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Associated microflora of medicinal ferns: biotechnological potentials and possible applications

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Abstract: Plant associated microorganisms that colonize the upper and internal tissues of roots, stems, leaves and flowers of healthy plants without causing any visible harmful or negative effect on their host. Diversity of microbes have been extensively studied in a wide variety of vascular plants and shown to promote plant establishment, growth and development and impart resistance against pathogenic infections. Ferns and their associated microbes have also attracted the attention of the scientific communities as sources of novel bioactive secondary metabolites. The ferns and fern alleles, which are well adapted to diverse environmental conditions, produce various secondary metabolites such as flavonoids, steroids, alkaloids, phenols, triterpenoid compounds, variety of amino acids and fatty acids along with some unique metabolites as adaptive features and are traditionally used for human health and medicine. In this review attention has been focused to prepare a comprehensive account of ethnomedicinal properties of some common ferns and fern alleles. Association of bacteria and fungi in the rhizosphere, phyllosphere and endosphere of these medicinally important ferns and their interaction with the host plant has been emphasized keeping in view their possible biotechnological potentials and applications. The processes of host-microbe interaction leading to establishment and colonization of endophytes are less-well characterized in comparison to rhizospheric and phyllospheric microflora. However, the endophytes are possessing same characteristics as rhizospheric and phyllospheric to stimulate the *in vivo* synthesis as well as *in vitro* production of secondary metabolites with a wide range of biological activities such as plant growth promotion by production of phytohormones, siderophores, fixation of nitrogen, and phosphate solubilization. Synthesis of pharmaceutically important products such as anticancer compounds, antioxidants, antimicrobials, antiviral substances and hydrolytic enzymes could be some of the promising areas of research and commercial exploitation.

Key words: Ferns, Biodiversity, Rhizospheric microflora, Phyllospheric microflora, Endophytic microflora, Biotechnological potential.

Introduction

The magical and curative properties of various plants have been narrated in the history and religion. The effectiveness of these plants for different ailment was learnt based on speculations and superstitions (Hamayun *et al.*, 2006). Plants, which contain active chemical constituents (viz. alkaloid, glycosides, saponins, essential oils, bitter principles, tannins and mucilages) in any of its parts like root, stem, leaves, bark, fruit and seed and produce a definite curing physiological response in the treatment of various ailments in humans and other animals are regarded as medicinal plants (Adhikari *et al.*, 2010). Several medical systems have evolved and prominent among these systems are Ayurveda, Siddha and the Unani Systems of medicine, which uses plants of diverse groups for the medicinal purpose. Pteridophytes played a very important role in ayurveda, homeopathy and allopathy because the bioactive compounds of pteridophytes belong to phenolic, alkaloid and flavonoids families of which flavonoids and other phenolic compounds have been demonstrated to be good antioxidants (Rathore *et al.*, 2011; Chai *et al.*, 2012). Pteridophytes commonly known as ferns are primitive vascular ornamental plants which grow in varied climatic zones of different phytogeographical regions including the threatened

areas. These fascinating groups of plants have attracted the botanist and naturalist all over the globe not only because of its beautiful and unique foliage but also because of their diverse useful aspects such as hyperaccumulation of arsenic by *Pteris vittata* L. (Ma *et al.*, 2001).

Moreover, their occurrence in different eco-geographically threatened regions from sea level to the higher mountains is of much interest. But the slow growth and limited root system makes the ferns inappropriate for the mass cultivation. Ma *et al.*, (2001), revealed that the rhizospheric microflora plays a very important role in plant growth because the metabolic activity of microbes present in the rhizosphere can enhance plant nutrition uptake, promote plant tolerance to heavy metals, and increase the synthesis of plant growth factors, etc. (Nazir and Fridaus 2011; Srivastava *et al.*, 2012a,b). While the current research activities have focused attention on symbiotic or parasitic plant-microbe interactions, other types of associations between plants and microorganisms, such as endophytic microbial associations have often been overlooked. Endophytes are microorganisms, mainly fungi and bacteria, which live inside the plant tissues without causing any disease symptoms (Azevedo and Araujo 2007; Petrini 1991). They represent a largely unexplored

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component of biodiversity, especially in the tropics. Endophytic bacteria possess an ecological niche similar to plant pathogens in that they are sometimes transmitted through seeds (Ferreira *et al.*, 2008), which allows them to be used as candidates for biocontrol agents.

In the last two decades increasing number of patents have been obtained on endophytic products because of substantial increase in the isolation of natural products, from endophytes, having important biological activities (Zhang *et al.*, 2012; Zilla *et al.*, 2013). Thus, numerous compounds with antimicrobial and anticancer activities have been characterized from endophytes and the search continues for novel natural products showing promise as potential drugs and for other uses (Kumar *et al.*, 2013; Zilla *et al.*, 2013). The previous research on endophytes mostly focused on the search for the host-plant metabolites in the endophytic partner with limited success (Kusari *et al.*, 2009; Priti *et al.*, 2009). It seems logical to acquire and study endophytes for genuine microbial products that are independent of their host plants (Zilla *et al.*, 2013). In case of pteridophytes, fern microbe interactions with reference to phyllosphere and rhizosphere have been studied, but the endosphere, microenvironment inside the fern (between plant cells) that is colonized by microorganisms have attracted the attention of the scientific communities only recently.

In this review, an attempt will be made to highlight the diversity of the common medicinal ferns with special emphasis on their ethnomedicinal uses and interaction with the phytosphere microbiota. Some recent approaches in the use of associated microbiota of the medicinal ferns with potential application of their metabolites will be discussed.

