., Gordonia sp., Dietzia sp., Gordonia sp., Mycobacterium sp., Nocardioides sp., Novosphingobium sp., Ochrobactrum sp., Polaromonas sp., Rhodococcus sp., Sphingomonas sp. etc. which are currently being explored for their ability to degrade organic compounds (Gkorezis et al. 2017). Certain endophytic fungus are also involved in phytovolatilization of organic and inorganic compounds, the most common of them are Alternaria alternate, Cladosporium cladosporioides, Cochliobolus sativus, oxysporum, Muscodor yucatanensis, Fusarium Talaromyces wortmannii, Trichoderma viride etc (Ningxiao et al. 2016). The impact of endophytes that enhances plant growth environmental sustainability by phytovolatilization is shown in Table 19.4.

19.5.8 Biofertilization

As previously mentioned biofertilization is a technique where living microorganisms in co-ordination with nutrients and minerals present in the surrounding, enhances the absorption properties of the plant without altering dynamic nature of the soil (Roychowdhury et al. 2017). The promotion of plant growth by increasing the accessibility or supply of major nutrients is termed biofertilization (Bashan 1998). The biofertilizers basically supply nitrogen, phosphorous, potassium along with certain ion scavenging molecules like siderophores and exopolysaccharides (Saha et al. 2016; Sanlibaba and Çakmak 2016). A well-studied form of biofertilization is nitrogen fixation, which is the conversion of atmospheric nitrogen to ammonia. Several plant growth promoting bacterial endophytes have been extensively evaluated for their efficiency to fix nitrogen including *Azospirillums*p (Hill and Crossman 1983), *Pantoea agglomerans* (Verma et al. 2001) and *Azoarcus* sp. (Hurek et al. 2002).

In the present scenario much significance is given to biofertilizers in comparison to the conventional fertilizers due to its ecofriendly nature. Generally these biofertilizers can be differentiated into azotobacter, phosphate solubilizers or rhyzobium on the basis of the major type of microorganisms they harbor and/or their solubilization property. Almost all the microorganisms in these ecofriendly fertilizers are symbiotic in nature moreover these organisms also act as endophytes in some cases.

Biofertilizers that harbors azotobacter that is a genus of motile oval bacteria belongs to a family of *Azotobacteriaceae* which are aerobic and heterotrophic in nature includes bacterial species like *A. beijerinckii*, *A. chroococcum*, *A. insignis*, *A.*

macrocytogenes and *A. vinelandii*. All of these bacterial endophytes are commonly found in crop plants like rice, wheat, maize etc. and are already have been proven to be efficient in crop improvement as well as sustenance of fertility of soil (Dursun et al. 2010; Roychowdhury et al. 2017). Inorganic and organic compounds solubilization is a distinct property bestowed by these biofertilizers is remarkable. Most common of these solubilization properties is phosphate solubilization where the bacterial species such as Azospirillum sp., Pseudomonas sp., Bacillus sp., Proteous sp. along with certain fungal species like Aspergillus flavus, A. niger, A. ochraceus, A. sydawi, A. terreus, A. versicolor, Chaetomium globosum, Fusarium sp., Mucor sp., Penicillium sp. etc. are involved in solubilization of insoluble inorganic phosphate such as hydroxyapatite, rock phospahate tricalcium phosphate and dicalcium phosphate, into ions (Selvi et al. 2017; Roychowdhury et al. 2017). Lastly, biofertilizers, composed rhizobacterial species that could fix atmospheric nitrogen to the soil or to the root nodules are under vigorous study. Common rhizobacterial species megaterium, Bradyrhizobium includes Bacillus japonicum, Rhizobium, Bradyrhizobium, Stenotrophomonas rhizophila etc. (Rajeswari et al. 2017; Tarekegn and Kibret 2017).

