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### Synthesis of Silver Nanoparticles by using *Irpex lacteus* (Meruliaceae) a wood rotting Aphylophore

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#### ABSTRACT:

Nanotechnology has immense applications in therapeutics of human diseases. Aphylophoraceous fungi are known to cause wood rot of tree species. These pathogenic fungi produce extracellular enzymes which degrade wood and damage plant. These fungi can be used in productive way for producing silver nanoparticles. A common wood rotting fungus *Irpex lacteus* (Meruliaceae) was collected from tropical moist deciduous forest of Ratnagiri district. The fungus was cultured on Sabouraud Dextrose Agar medium. The mycelial mass was harvested and centrifused at 10000 rpm for 20 minutes to get cell free extract. The supernatant obtained was used for biosynthesis of silver nanoparticles. 0.1mM AgNO<sub>3</sub> solution is used as substrate. 40 ml of 0.1mM AgNO<sub>3</sub> solution is incubated with 10 ml of cell free extract of wood rotting fungus in a 100 ml conical flask at room temperature for 120 hours on orbital shaker. After 120 hours O. D. of reaction mixture was measured on UV-Visible spectrophotometer by taking 0.1mM AgNO<sub>3</sub> solution as a blank. SEM of reaction mixture was prepared. Both Spectroscopy and SEM analysis shown synthesis of Silver nanoparticles. Thus wood rotting aphylophorales can be used in constructive way for biosynthesis of silver nanoparticles.

**KEYWORDS:** 0.1mM AgNO<sub>3</sub>; *Irpex lacteus*; wood rotting aphylophore; Silver Nanoparticles.

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## **INTRODUCTION:**

Nanotechnology is new interdisciplinary branch of science dealing with design and synthesis particles ranging from 1-200nm in diameter. These particles have applications in various branches of Science. Nanotechnology has applications in many fields such as food, cosmetic, environmental conservation, health care, industries, electronics, drug delivery etc.<sup>1</sup>.

The term “nanoparticles” is used to describe a particle whose size ranges from 1nm to several nanometers, at least in one of the three possible dimensions. In this size range, the physical, chemical, biological properties of the nanoparticles change from the properties of both individual atoms and molecules and from the corresponding bulk material. The nanoparticles can be made from various chemical materials such as metals, metals oxide, silicates, polymers, and biomolecules<sup>2</sup>. Shape of nanoparticles vary, they may be spherical, cylindrical, rod shaped or polygonal. Generally the nanoparticles are designed with tailored surface modifications to meet the needs of specific applications. The huge amount of variations in nanoparticles arises from their wide range of chemical nature, shape and size<sup>3</sup>.

Silver nanoparticles are of most sought after because of their unique properties, which can be used to get antimicrobial applications, cytogenetic superconducting materials, cosmetic products and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles<sup>4</sup>. The most accepted chemical method for the synthesis of silver nanoparticles is chemical reduction by using a number of organic and inorganic reducing agents, physicochemical reduction, and radiolysis.

Silver nanoparticles (SNPs) have attracted specific attention due to their potential applications, in electronics, biosensors, cloth manufacturing, food storage, paints, sunscreens, cosmetics and medical devices<sup>5</sup>. SNPs have also a potent bactericidal and fungicidal activity and general anti-inflammatory effects. Further, can be used to improve wound healing, to develop dressings for wounds and antibacterial coatings<sup>6</sup>. Although ultraviolet rays, aerosol technology, laser ablation and photochemical reduction have been used successfully to produce nanoparticles, they remain expensive and involve the use of hazardous chemicals<sup>7</sup>.

Recently, there is growing attention to produce nanoparticles using environmentally friendly methods. This approach includes mixed valence polyoxometalates, polysaccharides, biological and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Silver nanoparticles are the metal of choice as they hold the promise to kill microbe’s effectively and effect on both extracellularly as well as intracellularly.

The microbial synthesis of nanoparticles is a unique approach as it combines nanotechnology and microbial biotechnology<sup>7</sup>. Some microorganisms, including bacteria, filamentous fungi and yeast, plays important role in the remediation of toxic metals through the reduction of the metal ions; therefore, these microorganisms could be employed as a nanofactories for the production of nanoparticles<sup>1</sup>.

Biological method based on use of fungi to produce nanoparticles is based on the fact that fungi produce extra cellular enzymes, which can be used in the reduction of metal ions to synthesise nanoparticles. Wood rotting fungi are ideal candidates for this purpose. These fungi grow on easily available substrate and secrete large amount of extra cellular enzymes which can be used for synthesis of SNPs<sup>8</sup>.

Present paper discusses eco-friendly synthesis of silver nanoparticles by using wood rotting aphyllphoraceous fungus *Irpex lacteus* (Meruliaceae) (Figure 1) and their characterization by using UV-Vis spectroscopy, and SEM analysis.

## **MATERIALS AND METHODS:**

### ***Isolation and culture of Irpex lacteus***

A segment of *Irpex lacteus* basidiocarp was surface sterilized by sequential rinsing into 70% ethanol for 30 seconds, 0.1% mercury chloride for 30 seconds, 0.5% sodium hypochlorite for 30 seconds, and 2-3 minutes with sterile distilled water. Then cut segment was placed on Petri dish containing Sabouraud Dextrose Agar (SDA). It is incubated at 28<sup>0</sup>C for 3-4 days, and monitored every day for the growth of fungal colony (Figure 2). The pure fungal culture was obtained on PDA plates.

