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Endophytic Microbes: As a Source of Antibiotics and Anticancer Agents

Abstract

Endophytes are the microorganisms which reside inside the plant tissues. They are the great source of biologically active compounds. They can also be the reason for a special property in the plant. Several biologically active molecules have been reported from endophytes. They can be good source of therapeutic molecules, drugs against serious diseases, plant growth promoting molecules, antioxidants and many more. Endophytes are now being considered as treasurer of beneficial compounds. Looking at the limitations regarding isolation of biologically active molecules from plants, endophytes are gaining more attention for the same purpose. A lot of research is being done on endophytic bacteria and fungi in order to procure good amount of beneficial compounds. It is expected that almost all plants possess good number of endophytes. In order to face the challenges in health and agriculture sectors more investigation is required to harness the hidden potential in endophytes.

Keywords: Endophytes, biologically active compounds, bacteria, fungi

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Introduction

Natural products obtained from endophytic microbes have great potential in the pharmaceutical and agrochemical industries. A significant number of interesting molecules such as steroids, alkaloids, isocoumarins, quinones, flavonoids, phenylpropanoids, lignans, peptides, phenolics, aliphatic, and volatile organic compounds, many of them biologically active have been obtained from endophytic microbes. (Tan and Zou, 2001; Gunatilaka, 2006; Zhang *et al*., 2006). According to Strobel (2002) endophytic fungi are a promising source of novel compounds. It has been estimated that about 51% of biologically active substances from fungal endophytes were previously unknown. Endophytes also produce extracellular hydrolyases which provides resistance mechanism against plant invasion and some of the extracellular enzymes like cellulases, proteinase, lipases and esterases. The actions of these enzymes support the hypothesis of co-evolution between endophytes and their respective hosts. Many secondary metabolites produced by fungal endophytes are larger than that of any other endophytic microbes (Zhang *et al.,* 2006). Endophyte opens up with new and different areas for the biotechnological exploitations. Crude extracts from culture broth of endophytes shows antibacterial, antifungal, antiviral, anti-inflammatory and antitumor activities (Silva *et al.,* 2007). It has been reported that plant derived natural product is not up to the desired level, as it is produced at a specific development stage or under specific environmental condition, stress or nutrient availability. Moreover, plants may take many years to reach a suitable growth phase for product accumulation. Hence, considering these limitations, endophytes are the alternative source of bioactive natural compounds. There are number of endophytes which are the members of common soil bacteria genera like *Pseudomonas*, *Burkholderia* and *Bacillus* species (Lodewyckx *et al.,* 2002). Wide range of biological active compounds have been isolated from endophytic microorganisms. These microorganisms are still remaining a relatively untapped source of natural products. Current interest in natural bioactive compounds from endophytes especially endophytic fungi is evident from the no of review articles which have been appeared in recent literatures (Hasegawa *et al.,* 2006; Zhang *et al.,* 2006; Gunatilaka 2006; Guo *et al.,* 2008; Staniek *et al.,* 2008; Ryan *et al.,* 2008).

Endophytic Microorganisms as a Source of Antibiotics

Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful disease causing agents including phytopathogens, bacteria, fungi, viruses and protozoans that affect humans

and animals. *Cryptosporiopsi quercina* was isolated as an endophyte from *Tripterigeumwil fordii*, a medicinal plant native to Eurasia (Strobel *et al*., 1999). *C. quercina* showed an excellent antifungal activity against some important fungal pathogens such as *Candida albicans* and *Trichophyton* sp. Reports suggested that a unique peptide antimycotic, termedas cryptocandin, was isolated and characterized from *C. quercina* (Strobel *et al.,* 1999). The antibiotic ecomycins are produced by *Pseudomonas viridiflava*, member of a group of plant-associated fluorescent bacteria (Miller *et al.,* 1998). The ecomycins are very active against human-pathogenic fungi as *Cryptococcus neoformans* and *Candida albicans.* Colletotric acid, a metabolite of *Colletotrichum gloeosporioide*s, an endophytic fungus in *Artemisia mongolica*, displays antimicrobial activity against bacteria and fungus *Helminthsporium sativum* (Zou *et al.,* 2000).