Biodiversity and Ethno-medicinal properties of Ferns

Pteridophytes are long known for their medicinal and therapeutical utility. First historical effort was made by Caius in 1935 used in the treatment of rheumatism and spermatorrhea while *Drynaria quercifolia* (L.) J. Smith is used for the cure of typhoid fever. Formulations of these plants are also advised as supplement of appetizer and stimulants; however, certased for the ailment of diuretic and gastric ulcer (Benjamin and Manickam 2007). In addition few of the pteridophytic species

are historically in practice in the homeopathy as well as ayurvedic system of medicines. *Selaginella bryopteris* (L.) Bak, *Lycopodium clavatum* L., *Angiopteris evecta* (G. Forst.) Hoffm., *Blechnum orientale* Linn etc. are well known for the homeopathic system of medicine, where in the *Selaginella* is prescribed for the cure of neurological disorder and heat stroke effects. Similarly, *Lycopodium clavatum* and *Angiopteris evecta* have been recommended to the patients of splinted bones, leprosy and skin diseases respectively. While, *Blechnum orientale* (Linn) and *Lycopodium pseudoclavatum* var. *yunnanense* Ching are used in the treatment of urinary disorder, *Helminthostachys zeylinica* Linn. commonly known as 'Kamraj' is well known for its herbal ayurvedic formulation to enhance sexual efficiency and as a source of stimulant and aphrodisiac. Few of the pteridophyte species have been screened out (Bharti 2011; Benniamin 2011) chemically and a number of active novel chemical compounds have been validated. *Gleichenia linearis* (Burm. f.) C.B. Clarke, *Hemionitis arifolia* (Burm. f.) T. Moore, *Hymenophyllum javanicum* Spreng., *Marsilea minuta* L., *Lycopodiella cernua* (L.) Pic. Serm., *Phlebodium aureum* (L.) J.Sm., *Pityrogramma calomelanos* (L.) Link, *Thelypteris caudipinna* Ching are used in psychopathy, diarrhea, cough, skin diseases, dyspepsia fever, high fever, hypertension, headache, asthma and insomnia (Benjamin and Manickam 2007; Rout *et al.*, 2009; Benniamin 2011). Many other fern species have been extensively explored and determined to exhibit great economic value. The *Acrostichum aureum* L., *Botrychum lanuginosum* Wall. ex Hook & Grev., *Dicranopteris linearis* (Burm.f.) Underw., *Hypodematum crenatum* (Forssk.) Kuhn & Decken, *Leucostegia immerse* (Wall.) Presl, *Parabemionitis arifolia* (Burm. f.), *Polystichum moluscens* (Bl.) T. Moore, *Ptilotum nudum* (L.) P. Beauv, *Pteris vittata* Linn., *Sphaerostephanos unitus* (L.) Holttum have also shown antimicrobial activities against number of pathogenic fungal and bacterial strains (Benjamin and Manickam, 2007; Benniamin 2011). Extract of *Ampelopteris prolifera* (Retz.) Copel. is used as a health tonic in ayurveda (Bharti 2011). Thus, pteridophytes having tremendous importance and vast medicinal scope would prove itself as the biological resource for the upliftment of human society. Biodiversity and ethnomedicinal properties of some important medicinal ferns have been presented in table 1.

Table 1: Biodiversity and ethnomedicinal properties of some common ferns

Fern	Medicinal use	References
<i>Adiantum philippense</i> L. <i>A. caudatum</i> L. <i>A. incisum</i> Forsk. <i>A. lunulatum</i> Burm. <i>A. capillus veneris</i> Linn. <i>A. poiretii</i> Wikstr <i>A. pedatum</i> <i>A. latifolium</i> Lam.	Treatment of fever, asthma, dysentery, menstrual disorder, leprosy, diabetes, chest complaints, skin diseases also used as antimicrobial agent and in the treatment of snake and dog bite.	Benjamin and Manickam 2007; Singh <i>et al.</i> , 2008; Rout <i>et al.</i> , 2009; Perumal 2010; Benniamin 2011; Bharti 2011; Chandrappa <i>et al.</i> , 2011; Britto <i>et al.</i> , 2012
<i>Alsophila brunoniana</i> (Wall ex Hook.) <i>A. spinulosa</i> (Wall ex Hook.) <i>A. contaminans</i> (Wall ex Hook.) <i>A. glabra</i> sensu Bedd.	Treatment of wound and cut healing, immediate clotting of blood, also used in the treatment of snake bite.	Rout <i>et al.</i> , 2009; Sen and Ghosh 2011
<i>Asplenium nidus</i> Linn. <i>A. polydon</i> G. Foster var bipinnatum (sledge) sledge <i>A. indicum</i> Sledge, Bull. <i>A. laciniatum</i> D. Don, Prod	Treatment of gonorrhoea and leucorrhoea, fever, elephantiasis, jaundice, cough chest diseases, and anticancer agents.	Benjamin and Manickam 2007; Rout <i>et al.</i> , 2009; Sen and Ghosh 2011; Benniamin 2011
<i>Cheilanthes farinose</i> (Forsk.) Kaulf <i>C. tenuifolia</i> (Burm.) SW	Used in urine problems and epilepsy. <i>C. tenuifolia</i> mostly used in sickness and applied on wounds	Singh <i>et al.</i> , 2005
<i>Cheilanthes tenuifolia</i> (Blume. f) Sw <i>C. farinose</i> (Forsk.) Kaulf <i>C. bicolor</i> (Roxb.in Griff.) Griff. ex Fras. Jenk.	Used as general health tonic and antiseptic. <i>C. farinose</i> mostly used in stomachache and menstrual disorder.	Benjamin and Manickam 2007; Rout <i>et al.</i> , 2009; Shil and Choudhury 2009; Bharti 2011 Benniamin 2011
<i>Cyathea gigantea</i> (Wall. ex. Hook.) Holttum. <i>C. contaminans</i> (Wall. ex Hook.) Copel. <i>C. henryi</i> (Bak.) Copel.	Mixture of rhizome black pepper seeds (<i>Piper nigrum</i>) and milk effective against white discharges. Also used in treating cuts and wounds.	Rout <i>et al.</i> , 2009; Shil and Choudhury 2009
<i>Dryopteris cochleata</i> (Ham.ex D. Don) C.Chr <i>D. sparsa</i> (D. Don) Kunze.	Used in swelling, ulcers, pains and snakebite. Rhizomes are antibacterial and antifungal, dried rhizome used in epilepsy, leprosy and blood purification.	Singh <i>et al.</i> , 2005; Benjamin and Manickam 2007; Rout <i>et al.</i> , 2009; Perumal 2010; Bharti 2011; Benniamin 2011
<i>Lygodium flexuosum</i> (L.) SW. <i>L. scandens</i> Sw. <i>L. microphyllum</i> (Cav.) R. Br. <i>L. japonicum</i> (Thumb) Sw.	Used in sprains, scabies, ulcers and cut wounds, piles, menorrhagia, fever, gonorrhoea, and spermatorrhoea. <i>L. japonicum</i> root paste effective against food poisoning.	Benjamin and Manickam 2007; Singh <i>et al.</i> , 2005; Rout <i>et al.</i> , 2009; Bharti 2011; Benniamin 2011; Sen and Ghosh 2011; Britto <i>et al.</i> , 2012
<i>Microsorium punctatum</i> (L.) Copel. <i>M. superficial</i> (Bl.) Ching.	Leaf and juice are used as purgative, diuretic and for healing wound.	Benjamin and Manickam 2007; Rout <i>et al.</i> , 2009; Shil and Choudhury 2009
<i>Nephrolepis cordifolia</i> (L.) Pr. <i>N. auriculata</i> (L.) <i>N. biserrata</i> (SW.) Schoott	Rhizome is antibacterial & antifungal used in cough, rheumatism, chest congestion, jaundice, nose blockage.	Benjamin and Manickam 2007; Benniamin 2011
<i>Ophioglossum reticulatum</i> L. <i>O. costatum</i> R. Br. <i>O. gramineum</i> Willd <i>O. nudicaule</i> Linn.	Used in menstrual disorders, applied on burns as cooling agent. <i>O. gramineum</i> has antibacterial, anticancerous, antiseptic, detergent and vulnerary properties.	Benjamin and Manickam 2007, Rout <i>et al.</i> , 2009; Bharti 2011; Sen and Ghosh 2011
<i>Oleandra musifolia</i> <i>O. wallichii</i> (Hook-Presl)	Rhizome is used in snakebite and plant is anthelmintic.	Benjamin and Manickam 2007; Benniamin 2011
<i>Osmunda hugeliana</i> presl. <i>O. regalis</i> L.	Fronds are used as tonic stypic and also for the treatment of rickets, rheumatism and for intestinal gripping.	Benjamin and Manickam 2007; Benniamin 2011
<i>Onychium siliculosum</i> (Desv.) C.chr. <i>O. japonicum</i> (Thunb.) Kunze	Fronds are used in the treatment of dysentery and hair fall.	Benniamin 2011
<i>Pteridium aquilinum</i> Kuhn <i>P. revolutum</i> (Blume) Nakai	Decoction of rhizome and fronds used in the treatment of worms. The infusion relieves stomach cramps and increases urine flow.	Benjamin and Manickam 2007; Rout <i>et al.</i> , 2009; Perumal 2010; Benniamin 2011