### ***Mass culture of fungi***

For synthesis of SNPs, the fungus was cultured on large scale by transferring pure inoculum of *Irpex lacteus* in to Sabouraud Dextrose broth in 250 ml conical flask. It is incubated at 28±0.5<sup>0</sup>C, for 3 days on orbital shaker. After that, the mycelial biomass was removed by filtering media to obtain cell free extract (Figure 3). The filtrate that is cell free extract was centrifused at 10000 rpm for 20 minutes and supernatant was used further for the synthesis of SNPs.

### ***Assay for SNPs synthesis***

The reduction reaction for biosynthesis of silver nanoparticles was carried out by adding 0.5ml of 0.1M silver nitrate in 49.5 ml of distilled water so as to get the 1mM volume as the final concentration of silver nitrate in the reaction mixture. 40 ml of 1mM AgNO<sub>3</sub> solution is incubated with 10 ml of cell free extract of wood rotting fungus in a 100 ml conical flask at room

temperature for 120 hours on orbital shaker. After 120 hours O. D. of reaction mixture was measured on UV-Visible spectrophotometer by taking 1mM AgNO<sub>3</sub> solution as a blank. The formation of silver nanoparticles was monitored visually by observing the change in colour of the reaction mixture (Figure 4).



**Figure 1: *Irpex lacteus* in natural habitat**



**Figure 2: *Irpex lacteus* in culture**



Figure 3: Cell free fungal extract



Figure 4: Synthesis of SNPs

## RESULTS AND DISCUSSION:

Synthesis of SNPs is characterised by following way

### *Visual observations*

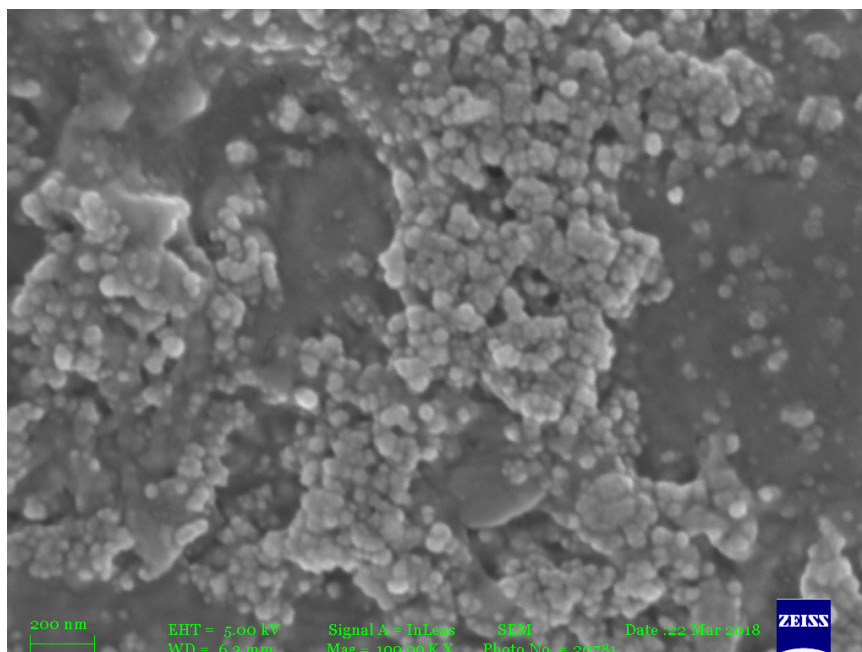
The synthesis of nanoparticles was initiated once the fungal culture was introduced into 1mM aqueous  $\text{AgNO}_3$  solution. Silver ions were reduced to silver nanoparticles. Reaction mixture develops dark colour in response to synthesis of SNPs. The intensity of colour change is due to rate of reduction reaction potential.

### ***UV visible spectroscopy***

The initial characterization of formation of silver nanoparticles was carried out by UV-Visible spectroscopy using a double beam spectrophotometer. Spectroscopy plays a very important role in the identification of different compounds and their qualitative and quantitative determination. Nothing can complement these characterizations now a days. The reduction of the  $Ag^+$  ions by the supernatant of the fungal culture in the solutions leads to formations of silver nanoparticles. The Optical Density (O. D.) was measured at 300nm-600nm wavelength. SNPs show maximum O. D. in between 400nm to 500nm wavelength.

### ***Scanning electron microscopy***

The morphological feature of synthesized silver nanoparticles from wood rotting fungal extract was studied by scanning electron microscopy. The shape and diameter of synthesized nanoparticles was identified using SEM analysis. SEM analysis done after drying the extract and dried powder was used for SEM images. Experimental result showed that the diameter of prepared nanoparticles was in between 90nm to 120 nm. The result showed that the particles were polygonal to irregular in morphology.



**Figure 5: SNPs in SEM**

### **CONCLUSIONS:**

SNPs synthesized by *Irpex lacteus*, a wood rotting Aphyllophoraceous fungus have variable size and shape. SEM analysis shown that SNPs are mostly polygonal in shape. Their size ranges from 90nm-120nm in diameter. UV-Vis spectroscopy results had shown that SNPs show

maximum O. D. in 400nm-500nm wavelength. Thus it is concluded that wood rotting aphylloraceous fungi can be potentially employed for synthesis of silver nanoparticles. Being an eco-friendly approach this method serves as a promising alternative to the traditional reduction routes to avoid usages of toxic chemicals. These nanoparticles have immense applications in pharmaceutical drug delivery systems and might be the future thrust in the field of medicines.

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