SYNTHESIS OF NANOPARTICLES FROM ENDOPHYTIC FUNGI - A REVIEW

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ABSTRACT: *Endophyte means organism which is growing from inside tissue of the plant.They are also defined as microorganism that grow from the healthy plant tissues without being harmful to the host cell.The active substance produced by them can also be used as antifungal, antibacterial, antitumor, antidiabetic agents.Nanoparticles are compounds or particles which can literally manipulate onto a macromolecule and condenses into smaller particles so that it can be used anywhere and can be stored anytime and anywhere. The concept of the nanotechnology is making the molecules smaller so that it is easily penetrable and is easy to use.Biological method includes nanoparticles synthesized from plant extracts and micro organisms.The fungi Fusarium oxysporum is majorly capable of producing silver nanoparticles. Currently some other species of fungi have also been studied such as Penicillium fellutanum, Fusarium acuminatum, Aspergillus niger, Fusarium semitectum, Aspergillus flavus and found to have the capability of reducing the metal ions to their respective nanoparticles. These nanoparticles synthesized from endophytic fungi are found to have anticancer, antiviral, antidiabetic, antimicrobial and immunosuppressive activity. But the activities and appications of endophytic fungi are still less explored and experimented. Thus this vast variety of source can serve as a good drug compound, a good supplier of medication via nanoparticles, their derivaties can also be used as product in medicinal and technological application. In future if they are taken under preference in formulation of new drug compound using their bioactivity it will be a product with eco friendly nature and with less time consumption. .*

Key Words: *endophytic fungi, nanoparticles, plant extract, bioactivity, biosynthetic method.*

1. INTRODUCTION

The term endophyte means organism growing from inside tissue of the plant. Endophytic are of two groups one do not generate any external structures from host and the other one is which generate external structures like nodules [1]. They are nothing but microorganism, which are grown from the healthy plant tissues without being harmful to the host cell. They have special ability to produce potential metabolites to enhance the power of host resistant against herbivores. From the information provided by molecular data it is proven that fungi are very much older, more than one billion years ago.This whole process is majorly depend on the bio activity of the endophytes. In recent most of the researchers are focused on the activity and ability of endophytic fungi which can produce various industrial, agricultural, pharmaceutical compounds [2-3]. The active substance produced by them can also be used as antifungal, antibacterial, antitumor, antidiabetic agents. The working on microbial products production of drugs has started once after invention of anticancer drugs form *Penicillium* [4]. The major relationship exist in between the endophytic fungi and the host plants are ranged from symbiotic to antagonistic or pathogenic or mutualistic[5].

Endophytic fungi represent an important and quantified component of fungal biodiversity and are known to affects plant diversity. Approximately, all vascular plant species established to harbor endophytic bacteriaor fungi. Moreover, the colonization of endophytes has already been recognized in marine algae and mosses and ferns [6]. The population of the endophyte is influenzed by the environment of the of the host from where they are growing. At present most of the plants like lichens, palms, large trees and even sea grasses can produce endophytic fungi [7]. Basidiomycets, Ascomycets and anamorphs are the mostly isolated endophytes. The fungi associated with Ericacea and Orchidaceae plants family are referred to as endophytes, mycorrhizae are one of the indistinct fungal species and some of the mutualistic fungi from mycorrhizal family which are identified to be isolated from the above plants. They are ubiquitous and occur in all known plants, including a broad range of host orders, families, genera and species, in ecosystems which is shrubs, ferns , mosses, lichens, grasses and deciduous and coniferous trees [8]. Based on the association of fungi with the plants the total number of fungi can be estimated using several methods. The magnitude of fungal diversity estimated about 1.5 million species, later revised by to 2.27 million. The fungal

species can vary diversely from each other but the endophytic fungi can be identified by using the modern techniques and tools[9-10].

2. CLASSIFICATION AND FEATURES OF ENDOPHYTIC FUNGI:

2.1.1 Class I

This class include Clavicipitaceous endophytes which represent a small number of phylogenetically related Clavicipitaceous species that are fastidious in culture and restricted to some grasses.

2.1.2 Class II

The fungi which do not form any mycorrhizal structures intracellularly and which forms a mycorrhizal colony in all parts of the plants also in seed coat are referred to as class II endophytes.

