18 Bioprospecting of Endophytic Microbes from Higher Altitude Plants: Recent Advances and Their Biotechnological Applications

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Abstract

Endophytes are plant beneficial microbes that inhabit inside the plants and can enhance plant growth and development and tolerance to various biotic and abiotic stresses. Endophytes ubiquitously present in almost all the plant species grown worldwide and these microbes are known to provide direct benefits to the host plant by improving nutrient uptake by plant and controlling plant growth by synthesis of phytoharmones. In addition, they also produces wide range of natural compounds namely antibiotics, hydrolytic enzymes, peptides, alkaloids, etc. These novel compounds/metabolites can be prospective candidates for agriculture and pharmaceutical industries. In this chapter, efforts were made to comprehend the microbial diversity at high altitude plants and their functional traits in the plant hosts with regard to their significance and impacts on environment and humans.

Keywords

Bioprospecting · Endophytes · Enzymes · Secondary metabolites · Himalayan region

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18.1 Introduction

The plant microbiome have been considered as key determinants of plant growth, health and productivity for over the decades (Berg et al. 2016). Endophytes can be defined as the microorganisms which colonize the plant tissues and live inside without causing any apparent harm to the host plant (Petrini 1991). The association between bacterial endophytes and their host plants has been established very early in evolution (Kawaguchi and Minamisawa 2010). For maintaining stable symbiosis, endophytic microbes produce various natural compounds which helps in promotion of plant growth and better adoption in the environment. In turn endophytes are well protected from microbial competition and extreme environmental conditions by the host plants. Endophytic microbes has been isolated from numerous plant species including both monocotyledons and dicotyledons and plant tissues viz., roots, leaves, stems, flowers, seeds and fruits (Kobayashi and Palumbo 2000). The holobiont theory opened a newer source of genetic variation, produced by plant microbiome and mainly from the endophytic compartment which is heritable in nature (Nogales et al. 2016). The plant (host) genome and cluster of the genes of the microbial communities (microbiota) which inhabited in various tissues of the host plant (plant microbiome form the holobiome also known as 'the holobiont'. A holobiont refers to as an aggregation of the organism and its microbial symbionts live and functions together as unit of biological association and have the ability to duplicate and transfer its genetic information/composition and the collective genomes of the holobiont form a 'hologenome' (van Opstal and Bordenstein 2015; Theis et al. 2016). Further advancement in the knowledge of microbiome revealed that the genetic diversity in the parents required for breeding of varieties for tolerance to various biotic and abiotic stresses is not only contributed by the parental genome but also by the associated microbes. The multifaceted symbiotic and beneficial interactions of the plant holobiont is governed by its holobiome (composed of the genomes of plant host their microbiota. Interaction of plant and microbial endophytes have demonstrated to have a crucial impact on maintaining integrity, sustainability and proper functioning of agro-ecological systems (Nagarajkumar et al. 2004).

The Himalayas and its foothills represents as the main hot spot for the biodiversity in the world (Hanson et al. 2009). Himalayan region of India along with alpine, sub alpine zones of the temperate, glaciers and cold deserts providing an unexploited spots for the isolation and characterization of novel microbial diversity adapted at cold or low temperature (Pandey et al. 2018).

Bioprospecting of microbial endophytic resources mainly bacteria (Rodrigues et al. 2018), fungi (Kumar et al. 2019) and actinomycetes (Shan et al. 2018) and their capability to inhabit in the various environments, genetic diversity and discovery of novel bioactive compounds research needs more attention to understand functional diversity, species richness and response under changing biotic and abiotic stress conditions. Exploitation and proper management of microbial diversity inhabiting crop/plants, plays a significant role in sustainable development towards industrial applications. Majority of endophytes particularly in extreme habitats still remains hidden and they must be explored for better use of humankind and the environment across the world.

18.2 Significance of Endophytes

The endophytic microbes have various significant contributions in environment and agroecosystems. The following paragraphs elaborate the various bioprospectings of endophytic microbes. The various properties of these microbes are also depicted in Fig. 18.1.

