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Comparative analysis of antibacterial potency of AgNPs synthesized from airborne fungi with Oxacillin drug

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ABSTRACT

Silver nanoparticles are used to prevent and eradicate different bacterial disease are well known for long time. During recent scenario, bacteria mould themselves to resist varied antibiotics based on their peculiar characteristics and known as super bugs. In the present study, a supportive approach was carried out to synthesize silver nanoparticles (AgNPs) from two airborne allergenic fungi; Alternaria chlamydospora and Alternaria sp. The occurrence of yellowish brown color in the conical flask suggested the formation of AgNPs. The fungal culture filtrate with $AgNo_3$ altered the solution into brownish color during the reaction. The silver nanoparticles were characterized by Uv-Vis spectrophotometry, Field emission scanning electron microscope (FESEM). Size of the nanoparticles measured between 20nm to 40nm by FESEM. The synthesized silver nanoparticles were subjected to their characterization by X-ray diffraction (XRD) technique to determine the metallic nature of nanoparticles. The pattern showed that silver nanoparticles have been formed resulting in the diffraction peaks in between 38 to 77 confirming the metallic nature of nanoparticles and peaks were specific for the silver nanoparticles. AgNPs synthesized from both Alternaria species showed good antimicrobial activity against the selected bacterial species, Bacillus cereus, Staphylococcus aureus, E. coli, Proteus vulgaris and Vibrio cholerae. The antibacterial efficacy of AgNPs combined with Oxacillin was found prominent in the study, the combined formulation of AgNPs of A. chlamydospora and Oxacillin was recorded with the best in comparison to the combined form between Oxacillin and AgNPs of Alternaria sp.

Key words: AgNPs, *Alternaria chlamydospora*, Oxacillin, Antibacterial potency

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INTRODUCTION

Silver nanoparticles combined with antibiotics are found to be good antibacterial drugs in order to retard the bacterial growth and epidemics. Silver generally used in medicine for its antimicrobial properties from the early of twenty first century and more recently has been used in wound dressings and catheters. As the antimicrobial properties of silver have been known for long time, but we have only recently begun to understand the mechanisms by which silver inhibits the bacterial growth. The current challenge is to measure its (AgNPs) activity, to understand the mechanism of action at molecular level and also to determine the effective concentrations for therapeutical use. Based on the current report, bacteria are becoming resistant to antibiotics and within no time will lead to outbreaks of superbugs like methicillin resistant *Staphylococcus aureus* in hospitals and communities. Lack of new antibiotics being licensed for use and so alternative therapies are on research for their alternate antibacterial properties. Nanomaterials have put a great attention in recent days by their promising interdisciplinary fields of science which offers valuable nanomaterials of wide application in the range of areas, including catalysis, mechanics and

biomedical sciences [1]. Comparison to physical and chemical process, biological synthesis has an interest because of the necessity to develop new cost-effective as well as efficient techniques. More people categorized that the biological synthesis of nanoparticles by bacteria, fungi, yeast and several plant extracts have original ability to reduce metal ions [2] in easy way. Silver nanoparticles (AgNPs) have drawn special attention owing to its immense potential as antimicrobials in health care applications. It is good to synthesize silver nanomaterial, since they are nontoxic to the human body at low concentrations and they have broad spectrum antibacterial efficacy [3]. Silver ions are very sensitive and are known to bind to the vital cell components, inducing cell death [1]. Fungal isolates have the ability to resist environmental stresses and have the capability to grow in presence of high metal concentrations [4]. In our study, biosynthesis of silver nanoparticles by extracellular method from two airborne fungi; *Alternaria chlamydospora* and *Alternaria* sp. was made and their characterization was also done with the evaluation of antibiogram against different bacteria, *Bacillus cereus, Staphylococcus aureus, E. coli, Vibrio cholerae* and *Proteus vulgaris* with their AgNPs. The antibacterial assay of synthesized silver nanoparticles combined with Oxacillin was also carried out comparing the effects of nano-silver alone and the combined form against the above said microbes.

MATERIALS AND METHODS

Isolation of *Alternaria* spp

Enumeration and identification of airborne fungi were made from indoor and outdoor environments of vegetable market of Vaniyambadi by Burkard's volumetric sampler on agar plates. Appropriate pure culture method was formulated to isolate and identify *Alternaria chlamydospora* and *Alternaria* sp. from the mixed fungal culture on Sabouraud Dextrose agar plates [5, 6]. The plates containing both the *Alternaria* spp were incubated at 25±3^{o}c for 3-7 on pure culture and then stored in refrigerator at 4°c for further studies.