2.1.3 Class III

They differ from other class by transmission and occurrence. They follow horizontal transmission. They includes endophytic fungi isolated from woody and herbaceous angiosperms, vascular and non vascular plants in tropical forest.

2.1.4 Class IV

These endophytes are restricted towards plant roots and they have melanized septa. The melanized structure is nothing but a formation of inter and intra cellular hyphae in roots. This is majorly done by conidial ascomycetous fungi.

2.2 Natural Products from Endophytic Fungi

In early 1990's the anticancer drug called "Taxol" was discovered from *Taxomyces andreanae*. Before it was isolated from *Taxus brevifolia* and *Taxus wallinchiana.*And the drug compound penicillin was discovered from *Penicillium notatum* in 1928 by W.Flemming. These both compounds are isolated from fungi. This payed the way for search and discovery of drugs and other medicinal products from microbial origin [11]. These antibiotic and anti cancer drug influenced the new way isolating and discovering new drug compounds in biological origin [12]. From that instant scientist started searching the source of natural products to produce endophytes for example an antifungal agent Micafungin isolated from *Coleophoma empetri,* Rosuvasatatin used to treat dyslipidemias is isolated from*Penicillium brevicompactum andPenicillium citrinum*andMycophenolate used to prevent renal transplant rejection is isolated from *Penicillium brevicompactum*[13-15].

2.3 Biological Roles of Endophytic Fungi

The production of new bioactive secondary metabolites are defined as the most important ability of an endophytic fungi. Those secondary metabolites of endophytic fungi are used as important source in industrial, agricultural, and pharmaceutical industries. These natural products are unique in structure and they bioactively prevents from various diseases [15].

2.4 Endophytic fungi in tissue culture

In plant tissue culture endophytes play major role as a important product to the host plant. Producing axenic plants are the highly aimed result in tissue culture. Endophytic fungi starts growing from the cultured explant or tissue in the nutrient medium this is even possible after surafce sterilization by autoclaving and UV treament of the explant. This may look like a contaminants in tissue culture media but sometimes result in growth of some endangered species of microbes which can be conserved and preserved by tissue culture techniques [14,15].

2.5 Antiviral activity

Some of the antibiotics used to inhibit the viral growth are produced from endophytic fungi. These includes compounds which are clearly explained by NMR and Mass Spectrometry to be effective against virus growth like cytomegalovirus protease inhibitors and cytonic acids A and B. Some desert plants are capable of producing potent inhibitors of HIV-1replication source containing endophytic fungi [16-19].

2.6 Anticancer activity

Endophytes are capable of producing major group of anticancer drug such as Paclitaxel and some of its derivative. During cell division process the paclitaxel act as a guard and prevents the tubulin molecule from depolymerizing [16]. It is used to treat many human tissue damaging diseases and diseases with continuously proliferation and it is referred as the first billiondollar anticancer drug in the world. Another fermentation product from *Taxomyces andreanae* used as an alternative source for taxol. Some other bioactive metabolites such as kaempferol, podophyllotoxin and diterpenoids, guanacastane are found to be produced from endophytic fungi *M. fragilis* and *Cercospora sp*for the first time and are also used as a compound having anti cancer activity [17].

2.7 Antidiabetic activity

There are so many natural products which can be used as medicine is needed to be explored which is provided to us by the nature. The same way scientist have identified the endophytic fungi with hypolipidemic and antidiabeticactivity. This include the endophyic fingi produced by *Salvadoraceae* majory in *Salvadora oleoides.* The experiment was made possible with glucose loaded alloxan induced diabetic Wistar albino rats making them undergo fasting and the investigation proved to gain new antidiabetic from endophytic fungi in glucose tolerance test. They tends to reduce blood glucose level significantly[18].

2.8 Immunosuppressive activity

The immunosuppressive agent are the drugs used to prevent the immune sysytem which includes compounds like subglutinol A and B, which are produced by the endophytic fungus *Fusarium subglutinans*isolated from *T. wilfordii.* Nowadays these drugs are majorly used in transplant patients to prevent the allograft rejection. In future they are believed to be good source to treat autoimmune diseases likeinsulin dependent diabetes, and rheumatoid arthritis. The compounds isolated from *P. microsporepossess* such as the two pyrones: pestalopyrone and hydroxyl pestalopyrone and Pestaloside has phytotoxicproperties. Another good example is *Pseudomycins* which is an very effective antifungal compound and it majorly act against human pathogen, Candida albicans. The ingredients isolated from *streptomyces* are bioactive compounds also called as peptide antibiotics and they are very effective against both gram negative and positive bacteria because they naturally contains unusual aminoacids like L-hydroxy aspartic acid, L-chlorothreonine and both D-and L-diaminobutyric acid[18,19].