18.2.1 Agriculture

Endophytic microbes (bacteria and fungi) have recently drawn attention as a group of prospective plant growth promoters (biofertilizers) and controlling the biotic stresses caused by the pathogens (biocontrol agents). An opportunity of exploring and application of endophytes for development of microbial bioinoculants is evolving as a realistic move towards sustainable agriculture (Rai et al. 2014). Endophytes are well-known to play a crucial role in promoting plant growth and suppressing and/or inhibiting pathogen growth and eliciting induced systemic resistance (ISR) or defence against pathogenic microbes and herbivore insects (Van Oosten et al. 2008). Endophytic bacteria have been explored as possible microbial inoculants for increasing plant productivity (Hallmann et al. 1997). Secondary

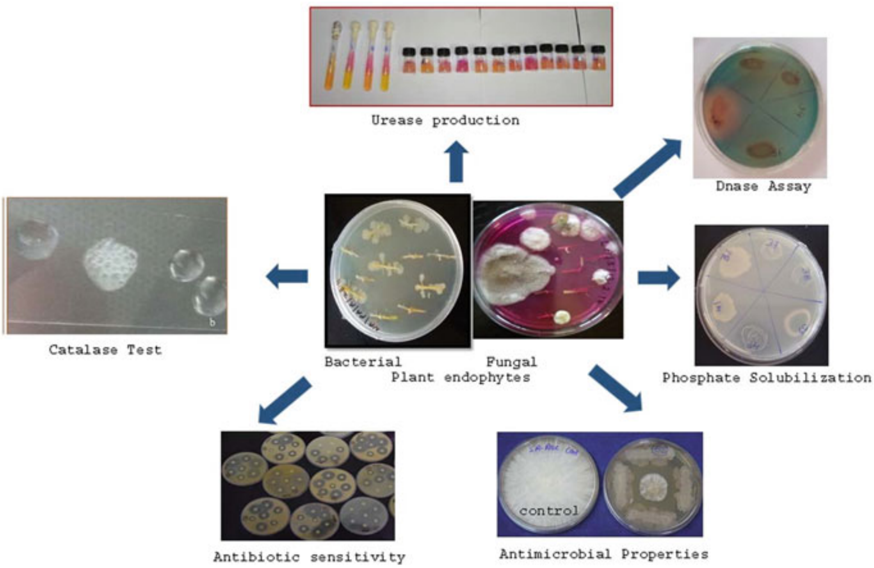

Fig. 18.1 The various properties of the microorganisms

metabolites or natural compounds synthesized by the endophytes play a vital role in various metabolic interactions between microbes and their host plants namely regulation of the symbiosis, signalling and defence mechanism (Schulz and Boyle 2005). Plants colonized with specific endophytes are often growing faster due to the synthesis of phytohormones and as a result they dominate in a specific environment hence they are also considered as chemical and metabolite synthesizers within the plants (Owen and Hundley 2004). Information about the microbial communities accompanying with seeds is crucial, because seed is considered as the basis or unit for the transmission (vertical) of the microbes to next generation. The seeds also accumulate the plant microbiome that have beneficial effect on growth and development of the plant (Hardoim et al. 2015).

Fusarium oxysporum, a fungal endophyte has been recovered from a mangrove plant *Rhizophora annamalayana* and analysed for the production of taxol. Endophytes synthesize secondary metabolites via several metabolic pathways like isoprenoid, amino acid origin and polyketide (Tenguria et al. 2011). Endophytic microbes are being considered as a key constituent of the biodiversity, as the distribution of endophytic microflora varies with the host. There is an increasing requirement of novel and effective chemotherapeutic agents, agrochemicals (insecticides and pesticides) and antibiotics to cope up the emerging agricultural, environmental and medical problems faced by mankind, turns the interest for understanding chemistry of endophytic microbes.

