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Bioactive Compounds from Endophytes and their Potential in Pharmaceutical Effect: A Review

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ABSTRACT

Endophytes are microorganisms that live inside the host plant tissues which have novel metabolites exhibiting a variety of biological activities against different diseases. In fact, a significant number of interesting natural bioactive compounds have been reported in the last years. The microbial biotransformation process is a novel alternative method to obtain bioactive compounds. In this review, some aspects in the phytochemistry of endophytes producing Phytochemicals and its pharmaceutical effects are discussed.

Key words: Bioactive compounds, endophytes, antimicrobial effect, antioxidant effect, anticancer effect, insecticidal effect, antidiabetic effect, immunosuppressive effect

INTRODUCTION

Many complementary and alternative medicines have enjoyed increased popularity in recent decades (Joseph and Priya, 2011). Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer (Cragg and Newman, 2005). More than 90% of the terms recorded in Indian medical literature are derived from plant sources (Joseph and Priya, 2010). For the fast two years, there has been an increasing interest in the investigation of different novel natural bioactive products from plants (Joseph *et al*., 2010). Recent trends, however, show that the discovery rate of active novel chemical entities is declining (Lam, 2007). Therefore, there is a need to bio-prospect new sources and if possible from less explored regions and habitats to maximize the discovery of novel bioactive metabolites.

Endophytes are microorganisms that include bacteria and fungi living within plant tissues without causing any immediate overt negative effects have been found in every plant species examined to date and recognized as the potential sources of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products isolated from the microorganisms (Bacon and White, 2000; Strobel and Daisy, 2003; Kumar and Sagar, 2007). Endophytes are ubiquitous with rich biodiversity, which have been found in every plant species examined to date. It is noteworthy that, of the nearly 3, 00,000 plant species that exist on the earth, each individual plant is the host to one or more endophytes (Strobel and Daisy, 2003). In this view of the special colonization in certain hosts, it is estimated that there may be as many as

1 million different endophyte species. However, only a handful of them have been described (Andrew and Hirano, 1991), which means the opportunity to find new and targeting natural products from interesting endophytic microorganisms among myriads of plants in different niches and ecosystems is great. Some of the endophytes are the chemical synthesizers in inside the plants (Owen and Hundley, 2004).

Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against human pathogens and some of these compounds have been proven useful for novel drug discovery. Recent studies have reported hundreds of natural products including substance of alkaloids, terpenoids, flavonoids, steroids, etc. from endophytes. Up to now, most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents and other bioactive compounds by their different functional roles. Thus far, they have not been widely explored for therapeutic properties. A single endophyte may be able to produce not one but several bioactive metabolites. As a result, the role of endophytes in the production of novel structures for exploitation in medicine is receiving increased attention (Wang *et al.*, 2000; Ezra *et al.*, 2004a; Gunatilaka, 2006).

A small amount of endophytes have been studied, recently, several research groups have been motivated to evaluate and elucidate the potential of these microorganisms applied on biotechnological processes focusing on the production of bioactive compounds. The 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, allowing them to better adapt to plant host and carry out some functions such as protection from pathogens, insects and grazing animals (Strobel, 2003). Endophytes are chemical synthesizer inside plants (Owen and Hundley, 2004), in other words, they play a role as a selection system for microbes to produce bioactive substances with low toxicity toward higher organisms (Strobel, 2003). Bioactive natural compounds produced by endophytes have been promising potential usefulness in safety and human health concerns, although there is still a significant demand of drug industry for synthetic products due to economic and time-consuming reasons (Strobel *et al.*, 2004). Problems related to human health such as the development of drug resistance in human pathogenic bacteria, fungal infections and life threatening virus claim for new therapeutic agents for effective treatment of diseases in human, plants and animals that are currently unmet (Strobel and Daisy, 2003; Strobel, 2003; Zhang *et al.*, 2005). Recent review by Cragg and Newman (2007) presented a list of all approved agents from 1981 to 2006, from which a significant number of natural drugs are produced by microbes and/or endophytes. 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).

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).

The intent of this review is to provide insights into the phytochemistry of endophytes producing phytochemicals, biological effects of endophytes producing bioactive compounds, the importance of including endophytic microbes for novel drugs and the microbial biotransformation process as a novel alternative method to obtain bioactive compounds. This review, however, also describes these compounds by different functions and pharmaceutical potential for human use.

