Endophytic Fungi, A Novel source in the treatment of Oral infections

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REVIEW ARTICLE

Endophytic Fungi, A Novel source in the treatment of Oral infections

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ABSTRACT:
Living organisms in nature are in constant relationship with each other. The high diversity of endophytic fungi that has been present in varied host plants contributes to the appeal of endophytic fungi for ecological studies. Bioactive secondary metabolites from endophytes represent a chemical reservoir for antibiotic, antioxidant, immune modulating, anticancer and anti-parasitic compounds. Endophytic fungal sources have proved to be promising sources of various oral infections. This chapter explores the diversity of endophytic fungi from various medicinal plants and their activity against two major oral pathogens *Streptococcus mutans* and *Candida albicans.*

KEYWORDS: Endophytic fungi, secondary metabolites, oral infections, *Streptococcus mutans*, *Candida albicans.*

INTRODUCTION:
Living organisms in nature are in constant relationship with each other. Plant-endophyte relationships have been understood by more than 100 years of research on fossil records [1]. Fungal association with plants either as mycorrhizal or as endophytes exists from more than 400 million years which plays an important role in understanding the evolution of life on land [2]. Fungal symbionts have enormous effects on plant communities by providing stress tolerance, resource allocation and thus help in understanding fitness and evolution of plant communities [3]. Endophytic fungi are considered as an important component of biodiversity since endophytic mycoflora distribution differs with the host [4]. The high diversity of endophytic fungi that has been present in varied host plants contributes to the appeal of endophytic fungi for ecological studies [5].

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Endophytic fungi are able to produce secondary metabolites with activities similar to or more than that of their respective hosts. They are considered as an outstanding source of pharmaceutically important products for their ability to inhabit unique higher plants growing in unusual environments [6]. Thus, screening of endophytic fungi for novel bioactive products forms a major part of discovery in medicine, agriculture and industry as they produce a plethora of substances with low toxicity towards higher organisms [7].

This chapter focuses on the potential of endophytic fungi in treating oral problems which focuses on *Streptococcus mutans* and *Candida albicans* as the major causative agents. It has become very difficult to control these organisms because of their tolerance towards various antimicrobial agents in routine use during the course of therapy. Natural products make excellent leads for new drug development which are safer and biodegradable.

Endophytes- An overview:
The widely accepted definition of endophytes was given by Stone *et al*., (2000) which states that microbes that colonize living plants in their internal tissues without causing negative effects to the host plant. Endophytes in

contrast to the epiphytes are present entirely within the host plant and may have either a parasitic or symbiotic association with their host. Microfungi which reside in the internal tissues of plant parts whose infections are inconspicuous and symptom-less are termed as endophytes [8,9].

Diversity of endophytic fungi:

More than 1 million Species of fungal endophytes have been estimated so far which represents an important component of fungal diversity [10]. Tan and Zou (2001) reported that almost all plant species harbor one or more endophytic species [11]. Endophytic colonization has been found in wide variety of plants growing in temperate, tropical and boreal forests and from various habitats including extreme arctic, alpine and xeric environments [12]. Research on the internal mycobiota of plants mostly uncover novel taxa and reveal new distributions of known species. Relatively small portion of potential hosts have been studied for their endophytes. Endophytic species composition varies according to host species, host site, tissue type and tissue age [5]. More diverse ecosystem possesses more diverse endophytic microorganisms with novel biological properties. Tropical and temperate rainforest cover about 60% of the world's biodiversity and thus diverse endophytes from these regions are a source of novel molecular structures and biologically active compounds [13]. Foliar endophytes are either host specific or ubiquitous in nature. Intra-specific and interspecific host specificity exists between the endophytes which strongly depend on the symbiotic relationship between the host and the fungal endophyte [14]. Seasonal variation also has a greater impact on endophytic fungal diversity as the frequency of endophyte isolation is more in winter than in other seasons [15].

