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Secrets of plants: Endophytes

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Abstract

Endophytic fungi are an important component that colonizes in healthy tissues of living plants and can be readily isolated from any microbial or plant growth medium. They act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties. Their huge diversity and particular habituation, they can provide a good area for research in the field of making new medicines and novel drug-like molecules. Because of the impact of endophytes on host plant by enhancing their growth or increasing their fitness, also making them tolerant to abiotic and biotic stresses and holding the secondary metabolites, endophytes are gaining attention as a subject for research. This review aims to comprehend the contribution and uses of endophytes and relationships between endophytic fungi and their host medicinal plants.

Introduction

Endophytes are microorganisms that reside within study plant tissues intercellularly and/or intracellularly but usually remain asymptomatic and does not show any noticeable damage to the host. Endophytic fungi inhabit plant tissues without destroying or producing substances that cause an infection in the host cell. They exhibit complex interactions with their hosts, which involves mutualism and antagonism.[1,2] Plants strictly limit the growth of endophytes, and these endophytes use many mechanisms to gradually adapt to their living environments.[3] Unlike mycorrhizal fungi that colonize plant roots and grow into the rhizosphere, endophytes reside entirely within plant tissues and may grow within roots, stems and/or leaves, emerging to sporulate at plant or host□tissue senescence.[1,4] Symbioses have been responsible for the origin of major ecological and evolutionary transitions in the history of life. Plants are hosts to complex communities of endophytes that colonize the interior of both below- and above-ground tissues. The endophytic fungus grows asymptomatically in aerial plant tissues and is vertically transmitted from the plant to its offspring via seeds and tillers. Endophytes are thought to interact mutualistically with their host plants mainly by increasing host resistance to herbivores[1] and have been termed *acquired plant defenses*.[5] In return, plants provide spatial structure and protection from desiccation, nutrients, and photosynthate and, in the case of vertical-transmission, dissemination to the next generation of hosts. Endophytic fungi represent an important and quantifiable component of fungal biodiversity in plants that impinge on plant community diversity and structure.

They produce a wide range of compounds useful for plants for their growth, protection to environmental conditions, and sustainability, in favor of a good dwelling place within the hosts. Due to its ability of producing bioactive secondary metabolites similar or same compounds as their host plant, promoting growth, good yield, inducing host plants to tolerate both biotic and abiotic stresses and disease resistance, endophytes is currently attracting a lot of research interest and therefore, can be used as a potential source of novel natural products for food, industrial, medicinal and agricultural industries.

Classification of endophytes

There are mainly two classes in which endophytic fungi are classified as Class I Clavicipitaceae and Class II Nonclavicipitaceous. The Class 1 endophytes are the clavicipitaceous endophytes associated with warm- and cool-season grasses. They are host specific, mainly in the grass family poaceae and rarely in cyperaceae, and are often vertically transmitted through seeds. Transmission of Class 1 endophytes is primarily vertical, with maternal plants passing fungi on to offspring via seed infections.[6] Class 1 endophytes frequently increase plant biomass, confer drought tolerance, and produce chemicals that are toxic to animals and decrease herbivory.[5] However, the benefits conferred by these fungi appear to depend on the host species, host genotype and environmental conditions.[7,8]

Compared with Class 1 endophytes, Class 2 endophytes are highly diverse and comprised of species from pezizomycotina (ascomycota) to agaricomycotina and pucciniomycotina (basidiomycota).[9] They colonize roots, stems, leaves, or the whole plant. They can be vertically or horizontally transmitted. However, NC-endophytes represent three distinct functional classes class 2, 3 and 4, based on host colonization and transmission, in plant biodiversity and fitness benefits conferred to hosts while the C group has just one class. Class 2 endophytes have been the most extensively researched and has been shown to enhance fitness benefits of their plant host as a result of habitat-specific stresses such as pH, temperature, and salinity. Class 3 endophytes are restricted to growth in belowground plant tissues and form in localized areas of plant tissue. Class 4 endophytes are also restricted to plant tissues below ground but can colonize much more of the plant tissue.[10] NC-endophytes have been recovered from every major lineage of land plants, and from all terrestrial ecosystems, including both agro-ecosystems and biomes ranging from the tropics to the tundra.[11]

The scale of their diversity, their ecological roles, the insights they provide into the evolution of various ecological modes in fungi, their potential applications, and the ability of many fungi to switch between endophytic and free□living lifestyles[12,13] are becoming more apparent, engendering growing enthusiasm from mycologists, ecologists, physiologists and applied scientists.

