Journal of Advances and Scholarly Researches in Allied Education
Vol. 16, Issue No. 9, June-2019, ISSN 2230-7540

A Review of Endophytic Fungi

Brajesh Ku Pandey[1]* Sanjiv Kumar Sahw[2]

[1] Research Scholar

[2] Associate Professor, Department of Chemistry

Abstract – Endophytes are set against pathogenic substances and therefore asymptomatically invade plants. As is shown, however, endophytic fungi may act differently under diverse biotic and abiotic conditions where the host plant can also play a defining role. The genetic differences between an endophytic fungus and a pathogenic fungus may be substantially different. However, endophytic fungi have often been isolated throughout the years and in different host plants they never produce disease signs.

Keywords – Endophytic Fungi, Identification, Morphological, Molecular, DNA

----------X----------

1. INTRODUCTION

Endophytic fungi are microorganisms that live symbiotically inside the stable tissues of higher host plants, imitate the chemistry of their hosts, and contain the same bioactive natural products, medicines, and derivatives as their hosts while causing no harm or disease to them.

Endophytes have been found in abundance in the majority of plant species studied, with over a million fungal endophytes known to occur in nature. 73 % Plant-associated microbes have been found in fossilized tissues of plant roots, branches, and leaves, suggesting the endophytic contact is almost as ancient as the moment higher plants first emerged on the face of the planet millions of years ago. Endophytes are thought to be linked to certain phytopathogens present in the atmosphere at the time, and have common sources, according to the literature These microorganisms successfully invaded plant tissues via wounds and openings, as well as proactively rupturing plant cell walls with hydrolytic enzymes such as pectinase and cellulase.

Endophytes evolved to safely live inside their hosts and be an essential component of the host plant's micro-ecosystems via evolutionary genetic variation across the long course of co-evolution. 81, 94, and 120 are three numbers that can be used to make a number of different combinations This entailed the uptake of certain plant DNA segments into their own genomes, as well as the addition of their own DNA segments into the host genomes; this is known as biotransformation. Over time, the relationship between endophytes and host plants evolved into a mutualistic one in which all parties benefited. Endophytes depend on the host for nutrients, shelter, and defense, and they produce bioactive compounds that aid the host's resistance to external biotic and abiotic stresses, provide immunity to diseases, parasites, insects, and other pests, and ensure the host's health and survival. Endophytes also evolved the capacity to biosynthesize certain "phytochemicals" that are identical to those formed by their hosts in an effort to imitate their hosts' chemistry.

The possibility that the plant hormone gibberellin may be generated not just from the plant but also from endophytic fungi associated with it piqued researchers' interest, which was further piqued when paclitaxel (taxol), the wonder drug for cancer, was produced in 1993 from the endophytic fungi Taxomyces andreanae isolated from the bark of the Pacific Yew plant Taxus brevi. 16 and 87 Due to significant advancements in genetic engineering, microbial fermentation technology, and other fields, the number of researchers working to further explore endophytic fungal diversity and better understand the relationships between endophytic fungi and their host plants has increased dramatically in the last two decades, in an attempt to use endophytes to obtain valuable components.

Endophytic fungi have been used to synthesize a variety of useful plant-based bioactive compounds with antimicrobial, insecticidal, antiparasitic, cytotoxic, immunosuppressive, and anticancer properties ever since. Secondary metabolites such as podophyllotoxin, camptothecine, vinblastine, vincristine, hypericin, diosgenin, toosendanin, -irone, -irone, huperzine, among

others may be categorized as alkaloids, peptides, terpenoids, hormones, flavonoids, quinones, lignans, phenols, lactones, and lactones. 108, 113, 120, 122, 125, 127) (14, 46, 49, 56, 108, 113, 120, 122, 125, 127) (14, 46, 49, 56, 108, 113, 120 As a possible alternative, eco-friendly source to effectively process valuable bioactive composition with a variety of uses in the medical, food industry, irrigation, pesticide control and other areas of research and usage, endophytes are thus very promising.

