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Endophytes Isolated from the Plants of Himachal Pradesh and their Importance

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A B S T R A C T

The term, "endophytes" includes a suit of microorganisms that grow intra and/ or inter cellularly in the tissues of higher plants without harming host plant and have proven to be rich source of bioactive natural compounds. This mutualism between endophyte and the host plant results in fitness benefits to both the partners. Endophytes provide protection and survival conditions to the host plant by producing plethora of substances. These substances produced by endophytes are very useful in industry, agriculture and medicine. Considering that only a small amount of endophytes have been studied, recently, several research groups have been motivated to evaluate and elucidate the potential of these microorganisms in Biotechnological processes focusing on the production of bioactive compounds. Production of bioactive substances by endophytes is directly related to the independent evolution of these microorganisms which may have incorporated genetic information from higher plants, to better adapt to the plant host and carry out some functions like protection from pathogens, insects and grazing animals. Endophytes play a role as a selection system for microbes to produce bioactive substances with low toxicity towards higher organisms. This review summarizes part of the work being done on the endophytes isolated from the plants of Himachal Pradesh, India.

Introduction

Nearly all the plants existing in this world were found to be associated with endophytes (Begum and Tamilselvi, 2016). Endophytes are microorganisms including bacteria and fungi, living within the plant tissues without causing any negative effect to each other. Endophytes have been found in every plant species examined to date and recognized as the potential source of novel natural bioactive compounds for exploitation in medicine, agriculture and industry (Bacon and White, 2000; Strobel and Daisy, 2003; Kumar and Sagar, 2007). Host plant interaction with endophytes leads to plant productivity, enhance soil health and nutrient uptake by the plant (Mei and Flinn, 2010) and defence against plant stress, diseases and insects (Gao *et al*., 2010). Endophytes are capable of synthesizing bioactive compounds that can be used by the

plants for defence against human pathogens and some of these compounds have been proven useful for novel drug discovery. Hundreds of natural products like flavonoids, terpenoids, alkaloids, steroids etc have been reported from endophytes. Most of the natural products isolated from endophytes are antibiotics, antioxidants, antimicrobial agents, insecticidal agents, anticancer agents, antidiabetic agents, immunosuppressive agents etc (Wang *et al.,* 2000; Ezra *et al.,* 2004; Yu *et al.,* 2010; Huang *et al.,* 2007; Wall *et al.,* 1966; Demain 2000; Zang *et al.,* 1999; Lee *et al.,* 1995). Endophytes play a role in shaping plant community structure by increasing the mineral supply to plants, improving water uptake and retention and thus drought tolerance. Endophytic bacteria have the ability to produce regulators of plant growth such as auxins, cytokinins and gibberellins. Endophytes help in phosphorus solubilization (Rosenblueth and Romero, 2006), nitrogen fixation, and the suppression of stress related ethylene synthesis in plants by producing 1-aminocyclopropane 1 carboxylate deaminase (Hardoim *et al.*, 2008). Biocontrol activity of endophytes, protect plants against pathogens by inducing the plants defense mechanisms, producing pathogen antagonizing substances (hydrogen cyanide (HCN), siderophores, antibiotics and insecticidal compounds) or by competing with pathogenic organisms for colonization sites and nutrients (Rosenblueth and Romero, 2006).The intent of this review provides insights about the endophytes isolated from the different regions of Himachal Pradesh, a diverse climatic region which is also a part of Himalayan system.

Diversity among Endophytes isolated from Himachal Pradesh

Mycorrhiza is the most important member of functional soil microbial community in natural ecosystem and creates an intimate link between plant roots and soil. It is reported that biodiversity of AM fungi differ in different plants of Himachal Pradesh, India. Twelve medicinal plants listed in Table 1 are reported for arbuscular mycorrhizal (AM) spore number and root colonization. Among these plants, *Adhatoda vesica* showed maximum root colonization and *Achyranthus aspera* lack mycorrhizal root colonization. The spore number ranged from 25.66±3.00 to 388.33±8.50. The highest mycorrhizal spore count was observed in *Aster thomsonii* and lowest in *Aloe vera*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization (Kumar *et al*., 2013).

