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Combined effects of antibiotics and AgNPs biosynthesized from *Aspergillus ustus* studied against few pathogenic bacteria

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Abstract: From the last decade the synthesis of silver nanoparticles has much value, because of its application in biomedical field.Green synthesis possesses major importance in thestudy of nanoparticle synthesis, rather than chemical and physical approaches.In recent years certain bacteria are found to resistant towards various antibiotics. Production of silver nanoparticles (AgNPs) by biological way was examined using a sand dune fungal isolate. The isolated strain was identified as Aspergillus ustus based on morphological traits and molecular identification technique. The silver nanoparticle formation was monitored by UV-spectrophotometer that records the maximum absorbance peak at 400nm that indicates the presence of silver nanoparticles. The nanoparticle shows enhanced antimicrobial susceptibility towards the certain gram positive and gram negative bacteria. The present study was mainly focused on the biosynthesis of silver nanoparticle from the fungus Aspergillus ustus isolated form the coastal sand dunes of Puducherry.The antibacterial activities of ampicillin, vancomycin were enhanced in the presence of silver nanoparticle against test strains. The silver nanoparticle alone and the combination of antibiotics with silver nanoparticle shows increased antimicrobial effects.
Key words: *Aspergillus ustus*, AgNPs, UV-Vis Spectrophotometer, Vancomycin,Ampicillin.

Introduction

Nanotechnology is the current emerging technology, which has a wide application in various fields. It has unique properties and variation in specific characteristics like size, shape[1]. The nanoscale covers the range from 1-100nm. The Nanoparticles are the fundamental building blocks of nanotechnology, environmentally friendly and anticipated that do not produce toxic wastes in their synthesis process[2, 3].Nanoparticle can be synthesized by many methods. The recent process is use of microorganisms like bacteria, fungi, herbal extracts and yeasts for the biosynthesis of nanoparticles.These organisms play an important role in remediation of toxic metals through reduction of the metal ions so they are not toxic in other ways[4]. Though fungi has the capacity of tolerating high metal nanoparticle concentration in the medium, easy management in large-scale production of nanoparticles and higher amounts of protein expressions play a major role in nanofield[5].Silver nanoparticles has specific disinfectant properties are used as antimicrobial drugs, it has a wide application in health care sectors[6]. Silver nanoparticles are also used as catalysts in chemical reactions[7].

The aim of the present study is on the biological synthesis of AgNPs by extracellular method using the filamentous fungus Aspergillus ustus isolated from the sand dunes of Puducherry coastal areas.The UV-Vis spectroscopy shows the maximum absorbance peak that reveals the presence of silver nanoparticle. The antimicrobial efficacy of the synthesized silver nanoparticle has been tested on the selected bacterial pathogens viz., *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Bacillus cereus.*

Materials and Methods

Source of fungal strain

The sand dunes soil samples were collected from Puducherry. The fungal strain was isolated by serially diluting 1gm of soil sample. 1 ml of diluted microbial suspension was transferred to Sabouraud Dextrose agar and potato dextrose agar plates. The plates were then incubated at 25±3°C for 3-7 days. *Aspergillus ustus*was isolated and identified from the mixed culture of sand dune fungi[8, 9, 10] put on pure culture and stored in a refrigerator at 4°C for further studies.

Preparation of silver nanoparticles

The production of silver nanoparticles was done in the isolated A*spergillus ustus*fungus. Fungal Biomass was grown aerobically in Potato dextrose broth medium (PDB) at 25±3°C and incubated at 25 °C under continuous mixing condition by a rotary shaker at 140 rpm for 72 hours. After 72 hours of incubation, the biomass was filtered using Whatman filter paper No.1 and washed with distilled water to remove the media components. The fungal biomass from the broth was taken out and washed thrice in rice in 100ml of deionized Milli-Q water in an Erlenmeyer flask.These are incubated again at 25 °C in a shaker at 140 rpm for 72 hours. The obtainedbiomass was again filtered with whatman filter paper No.1 and the cell free extract was used for the synthesis of silver nanoparticle. 1 Mm aqueous AgNo3 solution was prepared and added to the cell free extract. These were kept in a dark condition for 48 hrs.

Characterization techniques

The reduction of silver ions was occurred and leads to the color change of the solution after 24 hrs. 1ml of supernatant was subjected to measure the absorbance using UV- spectrophotometer.The maximum absorbance was analyzed between 300-600nm was recorded.

Antibacterial assay

The antibacterial activity of the obtained silver nanoparticle was tested against the pathogens by following disc diffusion method. Pathogens such as *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Bacillus cereus*were selected for antibacterial test. The activity of prepared silver nanoparticles from *Aspergillus ustus* weretested against thepathogenic bacteria. The AgNo3 and Ampicillin 10mcg were taken as control parallel tothe AgNPs to find a comparative assessment of the antibiotic efficacy over the pathogenic bacteria. After overnight incubation at 37°C the zone of inhibition was measured.

