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Biosynthesis of silver nanoparticles using *Sphaerulina albispiculata* and evaluation of antibacterial activity

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ABSTRACT

The present study six fungi were isolated from soil by spread plate technique. Among them Aspergillus terreus, Aspergillus sydowi, Aspergillus fumigatus, Penicillium funiculosum and Sphaerulina albispiculata and Masoniella grisea was identified by Lactophenol cotton blue mounting. To induce the biosynthesis of silver nanoparticles ($AgNO_3$) using Sphaerulina albispiculata and Masoniella grisea and evaluated their antimicrobial potential. The characterization of silver nanoparticles on these fungi by UV – FTIR and SEM analysis to confirm the reduction and it is believed that protein might have played an important role in the stabilization of silver nanoparticles. The synthesized $AgNO_3$) were found to be extracellular, polydispersed spherical or hexagonal particles ranging from 6-12 nm in size. Antimicrobial activity was performed using a agar well diffusion method against Vibrio cholerae ,Staphylococcus aureus and Salmonella typhi.

Keywords: Biosynthesis of silver nanoparticles ($AgNO_3$), UV – FTIR, SEM, Fungi, Antibacterial activity.

INTRODUCTION

Nanotechnology is emerging field of science which involves synthesis and development of various nanomaterials. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size, dependent interaction of silver nanoparticles with bacteria nanoparticles of silver have thus been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials. However the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth [6].

Biologically synthesized silver nanoparticles have many applications, as in spectrally selected coatings for solar energy absorption, as intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions and in bio-labelling. Silver has been recognized to have inhibitory action on microbes present in medical and industrial process [7].The most important application of silver and silver nanoparticles is in topical ointments to

prevent infection against burn and open wounds. Chemical synthesis of nanoparticles leads to presence of traces of toxic chemical adsorbed on the surface which is undesirable in the medical applications of nanoparticles.

The filamentous fungi possess some advantages over bacteria in nanoparticles synthesis, as most of the fungi are easy to handle, required simple nutrient, possess high wall-binding capacity, as well as intracellular metal uptake capabilities [1]. This study involves the biological synthesis of silver nanoparticles using fungi and the characterization of the synthesized silver nanoparticles by UV - Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopic (SEM) analysis.

MATERIALS AND METHODS

Collection of soil samples

The samples were collected from mangroves at Muthupet, Thanjavur district. Soil samples were collected employing sterile soil augers, hand trowel and polythene bags [3]. The soil was dug out using augers up to 0-20cm depth and was immediately scooped into sterile polythene bags using the hand trowel.

Isolation of Fungi

The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.5ml volumes were pipetted onto potato dextrose agar (PDA) and incubated at 30˚C for three days. Fungi was isolated from the mixed isolates from each plate and subcultured on PDA. Subculturing was continued until a pure isolate was obtained.

Characterization and Identification Isolates

Colony morphology and microscopic examinations of the various isolates of pure cultures were used to determine the reproductive and vegetative structures. Consequently, identification was done using [9]. Spore identification was achieved by reference to Spore atlases of [5, 4].

Biosynthesis of Silver Nanoparticles

The fungal mat was washed thrice in deionized water to remove the unwanted material. Approximately 3.5gm of fungal mat taken in a conical flask containing 100ml deionized water 10^{-1} mM $AgNO_3$ was added then it was incubated 37˚C for 3 days. After incubation period observed colour change. The nanoparticles were characterized by UV-vis spectroscopy, Fourier transform infra red (FT - IR) spectroscopy and scanning electron microscope (SEM) analysis.

Characterization of Synthesized Silver Nanoparticles

Ultra violet spectroscopy

The bioreduction of pure $AgNo_3$ are monitored using UV-Vis spectroscopy at regular intervals. During the reduction, 0.1ml of samples was taken and diluted several times with Millipore water. After dilution, it was centrifuged at 800 rpm for 5 minutes. The supernatant was scanned by UV-300 spectrophotometer (UNICAM) for UV – Vis 1601 Schimodzu spectophotometer, operated at a resolution of 420nm.