Endophytic fungi and their importance:

Fungal endophytes serve as a repository for novel compounds with immense value in agriculture, medicine and industry [16]. Bioactive secondary metabolites from endophytes represent a chemical reservoir for antibiotic, antioxidant, immune modulating, anticancer and anti-parasitic compounds [17]. Many ethno medicinal plants from unique environmental settings are likely to harbor distinct endophytes with novel biological properties. Currently plants having medicinal values are studied for their endophytic fungal diversity for the synthesis of distinct secondary metabolites with unique pharmacological values. Secondary metabolites of endophytic fungi have been known to inhibit or kill disease causing agents such as bacteria, fungi, viruses and protozoans. Endophytes are less studied group of microorganisms that have an enormous potential to be explored in the field of medicinal, agricultural and industrial arenas. [3]. Thus, exploring the endophytes in the hotspots of biodiversity is considered important to produce plethora of useful natural products.

Endophytic fungi from medicinal plants:

Medicinal plants are significant sources of bioactive compounds for treating various diseases and the traditional uses of medicinal plants are enormous. Endophytic fungi which harbor almost all plant species have the characters similar to that of the host plants in novel secondary metabolite production. It was proposed that plants with immense medicinal values and ethnobotanical uses house endophytes which produce novel bioactive products [3]. One such study was done by Jena and Tayung (2013) in which the antimicrobial potential of endophytic fungi isolated from three Ethno-medicinal plants growing in the Similipal Biosphere Reserve, India was explored. *Curvularia* sp., *Alternaria* sp., *Fusarium* sp. and *Penicilliium* sp. were the dominant species and they are host-specific. About 60 fungal endophytes were isolated in which 51.66% of them were filamentous forms and the remaining being yeast colonies. Screening of isolates against the pathogens showed that 13 isolates showed potent antimicrobial activity.

Endophytic fungi present in medicinal plants of the tropics form a rich source of functional metabolites [12, 13,14]. A diverse community of endophytic fungi was reported in the plants of the Western Ghats of India which is one of the hotspots of global biodiversity [15, 16]. A study conducted on the presence of endophytic fungi on the selected medicinal plants of the Western Himalayas showed that the plants *Pinus roxburgii*, *Cedrus deodara* and *Abies pindrow* showed high fungal diversity. A total of about 72 fungal strains were isolated of which some of them showed potent antimicrobial properties [17]. Endophytic fungal studies on the medicinal plant *Calotropis gigantea* from about 700 segments showed the presence of about 13 different fungal species. *Aspergillus niger* was isolated as the dominant species followed by *Aspergillus flavus* and *Alternaria pori* [18]. The medicinal plant Tulsi has been studied for the presence of endophytic fungi. About 40 endophytic fungal strains were isolated and some of them possessed antimicrobial activity against *Pseudomonas aeroginosa*, *Mycobacterium smegmatis* and *Candida albicans* [19].

The first study on endophytic fungi diversity from the medicinal plants of Chennai city was done by Gangadevi and Muthumary (2007). They studied the diversity of endophytic fungi from the leaves of the medicinal plant, *Ocimum basilicum* L. One of the major findings in their research was that the isolate *Phyllostica* sp. produced taxol in artificial culture media thus proving their potential to be a potential source of useful

bioactive products. Raviraja (2005) isolated fungal endophytes from the medicinal plants of Western Ghats in the Kundermukh range. About 18 endophytic fungi were isolated from five medicinal plants of the Kundermukh range. The fungi were isolated from the bark, stem and leaf segments of the medicinal plants of which *Curvlaria clavata* was the dominant species followed by *Curvularia lunata* and *Fusarium oxysporum*. The colonization frequency was found to be higher in leaf segments when compared to bark and stem segments. This shows that fungal endophytes are host and tissue specific.

Secondary metabolites of endophytic fungi:

Research on endophytic fungi gained interest with the discovery of an endophytic fungus *Taxomyces andreanae*, from *Taxus brevifolia*, producing the billion-dollar anti-cancer drug, taxol [21]. The endophytic fungi *Serratia marcescens* recovered from the aquatic plant *Rhyncholacis penicillata* produces oocydin A, a novel antioomycetous compound [22]. The common rainforest endophyte *Pestalotiopsis microspore* which is known for its biochemical diversity produces antifungal products such as ambuic acid and pestaloside. The endophyte *Phomopsis* sp. produces the first cytochalasin compound called phomopsichalasin which mainly exhibits antibacterial activity against *Bacillus subtilis* [23].