Calculation for Increase in fold area

The calculation of mean of increase in fold are measured by the mean surface area for the zone of inhibition of each antibiotics which used alone and antibiotic + AgNPs. The increase in fold area of different pathogens for antibiotics and antibiotics + AgNPs can be calculated by using this equation: $(B^2 – A^2)/A^2$, where A is the antibiotic alone and B is the antibiotic + AgNPs respectively[11].

Results and Discussion

A.Fungal biomass B.Cell free extract- without $AgNo_3$treatment C.With $AgNo_3$treatment

Fig 1: Synthesis of silver nanoparticles from *Aspergillus ustus*.

Fig 1.A shows a flask of fungal biomass.Fig. B shows the flask containing fungal cell free extract pale yellow color which can observe clearly before immersion in 1 mM $AgNo_3$ solution without addition of $AgNo_3$. Fig. Cshows the flask containing fungal cell free extract brownish color after 72 hours of reaction with $AgNo_3$can be observed. The formation of silver nanoparticle in the reaction mixture is clearly indicated by the appearance of yellowish brown color in solution.

The spectra reported by the UV-spectrophotometer are reported in **Fig 2.** The absorbance peak are observed between 300-600nm.The analysis of nanoparticles can be done by the techniques proved above[12] .UV-Vis spectra illustrated shows, a strong surface Plasmon resonance at420nmindicated the presence of silver nanoparticles

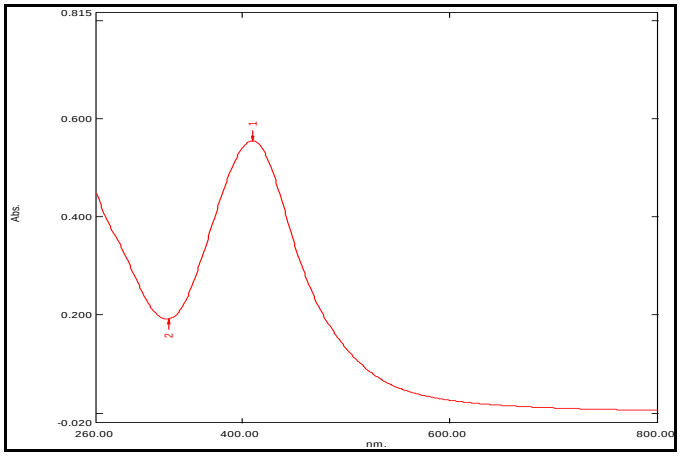

Fig. 2: UV–Vis spectrum of silver nanoparticles synthesized from *Aspergillus ustus.*

In the present study, the synthesized nanoparticle of the sand dune fungi *A. ustus* isolated was evaluated using the disc diffusion or Kirby-Bauer method [13],against the pathogens *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, and Bacillus cereus* The Zones of inhibition were measured after 24 hour of incubation at 37^{o}C. The comparative stability of discs containing vancomycinand ampicillinwas made. The inhibiting efficacy of AgNPs along with antibiotic and AgNPs alone and were recorded with the respective pathogens. The synergistic activity of AgNPs was evaluated by using a broad spectrum antibiotic Vancomycin (30 mcg) and a narrow spectrum antibiotic Ampicillin (10mcg). 20 µl of biosynthesized AgNPs combined with antibiotic Vancomycin (30mcg) and Ampicillin(10mcg) showed great activity against the gram positive pathogens *S. aureus, B. cereus* and gram negative pathogen *P. aeruginosa, K.pneumoniae*The maximum bacterial inhibition >20mm was observed in strains of *S. aureus and P. aeruginosa,* The maximum bacterial inhibition >17mm was observed in strains of *K.pneumoniae and E. coli.* It was found that the nanoparticle alone showed a high efficacy over the pathogens such as *E. coli*$^{-}$, *S. aureus*$^{+}$, *B. cereus*$^{+}$. Even thoughthe AgNPs combined with antibiotics are also showed more zone of inhibition (Table 1);(Fig 3).The highest increase in fold area was observed for ampicillin against*P. aeruginosa*(3.59%), *K. pneumoniae*(3.0%)(Table 1), whereas vancomycinshowed the highest fold area against *S.aureus*(1.46%), *K. pneumoniae* (1.25%). The present study carried out on enhanced antimicrobial activity of silver nanoparticles synthesized from *A. ustus* in combination with antibiotics.It is found that the nanoparticle along with antibiotic showed more zone of inhibition than the nanoparticle alone. The zone of inhibition was measured after 24 hour of incubation at 37^{o}C.

Table 1:Zone of inhibition (mm) of vancomycin and ampicillin against test pathogens.