Synthesis of silver nanoparticles

Both the *Alternaria* species were subjected to biosynthesis of silver nanoparticles. Fungal biomass was grown aerobically in Potato dextrose broth (PDB) and incubated at 25 ^{o}c in a shaker at 140 rpm for 72 hours. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed with distilled water to remove all residual media components. The resulting fresh and clean biomass was taken into the Erlenmeyer flasks, containing 100ml of deionized Milli-Q water. The flask was again incubated at 25 ^{o}c in a shaker at 140 rpm for 72 hours. The biomass was filtered again with Whatman filter paper No.1 and the cell free extract was used in the following experiment. 1mM $AgNo_3$ was prepared and 50ml was added to the cell free extract and kept in a dark condition for 48 hrs.

Characterization of silver nanoparticles (AgNPs)

The culture filtrate with the $AgNo_3$ in the flask was observed for color change and maximum absorbance was analyzed using Uv-Vis spectrophotometer. One ml of the sample supernatant was taken after 24hours and absorbance was measured by using UV-visible spectrophotometer between 300-600nm. FESEM analysis was also used to determine the surface morphology and particle size of the silver nanoparticle. The AgNPs were sonicated and followed by centrifugation at 15000 rpm for 20 minutes. In order to process the FESEM analysis, the samples were sonicated to get the uniformity and better observation. Later the supernatant were discarded and pellet was washed with the Milli-Q water for three to four times. Later on the sample were transferred into the Petriplate and dried for about two hours at 50^{o}c, after that the samples were subjected to FESEM analysis.XRD analysis was used to determine the crystalline and metallic nature and face centered cubic structure of silver nanoparticle. The sample was prepared by centrifugation of the silver nanoparticle solution at 15000 rpm for 20 minutes for XRD analysis. The pellet was washed only with Milli-Q water three to four times and dried in petriplates. The powder form of the sample was subjected to XRD analysis at International Research Centre, Sathyabama University, Chennai, India.

Antibacterial study of AgNPs

The silver nanoparticles were subjected for its antibacterial assay by disc diffusion method [7]. The antimicrobial activity of the prepared silver nanoparticles from both *Alternaria* spp was tested against the pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Proteus vulgaris* and *Vibrio cholerae*. The Oxacillin was taken separately as control parallel to the AgNPs to find a comparative assessment of the antibiotic efficacy over the pathogenic bacteria. The combined effects of both the AgNPs with antibiotics were used to find out combine effect against all bacterial strains.

RESULTS AND DISCUSSION

The airborne fungal isolates, *Alternaria chlamydospora* and *Alternaria* sp. used in this study were isolated from indoor air of the vegetable market and used for the biosynthesis of silver nanoparticles. AgNPs were synthesized by the reaction of Ag+ ions from $AgNo_3$ with the supernatants under dark conditions. After 48 h incubation, appearance of yellowish brown color in the conical flasks indicated the formation of AgNPs [8]. The supernatants of the *Alternaria* spp cultures changed the solution to a brownish color upon completion of the 24 h reaction with Ag+.

Characterization of AgNPs

In the recent study, AgNPs were characterized by Uv-vis spectroscopy, which is proved as very useful for the analysis of nanoparticles. Uv-Vis spectra, a strong surface plasmon resonance were centered at approximately 420nm and 430nm indicating the presence of silver nanoparticles. When the silver ions come in contact with the fungal biomass, the nitrate reductase enzyme secreted by the fungus may cause the reduction of silver ions into silver nanoparticles [9]. Field emission scanning electron microscopy (FESEM) was used to understand the surface topology and the size of silver nanoparticles. Analysis of AgNPs by FESEM showed spherical shaped silver nanoparticles which were well dispersed within the diameter ranges of 30 nm and 40 nm. The synthesized silver nanoparticles were further characterized by X-ray diffraction (XRD) technique to determine the metallic nature of nanoparticles. The XRD pattern clearly showed that silver nanoparticles have been formed resulting in the diffraction peaks in between 38 to 77 confirming the metallic nature of nanoparticles and peak was specific for the silver nanoparticles. Our obtained results were found similar to the earlier studies made by the following workers [10, 11, 12]. Abeer and his coworkers [13] reported the synthesis of silver nanoparticles from *Aspergillus terreus* strain KC46206. The biologically synthesized nanoparticles were characterized by UV-Vis spectroscopy which showed the absorption peak at 420nm. The nanoparticles were further characterized by FTIR, XRD, SEM and TEM, which showed that the particle size was in the range 20 to 40nm, spherical and well dispersed.