PHYTOCHEMISTRY

Tan and Zou (2001) believe the reason why some endophytes produce certain phytochemicals originally characteristic of the host might be related to a genetic recombination of the endophyte with the host that occurs in evolutionary time. This is a concept that was originally proposed as a mechanism to explain why the endophytic fungus *T. andreanae* may be producing paclitaxel (Stierle *et al.*, 1993). Thus, if endophytes can produce the same rare and important bioactive compounds as their host plants, this would not only reduce the need to harvest slowgrowing and possibly rare plants but also preserve the world's ever diminishing biodiversity. Furthermore, it is recognized that a microbial source of a valued product may be easier and more economical to produce, effectively reducing its market price (Strobel and Daisy, 2003).

All aspects of the biology and interrelatedness of endophytes with their respective hosts is a vastly under investigated and exciting field. Thus, more background information on a given plant species and its microorganismal biology would be exceedingly helpful in directing the search for bioactive products. Currently, no one is quite certain of the role of endophytes in nature and what appears to be their relationship to various host plant species. While some endophytic fungi appear to be ubiquitous (e.g., *Fusarium* species, *Pestalotiopsis* species and *Xylaria* species), one cannot definitively state that endophytes are truly host specific or even systemic within plants any more than one can assume that their associations with plants are chance encounters. Frequently, many endophytes (biotypes) of the same species are isolated from the same plant and only one of the endophytes will produce a highly biologically active compound in culture (Li *et al.*, 1996). A great deal of uncertainty also exists between what an endophyte produces in culture and what it may produce in nature. It does seem apparent that the production of certain bioactive compounds by the endophyte in situ may facilitate the domination of its biological niche within the plant or even provide protection to the plant from harmful invading pathogens. This may be especially true if the bioactive product of the endophyte is unique to it and is not produced by the host. Seemingly, this would more easily facilitate the study of the role of the endophyte and its role in the plant. Furthermore, little information exists relative to the biochemistry and physiology of the interactions of the endophyte with its host plant. It would seem that many factors changing in the host as related to the season and age, environment and location may influence the biology of the endophyte. Indeed, further research at the molecular level must be conducted in the field to study endophyte interactions and ecology. The ecological awareness of the role these organisms play in nature will provide the best clues for targeting particular types of endophytic bioactivity with the greatest potential for bio-prospecting (Strobel and Daisy, 2003).

BIOPHARMACEUTICAL EFFECTS OF ENDOPHYTES

The following section shows effects of bioactive compounds obtained from endophytic microbes and their potential in the pharmaceutical and agrochemical areas.

Antimicrobial effect: Metabolites bearing antibiotic activity can be defined as low-molecular-weight organic natural substances made by microorganisms that are active at low concentrations against other microorganisms (Guo *et al*., 2008). Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites (Tan and Zou, 2001). So far, studies reported a large number of antimicrobial compounds isolated from endophytes, belonging to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids (Yu *et al*., 2010). 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, probably due to relatively unfavorable returns on investment (Yu *et al*., 2010; Song, 2008). The antimicrobial compounds can be used not only as drugs by humankind but also as food preservatives 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, including antifungal agents, have been isolated from the genus *Xylaria* residing in different plant hosts, such as sordaricin with antifungal activity against *Candida albicans* (Pongcharoen *et al*., 2008), mellisol and 1,8- dihydroxynaphthol 1-O-a-glucopyranoside with activity against herpes simplex virus type 1 (Pittayakhajonwut *et al*., 2005), multiplolides A and B with activity against *Candida albicans* (Boonphong *et al*., 2001). The bioactive compound isolated from the culture extracts of the endophytic fungus *Xylaria* sp. YX-28 isolated from *Ginkgo biloba* L. was identified as 7-amino-4-methylcoumarin (Liu *et al*., 2008). The compound presented broad-spectrum inhibitory activity against several food-borne and food spoilage microorganisms including *S. aureus, E. coli, S. typhia, S. typhimurium, S. enteritidis, A. hydrophila, Yersinia* sp., *V. anguillarum, Shigella* sp., *V. parahaemolyticus, C. albicans, P. expansum* and *A. niger,* especially to *A. hydrophila* and was suggested to be used as natural preservative in food (Liu *et al*., 2008). Another strain F0010 of the endophytic fungus *Xylaria* sp., from *Abies holophylla* 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). They evaluated and reported high antifungal activity *in vivo* and *in vitro* of the endophyte-produced griseofulvin against plant pathogenic fungi, controlling effectively the development of various food crops. Aliphatic compounds, frequently detected in cultures of endophytes, often show biological activities. Four antifungal aliphatic compounds were characterized from stromata of *E. typhina* on *P. pratense* (Koshino *et al*., 1989). Two novel ester metabolites isolated from an endophyte of the eastern larch presented antimicrobial activity. One compound was toxic to spruce budworm (*Choristoneura fumiferana* Clem.) larvae and the other may serve as potent antibacterial agent against *Vibrio salmonicida, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Findlay *et al*., 1997a). Chaetomugilin A and D with antifungal activities were isolated from an endophytic fungus *C. globosum* collected from *Ginkgo biloba* (Qin *et al*., 2009). Cytosporone B and C were isolated from a mangrove endophytic fungus, *Phomopsis* sp. They inhibited two fungi *C. albicans* and *F. oxysporum* with the MIC value ranging from 32 to 64 mg Ml^{-1} (Huang *et al*., 2008).