Sl. No.	Pathogenic Bacteria	Vancomycin (30 mcg)	Vancomycin (30 mcg)+ 20µl AgNPs	Increase in fold area (%)	Ampicillin (10 mcg)	Ampicillin (10 mcg)+ 20µl AgNPs	Increase in fold area (%)	Ag NPs
1	*P. aeruginosa*$^{-}$	17	21	0.52	07	15	**3.59**	11
2	*S. dysenteriae*$^{-}$	13	16	0.51	06	11	2.36	12
3	*K. pneumoniae*$^{-}$	12	18	1.25	06	12	3.0	11
4	*E. coli*$^{-}$	08	11	0.89	15	18	0.44	22
5	*S. aureus*$^{+}$	14	22	**1.46**	11	16	1.11	13
6	*B. cereus*$^{+}$	14	17	0.47	06	12	3.0	13

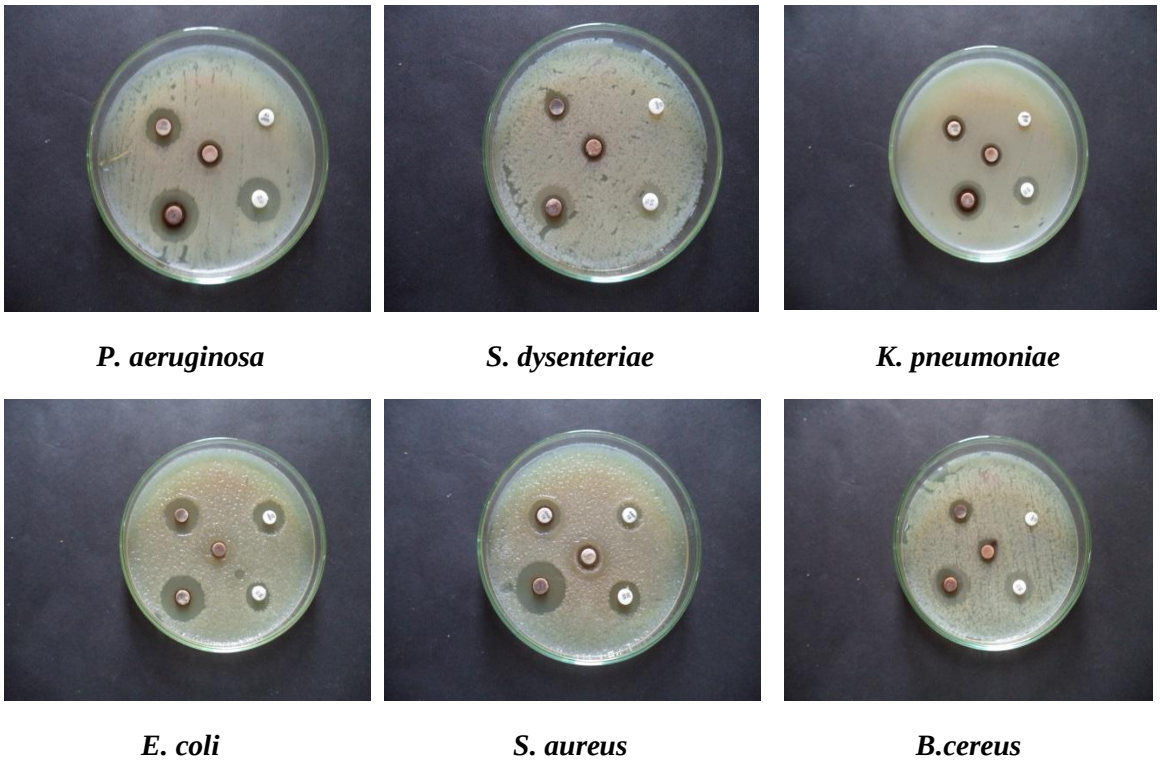

Fig 3: Synergistic activity of AgNPs from *A. ustus*combined with antibioticsVancomycinand Ampicillin, against bacterial pathogens.

Conclusion

From the current study, it is noted down the bioactive compounds present in the sand dune environment is still unexplored. The isolated fungus *A.ustus* from sand dunes of Puducherry and Karaikal coastal areas was used for the extracellular biosynthesis of silver nanoparticle. The reduction of silver ions were observed by the color change of the solution and measured by UV- spectrophotometer. The absorbance peak of UV-spectrophotometer were found between 300-600nm. The antimicrobial efficacy of the nanoparticle was evaluated against the pathogens *Pseudomonas aeruginosa, Shigella dysenteriae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus cereus.* It is concluded from the present study that the silver nanoparticles obtained from *A.ustus* showeda high antibacterial susceptibilitycombining with ampicillin and vancomycin antibiotics, against the pathogens. 20 µl of the biosynthesized nanoparticle was found to more effective against the selected gram positive and gram negative bacteria. Thus the sand dune fungus *A.ustus*acts as potent antibacterial agent and there is a need of study on the sand dune microbes.

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