The discovery of *Taxus andreanae* (endophytic fungi) from yew plant (*Taxus brevifolia)* which produces anticancer drug paclitaxel (Stierle et al. 1993, 1995) has established platform for future research areas in other plants for presence of paclitaxel and other important drugs producing endophytes, to use such them in industrial production of drugs. Microbial communities associated with plant such as plant growth promoting (PGP) bacteria and mycorrhizal fungi that enhances the growth and development of plants and improving plant health under multiple (biotic and abiotic) stresses (Vimal et al. 2017; Dash et al. 2019) and also helpful in improving environmental stability and sustainable agriculture (Kumar and Verma 2018). Bacterial endophytes can enhance uptake of nutrient, accumulation and metabolism by producing phytohormones involved in growth regulation of host plants (Afzal et al. 2019).

18.2.2 Peptide Production

A number of endophytic microbes reported for production of peptides demonstrating that endophytes can act as valuable source and exploited for manufacturing of peptide-based drugs. The antimicrobial peptides produced by microbes have huge attentiveness and are indispensable field for intensive research worldwide (Christina et al. 2013). Molecular screening of endophytes for non-ribosomal peptides which are produced by one or more specific non-ribosomal peptide synthetase enzymes exhibiting peptide producing capability. The major group of complex and cyclic lipopeptides with antifungal activities synthesized by *Bacillus subtilis* (Dunlap et al. 2015). The non-ribosomal peptide synthetase enzymes have ability to synthesize several peptide derivatives through one enzyme complex and genetic modifications in NRPS genes also offers the possibility to produce peptides with higher/better pharmacological activities (Abdalla and Matasyoh 2014). Lipopeptide fengycin was first reported as to have antifungal properties (Vanittanakom et al. 1986), recently cyclic lipopeptide, bacillomycin F was detected and isolated from *B. subtilis subsp. Inaquosorum* (Knight et al. 2018). Iturins produces several isomers which have potential broad spectrum antifungal activities, while surfactin is known as the most potent bio-surfactant lipopeptide (Falardeau et al. 2013).

18.2.3 Antimicrobial Activities

Fungal endophytes has been reported as a key source for novel and bioactive antimicrobial compound production namely alkaloids, peptides, steroids, flavonoids and phenol that possess numerous applications in medical sciences and agricultural industries (Strobel et al. 2004). Bioactive compounds synthesized by the fungal endophytes viz*., Fusarium* spp. and *Acremonium spp* exhibited potential antimicrobial property (Powthong et al. 2012), Fungal endophyte, *Pestalotiopsis species* was isolated from leaf tissues of *Pinus caneriensis* exhibited broad range of antibacterial properties against both gram negative and positive bacteria (Bagyalakshmi et al. 2012).

Bacterial endophytes inhabiting the plant tissues reported to provide defence/protection of host plant from plant pathogenic microbes namely bacterial, fungal and virus and virus like organisms. Endophytes provide protection to the host plant by triggering the induced systemic resistance (ISR) mechanism (Alvin et al. 2014). Endophytic microbes can also initiate induced systemic resistance by using phytoharmones (salicylic acid, jasmonic acid and ethylene) mediated pathways. Bacterial endophytes of genus *Serratia, Bacillus sps, Bacillus pumilus* and *Pseudomonas sps,* reported to protect their hosts through ISR (Pieterse et al. 2012; Kloepper and Ryu 2006). Plant defense mechanisms primed by ISR, protect unexposed/healthy plant tissues against future attack by insect pests.

18.2.4 Antibiotics Production

Antibiotics are the chemical substances/compounds synthesized by the microorganisms which have toxic/lethal effects on other microbes. They are mainly used to kill and or check growth of pathogenic microorganism and mode of action of antibiotics is highly specific and affect the vital biological processes such as synthesis of DNA, RNA, cell wall and proteins. In order to overcome the pressure of ever-increasing drug resistance in human and plant pathogens, substitutes are urgently required to be explored for isolation and identification of more effective novel natural products and secondary metabolites. The antimicrobial compounds are grouped in numerous structural classes namely alkaloids, flavonoids terpenoids, steroids, phenols, quinones peptides. New antibiotics are essentially needed to treat microbial pathogens which are becoming gradually resistant to various antibiotics available in the market. Endophytic microbes serve as an important source of novel bioactive compounds which can be used as an important source for antitumor, antiarthritic, antidiabetic drugs and other pharmaceutically important compounds to treat various diseases. They also produce various compounds and peptides which have antifungal, antibacterial and insecticidal activities to control pests and phytopathogens in agricultural sector (Jalgaonwala et al. 2011; Godstime et al. 2014).