Chlorinatedmetabolites such as (-) mycorrhizin A, (+)- cryptosporiopsin isolated from endophytic *Pezicula* strains were reported as strongly fungicidal and herbicidal agents and to a lesser extent, as algicidal and antibacterial agents (Schulz *et al.,* 1995). Similarly, two other new chlorinated benzophenone derivatives, Pestalachlorides A and B, from the plantendophytic fungus *Pestalotiopsis adusta,* proven to display significant antifungal activity against three plant pathogenic fungi, *Fusarium culmorum, Gibberella zeae* and *Verticillium albo-atrum* (Li *et al.,* 2008a). 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* sp., *aureus, Klebsiella pneumoniae* sp., *ozaenae, Pseudomonas aeruginosa, Salmonella enterica* sp., *Enteric* and *Escherichia coli* and fungal organisms *Aspergillus niger* and *Candida albicans* (Kusari *et al.*, 2008).

An endophytic *Streptomyces* sp., from a fern-leaved grevillea (*Grevillea pteridifolia*) 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). Another novel endophytic Streptomyces SUK 06 from *Thottea grandiflora* in Malaysia was reported bioactive secondary metabolites with ethyl acetate have killing activity against *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus, Pleisiomonas shigelloides* and MRSA. Nevertheless, there were some antifungal activity measured against *Fusarium solani, Aspergillus fumigatus, Pythium ultimum, Phytophthora erythroseptica* and *Geothrichum candidum* (Ghadin *et al.*, 2008). More than 50% of endophytic fungi strains residing in *Quercus variabilis* possessed growth inhibition against at least one pathogenic fungus or bacteria. *Cladosporium* sp., displaying the most active antifungal activity, was investigated and found to produce a secondary metabolite known as brefeldin A, a lactone with antibiotic activity. Results showed brefeldin A to be more potent than the positive control in antifungal activity (Wang *et al.,* 2007).

Coronamycin, a peptide antibiotic produced by an endophytic fungi *Streptomyces* sp., isolated from *Monstera* sp., is active against pythiaceous fungi, the human fungal pathogen *Cryptococcus neoformans* and the malarial parasite, *Plasmodium falciparum* (Ezra *et al.*, 2004b). Production of lipopeptide pumilacidin, an antifungal compound, by *B. pumilus* isolated from cassava cultivated by Brazilian Amazon Indian tribes was described for the first time (De Melo *et al.,* 2009). The compounds 2-hexyl-3-methyl-butanodioic acid and cytochalasin D were isolated from the endophytic fungus *Xylaria* sp., Isolated from Brazilian Cerrado and presented antifungal activity (Cafeu *et al.,* 2005). Two new bioactive metabolites, ethyl 2, 4-dihydroxy-5, 6-dimethylbenzoate and phomopsilactone were isolated from an endophytic fungus *Phomopsis cassiae* from *Cassia spectabilis* and displayed strong antifungal activity against two phytopathogenic fungi, *Cladosporium cladosporioides* and *C. sphaerospermum* (Silva *et al.,* 2005). The polyketide citrinin produced by endophytic fungus *Penicillium janthinellum* from fruits of *Melia azedarach,* presented 100% antibacterial activity against *Leishmania* sp. (Marinho *et al.,* 2005). Among the 12 secondary metabolites produced by the endophytic fungi *Aspergillus fumigatus* CY018 isolated from the leaf of *Cynodon dactylon,* asperfumoid, fumigaclavine C, fumitremorgin C, physcion and helvolic acid were shown to inhibit *Candida albicans* (Liu *et al.,* 2004). Endophyte *Verticillium* sp., isolated from roots of wild *Rehmannia glutinosa* produced two compounds 2, 6-Dihydroxy-2-

methyl-7- (prop-1E-enyl)-1-benzofuran-3(2H)-one, reported for the first time and ergosterol peroxide with clear inhibition of the growth of three pathogens including *Verticillium* sp., (You *et al.,* 2009).