FT - IR (Fourier Transform Infrared Spectroscopy)

A known weight of sample (1 mg) was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2 mm internal diameter micro-cup and loaded onto FTIR set at 26°C ± 1°C. The samples were scanned using infrared in the range of 4000–400 cm-1 using Fourier Transform Infrared Spectrometer (Thermo Nicolet Model-6700). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

Scanning Electron Microscopy (SEM)

For SEM, the silver nanoparticle synthesized using fungi was allowed to dry completely and grounded well to a powder specimen is normally required to be completely dry. Since the specimen is at high vacuum. Living cells and tissues and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize. Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutraldehyde. The fixed tissue is then dehydrated. The dry specimen was mounted on a specimen stub using an adhesive which as epoxy

resin or electrically-conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

Antibacterial activity of normal strain and silver nanoparticle synthesized strain (*Sphaerulina albispiculata*)
Preparation of Extract
1 gm of silver nanoparticle synthesizing fungi were kept in the 10ml of solvents namely aqueous, n-Butanol and methanol, silver nanoparticle synthesizing fungi was grounded well with the help of mortar and pestle. The grained fungus was filtered through whatmann No.1 filter paper and the supernatant was collected, and stored for antimicrobial screening purpose.

Screening of Antibacterial activity
The antibacterial activity of the aqueous and organic solvents (n-butanol and methanol) extracts from the normal strain and silver nanoparticle synthesized strain (*Sphaerulina albispiculata*) were tested against the selected human pathogenic bacteria. The sterilized Nutrient agar medium was poured into each sterile petriplate and allowed to solidify using a sterile cotton swab. Fresh bacterial culture was spread over the plates by following spread plate technique. A well was cut on the solidified agar. The solvent extract was added into the each well. All the plates were incubated at 37ºC for 24-48hrs. After the incubation, the zone of inhibition was observed.

RESULTS AND DISCUSSION

The present study, number of fungi isolated from the soil sample. Only six fungi were identified, such as *Aspergillus terreus, Aspergillus sydowi Aspergillus fumigates, Penicillium funiculosum, Masoniella grisea and Sphaerulina albispiculata. Masoniella grisea* and *Sphaerulina albispiculata* were predominantly found in soil sample (Plate-1). These fungi were selected for the biosynthesis of silver nanoparticles.

Biosynthesis of Silver Nanoparticle Production
Fungal mat of S*phaerulina albispiculata* and *Masoniella grisea* was mixed with silver nitrate solution and incubated in room temperature. The appearance of brown color was due to the excitation of surface Plasmon vibrations. The control shows no change in color of the mixture when incubated in the same conditions. The production of silver nanoparticles is higher in *Sphaerulina albispiculata* which sources dark brown colour than *Masoniella grisea* (Plate-2). [2] reported that, cell free filtrate of *Penicillium* sp. was mixed with silver nitrate solution and incubated in dark in rotary shaker samples shows changed in colour from almost colourless to brown this is a clear indication of the formation of silver nanoparticles in the reaction mixture. The intensity of the colour was increased during the period of incubation. The appearance brown colour was due to the excitation of surface Plasmon vibrations.

Characterization of Silver Nanoparticle by UV-Vis Spectroscopy
Biosynthesis of silver nanoparticles using S*phaerulina albispiculata* was monitored in the UV – vis spectrophotometer. The UV- vis spectra was recorded from the silver nitrate *Sphaerulina albispiculata*. UV- vis spectra was recorded at 420nm after 24, 48 and 72 and 96 hours incubation (Fig-1). In previous study synthesis of colloidal silver nanoparticles was initially performed by UV-visible spectroscopic analysis. In UV- visible spectrum, a strong peak was observed between 400-200 nm, indicate the presence of silver nanoparticles [10].