In addition to plants such as *A. annua* producing antimalarial compounds, some endophytes have also shown strong activity against protozoan diseases as well. Wide-spectrum antibiotics are produced by *Streptomyces* sp. strain NRRL 30562, an endophyte in *Kennedia nigriscans*. These antibiotics, called munumbicins, possess widely differing biological activities, depending on the target organism. The munumbicins demonstrate activity against *Bacillus anthracis* which is gram positive bacteriaum and multidrug-resistant *Mycobacterium tuberculosis, Plasmodium falciparum* and other drug-resistant bacteria (Castillo *et al.,* 2002). The endophytic *Chloridium* sp. from *A. indica* produces Javanicin which is known to be highly effective and active against *Pseudomonas* sp. (Kharwar *et al.,* 2008). A strain of *P. microspora* was isolated from the endangered tree *Torreya taxifolia* which produces several bioactive compounds having antifungal activity, including pestaloside, an aromatic ß glucoside, and two pyrones such as: pestalopyrone and hydroxypestalopyrone. These products possess phytotoxic properties (Lee *et al*., 1995). According to Strobel *et al.,* (1996 & 2002) *Pestalotiopsis microspora* is a common rainforest endophyte which has an enormous biochemical diversity which exists in this endophytic fungus and produces many secondary metabolites. One such secondary metabolite is ambuic acid, which is an antifungal agent and has been recently described from several isolates of *P. microspora* (Li *et al.,* 2001). According to Horn *et al.,* 1995, phomopsichalasin, a metabolite obtained from an endophyte of *Phomopsis* sp., represents the first cytochalasin type compound with a three ring system replacing the cytochalasin macrolide ring. Phomopsichalasin metabolite mainly exhibits antibacterial activity against *Salmonella enterica* serovar Gallinarum, *Bacillus subtilis* and *Staphylococcus aureus*. It shows moderate activity against the yeast *Candida tropicalis.* Colletotric acid, a metabolite of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mogolica*, displays antimicrobial activity against bacteria as well as against the fungus *Helminthosporium sativum* (Zou *et al.,* 2000). Another *Colletotrichum* sp., isolated from *Artemisia annua*, produces bioactive metabolites that showed varied antimicrobial activity. The *Colletotrichum* sp. found i*n A. annua* produced not only metabolites with activity against human pathogenic fungi and bacteria but they were also metabolites that were fungistatic to plant pathogenic fungi *(*Liu *et al.,* 2000*).*

Endophytic Microorganisms as Source of Anticancerous Agent

According to Suffness, 1995, Paclitaxel is a highly functionalized diterpenoid which is found in each of the world's yew (*Taxus*) species. Paclitaxel and its derivatives represent the first major groups of anticancerous compounds that are produced by endophytes. It has been reported that a novel paclitaxel-producing endophytic fungus, *Taxomyces andreanae*, was discovered in *Taxus brevifolia* (Strobel *et al.,* 1993). Most commonly found endophytes of the world's yews are *Pestalotiopsis* sp. (Strobel, 2002). Endophytes of *Taxus wallichiana* yielded *P. microspora*, and a preliminary monoclonal antibody test indicated that it might produce paclitaxel (Strobel *et al.,* 1996). This clearly showed that endophytes residing in plants other than *Taxus* sp. were producing paclitaxel. Chaetopyranin is a benzaldehyde derivative isolated from the endophytic fungus *Chaetomium globosum* associated with the marine red alga *Polysiphonia urceolata*. Chaetopyranin exhibited moderate to weak cytotoxic activities against three human tumor cell lines HMEC (human microvascular endothelial cells), SMMC-7721 (hepatocellular carcinoma cells) and A54 (human lung epithelial cells) (Wang *et al.,* 2004). Some of the most potent plant-derived antileukemic alkaloids have also been reported from endophytic fungi. Another important compound is camptothecin, which is a chemotherapeutic agent obtained from medicinal plants and was firstly isolated from *Camptotheca acuminate* (Wall *et al*., 1966). It has an unusual efficacy against lung, ovarian, and uterine cancers. Camptothecin basically acts as strong inhibitors of topoisomerase I by trapping the cleavage DNA-topoisomerase I complex (Torck and Pinkas, 1996). The endophytes *Rhinocladiella* sp. from *Tripterygium wilfordii* produces three new chalasins that inhibit cell division of colon and ovarian tumor cell lines (Wagenaar *et al*., 2000). Asparaginase an antileukaemic agent is produced from a variety of microbial sources including fungi (Sarquis *et al*., 2004), yeasts (Ferrara *et al*., 2006) and bacteria (Geckil and Gencer, 2004). Theantana *et al*., 2009 produced asparaginase from wild medicinal plants in Thailand namely, *Adenanthera microsperma, Betula alnoides, Cassia alata, Houttuynia cordata* and *Hiptage benghalensis*. Asparagine amino acid is a nutritional requirement of both normal cells and cancer cells. Normal cells have the ability to produce enzyme asparagine synthetase, which can synthesize asparagine from aspartic acid, whereas in cancer cells this enzyme is present in a very low level (Nakamura *et al*., 1999). Very low levels of these nonessential amino acid asparagines will only affect