Another fascinating use of antibiotic products from endophytic fungi is the inhibition of viruses. 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 *Pestalotiopsis theae* of an unidentified tree on Jianfeng Mountain, China, was capable of producing Pestalotheol C with anti-HIV properties (Li *et al.,* 2008b). It is apparent that the potential for the discovery of compounds, from endophytes, having antiviral activity is in its infancy. The fact, however, that some compounds have been found is promising. The main limitation in compound discovery is probably related to the absence of appropriate antiviral screening systems in most compound discovery programs (Strobel and Daisy, 2003).

Another interesting aspect of this research, newly described endophytic fungus *Muscodor albus* from small limbs of *Cinnamomum zeylanicum* effectively inhibits and kills certain other fungi and bacteria by producing a mixture of volatile compounds (Worapong *et al.,* 2001; Strobel *et al.,* 2001). This mixture mimicked the antibiotic effects of the volatile compounds produced by the fungus. It was also used to gain positive identification of the ingredients of the fungal volatile compounds (Strobel *et al.,* 2001). Each of the five classes of volatile compounds produced by the fungus had some inhibitory effect against the test fungi and bacteria, but none was lethal. The most effective class of inhibitory compounds was the esters, of which isoamyl acetate was the most biologically active. The ecological implications and potential practical benefits of the mycofumigation effects of *M. albus* are very promising given the fact that soil fumigation utilizing methyl bromide. This fungus is just as effective in causing inhibition and death of test microbes in the laboratory as *M. albus* (Worapong *et al.,* 2002). In addition, for the first time, a non-muscodor species, a *Gliocladium* sp., was discovered to be a volatile antibiotic producer.

Another important aspect of this research is inhibition of plant pathogens which is relevant in agriculture field. Many endophytic species produce antibiotic substances (Schulz and Boyle, 2005; Strobel *et al.,* 2002; Wang *et al.*, 2007). Liquid extracts from endophyte cultures have been found to inhibit the growth of several species of plant pathogenic fungi (Liu *et al.*, 2001; Park *et al.*, 2005; Inacio *et al.*, 2006; Kim *et al.*, 2007). If such compounds where produced by endophytes in plants, this could constitute a defense mechanism against fungal pathogens. Experiments where plant protection against pathogenic fungi is observed after the inoculation of plants with endophytes, as well as after the application of endophytic culture filtrates, suggest that the endophyte may produce an antifungal compound or a substance that induces plant defense mechanisms in the plant. This is the case with *Chaetomium* and *Phoma* endophytes of wheat, when these fungi were previously inoculated in plants, reduced severity of foliar disease caused by *Puccinia* and *Pyrenophora* sp., was observed and, the same protective effect was observed when only endophytic culture filtrates were applied to the plants (Dingle and McGee, 2003; Istifadah and McGee, 2006). Rajendran *et al.* (2008) reported that the management of Basal Stem Rot disease sixty endophytic, rhizosphere strains was isolated from coconut, other crops and virgin soils. The strains showed high growth promotion were subjected to *Ganoderma* mycelium inhibition study *in vitro*. The strains EPC5 and EPC8 were showed high growth promotion and strong inhibition to *Ganoderma* pathogen.

When a mixture of six species of endophytes frequently isolated from cacao (*Theobroma cacao* L.) trees was used to inoculate leaves of endophyte-free seedlings of this plant species, the severity of a leaf disease caused by a *Phytophthora* sp., was significantly reduced in endophyte-inoculated leaves. A mechanism of induced plant resistance did not seem to be involved, because differences in disease severity were observed between endophyte-inoculated and non-inoculated leaves of the same plant. In this case, the protection against a pathogen could be the result of direct competition among endophytes already present in leaves and the pathogen (Arnold *et al*., 2003). For instance, most tissue available for infection may be already occupied, or endophytes may produce zones of inhibition restricting the entry of other fungi.

Endophyte infection may alter plant biochemistry in a way that defense mechanisms against pathogens are induced. *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost and P. Franken are a root endophyte with a wide host range, including several species of cereals and *Arabidopsis*. Barley plants inoculated with this endophyte have shown resistance to a vascular (*Fusarium culmorum* (W.G. Sm.) Sacc.) and a leaf pathogen [*Blumeria graminis* (DC) Speer], in addition to an increase in yield and salt stress tolerance (Waller *et al*., 2005). The protection against the leaf pathogen appears to be mediated by a mechanism of induced resistance, because in the pathogen-inoculated plants there is a defense response involving the death of host cells.