18.2.5 Bioactive Compounds and Other Metabolites

Microorganisms are the rich and important source of biologically active compounds and able to produce >20,000 compounds, which are influencing the survival and performance of other organisms (Demain and Sanchez 2009). Endophytic bacterial microbiome exhibited beneficial effects and promotes plant growth and health in several cases mediated by metabolic interactions (Nter Brader et al. 2014). It was reported that microsymbionts associated with plants may produce an array of diverse metabolites that may play a crucial role in defence, required for precise communication and interaction with the plant host. Microbial endophytes mainly grouped as bacterial, fungal and actinobacterial and are considered as highly metabolically active than their counterparts (present in phylosphere or free) due to their specific nature of living and functions inside the plant tissue in turn activation of various metabolic pathways (Strobel 2006; Riyaz-Ul-Hassan et al. 2012).

Plants are highly dependent on its microbiome present in the rhizosphere and phylosphere for their functioning. In some cases, plants were unable to culture as transplants and/or unable to induce seed germination in the absence of specific endophytic microbes (Hardoim et al. 2008). Several functions of the plant microbiome is crucial for the host for instance seed germination, is the first and most critical stage of a plants' life cycle and it was noticed that in several plant species, seed germination is not possible without microorganisms. Plant produces a range of phytohormones including auxins, cytokinins, abscisic acid, ethylene, gibberellin, salicylic acid and jasmonic acid plays an essential role in the growth development, signalling and stress responses. Besides providing benefit the plants and environment; endophytes represent a huge natural resource to be explored for human welfare. They are also known to communicate with their host plants via chemical messengers/phytohormones signalling which play a crucial role in promoting plant growth (Kusari et al. 2012). Endophytes have capability to synthesize a wide range of secondary metabolites/natural compounds which are involved in the plant-endophyte interaction. They are probable sources of novel and diverse natural compounds to be discovered for pharmaceutical and agricultural industry (Bacon and White 2000).

18.2.6 Enzymes Production by the Endophytes

The enzymes produced by the endophytes are of two types namely intracellular and extracellular enzymes. Intracellular enzymes are those which produced inside the microbial cell and remain inside whereas extracellular enzymes produced by the growth and multiplication of microbial cells, they have ability to perform their functions outside the cell in numerous biological pathways and/or environmental processes. Endophytes (fungi and bacteria) have been described to produce certain enzymes viz., chitinase, cellulases, xylanases, pectinases, proteases, amylases, hemicellulases, gelatinase, phytases, tyrosinase, etc. Majority of the reported enzyme produced by plant endophytes have been screened or detected using agar-based methods. The chapter summarized the type of enzymes and bioactive compounds produced by the endophytes and their source and their application for further studies and biotechnological applications (Table 18.1). *Acremonium zeae,* endophyte recovered from maize exhibited the production of hemicellulase enzyme extracellularly (Bischoff et al. 2009). Production of various enzymes by endophytes and their biotechnological applications has been mentioned in earlier report of Dhanya and Padmavathy (2014). In the last two decades, numerous chemical entities have been obtained from such microorganisms that are potential leads for pharmaceutical, industrial and agricultural applications.

18.3 Endophytic Microbes Isolated from High Altitude Plants

Microbial diversity is the key and functional backbone of any ecosystem and is essential for life because they perform several functions which are vital for the biosphere. About 3,00,000 plant species exists on earth, and every individual plant hosts of one or more endophytic microorganisms (Strobel and Daisy 2003). Plants capable of growing at high altitudes about 5000 m or more have their highly specialized physiological processes which modulate the biochemical response of the plant, ranging from modifying cell membranes for water permeability and flexibility and synthesis of lipids molecular, anti-freeze carbohydrates, strong antioxidants and scavengers for free radical which are generally not observed in the plants grown at low altitude (Alonso-Amelot 2008). The Indian Himalayan region (IHR) represents great diversity, particularly with respect of geographic, topographic, and climatic conditions and supports a wide range of habitats involving the colonization of microorganisms. Thus plant grown at high altitude region offers an opportunity to identify and explore novel and useful endophytes among diverse plants in the diverse locations and ecological systems.

