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BIOSYNTHESIS OF SILVER NANOPARTICLES USING SOIL FUNGI

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ABSTRACT

Bio synthesis of silver nanoparticles is a rapidly processing area of nanobiotechnology. In this paper Aspergillus flavus assisted extracellular synthesis of silver nanoparticles is reported. The silver nanoparticles were characterized by UV-Visible spectrophotometer. SEM studies showed the size of the silver nanoparticles to be in the range of 2-500nm. The nanoparticles showed antimicrobial activity against human bacterial cultures Proteus myxofaciens, Vibrio cholera, staphylococcus aureus.

KEYWORDS: AgNo3, *Aspergillus flavus*, UV-Visible spectrophotometer, *Proteus myxofaciens, Vibrio cholera, staphylococcus aureus.*

INTRODUCTION

Nanobiotechnology and nanotechnology are terms that refer intersection of nanotechnology and biology. Given that the subject is one that has only emerged very recently, nanobiotechnology serve as blanket terms for various related technologies. This discipline helps to indicate the merger of biological research with various field of nanotechnology. Concept that enhanced through nanobiology include nanodevices, nanoparticles and nanoscale phenomena that within the discipline of nanotechnology.

Nanotechnology is the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale. The concepts that seeded nanotechnology were first discussed in 1959 by renowned physicist Richard Feynman in his talk there plenty of room at the bottom, in which he described the possibility of synthesis via direct manipulation of atom. The term 'nano-technology' was first used by Norio Taniguchi in 1974 (sad syed 2013), thought it was not widely known. Nanomaterials are the field that takes a materials science-based approach on nanotechnology.

The application of silver nanoparticles greatly depends on their size. Aspergillus niger is one of local common fungal strains abundant in warmer soils of south india. Hence, in the present study an attempt is made to synthesize silver nanoparticles of very small size using Aspergillus niger **[1].** The possible mechanim of silver nanoparticles formation through nitrate reductase, characterization and the antibacterial and antifungal activity of the silver nanoparticles are reported **[2]** in the present investigation. A fungus is a large group of eukaryotic organisms that include microorganisms such as yeast and molds. As well as the more familiar mushrooms. These organisms are classified as kingdom fungi, which separate from plants, animals, protists and bacteria.

Fungal reproduction is complex, reflecting the differences in the lifestyles and genetic makeup with in this diverse kingdom of organisms **[3]**. Reproduction may occur in two well- differentiated stages with in the life cycle of a species, the teleomorph and theanamorph. ***Aspergillus flavus*** found worldwide. Ubiquitous in nature and is the second common cause of invasive aspergillosis next to *Aspergillus fumigates*. Has been implicated in pulmonary, systemic, sinus, ear and other infections. Most widely reported food-borne fungus and can be found colonizing decaying vegetation, crops and seeds.

MATERIALS AND METHODS

Chemicals

potato Dextrose agar [PDA], silver nitrate, Lactophenol Cotton Blue Stain, Potasium bromide [FTIR grade], Potasiumdihydrogen phosphate [KH2PO4], dipotassium hydrogen phosphate [K2HPO4], magnesium sulphateheptahydrate [MgSO4.7H2O], Ammonium sulphate [NH4]2SO4, yeast extract and glucose.

Sample collection

Soil sample were collected from different fields in Thanjavur district, Tamil Nadu, India .Soil samples were collected from 3 to 4 cm depth with help of sterile spatula. Samples were transferred in to sterile plastic bags and brought to the Molecular and Microbiology Research Laboratory and stored in a refrigerator at 4^0C up to further processing.

Media preparation

PDA Medium
Potato - 200gram
Dextrose - 20gram
Agar - 20gram
Distilled water - 1litre

Isolation of fungal cultures

Isolation of soil fungi was performed by serial dilution and spread plate method. One gram of soil sample was serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6}. A volume of 0.1 ml of each dilution was transferred aseptically to PDA plates. The sample was uniformly distributed by using a sterile glass spreader. The plates were incubated at room temperature for 3 days. The fungal isolate were further subcultured on the PDA plates in order to obtain pure culture. Pure isolates were maintained at 4^0C in refrigerator for further studies.

