Bacterial Mediated Synthesis and Characterization of Silver Nanoparticles using *Planomicrobium* sp., and its Antimicrobial Activity.

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Abstract:

Microbial synthesis of nanoparticles are a better choice when compared to conventional physical and chemical routes as it is environment-friendly, low cost consuming and analysis can be done at, room temperature. In the present study extracellular synthesis of silver nanoparticles are carried out by using *Planomicrobium* bacterial species (Pl-AgNPs) and characterized by Transmission Electron Microscopy (TEM) and X-ray Diffraction (XRD) analysis. The Pl-AgNPs are analyzed for its antimicrobial activity. The surface morphology and the size of the Pl-AgNPs was identified as spherical shape with a diameter of 10 – 25 nm by using a Transmission Electron Microscopy. The XRD diffractogram revealed the crystal lattice and the face centered cubic structuresof the Pl-AgNPs and SAED pattern showed the crystalline nature of Pl-AgNPs. Antimicrobial assay revealed an excellent antimicrobial activity towards *Pseudomonas*, *E.coli* (Gram Negative) *S aureus*, *E. faecalis* (Gram Positive) and fungi *C. albicans*. Therefore, the Pl-AgNPs could be a good choice of antimicrobial agent in future with still more required and relevant investigation and may be used in the field of nano biomedicine.

Keywords: Microbial Synthesis, Silver nanoparticles, TEM, XRD, Antimicrobial Activity.
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Introduction:

Silver is the 47th element in the periodic table, it is a transmission metal and appears white in colour. The symbol Ag for silver was derived from ancient Roman language Argentum which means white and shiny (Fromm, 2011). Silver metal has the highest thermal and electrical conductive properties(Xia, 2019). The use of silver is as old as civilization of human being but the use of silver as nanoparticles have been identified only few decades before (Siddiqi, Husen and Rao, 2018). Silver has gained its value in the field of nanotechnology because of its diversified biomedical application that has been approved clinically for infections like Diabetic ulcers, anti-bio film activity, antimicrobial activity, antioxidant and anti-inflammatory properties (Paladini and Pollini, 2019), vaccines, silver coated medical devices, silver dressings (Singh *et al.*, 2017)and renal diseases etc. (Haider and Kang, 2015). Major contribution of silver

nanoparticles (AgNPs) in drug delivery, drug discovery, and new drug therapies paved way to cure many dreadfuldiseases, and also, they use body's natural mechanism of uptake of drug by the infected cell through natural transport pathway (David *et al.*, 2019).Silver nanoparticles have been synthesized by conventional physical and chemical routes but are not safe to the environment and also to the mankind (Naghizadeh *et al.*, 2021), (Ingarsal *et al.*, 2021).

Biological approach is the best amongst the conventional methods as they are safe to handle by human being and also non-hazardous to the environment (Patel *et al.*, 2016), (Ghosh *et al.*, 2012), (Rolim *et al.*, 2019). AgNPs are widely synthesized by different biological routes such as by using plants (leaf, stem, root, flower, fruit, and seed etc.), algae, fungi and bacteria (Simsek, Pehlivanoglu and Aydin Acar, 2021). Silver nanoparticle synthesized from the flowers of *Plumeria rubra*(Mandal, 2018), *Jasmine* flower (Aravind *et al.*, 2021), *Abelmoschus esculentus* (Devanesan and Alsalhi, 2021). Silver nanoparticles synthesized from leaves of *Carissa carandas*(Singh *et al.*, 2021), *Gymnema sylvestre*(Gomathi *et al*2020). Silver nanoparticles synthesized from stem *Piper nigrum*(Paulkumar *et al.*, 2014). Silver nanoparticles synthesized using the roots of *Avicennia marina* (Abdi *et al.*, 2018).