Antimicrobial activity of AgNPs

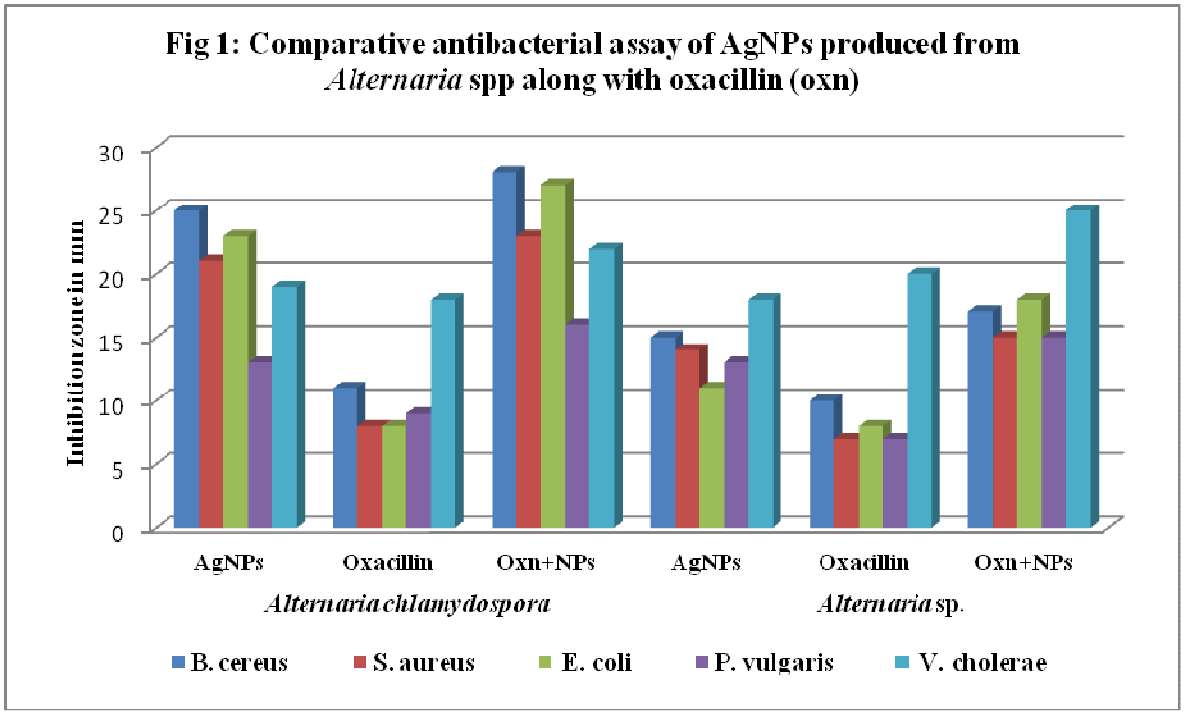
The antibacterial activity of synthesized silver nanoparticles by disc diffusion method against five clinically isolated pathogenic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Vibrio cholerae* is given in Table 1. Comparative antibacterial assay of AgNPs produced from *Alternaria* spp along with Oxacillin (oxn) is given in Fig 1. Both the synthesized silver nanoparticles showed good antimicrobial activity against all the selected pathogens. AgNPs synthesized from *Alternaria chlamydospora* were found to be the more effective drug in order to prevent the bacterial growth in comparison to *Alternaria* sp. combined with Oxacillin. During the study, Oxacillin on its own didn't show any impressive result over the test pathogens, but the combined formulations of AgNPs with Oxacillin showed remarkable results against all the pathogens (Table 1 and Fig 1). *Vibrio cholerae* was found to be more susceptible followed by *Bacillus cereus* in the combined formulation of Oxacillin and AgNPs. The antimicrobial studies showed that combination formulation of Oxacillin and AgNPs of *A. chlamydospora* was significantly effective compared to Oxacillin and AgNPs *Alternaria* sp. combination [1,9]. The studies confirmed that the biologically synthesized AgNPs from *Alternaria* spp amplified the antibacterial property of commercial antibiotics when used in combination. Some of the previous workers narrated their findings in the same field like this observation. Feng et al. [13] conducted a study to observe the effects of silver ions on gram positive and gram negative bacteria, *Staphylococcus aureus* and *Escherichia coli* respectively. They treated cells with $AgNO_3$, which is a source of Ag^+ in aqueous environments and looked at the structural and morphological effects of these silver ions on the cells. During the study, they exposed cells to $AgNO_3$ for 4 to12 hours before being prepared for microscopy and then fixed and sliced with an ultra-microtome to produce ultrathin sections for transmission electron microscopy (TEM). They observed that cells exposed to the Ag^+ ions seemed to have activated a stress response that led to the condensation of DNA in the center of the cell. They also observed that the cell membrane detachment from the cell wall, its damage and electron dense granules outside and in some instances, inside the cell. It was found that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell [14], however condensation of DNA could also prevent cell replication by preventing the DNA by transcriptional enzymes such as DNA polymerase. The dense electron granules which formed inside and outside the cell were extracted and subjected to X-ray microanalysis to determine the composition. It was also discovered that the granules were in part composed of silver and sulfur. This finding supports our idea that silver inactivates proteins by binding to sulfur-containing compounds [15]. It was also recorded that when treated with Ag^+, *E. coli*, a gram -ve bacterium, sustained structural damages than the gram +ve, *S. aureus* [14,18]. It was also been shown that treating cells with silver leads to cell shrinkage and dehydration leads to the death of the bacteria [16]. Feng et al. [14] confirmed that the cells that sustained extensive damage eventually ended up with cell wall and damage of cell