Seasonal fluctuation among the Fungal Associates of *Rhododendron campanulatum* D.Don was studied by Shilpa *et al.,* 2016 from Churdhar region of District Shimla and Sirmaur in Himachal Pradesh. Maximum numbers of endophytic and rhizospheric fungi were reported during the rainy season followed by summer and winter season while maximum numbers of VAM spores were observed during the rainy season followed by winter and summer season (Shilpa *et al*., 2016).

Plant Growth Promotion

Plant endophytes help in maintaining plant health by means of their biofertilizer and biocontrol attributes and, are currently being explored for their ability to produce novel biologically active compounds. Two bacterial endophytes, *Burkholderia* sp. and *Pseudomonas* sp. from the medicinal plants *Echinacea purpurea* and *Lonicera japonica* are reported to produce plant growth promoting agents. These medicinal plants were collected from the Botanical Garden of Shoolini University Solan, Himachal Pradesh. Viability counts in pea seedlings and (Random amplified polymorphic

DNA) RAPD analysis was used to confirm the endophytic nature of these isolates. Endophytes *Burkholderia* sp. and *Pseudomonas* sp. could be used as growth promoting agents and could control phytopathogens *in plantae* (confirmation by seed inoculation experiments). The presence of biofertilizer activities such as IAA production, nitrogen fixation and phosphate solubilization demonstrates that both *Burkholderia* sp. and *Pseudomonas* sp. could stimulate the plant growth. Both strains (*Burkholderia* sp. and *Pseudomonas* sp.) were reported to produce siderophores, which is another important trait of endophytes that may indirectly influence plant growth (Rosenblueth and Romero, 2006). Siderophores form bonds with the available form of iron Fe^{3+}, thus making it unavailable to phytopathogens and protecting plant health (Gupta *et al*., 2016). Plant growth promoting rhizobacteria are reported from the endophytic tissues of ginger plants from two different major growing locations of Solan and Sirmour districts of H.P. (India). The highest microbial count on ginger was reported at Sirmour (Narag), while the Phosphate Solubilisation Bacterial +ve colonies (87.72) and % P solublizers to PVK count (79.00) associated with ginger root was reported to be highest at Solan (Kandaghat), Himachal Pradesh (Kaundal *et al*., 2016).

Two hundred and six phosphate-solubilizing rhizobacteria (PSB) were isolated from rhizosphere soil (RS) and root endosphere (ER) of apple trees from different sites of four locations viz., Chamba, Shimla, Kinnaur and Kullu of Himachal Pradesh, Northern India. 50 isolates were reported to produce Indole Acetic Acid (24.2 %) and 53 isolates showed siderophore production (Mehta *et al*., 2013). A total of 247 bacterial endophytes were reported from five different sites of the northern hills zone of India. 16S rRNA gene based phylogenetic analysis revealed that 65, 26, 8 and 1 % bacteria belonged to four phyla, namely Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, respectively. Overall, 28 % of the total morphotypes belonged to *Pseudomona*s followed by *Bacillus* (20 %), *Stenotrophomonas* (9 %), *Methylobacterium* (8 %), *Arthrobacter* (7 %), *Pantoea* (4 %), *Achromobacter*, *Acinetobacter*, *Exiguobacterium* and *Staphylococcus* (3 %), *Enterobacter*, *Providencia*, *Klebsiella* and *Leclercia* (2 %), *Brevundimonas*, *Flavobacterium*, *Kocuria*, *Kluyvera* and *Planococcus* (1 %). Plant growth promoting traits, like solubilisation of phosphorus, potassium and zinc; production of ammonia, hydrogen cyanide, indole-3-acetic acid and nitrogen fixation were reported in the representative strains from each cluster (Verma *et al*., 2015). Cold-adapted isolates may have application as inoculants for plant growth psromotion and biocontrol agents for crops growing under cold climatic conditions (Verma *et al*., 2015). Bacterial endophytes B5 and B8 isolated from Strawberry plants of Solan, Himachal Pradesh possess biofertilzer activities like phytate production, ammonia production and nitrogen fixation (Sawhney *et al*., 2015)

