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Green Synthesis of Silver Nanoparticles using Airborne Actinomycetes

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Abstract: Green synthesis of nanoparticles are having an advantage as the physical and chemical process were costly and further green synthesis is ecofriendly. Synthesis of Silver nanoparticles using bacteria and fungi were already reported and very few reports are available on the synthesis of same using Actinomycetes. This study focus on the synthesis of silver nanoparticles by two different species of Actinomycetes isolated from the atomospheric air sample collected from Koyambedu, Chennai. The intracellular synthesis of silver nanoparticles using *Streptomyces* sp.and *Streptoverticillium* sp. is reported in this paper. The isolated actinomycetes were characterized by Physio-chemical and microscopic identification. These species were treated with silver nitrate solution of 3.5mM concentration and it was found that both the species showed their resistivity towards it. The formation of silver nanoparticles was confirmed using Transmission Electron Microscope (TEM). The size characterization showed the synthesis of silver nanoparticles were less than 70nm in *Streptoverticillium* sp. and the Streptomyces sp. showed deposition of silver nanoparticles within the cell of less than 8nm in size and were found to be spherical and monodispersed.
Keywords: Airborne Actinomycetes, Silver nanoparticles, *Streptomyces* sp., *Streptoverticillium* sp., TEM analysis.

Introduction:

New applications of Nanotechnology are developing rapidly and the need for biosynthesis of nanoparticles increased as the physical and chemical processes were costly. So in order to Synthesis nanoparticles in non-toxic, eco-friendly and less expensive way, scientists made use of microorganisms and plant extracts. *Biosynthesis* of Nanoparticles includes reduction or oxidation as main process. The enzymes and phytochemicals present in microorganisms and plant extracts are usually responsible for the reduction of metal compounds into their corresponding nanoparticles. The synthesis of silver nanoparticles using *Bacillus* sp. [1], plant extracts [2,3] and floral extracts [4,5] were already studied by this laboratory. Initially bacteria were used to synthesize nanoparticles and this was later overcome by the use of fungi, Actinomycetes and more recently plants. For example, the synthesis of magnetic nanoparticles by magneto-tactic bacteria was reported [6] and these nanoparticles have a wide range of application [7]. And also it has been shown that many plants and bacteria take up certain metals from the soil and solublize them in case of uranium or reduces them in case of iron or manganese. Microbial biomass can retain the absorbed metals by bioaccumulation (actively by viable cells) [8].

Fungi were found to be excellent candidates for metal and metal sulfide nanoparticle synthesis as they secrete a variety of enzymes [9].The use of Actinomycetes, group of gram positive filamentous bacteria, are less exploited in case of nanoparticle synthesis and the exposure of Actinomycetes to various metal oxide solutions reported either intracellular [10-12] or extracellular [13,14] metal ions to their corresponding nanoparticles.The present work reports the biosynthesis of silver nanoparticles from *Streptomyces* sp. and *Streptoverticillium* sp. isolated from the air sample collected from Koyambedu market, Chennai.

Materials and Methods:

Sample collection and isolation:

Anderson 2–stage air sampler was used to collect air sample from the atmosphere of vegetable market. The fractionating units were sterilized in hot air oven at 140˚C for 1 hour before using. The sampler was positioned at a height of 1m above the ground level and it was operated for 5mins at each site. Petridishes of 9cm diameter with solidified Starch Casein Agar (SCA) were used for isolating Mesophilic Actinomycetes.

Characterization and Identification of Actinomycetes

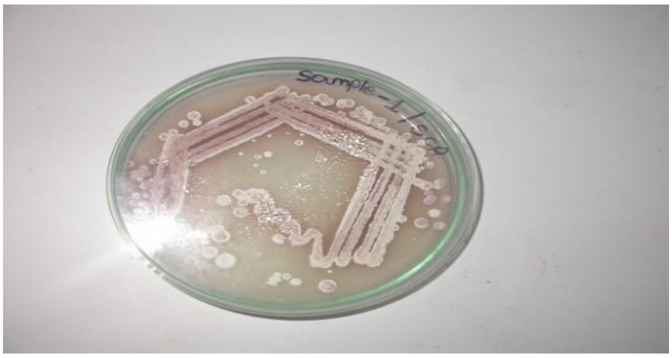
Microscopic identification:

Gram staining and Lacto-Phenol Cotton blue staining was performd to check the morphology of the cell and the morphology of the spore chain were identified by coverslip culture technique.