Some endophytes may be mycoparasites. *Acremonium strictum* W. Gams is an endophyte which has been frequently isolated from *Dactylis glomerata* L. and other grasses (Marquez *et al*., 2007), recently it has been shown that this fungus is a mycoparasite of *Helminthosporium solani* Durieu and Mont., a potato pathogen (Rivera-Varas *et al*., 2007). A significant increase in resistance to dollar spot disease, caused by *Sclerotinia homoeocarpa* F.T. Benn., has been observed in *Festuca rubra* L. cultivars infected by *Epichloë festucae* Leuchtm., Schardl and M.R. Siegel. (Clarke *et al*., 2006). Cultivars of several turfgrass species infected by *Epichloe* and *Neotyphodium* endophytes are commercially available at the present time. The efficient vertical transmission of these endophytes has allowed the production of infected seed at a commercial scale. Since *Neotyphodium* and *Epichloe* infected cultivars have shown increased resistance to herbivores, plant pathogens and some conditions of abiotic stress, the use of such symbiotic cultivars can result in a reduction in the use of insecticides and fungicides in lawns (Brilman, 2005).

Antioxidant effect: Many antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities in higher or lower level (Owen *et al.,* 2000; Cozma, 2004; Halliwell, 1994; Mitscher *et al.,* 1996; Sala *et al.,* 2002). Natural antioxidants are commonly found in medicinal plants, vegetables and fruits. However, it has been reported that metabolites from endophytes can be a potential source of novel natural antioxidants (Liu *et al.,* 2007) evaluated the antioxidant activity of an endophytic *Xylaria* sp., isolated from the medicinal plant *Ginkgo biloba*. The results collected indicated that the methanol extract exhibited strong antioxidant capacity due to the presence of phenolics and flavonoids compounds among 41 identified compounds. Huang and coworkers investigated the antioxidant capacities of endophytic fungal cultures of medicinal Chinese plants and its correlation to their total phenolic contents. They suggested that the phenolic content were the major antioxidant constituents of the endophytes (Huang *et al.,* 2007). Pestacin and isopestacin, 1, 3-dihydro isobenzofurans, were obtained from the endophytic fungus *Pestalotiopsis microspora* isolated from a plant growing in the Papua New Guinea, *Terminalia morobensis* (Harper *et al.,* 2003; Strobel *et al.,* 2002). Besides antioxidant activity, pestacin and isopestacin also presented antimycotic and antifungal activities, respectively. Pestacin is believed to have antioxidant activity 11 times greater than Trolox, a vitamin E derivative, primarily via cleavage of an unusually reactive C-H bond and to a lesser extent, O-H abstraction (Harper *et al.,* 2003). Isopestacin possess antioxidant activity by scavenging both superoxide and hydroxy free radicals in solution, added to the fact that isopestacin is structurally similar to the flavonoids (Strobel *et al.,* 2002).

Liu *et al.* (2009) reported for the first time, the capacity of endophytic microorganisms to produce polysaccharides with antioxidant. The bacterium endophyte *Paenibacillus polymyxa* isolated from the root tissue of *Stemona japonica* Miquel, a traditional Chinese medicine, produced exopolysaccharides (EPS) that demonstrated strong scavenging activities on superoxide and hydroxyl radicals. Graphislactone A, a phenolic metabolite isolated from the endophytic fungus *Cephalosporium* sp., IFB-E001 residing in *Trachelospermum jasminoides*, demonstrated to have free radical-scavenging and antioxidant activities *in vitro* stronger than the standards, Butylated Hydroxytoluene (BHT) and ascorbic acid, coassayed in the study (Song *et al.,* 2005).

Anticancer effect: 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). The anticancer properties of several secondary metabolites from endophytes have been investigated recently. Following, some examples of the potential of endophytes on the production of anticancer agents are cited.