the viability of abnormal cells as these cells have abnormal requirements for asparagine (Haley *et al.*, 1961; Mitchell *et al.,* 1994).

Table 1: Natural Products Reported From Endophytes of Different Host Plants (Ref. Shodhganga)

Host plant	Endophytic fungi	Bioactive compound	Activity	References
Garcinia dulcis	*Phomopsis* sp.	Phomoenamide	Antimicrobial	Rukachaisirikul et al., 2008. Phytochemistry 69: 783-787
Artemisia mangolica	*Colletotrichum gloesporides*	Colletotric acid	Antifungal & Antibacterial	Zou et al., 2000. J Nat. Prod 63:602-604
Cinnamomum zeylanicum	*Muscodor albus*	Volatile organic compounds	Antifungal/ Antibacterial	Ezra et al., 2004 Microbiology. 150:4023-4031
Taxus brevifolia	*Taxomyces andreannae*	Paclitaxel	Anticancer	Stierle et al., 1993.Science 260:214-216
Taxus wallichana	*Pestalopsis microspora*	Paclitaxel	Anticancer	Strobel, 1996. Journal of Industrial Microbiology 142:435-440
Taxus cuspidate	*Periconia sp*	Periconicins A&B	Antibacterial	Kim *et al.,* 2004. J.Nat Prod 67:448-450
Taxus chinensis	*Fusarium solani*	Tax3	Anticancer	Strobel 1996. Microbiology 142:435-440
Tripterygium wilfordii	*Rhinodadiella species*	Cytochalasin	Antibiotic	Wagenaar et al., 2000 J.Nat Prod. 63:1692-1695
Terminalia morobensis	*Pestalotiopsis microspora*	Pestacin & Isopestcin	Antioxidant & antimicrobial	Strobel *et al.,* 2002 Crit Rev Biotechnol 22:315-333
Woody plant	*Nodulisporium sp*	Nodulisporic acid	Insecticidal	Findlay *et al.,* 1997. J.Amchem Soc. 119:8809-8816
Paullinia Paulliniodes	*Muscodor 4TJKKKKTTTvitigens*	Naphthalene	Insecticidal	Daisy *et al.,* 2002.Mycotaxon. 84:39-50
Tripterygium wilfordii	*Fusarium subglutians*	Subglutinol A&B	Immunosuppressive	Lee *et al.,* 1995.J. Org.Chem, 60:7076-7077
Eucryphia cordifolia	*Muscodor albus &Gliocladium sp*	Volatile organic compound	Antibiotic	Stinson *et al.,* 2003 Plant science 165:913-922
Xylopia aromatic	*Periconia atropurpurea*	Perconian B	Cytotoxic activity	Teles *et al*., 2006, 67:2686-2690
Taxodium distichum	*Pestalotiopsis microspora*	Taxol	Anticancer	Li *et al*.,1996. Microbiology142:2223-2226
Torreya grandiflora	*Perconia species*	Taxol	Anticancer	Li *et al*.,1998.J.Ind Microbiol Biotechnol 20:259-264
Wallemia nobilis	*Pestalotiopsis guipini*	Taxol	Anticancer	Strobel *et al*., 1997.Aust J. Bot 45:1073-1082

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