Morphological identification:

The isolated Actinomycetes were observed under high power magnifying lens and the colony morphology was recorded with respect to their colour, nature of the colony, aerial mycelium and reverse side pigmentation.

Figure 1: Colonial morphology of Actinomycetes



Exposure of Actinomycetes to 3.5mM concentration of Metal solution:

Ten milliMolar concentration of silver nitrate solution was prepared using 0.169g of Silver Nitrate dissolved in 100ml of distilled water. From the above solution 3.5ml was taken and added with 6.5ml of SCA media to make a final concentration 3.5mM of Silver Nitrate. The media amended with Silver Nitrate were sterilized and poured into petriplates in duplicates. The cultures were inoculated by streaking method and incubated for 7 days.

Characterization using TEM:

The resistant colonies of Actinomycetes were fixed in 2.5% (w/v) aqueous glutaraldehyde in 1.5ml of 0.1 M phosphate buffer(pH-7.4)at 4°C and post fixed in 1% Osmium tetraoxide at 4°C in 0.1 M phosphate buffer (60 min) for TEM.Samples were dehydrated using a growing series of methanol. 20µl of the sample is treated with 10µl of 1% PTA and are mounted over the carbon-coated copper grids.The nanoparticle deposits were observed using Philips Technai (10) Transmission Electron Microscope with electromagnetic lens at 120 kV.

Results and Discussion:

Table 1: Characteristic Identification of Genus

Characeristics	***Streptomyces* sp.**	***Streptoverticillium* sp.**
Aerial mass colour	white	White
Reverse-side pigmentation	Coral pink to red colour	No pigmentation
Soluble pigments	Produced	Not Produced
Nature of Mycelium	Discrete	Verticils or Barbed hair like structure
Type of spore	Linear chain	Linear chain
Microscpic appearance	Coiled or hook like structure	'V' shaped linear chain of spore at the end of mycelium

The genus of the isolated Actinomycetes was identified using microscopical and morphological studies (Table-1) using Bergey's Manual of Determinative Bacteriology, 9th edition[15]. TEM analysis revealed that the silver nanoparticles produced by *Streptomyces* sp. And *Streptoverticillium* sp. was spherical in shape and was found to be well dispersed within the cell. (Figure- 3) shows the deposition of silver nanoparticle in *Streptoverticillium* sp., which is less than 70 nm in size.From the image it is clear that the nanoparticles are present in the cytosolic region which can be confirmed by the presence of cytosolic granules. (Figure- 4, 4a & 4b) clearly shows the deposition of silver nanoparticles within the cell of *Streptomyces* sp.less than 8nm in size and were found to be spherical and monodispersed. The presence of cytoplasm confirms that the nanoparticles were synthesized intracellularly.

Figure 2: Microscopic appearance of *Streptomyces* sp.

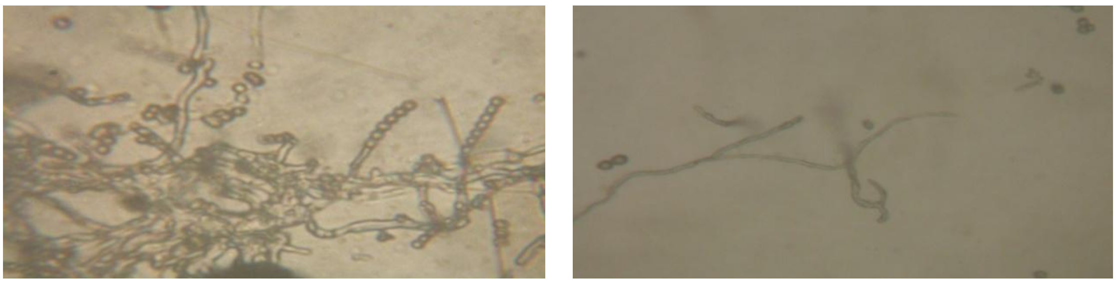


Figure 3: Microscopic appearance of *Streptoverticillium* sp.