<i>Pteris biaurita</i> L.		
<i>P. cretica</i> L.		
<i>P. pellucida</i> Presl, Rel.		
<i>P. quadrianrita</i> Retz.	Juice of frond is applied on cuts and bruises fronds are antibacterial and applied on wounds. Fresh rhizomes and leaves extract used in the treatment of glandular swellings	Benjamin and Manickam 2007;Rout <i>et al.</i> , 2009; Bharti 2011; Benniamin 2011; Sen and Ghosh 2011; Britto <i>et al.</i> , 2012
<i>P. vittata</i> L.		
<i>P. wallichiana</i> J. Agardh.		
<i>P. ensiformis</i> Burn. F.		
<i>P. semipinnata</i> L.		
<i>Pyrrosia heterophyll</i> (L.) Price		
<i>P. lanceolata</i> (L.) Farewell	Used as a cooling agent for the treatment of swelling, sprains, and in the preparation of itch guard.	Benjamin and Manickam 2007; Benniamin 2011
<i>P. adnascens</i> (Sw.) ching.		
<i>Selaginella bryopteris</i> (L.)		
<i>S. repanda</i> (Desv. ex Poir.) Spring		
<i>S. tenera</i>	Used in stomachache, urinary tract inflammation in children. Treatment of leprosy, diuretic gonorrhoea and hallucination, also used in the prolapsed of rectum, prevents cough, bleeding piles, gravel amenorrhoea and fever.	Singh <i>et al.</i> , 2005; Benjamin and Manickam 2007 Rout <i>et al.</i> , 2009; Perumal 2010; Bharti 2011; Benniamin 2011
<i>S. bryopteris</i> (L.) Bak.		
<i>S. indica</i> (Milde) Trayon		
<i>S. radiata</i> (Hook and Grev.)		
<i>S. delicatula</i> Desv.ex.poir.		
<i>S. involvens</i> (Sw.) spring		
<i>S. radicata</i>		
<i>S. palustris</i> (Burm.f.) Bedd.		
<i>Tectaria coadunata</i> (J.Sm.) C. Chr.		
<i>T. macrodonta</i> (Fee) Chr.	Plant is antibacterial, used in asthma, bronchitis and diarrhea. Cooked tender portion is used for curing stomach trouble.	Singh <i>et al.</i> , 2005; Benjamin and Manickam 2007; Bhari 2011, Benniamin 2011
<i>T. wightii</i> (Clarke) ching		
<i>T. polymorpha</i> (Wall ex Hook) Copel.		

Rhizosphere and associated microbiota of ferns

The rhizosphere is the narrow region of [soil](#) that is directly influenced by [root](#) secretions and associated soil [microorganisms](#). It is an area of intense biological and chemical activity influenced by compounds exuded by the root, and by microorganisms feeding on the compounds. The rhizosphere contains many [bacteria](#) that feed on plant cells, termed *rhizodeposition*, and the proteins and sugars released by roots. The rhizosphere is also a dynamic region where multiple interactive processes take place (Darrah *et al.*, 2006). Plants secrete many compounds into the rhizosphere which serve different functions. Dynamic rhizosphere system contains different components like root architecture and physiology, root-induced changes in water and nutrient availability, root exudates, and fungal and bacterial associations (Gahoonia and Nielsen 2003).