Isolation and identification

Endophytic fungi are any fungi that grow all or part of their life cycles symptomlessly in the intercellular spaces of living and apparently healthy host plant tis-

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sues, while the inhabited host tissues remain intact and functional. The diversity and numbers of fungal endophytes vary considerably and their detection depends on biotic, abiotic and experimental factors. Detection and recovery of endophytic fungi have relied heavily on dissection of plant organs into small fragments followed by their surface sterilization and subsequent plating of fragments onto a nutrient-rich agar medium.[4,14]

Traditionally, endophytic fungi inside plant tissues can be recognized by two basic techniques, *i.e.* direct observation and cultivation-dependent methods. In the direct observation method, endophytic fungal structures within living plant tissues are directly examined under a light and electron microscope, which can show all endophytic mycobiota within the plant tissue.[15] In contrast, cultivation-dependent techniques have been routinely employed in endophyte diversity studies.[1] It is important to isolate endophytic fungi for further detailed studies into their characterization, population dynamics, species diversity, or as inoculate to improve plant growth and health, or screening for novel biologically active secondary metabolites (Figure 1).

Endophytes can also be isolated by culturing from ground tissue extract[16] or by direct culturing of plant tissues[17] on media suitable for bacteria or fungi or actinomycetes. Conventionally, identification of endophytes is based on morphological characteristics for bacteria, fungi, and actinomycetes and with the help of biochemical tests for bacteria and actinomycetes. With the development of molecular biology, ribosomal DNA Internal Transcribed Spacer (ITS) sequence analysis is widely used for the identification of microorganisms. Ribosomal DNA (rDNA) ITS was proved to be a valuable source of evidence to resolve phylogenetic relationships at lower levels, such as among genera or species.[18]

Microorganisms are important mediators of plant-herbivore interactions. The role of endophytic microorganisms in plant associations has been extensively discussed. Endophytes are considered plant mutualists. They receive nutrition and protection from the host plant while the host plant may benefit from enhanced competitive abilities and increased resistance to herbivores, pathogens, and various abiotic stresses.[7] As endophytes colonize ecological niches similar to those colonized by phytopathogens, interactions between these two groups are possible, and in fact, many studies have shown that endophytic microorganisms isolated from surface disinfected plant tissues exhibit potential as biocontrol agents against phytopathogens and insects.[19] Reports of molecular studies on endophytic bacterial diversity have revealed a large richness of species. Endophytes promote plant growth and yield, suppress pathogens, may help to remove contaminants, solubilize phosphate, or contribute assimilable nitrogen to plants.[20] The endophyte-host relationship is believed to be complex and probably varies from host to host and microorganism to the microorganism.[21] Many experiments have been conducted to compare how endophyte-infected plants and non-infected plants behave in relation to environmental stress, and attack by insect and animal predators.[22] Therefore, search for interesting biological activities within the natural biodiversity has been the basis for the development of various applications in biotechnology, agriculture, production of pharmaceuticals compounds and other fields.

Application of endophytes

Medicinal plants and their endophytes are important resources for the discovery of natural products. Since endophytes residing inside healthy plant tissues without any discernible infectious symptom and exhibit mutualism and antagonism with the host, they could be a potential source of novel natural products for medicinal, agricultural, and industrial uses. Endophytes provide a wide variety of structurally unique bioactive natural products, such as alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others.[23]

Antioxidants and phenolic content of endophytes

Antioxidants are thought to be highly effective in the management of ROS-mediated tissue impairments. Many antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities to a greater or lesser extent. Afra *et al.*, in 2015, revealed that endophytic fungus *Aspergillus* sp. from *Trigonella foenum-graecum* seeds demonstrated the highest both total phenolic content in term of gallic acid equivalent and antioxidant activity for free radical scavenging assay.[24] The antioxidant activity and total phenolic content (TPC) of ethyl acetate extracts of endophytic fungi isolated from *Eugenia jambolana* were studied by Manila *et al.*, in 2014.[25] The study showed that 36% endophytic extracts of *Chaetomium* sp., *Aspergillus* sp., Aspergillus *peyronelii* and *Aspergillus Niger* was having high phenolic content exhibited potent antioxidant activity. The