The *Dysosma versipellis*, plant species is endemic to China and spread at high altitudes and contains fungi belongs to three different orders Pleosporales, Capnodiales and Venturiales comprising eight generas namely *Cladosporium, Alternaria, Phoma, Ochroconis, Microsphaeropsis, Pyrenochaeta*, *Pseudocercospora* and *Ramichloridium* (Tan et al. 2018). Kaul et al. (2013) reported that about 35 fungal endophytes were present in asymptomatic parts of *Digitalis*

Table 18.1 List of important secondary metabolites/bioactive compound produced by the fungal endophytes

S. No.	Secondary metabolites/ natural compound	Endophytic fungi	Host plants	Application/uses	Reference
1.	Taxol and Taxane	*Taxomyces andreanae*	*Pacific yew*	Anticancer	Stierle et al. (1993)
2.	Camptothecin	*Fusarium solani*	*Apodytes dimidiata*	Antitumor	Shweta et al. (2010)
3.	Camptothecin	*Entrophospora*	*Nothapodytes foetida*	Antitumor	Rehman et al. (2008)
4.	Paclitaxel	*Taxomyces andreanae*	*Taxus brevifolia*	Antitumor	Stierle et al. (1995)
5.	Paclitaxel	*Fusarium solani*	*Taxus chinensis*	Antitumor	Deng et al. (2009)
6.	Paclitaxel	*Cladosporium*	*Taxus media*	Antitumor	Zhang et al. (2009)
7.	Paclitaxel	*Aspergillus niger*	*Taxus cuspidata*	Antitumor	Kim and Ford (1999)
8.	Paclitaxel	*Phyllosticta dioscoreae*	*Hibiscus rosasinensis*	Antitumor	Kumaran et al. (2009)
9.	Podophyllotoxin	*Monilia species*	*Dysosma veitchii*	Antitumor	Yang et al. (2003)
10.	Podophyllotoxin	*Penicillium implicatum*	*Diphylleia sinensis*	Antitumor	Zeng et al. (2004)
11.	Podophyllotoxin	*Fusarium oxysporum*	*Juniperus recurva*	Antitumor	Kour et al. (2008)
12.	Quercetin	*Aspergillus oryzae* and *Aspergillus nidulans*	*Ginkgo biloba*	Anti- inflammatory	Qiu et al. (2010)
13.	Diosgenin	*Cephalosporium species*	*Paris polyphylla*	Antitumor, anti-inflammatory	Cao et al. (2007)

14.	Hypericin	*Chaetomium globosum*	*Hypericum perforatum*	Anti-depressant	Kusari et al. (2008)
15.	Chlorogenic acid	*Sordariomycete species*	*Eucommia ulmoides*	Antitumor and antimicrobial	Chen et al. (2010)
16.	Piperine	*Colletotrichum gloeosporioides*	*Piper nigrum*	Anticancer, anti-inflammatory and antimicrobial,	Chithra et al. (2014)
17.	Cajaninstilbene acid	*Fusarium oxysporum, F. solani, F. proliferatum and Neonectria macrodidym*	*Cajanus cajan*	Antioxidant and hypoglycemic	Zhao et al. (2012)
18.	Ginkgolide B	*Fusarium oxysporum*	*Ginkgo biloba*	Anti-inflammatory and antiallergic	Cui et al. (2012)
19.	Borneol	*Cochliobolus nisikadoi*	*Cinnamomum camphora*	Anti-inflammatory, antioxidant	Chen et al. (2011)
20.	Griseofulvin	*Xylaria sp.*	*Abies holophylla*	Antimicrobial	Park et al. (2005)
21.	Graphislactone A	*Cephalosporium sp.*	*Trachelospermum jasminoides*	Antioxidant compounds	Song et al. (2005)