Colletotric acid, an antimicrobial metabolite from the endophyte *Colletotrichum gloeosporioides* displays potent activity against the fungus *Helminthsporium sativum* [24]. *Cinnamomum zeylanicum* (cinnamon tree) harbors *Muscodor albus*, a xylariceous fungus which effectively inhibits and kills certain other fungi and bacteria by producing a mixture of volatile compounds [25]. Gangadevi and Muthumary (2007) studied that the isolate *Phyllosticta* sp. from *Ocimum basilicum* was found to produce taxol in artificial culture media. The endophytic fungus *Fusarium subglutinans*, isolated from *Tripterygium wilfordii*, produces the immunosuppressive but non cytotoxic diterpene pyrones subglutinol A and B [26]. The alkaloids commonly found in the fungal genera such as *Xylaria* sp., *Phoma* sp., *Hypoxylon* sp., and *Chalara* sp. produces large group of cytochalasins. Cytochalasins are a major group of anticancer agants which are produced in fungal endophytes such as *Xyalria* sp., *Hypoxylon* sp. and *Phoma* sp. They have not been used in pharmaceuticals due to their celluar toxicity. 22-oxa- [12]-cytochalasins were produced by the endophyte *Rhinocladiella* sp. from the plant *Tripterygium wilfordii* [27].

Computer-aided programs help in screening rare fungi with novel bioactivity from a large group of fungi producing known compounds. Thus, seeking out rare endophytes from interesting and uncommon hosts and environments is the current aim of mycologists. About 20,000 pharmaceutically useful bioactive metabolites of microbial origin were reported and fungi form the major source of clinically useful products. Some of the important drugs of fungal origin include griesofluvin, taxol, cyclosporine and beta lactum antibiotics [28,29]. The first fungal bioactive product was mycophenolic acid extracted from *Penicillium glaucoma* in 1896 followed by penicillin in 1940 [30]. The use of penicillin as an antibiotic increased the interest on microorganisms for natural product drug discovery. About 1500 secondary metabolites from fungi were found to have antitumour activity [31].

A large number of fungal endophytes possess anticancer property. A well-known anticancer drug paclitaxel was initially produced from the yew (*Taxus*) species. Paclitaxel prevents polymerization of tubulin molecules during the process of cell division and is helpful in treating a number of tissue proliferating diseases in human [32,33]. The usefulness of paclitaxel led to the extensive research on endophyte producing the compound which was identified as *Taxomyces andreanae* from the plant *T. brevifolia.* Suryanarayanan *et al.*, (2009) screened about 110 isolates of endophytic fungi for their anti-cancer activity against mouse fibroblast cell line L-929 [34]. Using the organic solvent extracts of the endophytes isolated, the cytotoxic effects were assayed using MTT colourimetric assay. The fraction of the extract showing highest anticancer activity was analyzed by HPLC-MS for the presence of potential compounds. The most remarkable activity was observed in *Chaetomium* sp. which produced chaetoglobosin that prevents actin polymerization.

Endophytic microbial products act as potent antimicrobial agents against various human pathogens. The common rainforest endophyte *Pestalotiopsis microspora* produces a range of bioactive secondary metabolites. Ambuic acid is one such antifungal agent produced by several strains of *Pestalotiopsis microspora* [35]. The isolate of *P. microspora* from the plant *Torreya taxifolia* produces several antifungal compounds such as pestaloside, pestalopyrone and hydroxy pestalopyrone and an aromatic glucoside. They also have phytotoxic properties. Phomopsichalasin extracted from the endophytic fungus *Phoma* sp. showed better antibacterial activity against various pathogens such as *Salmonella enterica*, *Bacillus subtilis* and *Staphylococcus aureus*. The compound also showed moderate antifungal activity against the human pathogen *Candida albicans* [23]. The endophytic *Fusarium* sp. from the plant *Selaginella pallescens* produces an antifungal metabolite with activity against *C. albicans*. A remarkable use of endophytes is that they are used to

inhibit viruses. Cytonic acids A and B from the endophyte *Cytonaema* sp. act as human cytomegalovirus protease inhibitors. These compounds from an endophyte were used to treat viral infection at their initial stages [36].