Antimicrobial Activity

It is reported that *Burkholderia* sp. and *Pseudomonas* sp. are effective bioagents that controls soil borne *Fusarium oxysporum* f. sp. *pisi*, *Fusarium. solani* f. sp. *pisi*, *Mycosphaerellapinodes*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* pathogens, which cause root rot complex in field peas (Gupta *et al*., 2016). 61 isolates (29.6%) from the apple tree of Himachal Pradesh showed percentage growth inhibition against *Dematophora necatrix* (Mehta *et al*., 2013). Biocontrol activity against *Fusarium graminearum*, *Rhizoctonia solani* and *Macrophomina phaseolina* was reported in the endophytic isolates belonging to genra

Pseudomonas, *Bacillus*, *Stenotrophomonas*, *Methylobacterium*, *Arthrobacter*, *Pantoea*, *Achromobacter*, *Acinetobacter*, *Exiguobacterium* and *Staphylococcus*, *Enterobacter*, *Providencia*, *Klebsiella* and *Leclercia*, *Brevundimonas*, *Flavobacterium*, *Kocuria*, *Kluyvera* and *Planococcus* respectively from the northern hills zone of India (Verma *et al*., 2015).

Bioremediation

The mutualism between the plant and endophytes can play a key role in the degradation of hazardous contaminants in the rhizosphere. Bacterial endophytes might function more effectively than bacteria added to the soil because they participate in a process known as bioaugmentation (Newman and Reynol, 2005). Chlorpyrifos, one of the most extensively used insecticides is also neurotoxic upon prolonged exposure as it inhibits the normal activity of the enzyme acetylcholine esterase needed for proper nervous transmission.

Table.1 Medicinal plants of Himachal Pradesh: Source of Endomycorrhiza.

S.No.	Botanical Name	Common Name	Family
1.	*Achellea millefolium*	Gandana, Millifoil	Asteraceae
2.	*Achyranthes aspera*	Puthkanda	Amaranthaceae
3.	*Adhatoda vesica*	Basuti	Acanthaceae
4.	*Agave americana*	Ramban	Amaryllidaceae
5.	*Ageratum conyzoides*	Gumdrya, Ujadu	Asteraceae
6.	*Allium coralinianum*	Ladam	Liliaceae
7.	*Aloe vera*	Kumar patha	Liliaceae
8.	*Arnebia benthamii*	Laljari	Boraginaceae
9.	*Arnebia hispidissima*	Ratanjot	Boraginaceae
10.	*Artemisia annua*	Maleria buti	Asteraceae
11.	*Asparagus filicinus*	Satavari	Liliaceae
12.	*Aster thomsonii*	Thomson's aster	Asteraceae

Two bacterial endophytes that could metabolize chlorpyrifos were reported from the roots of the strawberry plants collected from Solan district of Himachal Pradesh. The results strongly suggest that these strains possess useful bioremediation activities which can be used to improve the quality of food products (Sawhney *et al*., 2015).

Production of Bioactive Secondary metabolites

Polyketide synthases are an important source of naturally occurring small molecules used for chemotherapy (*Koehn and Carter 2005*). For example, many of the commonly used antibiotics, such as tetracycline and macrolides, are produced by polyketide synthases. Other industrially important polyketides are sirolimus (immunosuppressant), erythromycin (antibiotic), lovastatin (anticholesterol drug), and epothilone B (anticancer drug) (*Wawrik et al.,* 2005). Miller *et al*., (2012) demonstrated that screening for the presence of biosynthetic genes related to PKS (Polyketide synthases) or NRPS may be an effective initial step in identifying the potential of bacterial endophytes to produce the bioactive secondary metabolites (Miller *et al.,* 2012). *Burkholderia* sp. and *Pseudomonas* sp contained NRPS and PKS

genes, which supports their potential for the production of useful secondary metabolites (Gupta *et al*., 2016).

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