Bacterial synthesis of silver nanoparticles are in limelight as it is a simple one step procedure and involves only normal room conditions and have higher productivity (Parikh *et al.*, 2008). Bacterial mediated synthesis of silver nanoparticles have proved excellent antibacterial potential against *E. coli, S. typhi, S. aureus and M. luteus*(Fayaz *et al.*, 2010)and antifungal property (Monowar *et al.*, 2018), anti-biofilm property (Galvez *et al.*, 2019). The AgNPs synthesized from bacteria thus have a good potential to inhibit the growth of various clinically significant pathogens so, in the present study *Planomicrobium* sp was isolated and pure culture of the same was used to synthesize AgNPs extracellularly. The antimicrobial property was evaluated against the pathogens like *Pseudomonas*sp, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and fungi*Candida albicans*.

Materials and Methods

Chemicals:

Silver nitrate and other required chemicals were bought from Sigma Aldrich Chemicals Pvt Ltd, India. All the required agar medium and sterile Petri plates (plastic) were obtained from Hi Media Laboratories, India.*Planomicrobium* sp (bacterial sample) was collected and isolated from the marine sediment at Ennore coast Chennai India.

Isolation and Identification of Bacteria:

Marine sediment samples were collected from Ennore, Chennai, India. The samples were then serially diluted to minimize the load of bacterial biomass and inoculated on Nutrient agar plates by spread plate technique and incubated at 27^0C for a week to yield pure isolated colonies. After the incubation period was finished the isolated colonies grown on the plates were confirmed to be *Planomicrobium* sp.

Extra cellular Synthesis of Silver nanoparticles from*Planomicrobium* sp.

Planomicrobium sp. was isolated in pure colonies from the plates andwas inoculated in 100 mL of sterile Nutrient media broth and incubated at 35^0C for 24 hours. After the incubation period was finished the biomass culture broth was centrifuged at 7500 rpm for 15 minutes to get a cell free supernatant. Then the supernatant was taken in a clean and sterile Erlenmeyer flask and 1mM of silver nitrate salt ($AgNO_3$) was added to it and mixed well and incubated at 35^0C for 24 hours. The formation of Pl-AgNPs was carefully monitored by a double beam UV-Visible spectrophotometer at constant intervals.

Characterization of *Planomicrobium* sp mediated Silver Nanoparticles (Pl-AgNPs)

The bio reduction of silver ions to silver ($Ag^+ \rightarrow Ag^0$) by *Planomicrobium* sp.,and formation Pl-AgNPs was determined by colour changed before and after incubation. The size in diameter and surface morphology of the Pl-AgNPs was determined by using a Transmission Electron Microscope (TEM) model JEOL JEM-

1010. The crystalline structure and facets were obtained using X-ray Diffraction (XRD) spectrophotometer model HITACHI SU6600.

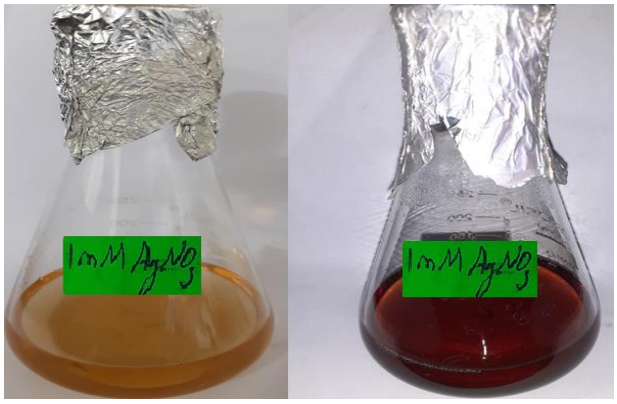
Result and Discussion

Visual observation

The formation of Pl-AgNPs is determined primarily by the colour change. The cell free supernatant solution obtained after centrifugating the pure broth culture of *Planomicrobium* sp. was pale yellow in colour. After adding 1 mM of $AgNO_3$to the supernatant the colour changed to dark brown after the incubation period. Similar colour change whitish yellow to brown was observed when silver nanoparticles were biosynthesized using *Enterococcus* sp(Rajeshkumar *et al.*, 2016a).