Colony characterization & Microscopic characterization

The fungal isolates were observed using hand lens and the colony morphology was recorded with respect to colour, shape, size and nature of colony. Fungal isolates were microscopically characterized by Lactophenol Cotton Blue mounting. The cell morphology was recorded with respect to spore chain morphology, hyphae and mycelium structure

Biosynthesis of silver nanoparticles

For the synthesis of silver nanoparticles, the biomass of each fungal isolates were prepared by growing aerobically in potato dextrose broth containing infusion of 250 g potato and 20 g dextrose per litre of distilled water. The inoculated flasks were incubated on orbital shaker (REMI – MODEL: RES 24) at 25 ± 2ºC and agitated at 120 rpm for 96 hr. The biomass was harvested after incubation by filtering followed by repeated washing with distilled water to remove any medium component from the biomass. 10g (wet weight) was brought in contact with 100 mL of sterilized double distilled water for 48 hr at 25 ± 2ºC in a 250 mL Erlenmeyer flask and agitated again at 120 rpm. The cell filtrate was obtained by filtering it through Whatmann filter paper No. 1 (GE Healthcare, Buckinghamshire, UK). The filtrate was treated with 1 mM AgNO3 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask. All experiments were done in three replicates.

The fungus was selected for the further studies for the production of silver nanoparticles. The fungus was inoculated in liquid media containing [g/l] KH_2PO_4 7.0, K_2HPO_4 2.0, $MgSO_4.7H_2O$ 0.1, $[NH_4]_2SO_4$ 1.0, yeast extract 0.6, and glucose 10.0. The flasks were incubated at 25^0C for 3 days in a rotary orbital shaker at a speed of 150rpm. The biomass was harvested after 72 hours of growth by sieving through a plastic sieve. The biomass was washed with sterilized distilled water to remove any medium component. 20g of biomass [fresh weight] was mixed with 200ml of deionized water in a 500ml Erlenmeyer flask and agitated in the same condition for 72h at 25^0C. After the incubation, the cell filtrate was obtained by passing it through Whatmann filter paper no.1. Filtrate was collected and used further for nanoparticles synthesis. For the synthesis of silver nanoparticles, 50ml of 1mM $AgNO_3$ solution was mixed with 50ml of cell filtrate in a 250ml Erlenmeyer flask and agitated at 25^0C in dark. Control [without the silver ion, only biomass] was also run along with the experimental flask. The aliquots were also characterised using microscopy methods as follows.

CHARACTERIZATION OF SILVER NANOPARTICLES.

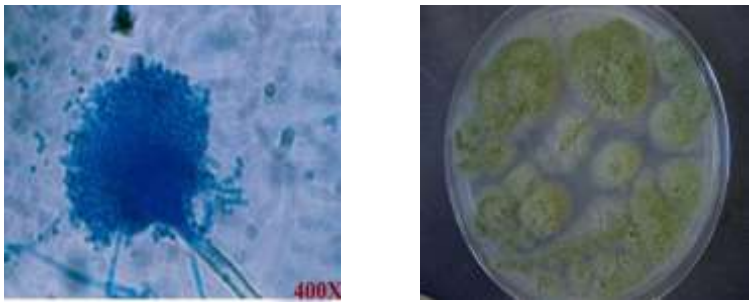
UV-visible spectroscopy analysis:

Change in colour of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. The bio-reduction of precursor silver ions was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (CARY-100 BIO UV-Vis Spectrophotometer; Varian Inc., Palo Alto, CA, USA) at a resolution of 1 nm. UV-Visible analysis of several days old samples was also carried out to check the stability of synthesized AgNPs.

RESULTS

FUNGAL ISOLATION AND IDENTIFICATION:

Soil samples were collected from various fields in Thanjavur District. Isolation of soil fungi was performed by serial dilution and spread plate method. One gram of soil samples was serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6}. A volume of 0.1 ml of each dilution was transferred aseptically to PDA plates. The plates were incubated at room temperature for 3 days. Pure isolates were maintained at 4^0c in refrigerator for further studies. The fungus morphological structure, shape and size were characterized under bright field microscope.