3.Nanooparticle

Nanoparticles are compounds or particles which can literally manipulate onto a macromolecule and condenses into smaller particles so that it can be used anywhere and can be stored anytime and anywhere [20]. They make the particles 10^{-3} times smaller than a normal molecule. These are most important tool of emerging science especially for biotechnology as it has made almost all our work simple and easier. It is truly multitasking as it cures disease it inhibits cancer and other vital disease. Nanomaterials are very important tools of medicine and biotechnology. Its whole concept is that shrinking the bulk material to a nano size which is more stable than the bulk materials. These nanoparticle possesses stable physical properties which makes then unique[21]

3.1 Nanotechnology

Nanotechnology is a widespread field in which the atoms or molecules are manipulated or the manipulation of matter with at least one dimension normally sized from 1 to 100 nm. The concept of the nanotechnology is making the molecules smaller so that it is easily penetrable and is easy to use .One nanometre (nm) is one billionth, or 10−9, of a meter. By examination, normal carbon-carbon bond lengths, or the dispersing between these particles in an atom, are in the range 0.12– 0.15 nm, and a DNA twofold helix has a width around 2 nm. Then again, the littlest cell living things, the microscopic organisms of the variety Mycoplasma, are around 200 nm long. These new marvels make nanotechnology particular from gadgets which are just scaled down forms of a comparable perceptible gadget; such gadgets are on a bigger scale and go under the depiction of microtechnology. Materials reduced to the nanoscale can indicate abundant properties contrasted with what they show on a macroscale and empowering novel applications. For example, substances which are opaque can be transformed into a transparent substance (copper); stable materials can turn flammable (aluminium); insoluble materials may wind up solvent (gold) [22].

A material, for example, gold, which is artificially inactive at ordinary scales, can fill in as a powerful synthetic impetus at nanoscales. A great part of the interest with nanotechnology originates from these quantum and surface wonders that issue shows at the nanoscale. Nanotechnology has adverse applications in medical and research fields it can transform the world of science and thinking to a different level it is used in drug delivery , imaging and as sensors it is also used as probes to detect any errors in machines. They are efficient in their biological activity and are used in purification of blood and as a good source for treating cancers. Nanotechnology has created a pathway for the cells to utilize medicines through nanoparticles. The general medication utilization and symptoms might be brought down essentially by storing the dynamic specialist in the bleak locale just and in no higher portion than required. Directed medication conveyance is planned to diminish the symptoms of medications with corresponding abatements in utilization and treatment costs. Medication conveyance centres around amplifying bioavailability both at particular places in the body and over some stretch of time. This can conceivably be accomplished by sub-atomic focusing by nanoengineered devices [23].

The major advantage of utilizing nanoparticles as therapeutic agentsis they are little gadgets which can be easily embedded inside the body and are less prominent in an intrusive way. Their biochemical reaction and response time are also considerably shorter. These gadgets are quicker and more touchy than regular medication delivery. The viability of medication conveyance through nanomedicine is to a great extent dependent on: an) effective exemplification of the medications, b) fruitful conveyance of medication to the focused-on locale of the body, and c) effective arrival of the medication.Nanotechnology might be utilized as a major aspect of tissue building to encourage duplicate or fix or reshape harmed tissue utilizing reasonable. nanomaterial-based frameworks and development factors. Tissue designing if fruitful may supplant regular medicines like organ transplants or counterfeit inserts. Nanoparticles, for example, graphene, carbon nanotubes, molybdenum disulphide and tungsten disulphide are being utilized as fortifying operators to create mechanically solid biodegradable polymeric nanocomposites for bone tissue building applications [23].