19.5.9 Biocontrol

The promotion of plant growth through protection from phytopathogens is known as biocontrol. The use of synthetic chemicals for controlling plant diseases is the major risk factor raised against ecological and environmental niches. The search for an ecofriendly way to fight against these diseases is the major public and research concern. This concern for a sustainable means has paved the way for an alternative approach that is, the potential use of endophytes, as biocontrol (Eljounaidi et al. 2016). Some of the recently evaluated endophytic bacterial species such as Achromobacter piechaudii, Enterobacter cloacae, Erwinia persicina, Pantoea agglomerans, P. fluorescens, Serratia plymuthica, S. marcescens, B. subtilis, S. iquefaciens, B. amyloliquefaciens, Paenibacillus sp., Stenotrophomonas sp., Enterobacter sp. etc. have shown promising results in against various plant diseases such as Verticillium, Fusarium, Eggplant and Verticillium wilt etc. (Eljounaidi et al. 2016; Shahzad et al. 2017; Egamberdieva et al. 2017). The main and common mechanism exploited by thes endophytes is the elevation of certain growth hormones, induced systemic resistance, signal interference, production of antimicrobial proteins, siderophores, antibiotics and inhibitory compounds (Eljounaidi et al. 2016).

Siderophore produced by a microorganism can bind iron with high specificity and affinity making iron unavilable for other microorganism; thereby limiting their growth. It may stimulate plant growth directly by increasing availability of iron in the soil sorrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron uptake system.. Siderophores, such as pyochelin and salicylic acid, chelate iron and can indirectly contribute to disease control by competing with phytopathogens for trace metals (Duffy and Defago 1999). Antimicrobial metabolites produced by plant growth promoting bacterial endophytes such as 2, 4 diacetylphloroglucinol (DAPG) can enhance disease suppression in plants. Endophytic microorganisms are regarded as an effective biocontrol agent, alternative to chemical control. *Beauveria bassiana* an endophytic fungi, was reported to control the borer insects in coffee seedlings, thus acts as an entomopathogen (Posada and Vega 2006) and sorghum (Tefera and Vidal 2009). The fungal pathogen *Botrytis cinerea* causes severe rotting in tomatoes during storage and shelf life can be well antogonised by endophytic bacteria *Bacillus subtilis* isolated from *Spaeranskiatuberculata* (Bge.) Baill (Wang et al. 2009). A new strain of *Burkholderia pyrrocinia* and *B. cepacia*, were identified as potential biocontrol agent against poplar canker (Ren et al. 2011). Bioactive compounds from endophytes and their use against pathogenic micro-organisms is shown in Table 19.5.

Other endophytic microorganisms like fungus and actinomycites have also been identified to play an indispensible role as a biocontrol in various cases. Some such endophytic fungus with biocontrol activity against woolly aphid, fusarium wilt, against various soil born bacterial pathogen and pests are *Gibberella fujikuroi*, *Aspergillus tubingensis*, *A. flavus*, *Trichoderma koningiopsis*, *Galactomyces geotrichum*, *P. simplicissimum*, *P. ochrochloron*, *Eupenicillium javanicum* (Potshangbam et al. 2017). Some endophytes and their use against pathogenic microorganisms are shown in Table 19.5.

19.6 Impact of Endophytes on Bioactive Compounds of Host Plant

Advancement proteomics, genomics studies have revolutionized the present prospective of evaluation biomolecules and their regulation, modulation as well as their impact on or within the host, both in active and in-active state. These advanced studies includes certain sophisticated instrumentations and certain gel- based approach such as 2D Gel Electrophoresis coupled with Edman Sequencing, Matrix-Assisted Laser Desorption/ionisation Time Of Flight (MALDI- TOF), Surface- Enhanced Laser Desorption/Ionization Time of Flight (MS), Electrospray Ionization (ESI)-MS/MS analysis, Multidimensional Protein Identification Technology (MudPIT), Isobaric Tags for Relative and Absolute Quantification (iTRAQ), Isotope- Coded Affinity Tag (ICAT) and Tendem Mass Tags (TMT) (Ahsan et al. 2009; Hu et al. 2015). The bioactive compounds of the host plants such as enzymes are one of the most significant phytocompounds that regulates almost every aspect of plant life. Much work has been done on the evaluation of enzymatic activity and their up or down-regulation based on the impact of the endophytic microbes the host plant harbor (Castro et al. 2014). Form these evaluation it can be speculated that all phyto enzymes that have a direct or indirect heterogeneous impact on plants growth and

