Review Article

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Secondary Metabolites & Application of Endophytic Organisms

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Abstract

Endophytic organisms live symbiotically within the plant and in turn helping the plant in number of ways like growth, protection to environmental conditions, and sustainability, in favour of the hosts. Some of the well-known examples of phytochemicals produced by endophytes are Taxol, camptothecin, azadirachtin, podophyllotoxin, vinca alkaloids, *cinchona* alkaloids rohitukine, and many others. It can be used as an antidiabetic, ulcerogenic, antiviral, anti-inflammatory and analgesic agent, and may protect against numerous chronic diseases.

Introduction

Plants have served as a source of medicinal bioactive compounds against numerous forms of materials for centuries. Ironically, in recent years, microorganisms associated with plants rather than plants themselves have proved to offer material and products with high therapeutic potential (Subbulakshmi et al., 2012). Endophytes are an endosymbiotic group of microorganisms – often bacteria or fungi – that colonize the inter- and/or intracellular locations of plants (Pimentel et al., 2011; Singh and Dubey, 2015). They are associated with almost all plants studied till date. Some commonly found endophytes are those belonging to the genera *Enterobacter* sp., *Colletotrichum* sp., *Phomopsis* sp., *Phyllosticta*sp., *Cladosporium* sp., and so forth.Endophyte resources could bring us a variety of benefits, such as novel and effective bioactive compounds that cannot be synthesized by chemical reactions. A large amount of bioactive compounds produced by them not only are useful for plants but also are of economical importance to humans. They serve as antibiotics, drugs or medicines, or the compounds of high relevance in research or as compounds useful to food industry. They are also found to have some important role in nutrient cycling, biodegradation, and bioremediation. In this review, we have tried to comprehend different roles of endophytes in plants and their significance and impacts on man and environment. They may improve the plant's ability to to survive in various types of abiotic and biotic stresses, and increase resistance plants to insects and pests. They produce phytohormones and other bioactive compounds of biotechnological interest like enzymes and pharmaceutical drugs (Joseph and Priya, 2011; Parthasarathi et al., 2012). Endophytes can colonize in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, seeds and also dead and hollow hyaline cells of plants (Hata and Sone, 2008; Specian et al., 2012; Stêpniewska and Kuzniar, 2013). Methods to obtain bioactive compounds include the extraction from a natural source, the microbial production *via* fermentation, or microbial transformation. Extraction from natural sources presents some disadvantages such as dependency on seasonal, climatic and political features and possible ecological problems involved with the extraction, thus calling for innovative approaches to obtain such compounds (Bicas *et al*., 2009). Hence, biotechnological techniques by using different microorganisms appear promising alternatives for establishing an inexhaustible, cost-effective and renewable resource of high-value bioactive products and aroma compounds. The biotransformation method has a huge number of applications (Borges *et al*., 2009), for instance, it has been extensively employed for the production of volatile compounds (Bicas *et al*., 2009; Bicas *et al*., 2008; Krings *et al*., 2006). These volatile compounds possess not only sensory properties, but other desirable properties such as antimicrobial (vanillin, essential oil constituents), antifungal and antiviral (some alkanolides), antioxidant (eugenol, vanillin), somatic fat reducing (nootkatone), blood pressure regulating (2-[E]-hexenal), anti-inflammatory properties (1,8-cineole) and others (Berger, 2009).

Isolation of Endophytes& their Study Methods

Endophytes have been distributed in all the parts of plants. In cactus they were isolated from stem,root and pulp. Endophytes can be easily isolated on any microbial or plant growth, such as agar, potato dextrose agar and any nitrogen- or carbon-containing media. The most frequent method used to detect and enumerate endophytes involves isolation from surface-sterilized host plant tissue. Apart from that Recent studies have confirmed the occurrence of endophytes by various cultivation-independent assays and by fluorescence *in situ* hybridization-confocal laser scanning microscopy studies (Mitter et al., 2013; Berg et al., 2014). Cultivation-based techniques, use the recovery and testing of isolates, whereas cultivation-independent techniques screen for variations in the total endophytic communities (Menpara and Chanda, 2013). With recent advances and developments, more studies at the molecular level are done with endophytes, which include metagenomic studies, use of molecular markers, molecular cloning, and genetic expression studies. Denaturing gradient gel electrophoresis (DGGE) profiles of 16S rRNA gene fragments amplified from total plant DNA were used to detect some nonculturableendophytic bacteria by comparing the profile with the bands obtained from the culturableendophytes from Citrus plant (W. L. Araújo, J. Marcon et al,2002). Bacterial automated ribosomal intergenic spacer analysis (B-ARISA) and Pyrosequencing was used to examine bacterial endophyte community of potato (D. K. Manter et al,2010) .

Types of Endophytes & Relatable Spp

Endophytes are associated with plants in various forms, including bacteria (actinomycetes or mycoplasma) or fungi that have been colonized inside the plant tissues. More than 200 genera from 16 phyla of bacterial species have been reported to be associated with endophytes and among them; most of the species belong to the phyla *Actinobacteria*, *Proteobacteria*, and *Firmicutes*(Golinska et al., 2015). The diversity of endophytic bacteria ranges from gram-positive to gram-negative bacteria, such as *Achromobacter, Acinetobacter, Agrobacterium, Bacillus*, *Brevibacterium*, *Microbacterium*, *Pseudomonas*, *Xanthomonas*etc. (Sun et al., 2013). Bacterial endophytes are diverse in nature and are known to produce different bioactive metabolites that act as antimicrobial and anticancer compounds, for example, with 76% of them reported from the single genus, *Streptomyces* (Berdy, 2012).