X-Ray Diffraction (XRD) Spectrophotometric Analysis of Pl-AgNPs

The X-Ray diffraction spectrophotometric analysis was performed to analyze the crystalline structure of silver nanoparticle synthesized from *Planomicrobium* sp. The data was taken at the 2Θ range between 20 to 80 degrees.The strong diffraction peaks of synthesized silver nanoparticles was seen in 28^0, 33^0 and in 46^0. The XRD data was compared with standard powder diffraction card of JCPDS, silver file No. 04-0783. Three peaks at 2Θ values 28^0, 33^0 and in 46^0in diffractogram was due to the presence of silver nanoparticles having face centered cubic structures of the nanoparticles. Similar reports have been found insilver nanoparticles synthesized from *Santalum album*(Mehta, Chhajlani and Shrivastava, 2017).



(A) (B)

Fig 1. Depicts the Colour Change of Cell Free Supernatant added with $AgNO_3$ from *Planomicrobium sp* (A) Initial Colour before Incubation (B) Colour change after 24 hours of incubation

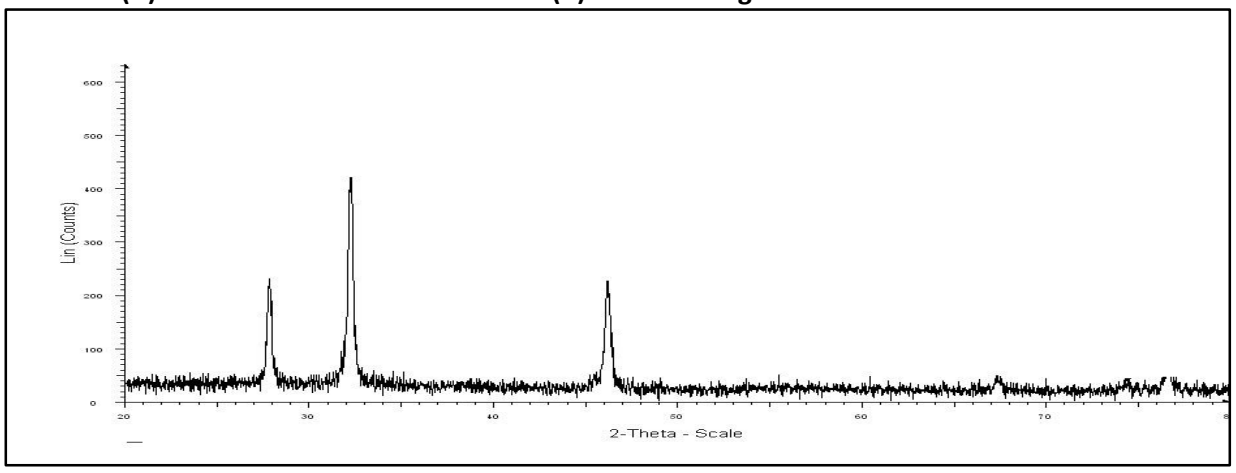
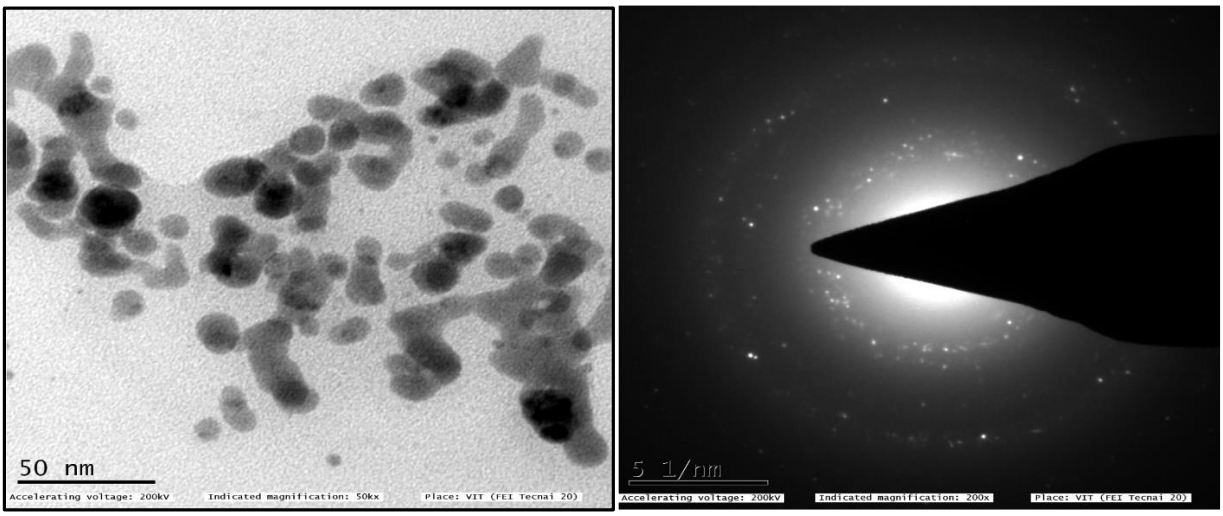