Antioxidant activity was observed in the compounds pestacin and isopestacin produced from the endophyte *Pestalotiopsis microspora* isolated from *Terminalia morobensis*. The antioxidant activity was due to the structural similarity of isopestacin with flavonoids [37]. Endophytes were known to possess anti-insect properties and the search for bio insecticides is increasing. *Nodulisporium* sp. isolated from *Bontiadaphnoides* produces nodulisporic acids and several other nodulisporic compounds which were known to possess insecticidial properties [38].

Oral pathogens:

The human mouth is more susceptible for formation of natural microbial biofilms which is uncontrollable due to its diverse niches and ample supply of nutrients. The oral microflora comprises of more than 700 bacterial species which makes it one of the most complex microbial flora of the human body. Study of bacterial diversity in the supragingival plaque has demonstrated *Streptococcus mutans* as the dominant species followed by other species of *Streptococci* such as *S. mitis*, *S. sanguinis*, *S. salivarius* along with *Lactobacilli* species. *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Prevotella intermedia* are some gram negative bacteria present in the oral cavity. The oral bacteria have metabolic communication between them where one organism provides nutrient and substrate for other organism to survive [39,40]. The oral flora forms a community in such a way that the primary and secondary colonizers stick to each other generating an exopolysaccharide matrix. The pathogenic oral microbes *Streptococcus mutans* and *Candida albicans* form oral biofilms which provides them antibiotic resistance. Oral microbial biofilms are 3D structured bacterial communities on a solid surface like the enamel of the teeth, the surface of the root or dental implants and are embedded in an exo-polysaccharide matrix. *Streptococcus mutans* is found to be the dominant species with elevated levels in the supragingivial plaque in addition to *S. sanguinis*, *S. mitis*, and *S. salivarius* whereas *Candida albicans* is the major causative agent of oral candidiasis, the most common oral fungal disease [41,42].

***S. mutans* and dental caries:**

S. mutans, a gram positive bacterium is a major cariogenic organism which resides in the multispecies biofilms on the surface of the teeth. *S. mutans* modulate sugar metabolism and produce an acidic environment to promote irreversible binding on the dental surfaces. Ultimately, the bacterium reaches the final stages of dissolution of hydroxyapatite crystals in enamel and dentin which results in the cavitation of teeth. If not prevented, this cavitation provides an excellent niche for a protected biofilm enabling caries to progress gradually. Various *Streptococci* are present in the oral microflora and these multiple interactions are based on the biology as well as on the physical outcomes of previously formed attachments, and the colonization regulate biofilm formation using multiple species of *S. mutans* and other commensal *Streptococci* in the oral cavity ecosystem [43].

***C. albicans* and oral candidiasis:**

Candida albicans is a commensal fungal species present in the human mucosal surfaces but they become opportunistic pathogens under conditions of immune dysfunction such as HIV infection and in malnourished individuals [44]. Available options for antifungal therapy are very limited when compared to bacterial antibiotics. However, prolonged antifungal therapy is linked with a significant risk of hematologic, hepatic, and/or renal toxicity. *Candida albicans*, causes both superficial and systemic disease. Further, with the use of commercial drugs for current antifungal therapy, invasive candidiasis has a mortality rate of about 40%. Candidiasis is usually associated with the attached medical devices (e.g., dental implants, catheters, heart valves etc.) which can act as substrates for biofilm growth. Biofilm formation is also critical in the development of denture stomatitis affecting 65% of toothless individuals. Infection is reestablished with the use of antifungal drugs. These clinical observations reflect the biofilm formation to both superficial and systemic circulation and the inability of current antifungal therapy to cure such diseases [45].