Plant-microbe-soil interactions: Plant root-microbe-soil interactions in the rhizosphere is vital to many areas such as plant nutrition, ecosystem diversity, rehabilitation of degraded soil, water quality, and bioremediation including phytoextraction, phytostabilization and phytovolatilization (Figure 1) (Hinsinger and Marschner 2006). The rhizosphere is the centre of biological activity due to the food supply provided by the root exudates of plants. Bacteria, fungi, protozoa, slime moulds, algae, nematodes, earthworms, millipedes, centipedes, insects, mites, snails, small animals and soil viruses compete constantly for water, food and space. Soil chemistry and pH can influence and control the

species mix and functions of microbes in the rhizosphere.

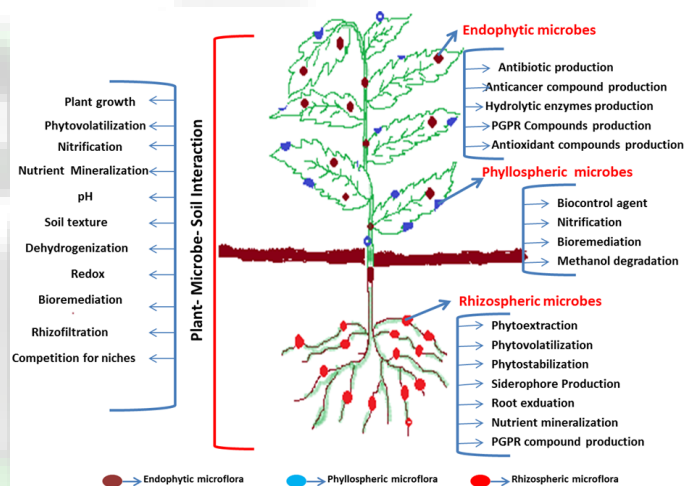


Figure 1: Represents the rhizospheric, phyllospheric and endospheric regions of the plant showing plant-microbe-soil interaction and their functional roles.

The specificity of the plant-microbe interaction is dependent upon soil conditions, which can alter contaminant bioavailability, root exudate composition, and nutrient availability. In addition, the metabolic requirements for heavy metals remediation may also dictate the form of the plant-bacteria interaction i.e., specific or nonspecific. Along with metal toxicity, there are often additional factors that limit plant growth in contaminated soils including arid conditions, lack of soil structure, low water supply and nutrient deficiency. The phytoremediation of metals can be influenced by the variation of rhizospheric microbe; and the interactions between microbes

and plant roots, including microbial and plant root exudates (Tang *et al.*, 2001).

Many of the soil microorganisms do not interact with plant roots, possibly due to the constant and diverse secretion of antimicrobial root exudates (Abhilash *et al.*, 2011). Fitz and Wenzel (2002) proposed that hyperaccumulators may enhance metal solubility in the rhizosphere via root exudation, consequently increasing plant metal uptake. Tu *et al.*, (2004) demonstrated that when exposed to higher arsenic in the solution, *Pteris vittata* released more root exudates. However, there are some microorganisms that do interact with specific plants. These interactions can be pathogenic, symbiotic, harmful, saprophytic or neutral. Interactions that are beneficial to agriculture include mycorrhizae, legume nodulation, production of antimicrobial compounds and plant growth promoters that inhibit the growth of pathogens. Microbes played some more important functions like nutrient mineralization in which they convert organic forms of nutrients into inorganic forms that plants roots can take up. In legumes, microbial root nodulations enable plants to fix nitrogen from the air. Rhizosphere microorganisms produce vitamins, antibiotics, plant hormones and communication molecules that all encourage plant growth. They are also capable to stabilize soil

aggregates like waste products and secretions from microorganisms help combine soil particles into stable aggregates around plant roots. These aggregates hold moisture within, but allow drainage between aggregates, so that root hairs do not get waterlogged.

In case of ferns only some reports are available related to rhizospheric bacteria. Recently, Yang *et al.*, (2012); Ghosh *et al.*, (2011) and Huang *et al.*, (2010) reported rhizospheric bacterial strains from the rhizosphere of *Pteris vittata* (Table 2). All the strains have the capability to cope with the metal toxicity. In some prior studies, Mills *et al.*, (1996) also observed rhizospheric bacterial population of leatherleaf fern (*Rumobra adiantiformis* [Forst.] Ching). A *Paenibacillus filicis* sp. strain was isolated from the rhizosphere region of fern in Daejeon, republic of Korea. This strain is capable of producing isoprenoid quinines and fatty acids (Kim *et al.*, 2009). Mycorrhizal interactions with ferns are much reported as compared to bacteria, yeast and fungi (Table-2). However, very less is known about microbial communities associated with the rhizosphere of seedless vascular plants, including ferns; although, they are widely distributed geographically from arctic to tropical habitats (Moran 2004; Anderson 2009). Some available reports are summarized in table 2.

Table 2: Check list of rhizospheric, phyllospheric and endophytic microflora of ferns.

Rhizospheric Microflora	Group	Fern Name	Reference
<i>Rhodococcus</i> sp. TS1	Bacteria	<i>Pteris vittata</i> L.	Yang <i>et al.</i> , 2012 Ghosh <i>et al.</i> , 2011 Huang <i>et al.</i> , 2010; Chang <i>et al.</i> , 2010
<i>Delftia</i> sp. TS33,			
<i>Comamonas</i> sp. TS37,			
<i>Delftia</i> sp. TS41,			
<i>Streptomyces lividans</i> sp. PSQ22			
<i>Pseudomonas</i> sp.,			
<i>Comamonas</i> sp.,			
<i>Stenotrophomonas</i> sp			
<i>Naxibacter</i> sp., AM774589			
<i>Mesorhizobium</i> sp., AM181745			
<i>Methylobacterium</i> sp., AM910536			
<i>Enterobacter</i> sp., Z96079			
<i>Pseudomonas</i> sp., AF368755			
<i>Bacillus</i> sp., FJ188297			
<i>Acinetobacter</i> sp., AM945567			
<i>Pseudomonas</i> sp., EF178450			
<i>Pseudomonas</i> sp., EF178450			
<i>Caryophanon</i> sp., AF385535			
<i>Pseudomonas</i> sp., EF178450			
<i>Pseudomonas</i> sp., AF368755			
<i>Pseudomonas</i> sp., EF178450			
<i>Acinetobacter</i> sp. strain MRI67 (DQ539027)	Mycorrhiza	<i>Equisetum</i> sp., <i>Marsilea</i> sp., <i>Nephrolepis</i> sp., <i>Adiantum</i> sp.	Sarwade <i>et al.</i> , 2012
<i>Buttiuxella</i> sp. strain MRI-65 (DQ539024)			
<i>Acaulospora</i> sp.			
<i>Gigaspora</i> sp.			
<i>Glomus</i> sp.			
<i>Sclerocystis</i> sp.			
<i>Glomus mosseae</i>			
<i>Glomus geosporum</i>			
<i>Glomus etunicatum</i>			
<i>Glomus mosseae</i>			
	Mycorrhiza	<i>Pteris vittata</i> L.	Liu <i>et al.</i> , 2009; Chen <i>et al.</i> , 2007
<i>Glomus mosseae</i> ,			Trotta <i>et al.</i> , 2006
<i>Gigaspora margarita</i>			Chen <i>et al.</i> , 2006.
<i>Glomus mosseae</i> ,			