The expansion of these nanoparticles in the polymer network at low focuses (~0.2 weight %) prompts huge enhancements in the compressive and flexural mechanical properties of polymeric nanocomposites Possibly, these nanocomposites might be utilized as a novel, mechanically solid, light weight composite as bone implants. For instance, a substance welder was exhibited to meld two bits of chicken meat into a solitary piece utilizing a suspension of gold-covered nanoshells enacted by an infrared laser. This could be utilized to weld courses amid surgery. Another model is nanonephrology, the utilization of nanomedicine on the kidney[24].

4. Synthesis of Nanoparticles

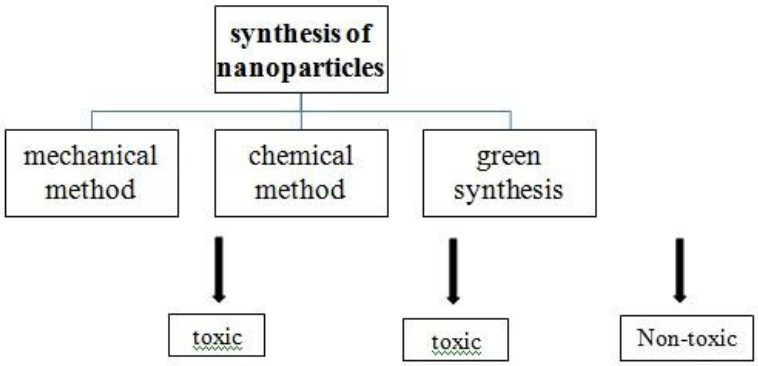
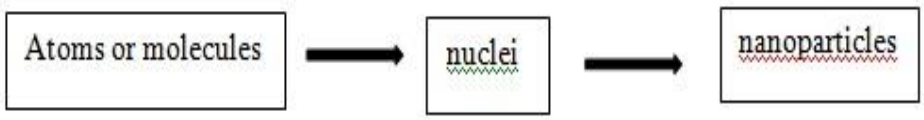


Fig.1: Various methods of nanoparticle synthesis

Mechanical methods includes ball milling, chemical etching, thermal or laser ablation sputtering. Chemical methods includes electrochemical precipitation, vapour deposition, atomic or molecular condensation, solid gel proces, aerosolpyrolysis, spray pyrolysis, laser pyrolysis. Green synthesis includes bacteria, fungus, plant extract [20-25]. Varius methods of nanoparticle synthesis is depicted in Figure 1.

4.1.Protocol employed in green synthesis of nanoparticles



This is the general protocol followed in green synthesis and chemical method of synthesis of nanoparticles.

4.2. Synthesis of nanoparticles from plant extracts

Currently usage of plant extracts or biological microoragnism for synthesis of nanoparticles are emerging these are considered as biosynthetic method with less toxicity when compared to chemical and physical method [25]. The synthesis of silver nanoparticles was the first successful result initially reported in *Pseudomonas stutzeri*, followed by this many works have been carried out on synthesis of semi conductor nanoparticles and metal ions from fungi, bacteria and plants also. In the need of eco friendly method for synthesizing noble metal ions, nanoparticles and product with less toxicity fungi and plant extracts have found to the best source of producing it they are very efficient in the production of silver nanoparticles [26]. In recent research one of the best gold nanoparticles was observed which are synthesized using the plant extract of lemongrass plant and it was found to have a triangular single crystal line with excellent shape-selective formation [27,28]. By modifying some experimental conditions the absorbance of those

nanostructures can be easily tuned and they posses strong near-infra red absorbance. Plants have many biological and medicinal application so their respective nanoparticles are referred to have same properties in nano size [29]. Thus by using the plant extracts and by varying the concentrations of them the shape of the nano structure is modified to spherical shape which leads to the optical properties with significant control of the nanoparticle in the solution[30-32]. Nanoparticle synthesis from plant extract is given in Figure 2.