The diterpenoid Taxol (also known in the literature as paclitaxel) have generated more attention and interest than any other new drug since its discovery, possibly due to its unique mode of action compared to other anticancer agents (Gangadevi and Muthumary, 2008; Firakova *et al.,* 2007). This compound interferes with the multiplication of cancer cells, reducing or interrupting their growth and spreading. FDA (Food and Drug Administration) has approved Taxol for the treatment of advanced breast cancer, lung cancer and refractory ovarian cancer (Cremasco *et al.,* 2009). Taxol was firstly isolated from the bark of trees belonging to Taxus family (*Taxus brevifolia*), its most common source (Wani *et al.,* 1971). Nevertheless, these trees are rare, slow growing and produce small amount of Taxol, which explain its high price in the market when obtained by this natural source (Gangadevi and Muthumary, 2008). Besides, in the context of environmental degradation, the use of plant source as unique option have limited the supply of this drug due to the destructive collection of yew trees (Guo *et al.,* 2008). Several reports about Taxol anticancer properties were published since its discovery (Lu *et al.,* 2007; Kakolyris *et al.,* 2006; Peltier *et al.,* 2006), as well as other sources for production of Taxol have been investigated in the last decade. The isolation of Taxol-producing endophyte *Taxomyces andreanae* has provided an alternative approach to obtain a cheaper and more available product *via* microorganism fermentation (Stierle *et al.,* 1993). After that, Taxol has also been found in a number of different genera of fungal endophytes either associated or not to yews, such as *Taxodium distichum* (Li *et al.,* 1996), *Wollemia nobilis* (Strobel *et al.,* 1997), *Phyllosticta spinarum* (Kumaran *et al.,* 2008), *Bartalinia robillardoides* (Gangadevi and Muthumary, 2008), *Pestalotiopsis terminaliae* (Gangadevi and Muthumary, 2009), *Botryodiplodia theobromae* (Pandi *et al.,* 2010).

Another important anticancer compound is the alkaloid Camptothecin, a potent antineoplastic agent which was firstly isolated from the wood of *Camptotheca acuminata* Decaisne (Nyssaceae) in China (Wall *et al.,* 1966). Camptothecin and 10-hydroxycamptothecin are two important precursors for the synthesis of the clinically useful anticancer drugs, topotecan and irinotecan (Uma *et al.,* 2008). Although it's potential use in medical treatments, the unmodified Camptothecin suffers from drawbacks that compromise its applications due to very low solubility in aqueous media and high toxicity (Li *et al.*, 2006; Kehrer *et al.*, 2001). On the other hand, some Camptothecin derivatives retain the medicinal properties and can show other benefits without causing over drawbacks in some cases (Kusari *et al.*, 2009a; Jew *et al.*, 1999). Therefore, it is desirable to develop strategies for isolation, mixture separation and production of Camptothecin and its analogues from novel endophytic fungal sources. The anticancer properties of the microbial products Camptothecin and two analogues (9-methoxycamptothecin and 10-hydroxycamptothecin) were already reported. The products were obtained from the endophytic fungi *Fusarium solani* isolated from *Camptotheca acuminate* (Kusari *et al.*, 2009b). Several reports have described other Camptothecin producing endophytes (Shweta *et al.*, 2010; Liu *et al.*, 2010; Rehman *et al.*, 2008; Amna *et al.*, 2006; Puri *et al.*, 2005). Since then, endophytes have been included inmany studies purposing new approaches for drug discovery. Ergoflavin, a dimeric xanthene linked in position 2, belongs to the compound class called ergochromes and was described as a novel anticancer agent isolated from an endophytic fungi growing on the leaves of an Indian medicinal plant *Mimusops elengi* (Sapotaceae) (Deshmukh *et al.*, 2009). Secalonic acid D, a mycotoxin also belonging to ergochrome class, is known to have potent anticancer activities. It was isolated from the mangrove endophytic fungus and observed high cytotoxicity on HL60 and K562 cells by inducing leukemia cell apoptosis (Zhang *et al.*, 2009).

Phenylpropanoids have attracted much interest for medicinal use as anticancer, antioxidant, antimicrobial, anti-inflammatory and immunosuppressive properties (Korkina, 2007). Despite the phenylpropanoids belong to the largest group of secondary metabolites produced by plants, reports showed the production of such compounds by endophytes. The endophytic *Penicillium brasilianum*, found in root bark of Melia azedarach, promoted the biosynthesis of phenylpropanoid amides (Fill *et al.*, 2010). Likewise, two monolignol glucosides, coniferin and syringin, are produced not only by the host plant, but were also recognized by the endophytic *Xylariaceae* species as chemical signals during the establishment of fungus-plant interactions (Chapela *et al.*, 1991). Koshino and coworkers characterized two phenylpropanoids and lignan from stromata of *Epichloe typhina* on *Phleum pretense* (Koshino *et al.*, 1988). Lignans are other kinds of anticancer agents originated as secondary metabolites through the shikimic acid pathway and display different biological activities that make them interesting in several lines of research (Gordaliza *et al.*, 2004). Although their molecular backbone consists only of two phenylpropane units (C6-C3), lignans show enormous structural and biological diversity, especially in cancer chemotherapy (Korkina, 2007). Podophyllotoxin and analogs are clinically relevant mainly due to their cytotoxicity and antiviral activities and are valued as the precursor to useful anticancer drugs like etoposide, teniposide and etopophos phosphate (Kusari *et al.*, 2009a; Kour *et al.*, 2008). The aryl tetralin lignans, such as podophyllotoxin, are naturally synthesized by *Podophyllum* sp., however, alternative sources have been searched to avoid endangered plant. Another study showed a novel fungal endophyte, *Trametes hirsute*, that produces podophyllotoxin and other related aryl tetralin lignans with potent anticancer and properties (Puri *et al.*, 2006). Novel microbial sources of Podophyllotoxin were reported from the endophytic fungi *Aspergillus fumigatus* Fresenius isolated from *Juniperus communis* L. Horstmann (Kusari *et al.*, 2009b), *Phialocephala fortinii* isolated from *Podophyllum peltatum* (Eyberger *et al.*, 2006) and *Fusarium oxysporum* from *Juniperus recurva* (Kour *et al.*, 2008).