membrane. Its damage would lead to the leaking of cytoplasm from the cell, which may result in dehydration and shrunken of cells agreed with Guggenbichler and his coworkers [16, 19]. Klueh and his team [15] opined that silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that may be involved in trans-membrane energy generation and ion transport. It is also believed that silver can take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R) [19]. Silver does this by catalyzing the reaction between oxygen molecules in the cell and hydrogen atoms of thiol groups: water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond [17]. The silver-catalyzed formation of disulfide bonds could possibly change the shape of cellular enzymes and subsequently affect their function leading to inactivate protein synthesis, which later inhibits the bacterial growth and also death. It was further proposed by Klueh et al. [15], that the mechanisms of the antimicrobial activity of silver might be presumed that Ag^+ enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule [15]. Whether this has yet to been proved, but it has been shown that silver ions do associate with DNA once they enter the cell according to Fox and Modak [18, 21]. It is necessary to study the cytotoxicity of the combined drug *in vivo* for accessing its biocompatibility before administrating as antimicrobial drugs for human therapeutic use.

Table 1: Antibacterial activity of AgNPs synthesized from *Alternaria* spp combined with Oxacillin drug against varied bacterial strains

Pathogens	***Alternaria chlamydospora*** (Zone of inhibition in mm)			***Alternaria* sp.** (Zone of inhibition in mm)		
	AgNPs	Oxacillin	AgNPs+ Oxacillin	AgNPs	Oxacillin	AgNPs+ Oxacillin
B. cereus	25	11	28	15	10	17
S. aureus	21	08	23	14	07	15
E. coli	23	08	27	11	08	18
P. vulgaris	13	09	16	13	07	15
V. cholerae	19	18	22	18	20	25

B. cereus: Bacillus cereus, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, P. vulgaris: Proteus vulgaris, V. cholerae: Vibrio cholerae,



CONCLUSION

There are a number of nanoparticles being produced by different techniques in the recent scenario having varied characters. Nanoparticles especially silver nanoparticles have unique physical and chemical properties, which are

clusters of silver atoms and have strong antimicrobial properties against pathogenic bacteria of resistant strains. In the present study, good antimicrobial activity was shown by extracellular biosynthesized silver nanoparticles against pathogenic bacteria from two *Alternaria* spp and the activity of the AgNPs produced from *Alternaria chlamydospora* was further enhanced in combination with Oxacillin in vitro condition, which may conclude that combined formulation of available drugs with silver nanoparticles would be an alternate approach in order to treat the multi drug resistant pathogenic bacteria and also to minimize the antibiotic doses to cure different diseases caused by these bacteria.

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