Left: Microscopic view on Aspergillus flavus
Right: plate view on Aspergillus flavus

SLILVER NANOPARTICLES PRODUCTION:

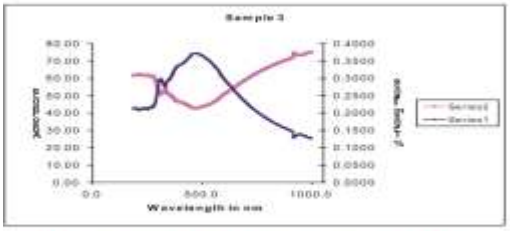
The fungus was selected for the further studies for the production of silver nanoparticles. The biomass was harvested after 72hours of growth by sieving through a plastic sieve. For the synthesis of silver nanoparticles, 50ml of 1Mm AgNO3 solution was mixed with 50ml of cell filtrate in a 250ml Erlenmeyer flask and agidated at 250c in dark. Silver nitrate (AgNO3), upon incubation with the fungal filtrate, turned dark brown color, while the positive and negative control flasks remain as such during the 72h incubation period. The colour change was observed on various time duration and the production of silver nanoparticles.

Synthesis of silver nanoparticles Days- 0-14

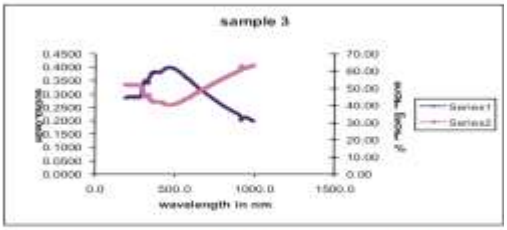
UV-VISIBLE SPECTROPHOTOMETER ANALYSIS:

Change in colour of the cell filtrate incubated with silver nitrate solution was visually observed over a period of time. The bio-reduction of precursor silver ions was monitored by sampling of aliquots at different time intervals. UV-Visible analysis of several days old samples was also carried out to check the stability of synthesized AgNPs.

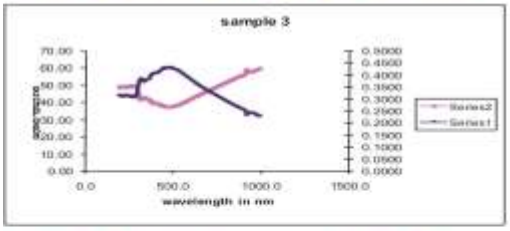
Characterization of Silver Nanoparticles by UV - visible Spectroscopy