phyllosphere include methanol degradation (Van Aken *et al.*, 2004) and nitrification (Papen *et al.*, 2002). Microorganisms on arrival in the phyllosphere have to establish and colonize the leaf to become a residual epiphyte. An uneven pattern of distribution of microorganisms is found on leaves. Epidermal cell wall junctions are the most common sites for bacterial colonisation (Davis and Brlansky 1991) especially in protected sites in grooves along the veins, at stomata (Mariano and McCarter 1993), and at the base of trichomes (Mariano and McCarter 1993). They are also found under the cuticle (Corpe and Rheem 1989), in depressions in the cuticle (Mansvelt and Hattingh 1987), near hydrathodes (Mew *et al.*, 1984) and in specific sites that only occur on particular plants such as stomatal pits in *Oleander* and pectate hairs in *Olive* (Surico 1993). In general, greater numbers of bacteria are found on lower than upper leaf surfaces (Surico 1993).

Although much work has been reported on phyllospheric microbes of various plant groups, very few reports are available on phyllospheric microflora of ferns. Rathinasabapathi *et al.*, (2006) isolated a phyllospheric arsenic resistant bacterium from the fronds of *Pteris vittata* grown in a site contaminated with copper, chromium and arsenate. This bacterium belongs to *Proteobacterium* group of bacteria. Recently, Kloeppe *et al.*, (2012) have also isolated *Pseudomonas* strain from *Rumohra adiantiformis*. Some more examples are mentioned in table-2.

Endosphere and associated microbiota of ferns

Endosphere is defined as the intercellular and intracellular region of higher plants and endophytes are microorganisms that grow inter or intracellular tissues of higher plants without causing any negative effects on the plants. The plants in which endophytes live have proven to be rich sources of bioactive natural products (Pimentel *et al.*, 2011). According to Qadri *et al.*, (2013) microorganisms are important sources of bioactive natural products with huge possibility for the discovery of new molecules for drug discovery, industrial use and agricultural applications. Studies based on estimation of microbial populations have revealed that only about 1% of bacteria and 5% of fungi have been characterized and the rest remain unexplored for their contribution to the human welfare (Staley *et al.*, 1997).

Endophytes may provide protection and survival conditions to their host plant by providing mutualistic interaction and producing a plethora of substances which on isolation and characterization might find potential use in industry, agriculture and pharmaceuticals (Strobel and Daisy 2003; Strobel *et al.*, 2004; Kogel *et al.*, 2006). The endophytes displayed diverse taxonomic positions

and bioactive potential. Conifers (*Cedrus deodara* (Roxb.) G.Don, *Pinus roxburgii* Sarg. and *Abies pindrow* (Royle ex D.Don) Royle) possessed a broad range of fungal endophytes, harboring about half of the total genera. These hosts produce bioactive essential oils (Qadri *et al.*, 2013) that may generate considerable selection pressure for the microbes to colonize inside the plant tissues. They can also survive for several hundred years (Qadri *et al.*, 2013); thus their microbial symbionts may undergo considerable evolutionary changes as their host plants grow and produce a variety of secondary metabolites during various stages of life cycle. Researchers like Yuan *et al.*, (2011); Qadri *et al.*, (2013) have been successful in isolating various endophytes of different conifers. Conifer group represents important ecological niches for novel microbial strains with useful bioactivities.

Fungal endophytes belonging to *Alternaria* and *Fusarium* species were mostly obtained from medicinal plants like *Ginkgo biloba* L., *Dysoxylum binectariferum* Hook.f, as compared to the other host plant (Qadri *et al.*, (2013). These plants are rich in medicinal properties and these fungal endophytes have the potentials to contribute to metabolic products to the host plant which act as anticancer and antimicrobial agents (Qin *et al.*, 2009; Xu *et al.*, 2010; Mohana *et al.*, 2012). Some strains of *Fusarium tricinctum* are known to produce different *enniatins* which have strong biological activities including antifungal properties (Meca *et al.*, 2010).

There are very few reports available on the endophytic microflora of pteridophytes. Some available reports are mentioned in table-2. These endophytes need to be studied in more detail and may be exploited for disease management for important agricultural crops and some human and animal diseases. In case of bacterial endophytes very less work has been reported in the plant group of pteridophytes, while pteridophyte group have so many medicinal properties which has been explored in the treatment of various chronic diseases. In case of endophytic bacteria only two reports are available with the ferns. *Bacillus* spp. *Paenibacillus* sp., *Amphibacillus* sp., *Gracilibacillus* sp., *Micrococcus* sp., *Stenotrophomonas* spp. have been isolated from the fern *Dicksonia sellowiana* Hook. and *Rhodococcus* sp. strain APG1 was isolated from aquatic fern *Azolla pinnata* R.Br (Barros *et al.*, 2010; Cohen *et al.*, 2004). In case of fungi very few reports are available which are mentioned in table-2. Actinomycetes have great potential for production of antibiotics and antioxidants. Castillo *et al.*, (2003) have reported the isolation of a *Streptomyces* sp. from the fern *Grevillea pteridifolia* Knight which have the capability to produce different antibiotics. While so many ferns are reported to produce antioxidants and antibiotics against several diseases but the isolation of

microbes from ferns have not been reported to that extent. The production of bioactive compounds by endophytes, especially those exclusive to their host plants, is not only important from an ecological perspective but also from a biochemical and molecular standpoint. Many recent studies provide compelling evidence that microbial interactions can play a major role in the onset of metabolite production in bacteria and fungi. These encounters may involve small, diffusible signaling molecules, such as quorum-sensing signals or other elicitors, which may trigger otherwise silent biosynthetic pathways (Kusari *et al.*, 2009).