Figure 4: TEM image of *Streptoverticillium* sp. (Intracellular synthesis of AgNPs – 70nm)

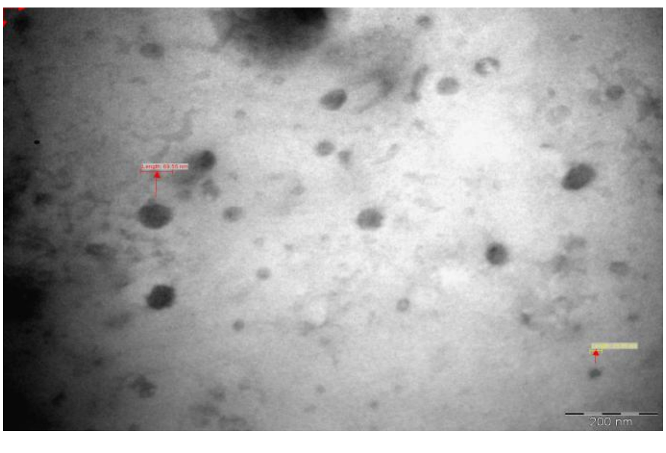


Figure 4a: TEM image of *Streptomyces* sp. (Intracellular synthesis of AgNPs – 5-8nm)

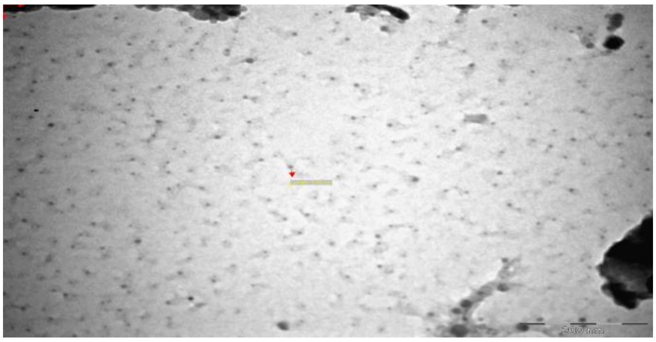
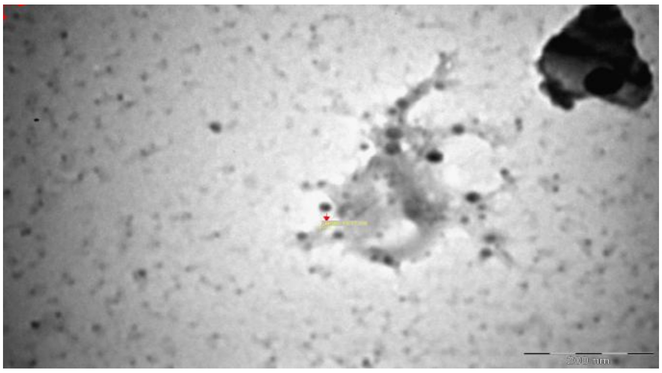


Figure 4b: TEM image of *Streptomyces* sp. (AgNPs – seen bounded to cell cytoplasm)



The mechanism which is widely accepted for the synthesis of silver nanoparticles is the presence of enzyme "Nitrate Reductase"[16,17]. The cell wall being negatively charged binds with the positively charged metal ions and the enzyme present in the cell wall reduces these metal ions to their nanoparticles and finally the smaller sized nanoparticles diffuses through the cell wall into the cell[18]. To the best of our knowledge, there are only few reports for the intracellular synthesis of nanoparticles using Actinomycetes. In earlier studies, the intracellular synthesis of Gold[16], Manganese and zinc nanoparticles[12] was reported in the size range of 10-20nm when treated with 1mM concentration of $HAuCl_4$,$MgSO_4$ and $ZnSO_4$. And also it was reported that the nanoparticles produced by Actinomycetes was found to be non-toxic to the cells which continued to multiply even after the formation of the nanoparticles and there are no regular reports of silver allergy [19]. To the best of our knowledge this is the first report on the intracellular synthesis of silver nanoparticles in Actinomycetes and also is the first report on nanoparticle synthesis at 3.5mM concentration of silver nitrate. And also this is the first report on the intracellular synthesis of monodispersed, spherical 5nm sized silver nanoparticle by the Actinomycetes. Thus the present study provides scientific evidence for the silver nanoparticle synthesis by different Actinomycetes species and this can be successfully exploited in Biosynthesis of Silver Nanoparticles.

Conclusion:

The present study showed the possibility of Actinomycetes to synthesize nanoparticles and also proved that the use of microorganism in nanoparticle synthesis is more essential than chemical process. This study may also help to increase the future, natural "Nano-Factories" which supports the emerging Nanotechnology field to come to screen with wide applications.

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