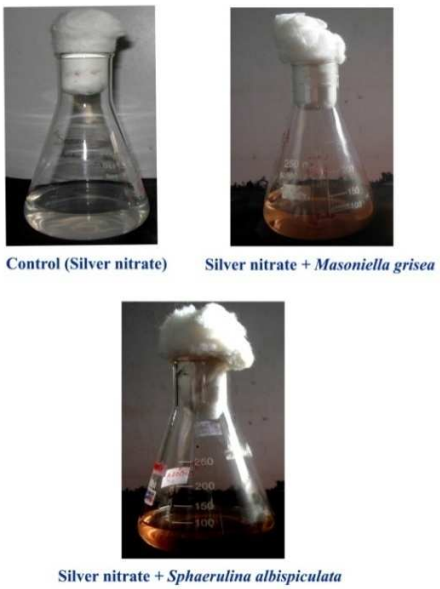
Identifications of functional groups using FT-IR
FT-IR measurements of the freeze dried samples were carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of silver nanoparticles. The amide linkages between amino acid residues in proteins give rise to well known signatures in the infrared region of electromagnetic spectrum (Table-1& Fig-2).The lyophilized nanoparticles samples were analyzed in FT - IR to identify the possible biomolecules responsible for the reduction of the Ag^{+} ions by the cell filtrate. The representative spectra of nanoparticles obtained manifest absorption peaks located at about 3843.68cm^{-1} (– NH group of amines). 3597.73cm^{-1}(- OH group of phenols), 2080 65cm^{-1} (aromatic – CH stretching) 1631.66 cm^{-1} (-NHCO of amide) and 767.16cm^{-1} (C – CI) [8].

Scanning Electron Microscope (SEM)
Fungal species were inoculated in the silver nitrate was selected and it was characterized by scanning electron microscope. Scanning electron microscope analysis was used measure the size of silver nanoparticles. In this

analysis the size of silver nanoparticles 80µm and120µm, size silver nanoparticle obtained as S*phaerulina albispiculata* (Fig-3). In previous study the size of synthesized nanoparticles was found to be 58.35 ± 17.88nm by SEM analysis [11].

Plate-1.Colony morphology and microscopic view of S*phaerulina albispiculata* and *Masoniella grisea*

Plate-2 Synthesis of silver nanaparticle Production from fungi



Screening of antibacterial activity by agar well diffusion method

The organic solvents extracts of S*phaerulina albispiculata* shows better zone of inhibition 12mm, 8mm, 6 mm in n-Butanol with *Vibrio cholerae*, *Salmonella typhi* and *Staphylococcus aureus* than methanol (Fig-4). There are no significant results in aqueous extracts of *Sphaerulina albispiculata*. The results were compared with silver synthesized strain S*phaerulina albispiculata*. Silver synthesized S*phaerulina albispiculata* shows better zone of inhibition (Fig-5). It was observed in n-Butanol as 10 nm in diameter for *Vibrio cholerae* and S*taphylococcus aureus* and the methanol extracts shows moderate activity against tested pathogens 10 mm, 5 mm in diameter. The aqueous extracts of silver synthesized S*phaerulina albispiculata* shows significant results as 7.5mm, 7.5 mm, and 6mm rather than n-Butanol and methanol.

In previous study, the biological synthesis of silver nanoparticles was found to be most active against the clinically isolated human pathogenic bacteria. The results proved that silver nanoparticles showed maximum activity at least concentration, which revealed silver nanoparticles as novel antibacterial agent [12].

Fig -1 Characterization of Silver Nanoparticles by UV-Vis Spectroscopy in 420nm

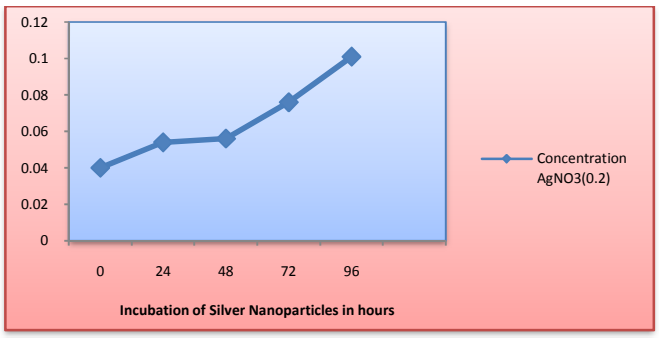


Fig-2 Detection of various functional groups by FT-IR from *Sphaerulina albispiculata*