lanata from Gulmarg, Jammu and Kashmir, India. They reported that the *Penicillium, Aspergillus* and *Alternaria* species of fungal endophytes were more abundant in comparison to other genera. Furthermore, an endophytic fungus was isolated from *Juniperus procera* from high altitude regions of Saudi Arabia and screened for its antibiotic activities (Gherbawy and Elhariry 2016). Similarly, Verma et al. (2015) screened 41 bacterial endophytes recovered from wheat culm and roots grown in north-west, Indian Himalayan region. These isolates have PGPR features with cold adaptability which suggest that these endophytes need to be exploited as beneficial bioinoculants for sustainable agroecosystem of high altitudes. A list of some important fungal endophytes isolated from the crop plants of high altitude is given in Table 18.2.

18.3.1 Importance/Functional Significance of Endophytic Microbes

Exploring the plants microbiome and its diversity is of great significance to identify new and effective microorganisms which can be used as biological control agent (BCAs) and production of bioactive substances/compounds. Microbial endophytes have ability to naturally colonize and live within the plants and plant tissues without showing any apparent damage or negative effect to their host. Endophytes attuned to their host in a balanced antagonism manner between both the associates (Schulz and Boyle 2005). Bacterial endophytes may be considered as potential candidates for BCAs, as they provide additional advantages over the other microbes owing to their ability to colonize plant tissues and able to enter inside the plant tissues through wounds and natural openings and also able to survive as epiphytes (Porras-Alfaro and Bayman 2011). It was confirmed that microbes initially isolated as endophytes may possess higher capabilities to colonize and enter in to the plant or seed when endophytes inoculated on plant or seed surface than the non-endophytes, thus escaping from UV rays, moisture and temperature fluctuations confronted on the plants (Hallmann et al. 1997). The endophytes exhibiting antagonistic activities, majority of them associated with the process of antibiosis, through the production of bioactive compounds, peptides and metabolites (Ulloa-Ogaz et al. 2015), and exploring endophytes may provide a better opportunity to isolate and identify BCAs as endophytes have been reported as better producers of antimicrobial compounds, metabolites than the plant epiphytes (Nongkhlaw and Joshi 2015) or soil isolates (Schulz et al. 2002). A recent report revealed that the plants inoculated by overnight soaking of their seeds or roots in plant growth promoting bacterial culture showed huge resistance to multiple biotic stresses (Ngumbi and Kloepper 2016).

Table 18.2 List of endophytic microbes isolated from the crops/plants grown at high altitude

S. No.	Name of host plant/crop	Name of the fungal endophytes	Location	Plant parts used	Reference
1.	*Picrorhiza kurroa*	*Chaetomium globosum, Valsa sordid, Thielavia subthermophila* and *Diaporthe phaseolorum*	Jammu & Kashmir, Western Himalaya	Stems or twigs of the plants	Qadri et al. (2013)
2.	*Cannabis sativa*	*Alternaria alternata, Schizophyllum commune, Alternaria* sp. and *Alternaria brassicae*			
3.	*Withania somnifera* (Ashwagandha)	*Gibberella moniliformis, Cochliobolus lunatus, Fusarium* sp., *Fusarium equiseti, Gibberella moniliformis, Hypoxylon fragiforme, Nigrospora sphaerica, Cercophora caudate* and *Cladosporium cladosporioides*			
4.	*Rauwolfia serpentine* (Sarpgandha)	*Alternaria brassicae, Cladosporium cladosporioides, Alternaria alternata, Fusarium proliferatum, Lasiodiplodia theobromae, Glomerella acutata* and *Diaporthe helianthi*			
5.	*Cedrus deodara*	*Sordaria humana, Talaromyces trachyspermus, Cochliobolus spicifer* and *Scleroconidiomas phagnicola*			
6.	*Abies pindrow*	*Daldinia fissa, Penicillium oxalicum, Polyporus arcularius* and *Apiosordaria otanii*			
7.	*Pinus roxburgii*	*Petriella* sp. *Bipolaris tetramera, Trichophaea abundans, Penicillium expansum* and *Ulocladium* sp.			
8.	*Nothapodytes nimmoniana*	*Phomopsis* sp. and *Petriella setifera*			
9.	*Platanus orientalis*	*Fusarium tricinctum, Fusarium solani* and *Gibberella* sp.			
10.	*Artemisia annua* (Artemisia plant)	*Fusarium tricinctum, Fusarium flocciferum, Sordaria superba, Fusarium redolens, Chaetomium* sp., *Alternaria alternata, Alternaria brassicae, Paraphoma* sp. and *Gibberella avenacea*			