Recent studies suggest that caries development is due to the interaction between fungal pathogen *Candida albicans* and oral bacteria as there is high prevalence of *Streptococci* where *Candida* resides [46].

Anti-oral infection property of endophytic fungi:

Zhao *et al*., (2010) reviewed the potential of endophytic fungi producing novel secondary metabolites similar to their host plants [47]. Some of the plant secondary metabolites such as paclitaxel, camptothecin, hypericin and vinblastine are produced by the endophytic fungi colonizing them. The antimicrobial activity of a large number of marine and terrestrial fungal isolates has been examined and some marine isolates have shown remarkable antimicrobial activity [48]. Kalyanasundaram *et al*., (2015) reported that about 70% of the endophytic fungi isolated from the salt marsh plant *Suaeda maritima* and *Suaeda monoica* showed

antibacterial and antifungal activity against the test pathogens [49]. Similar study was done by Gond *et al*., (2012) in which 75% of endophytic fungi isolated from the medicinal plant *Nyctanthes arbor-tristis* showed potent antibacterial activity and 56.25% exhibited antifungal activity against the fungal pathogens [50].

The crude mycelial extract of the endophytic *Aspergillus* sp. inhibited the human pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* [51]. Similarly, the ethyl acetate extract of the endophytic fungi identified as *Aspergillus* sp. isolated from *Azadirachta indica* also possesses good inhibitory activity towards *S. mutans*, *C. albicans* and other species of *non-albicans Candida* [52]. Elaasser *et al*., (2011) demonstrated that the crude extract of *A. candidus* showed potential activity against *Streptococcus pyrogens* and *C. albicans* [53]. The endophytic fungus *Talaromyces wotmannii* isolated from aloevera possess antibacterial activity and anti-inflammatory activity against *Propionibacterium acnes* which induces skin acne [54]. Fabry *et al.,* (1998) reported that endophytic fungi with low antimicrobial activity might possess the active compounds in a low concentration and the amount of these compounds could be enhanced by optimizing some of the factors of fermentation processes such as pH, temperature, inoculum concentration, solvents used for extraction and method of purification processes [55].

Estrela and Abraham (2016) reported the biofilm inhibition efficiency of several fungal metabolites. Fungal metabolites inhibit quorum sensing receptors and cell wall synthesizing enzymes of the bacteria, thus preventing their biofilm formation [56]. The fungal metabolite usnic acid has biofilm inhibition activity of *S. mutans* and *C. albicans* which was applied on bacterial biofilm as a surface coating agent [57]. Martin-Rodriguez *et al*., (2014) reported that the extracts of marine endophytes such as *Fusarium* sp., *Khuskia* sp., *Epicoccum* sp. and *Sarocladium* sp. have good quorum sensing inhibitor activity which in turn inhibits bacterial biofilm [58]. Some metabolites such as terreic acid from *A. terreus* and flavipesin from *A. flavipes* have inhibitory activity towards various bacterial and fungal biofilms [59,60].

***In silico* approach:**

Increasing interest in the natural sources of medicine has led to the screening of traditional compound for the treatment of a variety of diseases [61]. Increasing interest in the natural sources of medicine has led to the screening of traditional compounds for the treatment of a variety of diseases. Different natural compounds such as phenols, terpenoids, alkaloids, flavonoids, coumarins, saponins, quinones and xanthones have been reported to have antimicrobial properties. But screening large number of compounds is a limiting step in current researches. Virtual screening is an efficient method for preliminary searching of compounds using the molecular databanks [62]. Bioinformatics tools were applied to identify the potential targets for uncontrollable infections. Design of new compounds by modern strategies is done based on the known definition of therapeutic mechanism through modelling techniques. Although, prevention of oral problems is simple by following proper oral hygienic measures, it is still a global problem with high prevalence. The use of broad spectrum antibiotics and various chemicals like fluorides destroys the pathogenic oral microbes along with the beneficial microbes which may create some complications such as the development of opportunistic pathogens. Hence appropriate use of drugs targeting the pathogenicity of biofilm forming pathogens has to be considered. *In silico* approach helps in understanding the sites of molecular targets for specifically inhibiting these pathogens.