Selection of host plant should be very selective for endophytes isolation because the host plant played a very imperative role in endophytes metabolic production because according to Selim *et al.*, (2012) the endophytes may be metabolically aggressive by affecting host defense chemicals. Such a species of endophyte isolated from a plant host produces a bioactive compound but fails to do so when isolated from another plant species (Li *et al.*, 1996). The herbicidal activity of secondary metabolites of an endophytic *Phyllosticta capitalensis* differed with the plant host from which the endophyte was isolated. This probably indicates that the plant host and its metabolism influence the synthetic ability of an endophyte. As such bioprospecting for endophyte natural products should be host plant based. In this regard, the endophyte-plant host association could also be exploited in enhancing the production of useful metabolites by the plant host (Wang *et al.*, 2004).

Biotechnological potential and possible applications of associated microbiota

Examples of natural products obtained from microbes associated with the medicinal ferns and their biotechnological potential and possible applications are diverse. In this section attention has been focused on the role of associated microbiota in the fields of the medicine, agriculture and industry.

Antibiotics Production: Antibiotics by definition are the low molecular weight organic compounds produced by microorganisms that are active at low concentration against other microorganisms (Strobel and Daisy 2003). Antibiotics have enormous economic health values because these are used to cure many diseases caused by bacteria, virus, fungi and parasites. Although microbes are ubiquitous and can be found everywhere but rhizospheric microorganisms are more diverse and active than phyllospheric microorganisms (Krutz *et al.*, 2005). At present very few reports are available on antibiotic producing endophytic microflora of ferns. Although rhizospheric and phyllospheric microflora of ferns are reported (Table-2). However, some other groups of plants are

reported to be associated with the antibiotic producing microflora. The “ecomycins” are produced by *Pseudomonas viridiflava* a fluorescent bacterium that is generally associated with the leaves of many grass species and is located on and within the tissues (Miller *et al.*, 1998). Ecomycins are active against human pathogenic fungi such as *Cryptococcus neoformans* and *Candida albicans*.

In case of endophytic microbes a large number of antimicrobial compounds have been isolated and they belong to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids (Yu *et al.*, 2010). These antimicrobial compounds can be used not only as drugs by humankind but also a food preservative in the control of food spoilage and food borne diseases, a serious concern in the world food chain (Liu *et al.*, 2008).

Many bioactive compounds like “sordaricin”, “mellisol” and “1, 8-dihydroxynaphthol 1-O- α -glucopyranoside” have been isolated from the genus *Xylaria* residing in different plant hosts. The “sordaricin”, act as an antifungal agent against *Candida albicans*. “Mellisol” and 1, 8-dihydroxynaphthol1-O- α -glucopyranoside are active against herpes simplex virus-type 1 (Pittayakhajonwut *et al.*, 2005). The bioactive compound “7-amino-4-methylcoumarin” isolated from the culture extracts of the endophytic fungus *Xylaria* sp. YX-28 isolated from *Ginkgo biloba* L. presented broad-spectrum inhibitory activity against several food-borne and food spoilage microorganisms including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Aeromonas hydrophila*, *Yersinia* sp., *Vibrio anguillarum*, *Shigella* sp., *Vibrio parahaemolyticus*, *Candida albicans*, *Penicillium expansum*, and *Aspergillus niger*, especially to *Aeromonas hydrophila*, and was suggested to be used as natural preservative for food (Liu *et al.*, 2008).

An endophytic *Streptomyces* sp. isolated from a fern-leaved grevillea (*Grevillea pteridifolia* Knight) in Australia was described as a promising producer of novel antibiotics, “kakadumycin A” and “echinomycin”. Kakadumycin A is structurally related to echinomycin, a quinoxaline antibiotic, and presents better bioactivity than echinomycin especially against Gram positive bacteria and impressive by activity against the malarial parasite *Plasmodium falciparum* (Castillo *et al.*, 2003). An endophytic fungi *Fusarium* sp. isolated from fern *Selaginella pallescens* (Presl) Spring have potent activity against *Candida albicans* (Brady and Clardy 2000).

In case of ferns not much work has been reported for producing antibiotics or antimicrobial compounds by associated microflora, while various ferns like *Dryopteris cochleata* (Buch.-Ham. ex D.

Don) C. Chr., *Equisetum ramosissimum* Desr., *Selaginella bryopteris* (L.) Bak., etc. have been reported for antibacterial, antifungal and antiviral properties (Perumal 2010; Bharti 2011). The chances of having antibiotic producing microorganism associated with these ferns seem to be great. Extensive research is therefore essentially needed for the exploration of the future prospects and biotechnological importance of the microorganisms associated with ferns of economic values.

Antioxidant production: Antioxidants are compounds that worked against damage caused by reactive oxygen species (ROS) and oxygen free radicals, which contribute to a variety of pathological effects like DNA damage and cellular degeneration. Antioxidants are promising therapy for the prevention and treatment of ROS-linked diseases like cancer, cardiovascular disease, hypertension, diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis and ageing. Many antioxidants have antibacterial and antiviral compounds at higher and lower levels (Pimentel *et al.*, 2011).