1.1 Isolation of Endophytes

The plant materials are thoroughly washed and surface sterilized with different surface sterilizing agents such as mercuric chloride (HgCl2), ethanol, and others to remove epiphytic microbes. Cut into small pieces, these are then placed on a PDA (potato dextrose agar) plate. The hyphal tips of the fungi are removed and transferred to PDA slants, where they are screened for bioactive secondary metabolite production after a significant period of incubation ranging up to several days. Isolations of these fungi from surface sterilized tissues were used to determine whether they were epiphytic or endophytic. Due to the slow growth rate of endophytes, the most difficult part of most procedures is isolating and purifying these cultures for long periods of time. (4) A variety of stains have been used to identify these fungi in plants. To look for endophytic fungi in epidermal peels from stems, seeds, leaf sheaths, pith scrapings, and other places, Sampson used cotton blue or gentian violet, followed by Gram's iodine solution. Other researchers used lactophenol cottonblue, lactophenoltrypan blue, and aniline blue to stain mycelium. Clark et al. soaked the seeds in aniline blue-lactic acid. Saha et al. developed a rapid staining method for endophytes using rose bengal stains. This method was superior to trypan blue in terms of speed and safety. Since then, a large number of bioactive secondary metabolites from plant-associated endophytic fungal strains have been isolated, purified, and thoroughly characterized.

2. REVIEW OF LITERATURE

2.1 Fungi

Fungi can thrive in a variety of environments, including the weather, soil, living organisms, and even seafoam. Their propensity to colonise environmental niches and their capacity to metabolise different kinds of organics, to create new chemical structures and to remove inorganics have attracted attention in the areas of bioremediation and toxicity. The fungus were the majority of the five eukaryotic areas. The throne of the empire Based on the shape of their sexual reproductive systems, fungi are grouped into three groups: Zygomycetes (zygospores), Ascomycetes (ascospores), and Basidiomycetes (basidiospores). True fungi are heterotrophic, filamentous, and have chitin in their cell walls.

Hyphae are filaments with cross-walls that separate them into compartments, each with several nuclei. Mycelium, which is a set of filaments, is made up of them. These filaments can fuse and form a complex network of mycelia in Ascomycetes and Basidiomycetes, which can aid in the growth of fruiting bodies.

Saprophytes break down organic matter like cellulose and lignin into the building blocks that other heterotrophic species (like plants and microbes) need to develop. Some mushrooms, such as mycorrhazes, share nutrients with a host plant in a reciprocal relationship.

The symbiotic connection between fungus and plants has been recognised since Frank discovered mycorrhizae in 1885. Mycorrhizae are fungi that grow on plant roots, provide important minerals such as phosphate and nitrate and transport waters for glucose and other nutrients.

2.2 Endophytes

Fungi may also live on the plant's surface as epiphytes or endophytes. Endophytes reside within a plant's tissue, while epiphytes live on the leaves' top.

A fungus dwelling within the tissue of buds of the conifer Picea canadensis was described by Lewis (1924) (130) from a sample collected in Alberta (a synonym of P. glauca, white spruce). At the period, the effect of the fungus on the fescue grass was unknown, but the plant shows no signs of stress. The fungus was also discovered in the seeds of the infected F. rubra grass, implying that the endophyte infection was transmitted down vertically.

Cunningham (1949) and Pulsford (1950) discovered a mysterious disease in cattle in Australia and New Zealand, respectively, that was caused by tall fescue (1950). Bacon et al. (1977) related the occurrence of the endophyte E. typhina in tall fescue to symptoms, implying the development of a vasoconstricting mycotoxin. Cattle fed endophyte-infected fescue had gangrenous tail ends, ears, and hooves, indicating poor circulation. Other symptoms included a thick, "rough" coat and general malaise, as shown by reduced food intake and weight loss. Lower reproductive rates and milk yields have been linked to cattle consuming polluted fescue tainted with Acremonium coenophialum.

Since the early 1900s, rye grass staggers have been discovered in pasture animals in New Zealand eating annual ryegrass (Lolium perenna L.). According to Neill (1940), these symptoms

were linked to the presence of endophytic fungi, but this hypothesis was abandoned because the connection could not be established. The rye grass endophyte A. lolii has also been connected to the staggers.