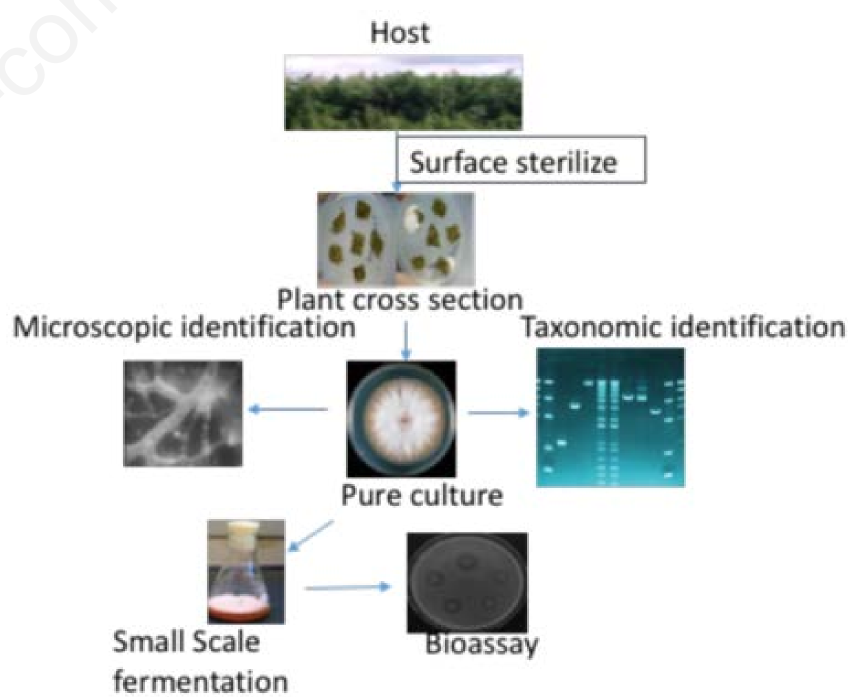

Figure 1. The interaction between endophytes and plant host.

free radical scavenging ability of phenols is attributed to the occurrence of hydroxyl groups. Phenols and alkaloids were the major phytochemical constituents of endophytes and were reported by Huang *et al*. *Cephalosporium* sp., an endophytic fungus isolated from the root of *Trachelospermum jasminoides* (Apocynaceae) produce a phenolic compound (graphislactone A) with strong free radical scavenging and antioxidant activity.[26] Cui *et al*., in 2015 isolated 315 endophytes from *Rhodiola crenulata*, *R. angusta*, and *R. sachalinensis* and reported that antioxidant assay by showing that the DPPH radical-scavenging rates of 114 isolates (63.33%) were >50%, and those of five isolates (Rct45, Rct63, Rct64, Rac76, and Rsc57) were >90%.[27] The culture filtrate of *Pestalotiopsis microspora*, which was isolated from combretaceaous plant *Terminalia morobensis*, showed promising antioxidant activity.[28]