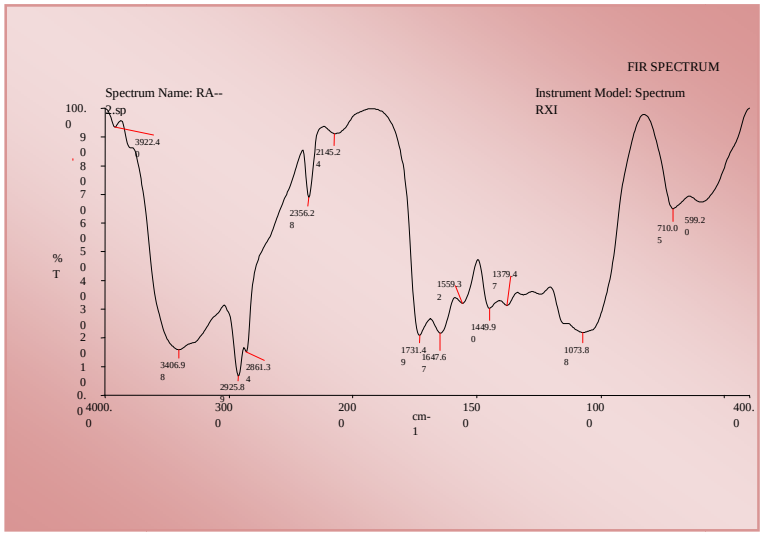


Table-1 Detection of various functional groups by FT-IR from *Sphaerulina albispiculata*

S. No	Group frequency cm^{-1} of the sample	Functional group assignment
1.	3406.98	N-H stretch, primary two bands, amine N-H stretching
2.	2925.89	Chelating compound Co-H stretching vibration free OH
3.	2861.34	C-H alkalines, C-H stretching vibrations two band (aldehyde)
4.	2356.28	Hydrocarbon chromophone, C-H stretching (Alkane)
5.	2145.24	-N=C=N- stretching vibrations, diamides
6.	1731.49	Cyclic, βlactams, dilute solution
7.	1647.67	C-C alkene / ketone stretching β dilution, -N=N- stretching
8.	1559.32	N-H, amine salt, βdiketone, primary amide – N-H, Coo- aromatic
9.	1449.90	Aromatic
10.	1379.47	Coo- anion, OH – Phenol (sulfonyl chlorides)
11.	1073.88	(C-F) Halogen Compound C- X stretching Vibrations
12.	710.05	(C-Cl) Halogen Compounds C-X stretching vibration
13.	599.20	C-Br Halogen compounds, C-X stretching vibration

Fig -3 Scanning Electron Microscope (SEM)

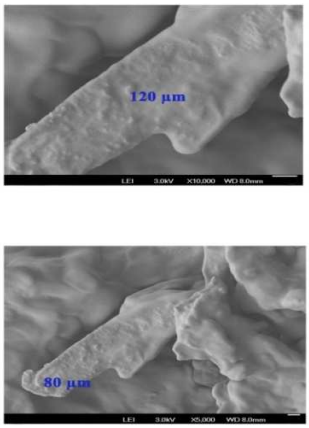


Fig -4 Antibacterial activity of normal strain *Sphaerulina albispiculata*

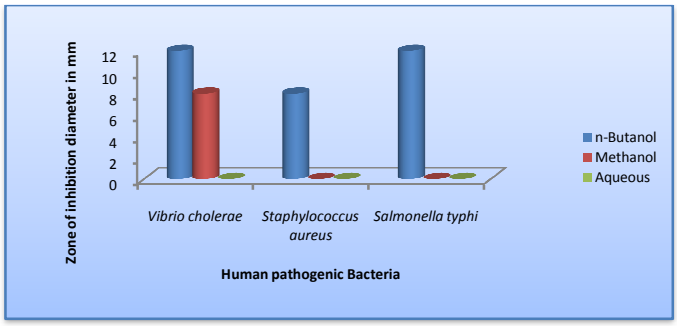
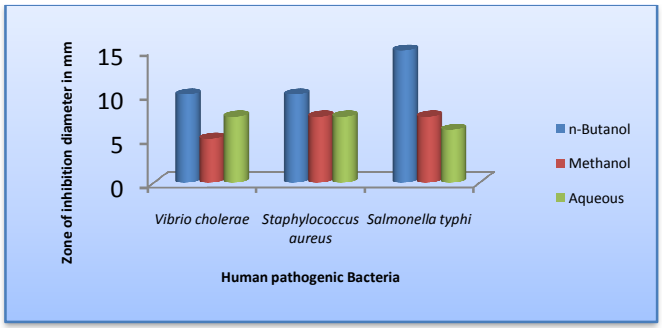


Fig -5 Antibacterial activity of silver nanoparticles synthesized *Sphaerulina albispiculata*



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