(continued)

Table 18.2 (continued)

S. No.	Name of host plant/ crop	Name of the fungal endophytes	Location	Plant parts used	Reference
11.	*Ocimum sanctum* (Indian medicinal plant)	90 endophytic fungi were isolated and 23 different endophytic fungal isolates characterized as *Meyerozyma guilliermondii, Colletotrichum sp., Penicillium crustosum, Fusarium proliferatum* and *Chaetomium coarctatum*	Mukteshwar, Uttarakhand		Chowdhary and Kaushik (2015)
12.	*Pinus roxburghii*	*Alternaria alternata, Geotrichium albida, Penicillium frequentans* and *Thielaviopsis basicola*	-do-	Spike	Bhardwaj et al. (2015)
13.	*Eugenia jambolana* (*Schizium cumini*) (Jamun)	24 fungal species isolated, Ascomycetes (20) and two each of Basidiomycetes and Zygomycetes *Aspergillus niger* and *Alternaria alternata* were most dominant and *Chaetomium globosum, Aspergillus japonicus*, *Aspergillus niger* strain, *Aspergillus fumigatus* strain were very rare species	-do-	Leaf, stem and petiole	Yadav et al. (2016)
14.	*Withania sominifera* (Ashwangandha)	6 fungal endophytes	Dehradun	Leaf tissue	Kapoor et al. (2018)
15.	*Ocimum basillicum* (Tulsi)	2 fungal endophytes	-do-	Stem and internal stem tissues	Kapoor et al. (2018)
16.	*Syzygium aromaticum* (Jamun)	6 fungal endophytes	-do-	Leaf, stem and internal stem tissues	Kapoor et al. (2018)
17.	***Dysosma versipellis*** (Podophyllum)	224 fungal endophytes were isolated were 4 belonged to at least 29 genera of 15 orders of Ascomycota (93%), Basidiomycota (6%), and Zygomycota (1%)	High altitudes region (200–2400 m) above sea level in China	Root, rhizome, stem and leaves	Tan et al. (2018)
18.	*Juniperus procera*	A total of 144 isolates were obtained and identified into 6 distinct operational taxonomic units *Aspergillus fumigatus, Penicillium oxalicum, Preussia*	Taif region (Saudi Arabia)	Twigs	Gherbawy and Elhariry (2014)

		sp., Peyronellaea eucalyptica, Peyronellaea sancta and Alternaria tenuissima			
Bacteria					
19.	Sugarcane varieties of Himalayan region	Seven different species of *Gluconacetobacter spp.*	Hilly areas of Uttarakhand		Singh et al. (2013)
20.	*Mussaenda roxburghii* Akshap	*Pseudomonas sp., Klebsiella sp. Acinetobacter sp.*	Arunachal Pradesh, eastern Himalayan province	Stem, leaf and root	Pandey et al. (2015)

18.4 Conclusion

In the past few years, significant increase in research on microbial endophytes related to the isolation and characterization of novel endophytic microbes and their bioactive metabolites have been noticed. Endophytic microbes hold an enormous potential to be deployed for product development to practical applications. They may be used as plant biofertilizer and biocontrol agents and for production of antimicrobial compounds. Looking into the importance of endophytes in the various sectors, it is high time to explore endophytes from extreme and diverse habitats and identify their natural products like antibiotics which can be effective against multidrug resistant (MDR) bacteria. Molecular studies is required for understanding of plant and endophyte interactions in a more comprehensive way and identification of potential endophytes for bioenergy crops, biodegradation of xenobiotics and bioremediation of toxic metals, etc. Unravelling the microbiome associated with plants offer genetic variability to the plants thus opening up novel possibilities for breeding of next-generation crops for developing high yielding, pest and disease resistant plants and resilient to climatic change.

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