The neurotoxins ergovaline and lolitrem B generated by the endophytes E. typhina, A. coenophialum, and A. lolii induce fescue-related toxicosis and tremorogenic staggers

3. IDENTIFICATION OF ENDOPHYTIC FUNGI

3.1 Morphological characters

The morphological features of the fungal isolates that showed promising results for antimicrobial and cytotoxicity were used to classify them. The fungal isolates were subcultured into fresh media (PDA) and incubated at 28oC for 2 weeks to analyze their cultural and morphological characteristics. Visual observation was used to establish cultural characteristics such as color and the essence of the colony's development.

3.2 Molecular characterization

3.2.1 Isolation of fungal genomic DNA

In 50 mL potato dextrose broth in 250 mL Erlenmeyer flask, fungal isolates were individually inoculated. After 7 days of incubation at 28°C, the mycelium from each mushroom was removed using the filter of the cheese cloth. CTAB was used to extract mycelial genomic DNA.

3.2.2 CTAB method for DNA extraction

1. Mycelium was ground into powder in liquid nitrogen in an autoclaved mortar and immediately transferred to 50 ml oak ridge tube and added preheated CTAB buffer to make slurry along with 200 µL β-mercaptoethanol. Incubated at 60ºC for 1h in water bath with mixing at regular intervals.

2. A 24:1 mixture of chloroform and isoamyl alcohol was applied, blended thoroughly for 5 minutes, and centrifuged for 10 minutes at 5000 rpm.

3. Aqueous phase was removed with wide-bore pipette (cut off tip from mouth) to clean oak ridge tube. Repeated chloroform extraction if extract was still colored.

4. DNA was precipitated with 0.66 volumes of cold isopropanol and incubated for 1 h at -20ºC.

5. Centrifuged at 10000x g for 15 min.

6. Supernatant was discarded and dissolved the pellet in 1 mL TE buffer (Appendix II) and taken solution in microfuge tube.

7. 2 µL RNase solution (10 mg/mL stock) was added and incubated at 37ºC for 1 h. RNase stock solution was preheated for 5 min at 60ºC before use.

8. Re-extracted with equal volume of phenol: chloroform (1:1 v/v). Centrifuged (1000 x g, 10 min) and retained aqueous phase.

9. 0.3 volume of 3M sodium acetate and 0.6 volume of chilled iso-propanol were added. Incubated for 1 h at -20ºC.

10. Centrifuged (10000 x g, 8 min) and retained pellet. Washed pellet with 30 µL of 70% EtOH and air-dried pellet.

11. Dissolved pellet in TE buffer and stored at -20ºC.

3.2.3 Checking of DNA (Agarose Gel Electrophoresis)

1. Agarose gel (0.8 % (w/v)) in TBE buffer was prepared and ethidium bromide (1.0 µL/30mL) was added after cooling down and poured the gel in mini gel tray with comb. Allowed the gel to solidify.

2. Placed gel tray in electrophoresis unit, filled the unit with 0.5X TBE buffer and removed the comb carefully.

3. Prepared samples by adding tracking dye to DNA samples and mixed.

4. Electrophoresis was carried out at 60 V for 30-45 minutes after filling the samples in wells, and the results were visualized using a UV transilluminator.

3.2.4 Quantification of DNA using Nano drop

A nanodrop spectrophotometer was used to determine the DNA concentration (Thermo Fisher Scientific Inc. USA). 1 l of sample was placed onto the lower optical surface for DNA quantification, and then the lever arm was lowered. The sample is engaged by the upper optical surface, forming a liquid column with a path duration defined by the distance between the two optical surfaces. The sample is analysed at both a 1 mm and 0.2 mm direction, giving the nucleic acid detection a wide dynamic range. The A260/A280 and A230/A260 ratios were used to determine the accuracy of the DNA. The A260/A280 ratio should be 1.8-2.0 in ideal conditions, whereas the A230/A260 ratio should be 0.3-0.9. Phenol contamination

(A260/A280) is shown to be below 1.8, while RNA contamination is shown to be over 2.0.