Endophytic fungi have been classified into two broad groups based on their phylogeny and life history traits. These include the clavicipitaceous, which infect some grasses confined to cool regions and the non-clavicipitaceousendophytes, which are from asymptomatic tissues of non-vascular plants, ferns and allies, conifers and angiosperms and are limited to the Ascomycota or Basidiomycota group (Jalgaonwala et al., 2011; Bhardwaj and Agrawal, 2014). Endophytic fungi produce some of the most broadly used antibiotic and anticancer drugs. Penicillenols, extracted from *Penicillium* sp., is cytotoxic to numerous cell lines. Taxol, isolated from *Taxomycesandreanae*, is the most effective and successful anticancer drug extracted from endophytic fungi to date. Clavatol (*Torreyamairei*), sordaricin (*Fusarium* sp.), jesterone (*Pestalotiopsisjesteri*), and javanicin (*Chloridium* sp.) are all known to possess strong antibacterial and antifungal properties against numerous foodborne infectious agents (Jalgaonwala et al., 2011). Pestacin, isolated from *P. microspora*, has excellent antioxidant properties

Implimentation of Endophytes

4.1) Phytohormons

Endophytic organisms produce a wide range of phytohormones, such as auxins, cytokinins, and gibberellic acids. *Burkholderiavietnamiensis*, a diazotrophicendophytic bacterium isolated from wild cottonwood (*Populustrichocarpa*), produced indole acetic acid (IAA), which promotes the growth of the plant (G. Xin, G. Zhang et al. 2009). A new strain of fungus *Cladosporiumsphaerospermum* isolated from the roots of *Glycine max* (L) Merr. showed the presence of higher amounts of bioactive GA3, GA4, and GA7, which induced maximum plant growth in both rice and soybean varieties(M. Hamayun et al. 2009).

4.2) Phytochemicals

Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones and others (Tan and Zou, 2001). Such bioactive metabolites find wide-ranging application as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants and anticancer agents (Gunatilaka, 2006).

4.3) Antimicrobial Activity

Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites (Tan and Zou, 2001). The discovery of novel antimicrobial metabolites from endophytes is an important alternative to overcome the increasing levels of drug resistance by plant and human pathogens, the insufficient number of effective antibiotics against diverse bacterial species and few new antimicrobial agents in development.Apart from that this agents are also useful in food industry as a preservatives in control of food spoilage and against food borne diseases(Liu et al 2008). Endophytic fungus *Xylaria* sp., from *Abiesholophylla* was characterized as a producer of griseofulvin, a spirobenzofuran antifungal antibiotic agent used for the treatment of human and veterinary animals mycotic diseases (Park *et al.*, 2005). The production of Hypericin, a naphthodianthrone derivative and Emodin believed to be the main precursor of hypericin by the endophytic fungus isolated from an Indian medicinal plant was reported. Both compounds demonstrated antimicrobial activity against several bacteria and fungi including *Staphylococcus aureus*, *Klebsiellapneumoniae* ., *Pseudomonas aeruginosa*, *Salmonella enterica* sp., *Enteric* and *Escherichia coli* and fungal organisms *Aspergillusniger* and *Candida albicans* (Kusari *et al.*, 2008). An endophytic *Streptomyces* sp., from a fern-leaved grevillea (*Grevilleapteridifolia*) 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 Grampositive bacteria and impressive activity against the malarial parasite *Plasmodium falciparum* (Castillo *et al.*, 2003). Two novel human cytomegalovirus protease inhibitors, cytonic acids A and B have been isolated from the solid-state fermentation of the endophytic fungus *Cytonaema* sp., Their structures as p-tridepside isomers were elucidated by mass spectrometry and NMR methods (Guo *et al.*, 2000). An endophytic fungus *Pestalotiopsistheae* of an unidentified tree on Jianfeng Mountain, China, was capable of producing Pestalotheol C with anti-HIV properties (Li *et al.*,2008).

4.5) Novel Properties

Endophytes can be a potential source of novel natural antioxidants. (Liu *et al.*, 2007). Some evidences that bioactive compounds produced by endophytes could be alternative approaches for discovery of novel drugs, since many natural products from plants, microorganisms and marine sources were identified as anticancer agents (Firakova *et al.*, 2007).Taxol is a drug used to cure cancer mainly breast cancer, ovarian cancer, and lung cancer. An endophytic microorganism *Metarhiziumanisopliae*, isolated from *Taxuschinensis*, was found to produce taxol in abundance in vitro .*Colletotrichumgloeosporioides* isolated from the leaves of a medicinal plant, *Justiciagendarussa*, also producestaxol .

4.6) BiocontrolAgent

Endophytic microorganisms are useful as an effective biocontrol agent, alternative to chemical control. Endophytic fungi have been describe to play an important role in controlling insect herbivory not only in grasses but also in conifers. An endophytic fungi *Beauveriabassiana* known as an entomopathogen was found to control the borer insects in coffee seedlings and sorghum. The endophytic bacteria *Bacillus subtilis,* isolated from *Speranskiatuberculata* (Bge.) ,was found to be strongly antagonistic to the pathogen*B. cinerea* in in vitro studies.

Apart from that they can be use tremendously in bioremediation and in biodegradation processes.

Conclusion

Endophytes are a poorly investigated group of microorganisms that represent an abundant of bioactive and dependable source and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial areas.Endophytes can be either bacteria or fungi or actinomycetes. In this review, we have come to a conclusion that it is mostly the actinomycetes which are involved in production of pharmaceutically important compounds within the plants. Generally, the fungi are involved in the role of phytoremediation, biodegradation, and nutrient cycling and thus reduce the debris load on the environment in a better way. By and large, it is the bacterial community of endophytes which helps the plants in their better growth by producing different growth hormones.

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