Wagenaar *et al.* (2000) reported identification of three novel cytochalasins, bearing antitumor activity from the endophyte *Rhinocladiella* species. Extensive experiments identified these new compounds as 22-oxa-12-cytochalasins. Torreyanic acid is an unusual dimeric quinone isolated from the endophytic fungus *Pestalotiopsis microspora* from *T. taxifolia* (Florida torreya) and was proven to have selective cytotoxicity 5 to 10 times more potent in cell lines that are sensitive to protein kinase C agonists and causes cell death by apoptosis (Lee *et al.*, 1996). Gliocladicillins A and B were reported as effective antitumor agents *in vitro* and *in vivo*, since they induced tumor cell apoptosis and showed significant inhibition on proliferation of melanoma B16 cells implanted into immunodeficient mice (Chen *et al.*, 2009). Crude Extracts of *Alternaria alternata*, an endophytic fungus isolated from *Coffea Arabica* L., displayed moderate cytotoxic activity towards HeLa cells *in vitro*, when compared to the dimethyl sulfoxide (DMSO) treated cells (Fernandes *et al.*, 2009). The investigation of endophytic actinomycetes associated with pharmaceutical plants in rainforest reported 41 microorganisms from the genus Streptomyces displayed significant antitumor activity against HL-60 cells, A549 cells, BEL-7404 cells and P388D1 cells (Li *et al.*, 2008c). The screening of endophytic fungi isolated from pharmaceutical plants in China showed that 13.4% endophytes were cytotoxic on HL-60 cells and 6.4% on KB cells (Huang *et al.*, 2001). Finally, other compounds with anticancer properties isolated from endophytic microbes were reported such as cytoskyrins, phomoxanthones A and B, photinides A-F, rubrofusarin B and (+)- epiepoxydon (Brady *et al.*, 2000; Isaka *et al.*, 2004; Ding *et al.*, 2009; Song *et al.*, 2004; Klemke *et al.*, 2004).

Insecticidal effect: Several endophytes are known to have anti-insect properties. Nodulisporic acids, novel indole diterpenes that exhibit potent insecticidal properties against the larvae of the blowfly, work by activating insect glutamate-gated chloride channels. The first nodulisporic compounds were isolated from an endophyte, a *Nodulisporium* sp., from the plant *Bontia daphnoides*. This discovery has since resulted in an intensive search for more *Nodulisporium* sp., or other producers of more-potent nodulisporic acid analogues (Demain, 2000). Insect toxins have also been isolated from an unidentified endophytic fungus from wintergreen (*Gaultheria procumbens*). The two new compounds, 5-hydroxy-2-(1'-hydroxy-5'-methyl- 4'-hexenyl) benzofuran and 5-hydroxy-2-(1-oxo-5-methyl-4- hexenyl) benzofuran, both show toxicity to spruce budworm and the latter is also toxic to the larvae of spruce budworm (Findlay *et al.*, 1997b). Another endophytic fungus, *Muscodor vitigenus*, from a liana (*Paullina paullinioides*), yields naphthalene as its major product. Naphthalene, the active ingredient in common mothballs, is a widely exploited insect repellant. *M. vitigenus* shows promising preliminary results as an insect deterrent and has exhibited potent insect repellency against the wheat stem sawfly (*Cephus cinctus*) (Daisy *et al.*, 2002a, b). As the world becomes wary of ecological damage done by synthetic insecticides, endophytic research continues for the discovery of powerful, selective and safe alternatives.