Fig 2. Shows XRD Diffractogram depicting strong peaks representing Pl-AgNPs

Transmission Electron Microscopy and SAED Pattern of Pl-AgNPs

Transmission Electron Microscopy revealed spherical nanoparticles with a diameter of 10 – 25 nm and also some elongated pseudo spherical shaped nanoparticles were identified with agglomeration. The Selected Area Electron Dispersion (SAED) patterns of Pl-AgNPs revealed three face centered cubic rings which exhibited the crystalline nature of the nanoparticles. The AgNPs synthesized by using Enterococcus sp. also showed similar morphology (Rajeshkumar *et al.*, 2016b).



(A) (B)

Fig. 3 (A) TEM images of synthesized spherical shaped Pl-AgNPs (B) SAED patterns shows the crystalline structure of the nanoparticles

Antimicrobial Activity of Pl-AgNPs

Agar Well Diffusion Assay was done to exhibit the antimicrobial potential of Pl-AgNPs. The antimicrobial activity was carried out against *Pseudomonas*sp, *E coli*(Gram –ve), *Enterococcus faecalis, S aureus*(Gram +ve) and *Candida albicans*fungi. The test was performed using sterilized Muller-Hinton agar media poured in sterile petri plates. The agar media was set to solidify. With a sterile polystyrene tip, wells were made in the agar plates and concentration value was marked as (25µL, 50µL, 100µL and standard drug). The standard drug used for bacteria was amoxicillin and for fungi it was Fluconazole. The pure broth culture of each microorganism were then swabbed properly on separate plates and labelled accordingly. In each well the Pl-AgNPs and the standard or control drugs were added according to the concentration value mentioned. The plates were incubated at room temperature for 24 hours. Once the incubation period was finished the zones were measured in millimeter and recorded. The antibacterial activity of Pl-AgNPs against *Pseudomonas* showed a good zone of inhibition which was at 25 µL 14 mm 50 µL 15 mm, 100 µL 21 mm and for standard 20 mm. *E.coli* revealed still more better zone of inhibition which was measured18 mm 25 µL, 22 mm in 50 µL, 27mm in 100 µL concentrations and 38mmfor standard drug. *Enterococcus faecalis*exhibited 25 µL 12 mm 50 µL 14 mm, 100 µL 19 mm and for standard 36 mm. Pl-AgNPs showed good antibacterial activity against *S.aureus* which was determined from the zone of inhibition. The zones measured in different concentrations was 25 µL 11 mm 50 µL 15 mm, 100 µL 20 mm and for standard 12 mm.*S.aureus* showed mild inhibition against the standard drug but more susceptible towards the Pl-AgNPs. Antimicrobial activityof Pl-AgNPs against *C.albicans*was also good which was revealed in the zones that are formed. The diameter of the zones formed are 13 mm in 25