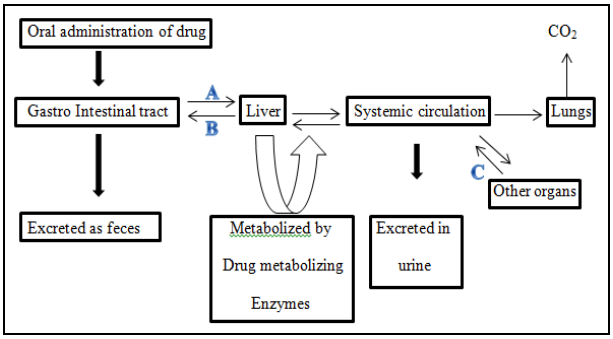
Docking studies:

In the modern era, molecular docking has emerged as a fundamental tool of research in the drug discovery process. It accurately predicts binding of the compound of interest with its potential target at the active site and correctly estimates the binding energy of the docked complex. This provides insights whether the predicted compounds can be used as drugs [63]. In dental caries, the major causative agent is *S. mutans* which binds to the already existing biofilm and by producing glycosyltransferase (GTF-SI) enzyme maintains dental plaque. Thus, inhibition of GTF-SI is an effective way of treating dental plaque [64]. Molecular docking studies aims at finding the potential compounds to bind in the active site region of GTF-SI enzyme with high binding energy. N-myristoyltranferase (NMT) enzyme in *C. albicans* has been the potential drug target for oral candidiasis. NMT has been involved in various signaling networks and is essential for *C. albicans* growth [65].

ADME and toxicity studies:

The compound should have a good absorption, distribution, metabolism and excretion (ADME) profile to be used as drugs apart from having good docking results. Large number of compounds fails to reach the drug market because of their poor pharmacokinetic (PK) and pharmacodynamic (PD) properties. The PD and PK characteristics depend upon the ADME properties of the compound [66]. The ADME profile of an oral drug is shown in Figure 1. A drug administered orally passes through gastrointestinal (GI) tract and liver. Some get excreted and some are metabolized by enzymes in the liver. The drug then enters the blood stream and

distributed throughout the body organs. Lipophilicity and solubility are the major factors which affect a drug's absorption in the body. More lipophilic drugs are absorbed at a greater rate in the blood stream due to the fact of metabolic enzymes having greater affinity towards lipophilic compounds [67]. Distribution of drug is greater when the drug is more lipophilic. The distributed drug gets metabolized by various cytochrome P450 (CYP450) enzymes. These enzymes convert the drug in to a more water-soluble compound which helps in their easy elimination from the body [68]. The excretory organs such as kidney and liver eliminate them from the body. The development of various softwares for ADME profile assessment helps in the early analysis of ADME profile which reduces risk, time and money when done at the end stages of drug development. In addition to ADME analysis, software for toxicity analysis have been developed which calculates the druglikeness score, carcinogenicity, mutagenicity, irritancy and reproductive effects of the compounds.



A – Absorption, B – Excretion, C – Distribution
Figure 1: ADME scheme of oral drug

CONCLUSION:

This chapter explores the diversity of endophytic fungi from various medicinal plants and to screen the endophytes against two major oral pathogens *Streptococcus mutans* and *Candida albicans*. Modern strategies of drug designing involves rational designing of new prototypes of compounds which helps in designing the chemical structure based on the proposed mechanism of therapeutic action. When a three dimensional structure of a protein is known, it becomes easy to identify a compound that binds with its active site using modeling techniques. For oral science, it is vital to understand the mechanism of formation, drug resistance and virulence of biofilms to develop compounds that can target dental biofilms to destabilize them. Hence, the endophytes could be explored as potential sources of useful bioactive compounds against *S. mutans* and *C. albicans* and further work on compound isolation from the endophytes to be carried out for treatment of oral diseases.

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