Many ferns like *Pteris vittata*, *Adiantum capillusveneris* Linn., are reported to have antioxidant properties (Singh *et al.*, 2010), but very few fern-associated microbes have been reported for antioxidant characteristics. An endophytic *Streptomyces* sp. from a fern-leaved *Grevillea pteridifolia* is reported for antioxidant production (Castillo *et al.*, 2003). It is well known that natural antioxidants are commonly found in medicinal plants, vegetables, and fruits. However, it has been reported that metabolic products from endophytes can be a potential source of novel natural antioxidants. Two metabolic compounds “Pestacin” (C₁₅H₁₄O₄) and “isopestacin”, 1,3-dihydroisobenzofurans, were obtained from the endophytic fungus *Pestalotiopsis microspora* isolated from a plant, *Terminalia morobensis* Coode (Strobel *et al.*, 2002; Harper *et al.*, 2003). These two compounds are also having antibacterial and antifungal activities. Polysaccharides from plants and microorganisms have been extensively studied and considered as potent natural antioxidants (Yu *et al.*, 2007). Exopolysaccharides (EPS) metabolic compound of the bacterial endophyte *Paenibacillus polymyxa* isolated from the root tissue of *Stemona japonica* Miquel, have been demonstrated to have strong scavenging activities on superoxide and hydroxyl radicals (Liu *et al.*, 2009). Similarly Song *et al.*, (2005) have reported that the endophytic fungus *Cephalosporium* sp. IFB-E001 residing in *Trachelospermum jasminoides* (Lindl.) Lem. produce “Graphislactone A”, a phenolic metabolite. It has been demonstrated to have free radical-scavenging and antioxidant activities *in vitro* stronger than the standards, butylated hydroxytoluene (BHT) and ascorbic acid. For more details of antioxidants and

antimicrobial and anticancer agent of endophytes the reviews of Fir'akov'a *et al.*, (2007) and Pimentel *et al.*, (2011) are recommended.

Antioxidant characteristics in ferns have played a major role in plant protection against toxic pollutants and diseases and we can predict that their associated microbes may be capable of producing good quality of antioxidants. So a broad and systematic study is needed to unravel this unique potential of the fern associated microbiota.

Anticancer compounds production: Cancer is a chronic disease which is characterized by unregulated cell growth and can result in death if not controlled in time as required. It is considered as one of the major causes of death every year throughout the world. Many anticancer drugs show various side effects and are found to be non-effective against many forms of cancer (American Cancer Society 2009; WHO 2009). Thus cure of cancer has become very costly due to chemical forms of medicines because chemical products are costlier than natural products. Recent evidences have indicated that there are some bioactive compounds produced by plants and microorganisms could be a better approach for discovery of novel drugs against cancer. Some more potential rhizospheric and endophytic microbes are also reported for the production of anticancer agents (Pimentel *et al.*, 2011). According to Devi *et al.*, (2012) mangrove actinomycetes are a prolific and underexploited source for the discovery of anticancer compounds.

However, anticancer compounds have been isolated and studied from many of the microbes with special reference to their antioxidant activities. Microbial L-asparaginase (L-asparagine amidohydrolase) has been used widely as a therapeutic agent in the treatment of certain human cancers, mainly in acute lymphoblastic leukemia. Several actinomycetes like *Streptomyces* species such as *S. karnatakensis*, *S. venezuelae*, *S. longisporusflavus* and a marine *Streptomyces* sp. strain PDK2 have been explored for L-asparaginase production (Anupa *et al.*, 2013).

The other compound “Taxol” (C₄₇H₅₁NO₁₄) have gain more attention because of their unique mode of action compared to other anticancer agents (Fir'akov'a *et al.*, 2007; Gangadevi and Muthumary 2008). This compound directly interferes with the multiplication of cancer cells and reducing their growth. It has been approved for the treatment of breast cancer, lung cancer and ovarian cancer (Cremasco *et al.*, 2009). “Taxol” was first extracted from *Taxus brevifolia* Peattie (Wani *et al.*, 1971), but this tree is very rare, slow growing and produces very small amount of taxol.

As an alternative approach the endophyte, *Taxomyces andreanae* has provided more taxol by fermentation process as a cheaper technique. Taxol has also been found in a number of different genera of fungal endophytes, such as *Taxodium distichum* (Li *et al.*, 1996); *Phyllosticta spinarum* (Kumaran *et al.*, 2008); *Bartalinia robillardoides* (Gangadevi and Muthumary 2008); *Pestalotiopsis terminaliae* (Gangadevi and Muthumary 2009); *Botryodiplodia theobromae* (Pandi *et al.*, 2010); *Fusarium redolens* (Garyali *et al.*, 2013).

Another anticancer compound “camptothecin” (C₂₀H₁₆N₂O₄), is an alkaloid which was first isolated from the wood of *Camptotheca acuminata* Decaisne (Nyssaceae) in China (Wall *et al.*, 1966). Following this, camptothecin were also obtained from the endophytic fungus *Fusarium solani* isolated from *Camptotheca acuminata* (Kusari *et al.*, 2009). After that several other anticancer compounds have been isolated from endophytic microbes. These include phenylpropanoids from *Penicillium brasilianum* (Fill *et al.*, 2010), podophyllatoxin from *Trametes hirsute* (Puri *et al.*, 2006) and cytochalasins from *Rhinocladiella* sp. (Wagenaar 2000) etc.

Among pteridophytes, a number of ferns like *Adiantum capillusveneris* Linn., *Asplenium polydon* G., *Ophioglossum gramineum* Willd., etc. have been reported for anticancerous properties (Benjamin and Manickam 2007), but so far there is no report on the discovery of anticancer compounds from microflora associated with ferns.

Production of plant growth promoting (PGP) substances: The use of plant growth promoting bacterial strains has increased tremendously because of increasing concern of environmental pollution caused directly or indirectly by pesticides, herbicides and fungicides (Glick *et al.*, 2007). The plant growth promotion effect by these bacterial has been tentatively attributed to the production of indol acetic acid (IAA), siderophores, 1-aminocyclopropane-1-carboxylic deaminase (ACC) and solubilization of phosphate. Other plant hormones such as gibberellins and cytokinins are also produced by many bacterial strains. In contrast, the role of bacteria in preventing ethylene synthesis has been better studied. Ethylene is a plant hormone that prevents the root elongation. Many plant growth promoting strains have the capability to produce 1-aminocyclopropane-1-carboxylic acid deaminase which inhibits the production of ethylene and facilitate elongation of root length more profoundly (Kumar *et al.*, 2009).