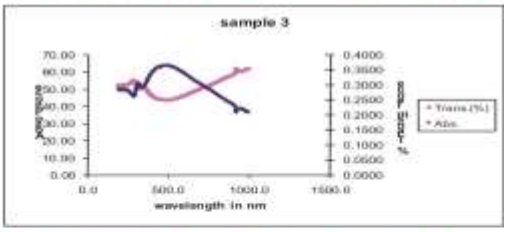
UV - Visible Analysis - 3rd Day



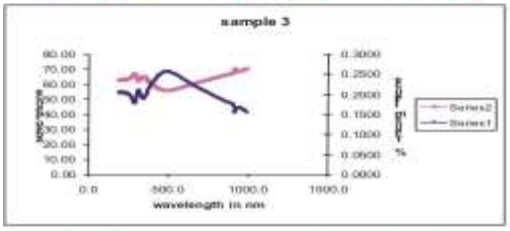
UV - Visible Analysis - 5th Day



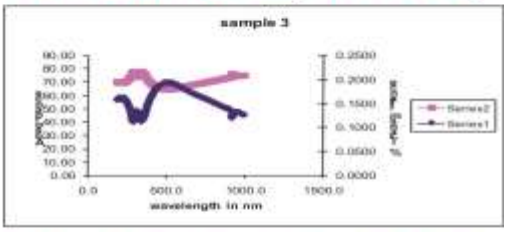
UV - Visible Analysis - 7th Day



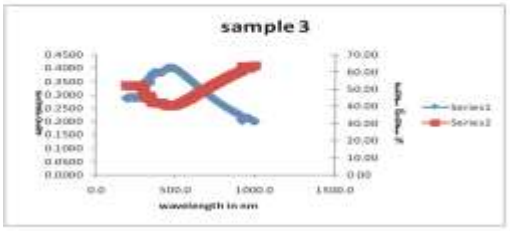
UV - Visible Analysis - 9th Day



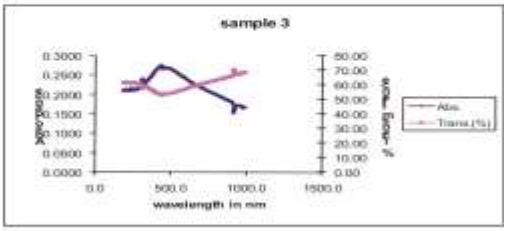
UV - Visible Analysis - 11th Day



UV - Visible Analysis - 12th Day



UV - Visible Analysis - 13th Day



UV - Visible Analysis - 14th Day

DISCUSSION

Fungal cultures were isolated from the soil samples collected from various agricultural lands in Thanjavur district, Tamil Nadu, India. The fungal isolates were characterized on the basis of colony characteristics and microscopic appearance **[4].** *Aspergillus sp.* was further selected for the biosynthesis of silver nanoparticles because of very few reports only reported the synthesis of silver nanoparticles using *Penicillium* sp. The present study deviates from **[5]** have reported the biosynthesis of silver nanoparticles using aqueous extract of *Penicillium brevicompactum* WA 2315.

Silver Synthesizing Fungi

The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp. or *Aspergillus fumigatus*has been reported. A novel biological method for synthesis of silver nanoparticles using *Verticillum* was proposed by **[6].** He was suggested two-step mechanism for synthesis of silver nanoparticles **[7].**

Recently has reported the synthesis of silver nanoparticles using white rot fungus *C. Verscolar* **[8].** Various fungi such as *Fusariumoxysporum*, *Trichodermareesei*and *Trichodermaviride*which contain the hydrogenase enzyme which was demonstrated with washed cell suspensions that had been grown aerobically or anaerobically in a medium with glucose and salts amended with nitrate. The nitrate reductase was apparently essential for ferric iron reduction. Many fungi that exhibit these characteristic properties, in general, are capable of reducing *Au* (III) or *Ag* (I).

The Erlenmeyer flasks with the isolated fungi *Aspergillus flavus*. The broth was pale yellow color before the addition of $AgNO_3$ ions and this change to a brownish color on completion of the reaction with Ag+ ions for 28 h. The appearance of a yellowish-brown color in solution containing the biomass was a clear indication of the formation of silver nanoparticles in the reaction mixture. The production of Nanoparticles by the fungi and their stability was continuously monitored on 3rd, 5th, 7th, 9th, 11th, 13th, 14th and 15th respectively.

The UV-Vis spectrum of the surface plasmon resonance *reaction* vessels at different times of reaction is presented in. **[9]** reported that marine bacterium also produce nanoparticles, it was observed that the NP–SH levels in the silver-exposed culture were consistently higher (261% on an average) than in the unexposed culture. The present investigation greatly deviates from the above mentioned work that the production of silver nanoparticles started after few hours from the addition of 1mM $AgNO_3$ and continued up to 15th day of incubation, so this leads to the confirmation that the production ability depends upon the environmental conditions where the plates are being incubated (37°C/Shaking incubator) and the nature of the organism. The present study also correlates with (**Saeed Moharrer, *et al.*, 2012),** who also reported that the *Aspergillus* species are efficient producers of Silver nanoparticles.

The present study correlates with **(Maribel G. Guzman),** reported UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The absorption spectrum of the pale yellow-brown silver colloids prepared by hydrazine reduction showed a surface Plasmon absorption band with a maximum of 418 nm indicating the presence of spherical or roughly spherical Ag nanoparticles.

SUMMARY AND CONCLUSION

Biologically synthesized nanoparticles are environmental friendly, cheap and completely safe, so used widely in different application aspects. Nanoparticles using soil derived fungus have received attention in the recent times as it is a simple and economical method. Several microorganisms from bacteria to fungi and higher plants have been reported to synthesize inorganic material either intra or extra cellularly and thus to be potentially utilized as eco-friendly nanofactories**.** In the present study, AgNPs were synthesized extra cellularly by using fungal extract of *Aspergillus flavus*. The ability to synthesize AgNPs as potential anti-bacterial agents using *Aspergillus flavus* is highly promising for the green, sustainable production of nano-metals. The unique properties of silver nanoparticles make them ideal for numerous technologies including biomedical, materials, optical and antimicrobial applications, as well as use in nano-toxicologystudies. To be potentially utilized as eco-friendly nanofactors.

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