3.2.5 Amplification of Internal Transcribed Spacer (ITS) region

The universal primers ITS1 (5′-TCCGTAGGTGAAC CTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used to amplify the ITS regions of the rDNA from genomic DNA of selected endophytic fungi, as well as the 5.8S rRNA gene, as defined by White et al (1990). In a final volume of 50 l, the PCR reaction mixture produced 1X PCR buffer (Fermentas, USA), 200 M dNTPs, 1.5 mM MgCl2, 0.1 M primers, 50 ng DNA, Taq DNA polymerase units and 2.5 units (Fermentas, USA). A GenAmp thermocycler was used to perform ITS area amplification from isolates (Applied Biosystem, USA). An initial denaturation at 94°C for 5 minutes was accompanied by 35 intervals of 94°C for 1 minute, 50°C for 1 minute (annealing), and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. Controls with no DNA template were used to ensure that the reagents and reaction buffer were not contaminated. By electrophoresis on a 1.5 percent w/v agarose gel and visualization with a UV trans-illuminator, effective amplifications were verified.

3.2.6 Purification of PCR products and sequencing

Augarose gel (0,8%) electrophorosis was used to purify amplified ITS products before to cloning. The DNA fragment was extracted using the QIAquick gel extraction kit following the manufacturer's instructions (Qiagen Inc., USA). Purified PCR products were utilised for elute with 30 l TE buffer (pH 8.0). Then PCR products have been sequenced with purification. The sequence was produced utilising the chain termination technique using an Applied Biosystems automated sequencing.

3.2.7 Sequence analysis

To find possible homologous sequences of the newly sequenced taxa for each fungus, the ITS nrDNA sequences of the isolates were compared with those available in GenBank databases using the BLAST search program (182). To reduce the number of inferred gaps, the sequences of closely related fungi retrieved from GenBank were aligned. The sequences were edited in BioEdit 5.0.6 (183) and aligned in ClustalW using multiple alignments (184). MEGA5.1 software was used to reconstruct the phylogenetic trees (185). The bootstrap was 1,000 replications to determine the tree node's reliable level. The NCBI GenBank database was used to store all of the sequences generated in this study.

4. FUTURE PROSPECTS

The discovery of the true potential of endophytes has been one of the major breakthroughs in science in the 21st century. Endophytes have very soon found a wide variety of applications in agriculture, medicine, therapeutics, food processing and preservation, etc. However, synthetically derived compounds, although most produce toxicity and side effects are still in huge demand and thus, studies on endophytes need to be ventured deeper still so as to increase their overall output and obtain many more valuable compounds of plant origin which may then benefit the society at a larger scale.

Exponential advances in engineering and science in the past two decades have further established techniques like microbial fermentation, metabolic technology, microscopy, chromatography, spectroscopy, etc. and have helped researchers dwell further into various unexplored regions of possibilities. Through gene manipulation, mutation and other recombinant DNA techniques, the best quality endophytic fungi samples can be chosen and their productivity increased by tampering with the relevant functional genes in their biosynthetic pathways and colonizing them for optimal production of bioactive compounds. Desired features may be attributed to these compounds and stronger derivatives may be obtained.

Microbial fermentation is a very simple and inexpensive process, occurs within a very short period of time, where the parameters can easily be controlled for the best growth and breeding conditions. Moreover, the efficacy of the fungal culture medium can be enhanced by using special anti-inhibitory enzymes, feeding precursors, addition of biotic and abiotic elicitors confirmed through metabolic studies, etc. All these developments will lead to an unlimited supply of cheap, completely safe and naturally derived medicines, flavor and aromatic compounds, wound healers, ointments, antiseptics, etc. which will be easily available to all. The entire process will be in complete synchronization with the environment with a view to conserve and preserve the balance of eco-system by preserving the trees which sustain it.

5. CONCLUSION

Endophytic mushrooms should be a possible source of new biological substances. The discovery of novel medicines against cancer and multidrug-resistant bacteria may also provide improved sources of endophytic fungus. Laboratory and fermenter are readily grown endophytic fungi. It helps prevent plant harvest and influence the biodiversity of the environment.