µL, 17 mm in 50 µL, 21 mm in 100 µL concentrations and 26 mm for standard drug.The results revealed a better antimicrobial activity towards Gram negative bacteria than that of Gram positive bacteria. This differences in susceptibility may be due to the differences in the cell wall composition and its thickness seen in Gram positive bacteria majorly because of Peptidoglycans and Lipotecoic acids compositions present in the cell wall (Tamboli and Lee, 2013). Also, the antimicrobial efficacy of Pl-AgNPs was may be due to the denaturation of proteins, inhibition of enzymatic activity and DNA denaturation(Singh *et al.*, 2015). Hence, *Planomicrobium* sp. mediated AgNPs proved to have an excellent antimicrobial potential and may be used as an antimicrobial agent with further necessary investigations required for the mechanism of action involved in antimicrobial activity of Pl-AgNPs.

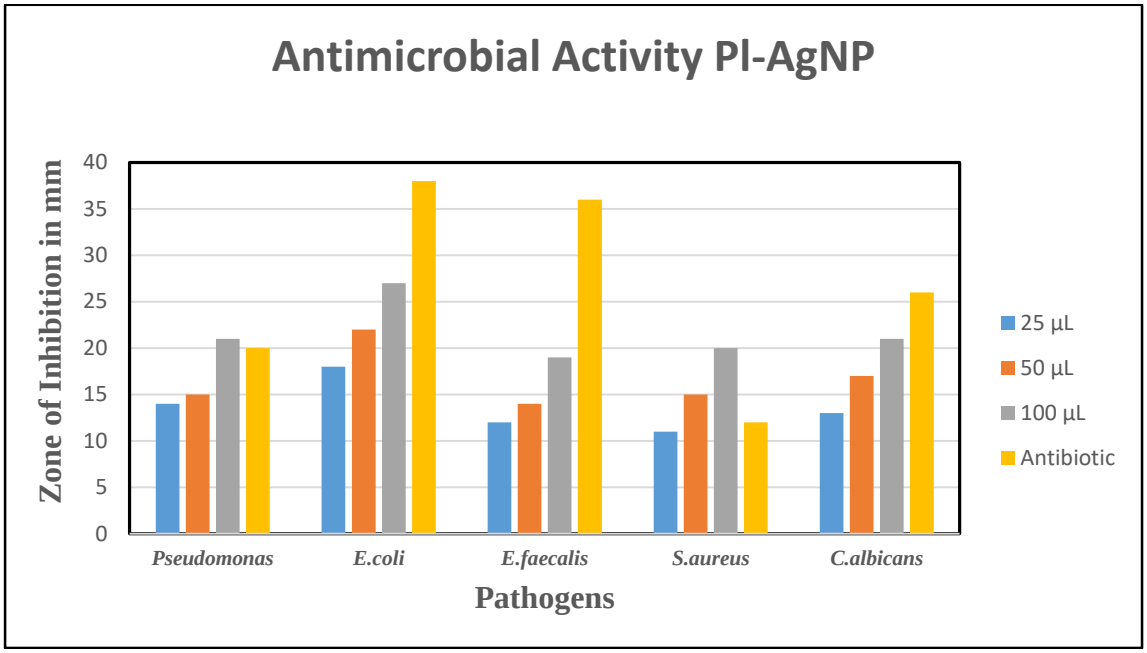


Fig 4.Represents the antimicrobial activity of Pl-AgNPs at different concentration against pathogens

Conclusion

We conclude thatthe extra cellular bacteria mediatedsynthesis of silver nanoparticles from*Planomicrobium* sp is an easy one step method, environment friendly and cost effective approach. The cellular metabolites of *Planomicrobium* sp present in the supernatant significantly reduced silver ions to silver and also involved in capping and stabilization of the synthesized nanoparticles. The Pl-AgNPs revealed an excellent antimicrobial activity against *Pseudomonas*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and fungi *Candida albicans*. Pl-AgNPs may be a better alternative for any chemically produced antimicrobial agents. Thus the Pl-AgNPs could be used in nano biomedicine field with further required analytical evidences for its antimicrobial potential.

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