Antidiabetic effect: A nonpeptidal fungal metabolite (L-783,281) was isolated from an endophytic fungus (*Pseudomassaria* sp.,) collected from an African rainforest near Kinshasa in the Democratic Republic of the Congo (Zhang *et al.*, 1999). This compound acts as insulin mimetic and unlike insulin, is not destroyed in the digestive tract and may be given orally. Oral administration of L-783,281 to two mouse models of diabetes resulted in significant lowering of blood glucose levels. These results may lead to new therapies for diabetes (Zhang *et al.*, 1999).

Immunosuppressive effect: The endophytic fungus *Fusarium subglutinans*, isolated from *T. wilfordii*, produces the immunosuppressive but noncytotoxic diterpene pyrones subglutinol A and B (Lee *et al.*, 1995). Subglutinol A and B are equipotent in the mixed lymphocyte reaction assay and thymocyte proliferation assay, with a 50% inhibitory concentration of 0.1 μM In the same assay systems, the famed immunosuppressant drug cyclosporine is roughly as potent in the mixed lymphocyte reaction assay and 104 more potent in the thymocyte proliferation assay. Still, the lack of toxicity associated with subglutinols A and B suggests that they should be explored in greater detail (Lee *et al.,* 1995). The Microbiology Department at Sandoz Ltd. developed a computer-aided evaluation program to screen and evaluate fungi for bioactivity. The program can recognize and eliminate from study common fungi producing known compounds and thereby direct attention to the evaluation of rare samples, which are more likely to produce metabolites with novel bioactivity. This approach resulted in the discovery of the fungus *Tolypocladium inflatum*, from which cyclosporine, a hugely beneficial immunosuppressant, was isolated (Borel and Kis, 1991). This example perfectly depicts the current aim of many investigators to seek out rare endophytes from interesting and uncommon hosts and environments (Strobel and Daisy, 2003).

BIOACTIVE COMPOUNDS PRODUCTION BY BIOTRANSFORMATION PROCESS

Biotransformation can be defined as the use of biological systems to produce chemical changes on compounds that are not their natural substrates (Borges *et al.,* 2008). The microbial growth, sustenance and reproduction depends on the availability of a suitable form of reduced carbon source, used as chemical energy, which under normal conditions of culture broth are the common sugars. Nevertheless, microorganisms are believed to have no limit to adapt to new environments and to metabolize various foreign substrates to carbon and nitrogen sources (Doble *et al.*, 2004). A molecule can be modified by transforming functional groups, with or without degradation of carbon skeleton. Such modifications result in the formation of novel and useful products not easily prepared by chemical methods (Borges *et al.,* 2009). The biotransformation process provides a number of advantages over chemical synthesis. The process can be carried out under mild conditions like ambient temperature and without the need of high pressure and extreme conditions, thus reducing undesired byproduct, energy needs and cost (Suresh *et al.,* 2006). The region-and stereo-selectivity of the process allows the production of enantiomerically pure compounds, eliminating the need for complicated separation and purification steps (Borges *et al.,* 2008). Besides, the reactions occur under ecologically acceptable conditions, with lower emission of industrial resides and production of biodegradable resides and products, thus reducing the environmental problems (Bicas *et al.,* 2009; Aleu and Collado, 2001).

Finally, the products obtained by biotransformation process can be labeled as natural. On the other hand, chemical synthesis often result in environmentally unfriendly production processes and lacks substrate selectivity, possibly causing the formation of undesirable reaction mixtures and reducing process efficiency and increasing downstream cost (Longo and Sanroman, 2006). Therefore, biotransformation is a useful method for production of novel compounds; enhancement in the productivity of a desired compound; overcoming the problems associated with chemical analysis; leading to basic information to elucidate the biosynthetic pathway (Suresh *et al.,* 2006). For this reason, biotransformation using microbial cultures and/or their enzymatic systems alone has received increasing attention as a method for the conversion of lipids, monoterpenes, diterpenes, steroids, triterpenes, alkaloids, lignans and some synthetic chemicals, carrying out stereospecific and stereoselective reactions for the production of novel bioactive molecules with some potential for pharmaceutical and food industries (Borges *et al.,* 2009; Figueiredo *et al.,* 1996; Pimentel *et al.,* 2010).

CONCLUSION

Endophytes are a poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural and industrial areas. The mechanisms through which endophytes exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek, study and spend time isolating micro floral components. This may facilitate the product discovery processes. Although work on the utilization of this vast resource of poorly understood microorganisms has just begun, it has al ready become obvious that an enormous potential for organism, product and utilitarian discovery in this field holds exciting promise. This is witnessed by the discovery of a wide range of products and microorganisms that already hold inkling for future prospects as mentioned in this report. However, the application of microorganisms by the food and pharmaceutical industries to obtain compounds of interest is still modest, considering the great availability of useful microorganisms.

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