Antimicrobial activities of endophytes

Many endophytic fungi have the ability to produce antimicrobial substances. An increasing number of compounds with antibacterial activity are being isolated from endophytic fungi, including *fumitremorgins* B isolated from *Phomopsis sp*., and periconicins A and B from *Periconia* sp.[29] Research has shown that the compound ophiopogon japonicus pill has displayed effective inhibition of *Staphylococcus aureus* and an extract from *O. japonicus* showed strong inhibition of malic mildew.[30] 8.3% of the strains isolated from *Dracaena cambodiana* and *Aquilaria sinensis* showed antimicrobial activity,[31] whereas 27.6% of strains isolated from *Camptotheca acuminata* displayed antimicrobial activity against some pathogens.[32] Antimicrobial activity of endophytic fungi from *Lippiasidoides Cham*. showed antimicrobial activity in an assay on a solid medium.[33] The steroid ergosta-7,9(14),22-triene-3β-ol, produced by the endophytic fungus *Nigrospora sphaerica* isolated from leaves of *Vinca rosea*, showed antifungal activity against *Cryptococcus neoformans* with an IC50 value of 14.81 μg/mL.[34] Maryam et al. reported Sixteen out of 23 bacterial isolates (69%) exhibited antimicrobial activity against the selected pathogenic bacteria, such as *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Shigella flexneri* and *Escherichia coli.*[35] Rodrigues *et al.,* in 2000 have reported that Guignardia sp. from Spondias mombin inhibited *E. coli, S. aureus, Saccharomyces cerevisiae, Geotrichum sp.* and *Penicillium canadensis.*[36] Antimicrobial activity was exhibited by some endophytic fungal isolates from *Cinnamomum. burmani, C. camphora*, and *C. insularimontanum* and *C. zeylanicum.*[37] Beauvericin, isolated from *Fusarium oxysporum*, which is associated with cinnamomum kanihirai, was reported toxic against *methicillin-resistant S. aureus* and *B. subtilis.*[38] The endophyte of *Magnolia x soulangeana, Fusarium dimerum*, was the source of enniatins which exhibited effectiveness against *Bacillus subtilis*, *Candida albicans*, *Trychosporom cutaneum* and *Cryptococcus neoformans.*[39]

Anticancer activities of endophytes

There is some evidence that bioactive compounds produced by endophytes could be substitute approaches for innovation of new drugs, as various natural products of plants, microorganisms, and marine sources were identified as anticancer agents. Firakova *et al.*, in 2007 reported diterpenoid that taxol also known as paclitaxel as a novel drug since it's due to the mode of action compared to other anticancer agents.[40] Taxol is beneficial for the cure of advanced breast cancer, lungs cancer, and refractory ovarian cancer. Taxol was from the bark of tree belonging to taxus family (*Taxus brevifolia*), its main general resource. Another significant anticancer compound is the alkaloid Camptothecin ($C_{20}H_{16}N_2O_4$), a powerful antineoplastic agent which was firstly isolated from the wood of Camptotheca acuminate Decaisne (Nyssaceae) in China.[41] Ergoflavin ($C_{30}H_{26}O_{14}$), a dimeric xanthene linked in position 2, belongs to the compound class called ergochromes and was described as a new anticancer agent isolated from an endophytic fungus rising on the *Mimuscop elengi* (Sapotaceae) plant leaves[42] Wu *et al*., in 2015 also reported leaf endophytes from *Morinda citrifolia* inhibited the growth of human carcinoma cell lines of lungs, prostate, and breast.[43] Bioactive compound sclerotiorin has been isolated from an endophytic fungus *Cephalotheca faveolata.*[44] Sclerotiorin was found to be potent antiproliferative against different cancer cells. An endophytic fungus *Colletotrichum gloeosporioides* (strain JGC-9) was isolated from *Justicia gendarussa*, a medicinal plant and screened for taxol production.[45] *Podophyllum hexandrum*, which produced podophyllotoxin, podophyllotoxin glycoside and demethoxypodophyllotoxin. The isolated metabolites exhibited cytotoxicity in U-87 cell line.[46] The production of podophyllotoxin from endophytic *Alternaria tenuissima* isolated from *Sinopodophyllum emodi* (Wall) was also reported by Liang *et al*.[47]

Conclusions

Endophytic fungi comprise a diverse group of species existing in various ecosystems and are capable of synthesizing bioactive compounds, which have proven to be useful for novel drug discovery. This group, which causes no apparent effects on plant performance but lives on the metabolites produced by the host, is presumably the most dominant functional group among endophytes by quantity. Since many microorganisms have developed resistance against the current drugs so it is necessary to search for new drugs. Much more work is essential to understand endophytes physiology, biochemical pathways, defensive role, secondary metabolite production, motivation and encouragement of researcher from life sciences to contribute research related to endophytes. It is vital importance to review and highlight the previous successes, on-going research and latest developments in research associated with endophytic microorganisms to draw the attention of the research community toward this emerging field and possible exploitation of the available sources for their therapeutic uses in various fields, such as the medical, pharmaceutical, food and cosmetics.

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