Production of N-acylhomoserine lactone that may be involved in plant growth promotion was also observed in some cases (Rothballer *et al.*, 2008). Many rhizospheric microfloras are reported in various agricultural crops and ornamental plants, which are also capable to cope with metal toxicity

in plants by the production of siderophores and phosphate solubilization (Srivastava *et al.*, 2012 a; b). Huang *et al.*, (2010); Ghosh *et al.*, (2011); Yang *et al.*, (2012) have also reported some rhizospheric bacterial strains of *Pteris vittata* which are capable to cope with metal toxicity. To date, there is still limited information related to the role of nitrogen-fixing bacteria as biocontrol agents in ferns. Symbiosis between the aquatic fern *Azolla* and *Anabaena azollae* is of particular interest because it is the only plant-prokaryote symbiosis known to persist throughout the reproductive cycle of the host plant (Lechno-Yossef and Nierzwicki-Bauer 2002).

Rhizospheric bacteria are well-known for beneficial PGP because of their role in plant growth, plant nutrition and antagonistic effect. Endophytic bacteria are also involved with host plants in mutual interaction. They promote plant growth directly or indirectly, via production of phytohormones, biocontrol of host plant diseases or improvement of plant nutritional status (Pandey *et al.*, 2005; Qin *et al.*, 2011). Endophytic bacteria are also producing siderophore to bind Fe³⁺ from the environment and help to improve nutrient uptake, supply of plant nutrients (nitrogen, phosphate and other mineral nutrients), or suppression of stress ethylene production by ACC deaminase activity (Compant *et al.*, 2010; Qin *et al.*, 2011). Several endophytic actinobacteria isolated from winter rye produced indolyl-3-acetic acid. Treatment of winter rye seeds with auxin producing strains increased the germination capacity and enhanced an intensive seedling growth *in vitro* (Merzaeva and Shirokikh 2010). Two compounds pteridic acids A and B were found from species *S. hygrosopicus* TP-A0451 isolated from a stem of bracken, *Pteridium aquilinum* (L.) Kuhn (Igarashi *et al.*, 2002; Igarashi *et al.*, 2006). Pteridic acids inhibited the rice germination at 100 ppm, but pteridic acid A promoted the root elongation at 20 ppm. Furthermore, pteridic acid A induced the adventitious root formation of the kidney bean hypocotyls as effectively as indole acetic acid (Qin *et al.*, 2011). According to Wagas *et al.*, (2012), two endophytic cultures, *Phoma glomerata* and *Penicillium* sp. also contained IAA. Lin and Xu (2013), also discovered IAA production by endophytic *Streptomyces* sp. isolated from medicinal plants. Zhang *et al.*, (2011) isolated ACC deaminase-producing endophytic bacteria obtained from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus* (L.). In case of nitrogen fixation process, endophytic bacterial strain *Acetobacter diazotrophicus*, was isolated from sugarcane tissue. This strain has nine biochemical and morphological characteristics, including acetylene reduction in air (Dong *et al.*, 1994). Elbeltagy *et al.*, (2001) also isolated endophytic bacterial strains from stem tissue of rice plant the endophytes were

close to bacterial genera *Herbaspirillum*, *Ideonella*, *Enterobacter*, and *Azospirillum* and have nitrogen fixation process. Several mycorrhizal fungi are reported for the expression of PGP properties on pteridophytes which are reported in table-2. Trotta *et al.*, (2006) also explained the effect of arbuscular mycorrhizal fungus on the growth of *Pteris vittata* L. plants. They also help in water uptake, thereby protecting the plants under mild drought stress and also help to prevent the activity of root pathogens. They produce growth-promoting substances such as indole acetic acid (IAA), cytokinins and gibberellin like substances. *Arbuscular mycorrhizal* fungi enhance the plant growth as a result of the improved phosphate nutrition and water supply of the host plant, likewise the fungus receives fixed carbon (Karthikeyan *et al.*, 2008).

Production of hydrolytic enzymes: Microbes are known producers of extracellular hydrolytic enzymes as a resistance mechanism against pathogenic invasion and to obtain nutrition from host. Such enzymes include pectinases, cellulases, lipases, laccase, xylanase, phosphatases and proteinase Caldwell *et al.*, 2000; Bailey *et al.*, 2006; Sunitha *et al.*, 2013).

Certain *Paenibacillus polymyxa* strains that are associated with many plant species have been used effectively in the control of plant pathogenic fungi and bacteria. *P. polymyxa* strains produce many hydrolytic enzymes, making them valuable antagonists to control plant pathogens and reflect the potential of these strains for further industrial application. The association between plants and *P. polymyxa* seems to be specific and to involve co-adaptation processes (Raza *et al.*, 2008). In ferns no rhizospheric and phyllospheric microbes have been reported with hydrolytic enzymatic activity. This objective wants some more intensive research. Caldwell *et al.*, (2000) reported two endophytic fungi *Philaophora finlandia* and *Philaophora fortinii* isolated from alpine plant communities which were able to breakdown the major polymeric forms of carbon, nitrogen and phosphorus found in plants. Maria *et al.*, (2005), studied the amylase activity of endophytic fungi from mangrove fern, *Acrostichum aureum* L. of south coast of India. At the beginning of colonization process, endophytes have to achieve at least partial degradation of cell wall. Extracellular enzymes, proteins that catalyze different types of chemical reactions, might be one of the main tools in that process. Fungal cellulases and pectinases can be very active during plant cell wall degradation (Chang *et al.*, 2009).

A wide range of literature survey predicts that not many have explored the possibility of associated microbes of medicinal ferns as biotechnological sources of industrially relevant enzymes. Hence

they can represent a new source in obtaining different enzymes with potentialities.

Conclusion and prospective

Pteridophytes having enormous potentiality can be exploited for the development of many allopathic, homeopathic and ayurvedic system of medicines are to be produced at large scale to meet the global requirement involving the common as well as tribal people. As it is well known that the herbal medicines do not have any side effect, these plants can be prescribed as herbal formulations to cure numerous diseases letting tremendous scope of economic earn.

Medicinally important fern associated microflora are a poorly investigated group of microorganisms that represent secondary metabolites having an immense crash on modern medicine. Isolation of microbes from medicinal ferns may result in methods to produce biologically active agents for biological utilization on a large commercial scale as they are easily cultured in laboratory and fermentor instead of harvesting plants and affecting the environmental biodiversity. Metabolic products of these associated microbes would be a good source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas.

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