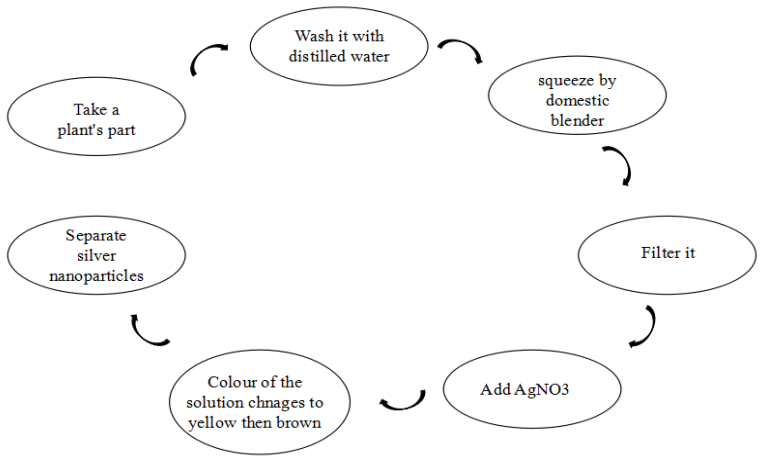


Fig.2: Protocol for synthesis of silver nanoparticles from plant extract

4.3. Synthesis of nanoparticles from endophytic fungi

At present the nano-revolution begins and made a huge need of nanoscale material with more safer side and which are needed to be synthesized eco-friendly [33]. The novel strategies are formulated for the synthesis of nanoparticles with the combination of many studies which includes biotechnology, materials chemistry, nanotechnologyto develop a product with exact control on its shape and size [34]. The emerge of microbial biosynthesisofnanomaterials is found to be he most eco-friendly method when compared to the chemicalsynthesis because the microbial synthesis of nanoparticles are reproducible and simple [35,36]. The major concern of biological synthesis of nanoparticles is for the overall toxicity caused or carried by the nanoparticles which is satisfied in this method thus they can be undoubtedly eligible intechnological and medical applications. In recent research many microoragnisms such as bacteria, algae and fungi can produce nanoparticles eitherextracellularly or intracellularly [37]. Figure 3 shows the production of silver nanoparticles from endophytic fungi. The fungi *Fusarium oxysporum* is majorly capable of producing silver nanoparticles with the cell asociated biosynthesis method. Currently some other species of fungi have also been studied such as *Penicillium fellutanum, Fusarium acuminatum, Aspergillus niger, Fusarium semitectum , Aspergillus flavus* and found have the capability of reducing the metal ions to their respective nanoparticles. Even with silver and gold nanoparticles endophytic fungi play major role in many application they are also capable of synthesizing some noble metals like zirconium and platinum nanoparticles in a biological manner[38,39,40].

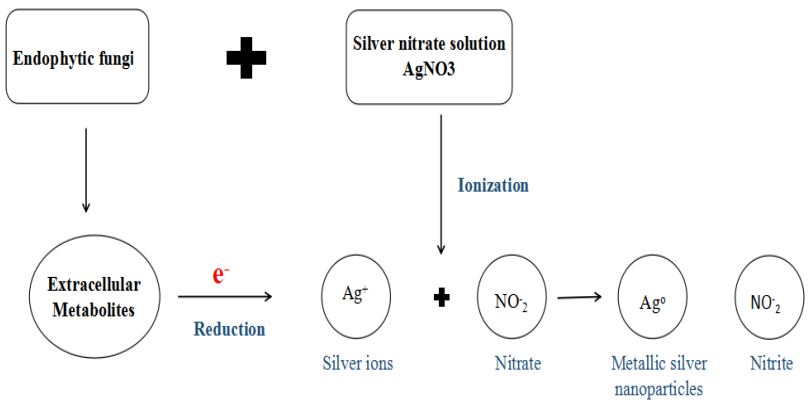


Fig.3: Protocol for synthesis of silver nanoparticles from endophytic fungi

5. CONCLUSION

Among all microbes fungi are referred to have the high potential for synthesis of nanoparticles using bio-synthetic method. They not only produce silver and gold nanoparticles but they are also capable of synthesizing platinum, zinc, silica nanoparticles. Sevaral species of *Fusarium, Aspergillus* and*Penicillium fellutanum*are referred as good source of producing silver and gold nanoparticles. Among these *Aspergillus species* are found to be capable of producing nanoparticles both extra and intracellularly. To elucidate the bioreduction by metabolic mechanism much resolution is needed. Thus at present much effort was taken to develop a process of green synthesis to synthesis nanoparticles. This vast variety of source can serve as a good drug compound, a good supplier of medication via nanoparticles, their derivaties can also be used as product in medicinal and technological application. In future if they are taken under preference in formulation of new drug compound using their bioactivity it will be a product with eco friendly nature and with less time consumption.

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