REFERENCES:

1. Bansal V, Ahmad A, Sastry M. (2006). Fungus-mediated biotransformation of amorphous silica in rice husk to nanocrystalline silica. Journal of the American Chemical Society 128: pp. 14059–14066.

2. Bastos DZL, Pimentel IC, De Jesus DA, De Oliveira BH. (2007). Biotransformation of betulinic and betulonic acids by fungi. Phytochemistry 68: pp. 834–839.

3. Bondy GS, McCormick SP, Beremand MN, Pestka JJ. 1991. Murine lymphocyte proliferation impaired by substituted neosolaniols and calonectrins--Fusarium metabolites associated with trichothecene biosynthesis. Toxicon official journal of the International Society on Toxinology 29: pp. 1107– 1113.

4. Borges KB, De Souza Borges W, Pupo MT, Bonato PS. (2008). Stereoselective analysis of thioridazine-2-sulfoxide and thioridazine-5-sulfoxide: an investigation of rac-thioridazine biotransformation by some endophytic fungi. Journal of Pharmaceutical and Biomedical Analysis 46: pp. 945– 952.

5. Cao L, Huang J, Li J. (2007). Fermentation conditions of Sinopodophyllum hexandrum endophytic fungus on production of podophyllotoxin. Food and Fermentation Industries. 33: pp. 28–32.

6. Cao X, Li J, Zhou L, Xu L, Li J ZJ. (2007). Determination of diosgenin content of the endophytic fungi from Paris polyphylla var. yunnanensis by using an optimum ELISA. Natural Product Research and Development. 19: pp. 1020–1023.

7. Carroll G. (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69: pp. 2–9.

8. Choi WY, Rim SO, Lee JH, Lee JM, Jee IJ, Cho KJ, Rhee IK, Kwon JB, Km JG. (2005). Isolation of gibberellins-producing fungi from the root of several Sesamum indicum plants. Journal of Microbiology and Biotechnology 15: pp. 22–28.

9. Clark EM, White JF, Patterson RM. (1983). Improved histochemical techniques for the detection of Acremonium coenophilum in tall fescue and methods of in vitro culture of the fungus. Journal of Microbiology Methods. 1: pp. 149–155.

10. Clay, K. and Jones JP. (1984). Transmission of Atkinsonella hypoxylo (Clavicipitaceae) by cleistogamous seed of Danthonia spicata (Gramineae). Canadian. Journal of Botany. 62: pp. 2893–2895.

11. Cole RA, Jarvis BB, Schweikert MA. (2003). Handbook of Secondary fungal Metabolites. Academic Press, NY, USA,, New York.

12. Cundliffe E, Davies JE. (1977). Inhibition of Initiation, Elongation, and Termination of Eukaryotic Protein Synthesis by Trichothecene Fungal Toxins. Antimicrobial Agents and Chemotherapy 11: pp. 491–499.

13. Degenkolb T, Dieckmann R, Nielsen KF, Gräfenhan T, Theis C, Zafari D, Chaverri P, Ismaiel A, Brückner H, Von Döhren H, Thrane U, Petrini O, Samuels GJ. (2008). The Trichoderma brevicompactum clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. Mycological Progress 7: pp. 177–219.

14. Desjardins AE. (2006). Fusarium Mycotoxins: Chemistry, Genetics and Biology- by Anne E. DesjardinsPlant Pathology. APS Press.

15. Desjardins AE, McCormick SP, Appell M. (2007). Structure-activity relationships of trichothecene toxins in an Arabidopsis thaliana leaf assay. Journal of Agricultural and Food Chemistry 55: pp. 6487–6492.

16. Eyberger AL, Dondapati R, Porter JR. (2006). Endophyte fungal isolates from Podophyllum peltatum produce podophyllotoxin. Journal of Natural Products 69: pp. 1121–1124.

17. Findlay JA, Bethelezi S, Li G, Sevek M. (1997). Insect toxins from an endophyte fungus from wintergreen. Journal of Natural Products. 60: pp. 1214– 1215.

Corresponding Author

Brajesh Ku Pandey*

Research Scholar