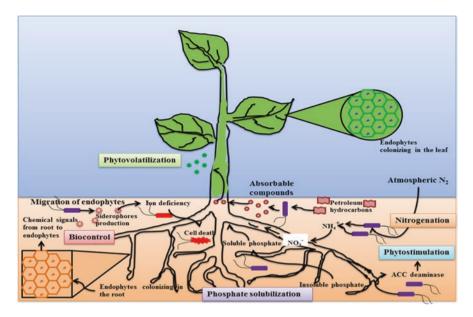


Fig. 19.1 Mechanism, interaction and beneficial effect, of endophytes

survivable under biotic and/or abiotic stress possesses four basic enzymatic activity that is proteolytic, amilolytic/endoglucanase, lipolytic and esterasic (Table 19.5) (Carrim et al. 2006; Castro et al. 2014). The mechanism, interaction and beneficial effect, of endophytes is summarized in Fig. 19.1.

19.7 Extracellular Enzymes from Endophytes

Endophytes exhibit a complex network interact in association with the host plants were widely studied as inexhaustible sources of new bioactive natural products. Enzymes of the endophytes degrade the polysaccharides available in the host plants. Fungal strains, isolated from various plants of medicinal importance viz., *Alpinia calcarata, Bixa orellana, Calophyiium inophyllum* and *Catharanthus roseus* have been reported to produce enzymes such as amylase, cellulose, laccase, lipase, pectinase, xylanase, -1, 4- glucan, phosphotases and proteinase e, -1, 4- glucan, without lyase, phosphotases and proteinase and protease extracellularly. The hydrolytic enzymes produced through endophytes differs from species to species and depends on the interactions with host and their ecological factors (Sunitha et al. 2013).

Source of endophytes	Bioactive compounds Munumbicins	Cure against pathogen Escherichia coli	Mode of endophyte transmission of the pathogen Ground meats,	Reference Golinska et al.
Boesenbergia rotunda Streptomyces coelicolor	Munumbicins	Escherichia coli	raw or under pasteurized milk	(2015)
Chloridium sp.	Javanicin	Pseudomonas sp.	Contaminated water or surgical instruments	Jalgaonwala et al. (2011)
Cytonaema sp.	Cytonic acids A and B	Human cytomegalovirus	Shellfish, berries or contaminated water	Bhardwaj and Agrawal (2014)
Diaporthe helianthi	Fabatin, tyrosol	Enterococcus hirae	Nosocomial infection	Godstime et al. (2014)
Fusarium proliferatum	Kakadumyci, beauvericin	Listeria monocytogenes	Raw or under pasteurized milk	Golinska et al. (2015)
Streptomyces hygroscopicus	Clethramycin	Cryptococcus neoformans	Lettuce harvested from tropical regions	
Streptomyces sp.	Kakadumycin A, hypericin	Shigella sp.	Contaminated food, water a	Golinska et al. (2015); Joseph and Priya (2011)
Streptomyces tsusimaensis	Valinomycin	Corona virus	Food or water contaminated with infected fecal matter	Alvin et al. (2014)
Thottea grandiflora	Streptomycin	Bacillus cereus	Uncooked meat and raw milk	Joseph and Priya (2011)
Xylaria sp. Ginkgo biloba	Sordaricin 7	Yersinia enterocolitica	Swine meat and meat products, milk and dairy products	Joseph and Priya (2011)
Fusarium proliferatum	amino-4- methylcoumarin, Beauvericin	Yersinia enterocolitica	Swine meat and meat products, milk and dairy products	Joseph and Priya (2011)

 Table 19.5
 Source of bioactive compounds from endophytes and their use against pathogenic microorganisms

19.8 Conclusion

Endophytes are capable of synthesizing number of bioactive metabolites which are mostly used as effective drugs against various diseases and are having profound impact on agricultural and environmental sustainability. These secondary metabolites were categorised into various functional groups, alkaloids, benzopyranones, flavonoids, phenolicsacids, quinones, steroids, saponins, tannins, tertaralones, xanthones and many others (Schulz et al. 2002; Strobel and Daisy 2003; Jalgaonwala et al. 2011; Pimentel et al. 2011; Godstime et al. 2014). Source of bioactive compounds from endophytes is presented in Table 19.5.

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