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Biosynthesis of *Streptomyces* sp. bactericidal silver nanoparticles against antibiotic-resistant bacteria

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Abstract

The study investigated isolation and screening of antimicrobial producing *Streptomyces* sp. from soil sample and also in the biological synthesis of silver nanoparticles and their antimicrobial properties. Isolation of *Streptomyces* sp. from five different soil samples and about 10 different *Streptomyces* sp. isolates were separated and screened for the antimicrobial potentiality. From that the admirable isolates were selected based on the maximum diverse potential of antibacterial zone of inhibition. Furthermore, best isolate (ISB1) was used for the biological synthesis of silver nanoparticles and their synergistic antibacterial role is observed. Hence, this has been chosen for further optimization studies and biosynthesis of silver nanoparticles and confirmation of silver nanoparticles was carried by EDAX, size and morphology of the nanoparticles was determined by TEM and it was spherical; ranged between 20-50 nm. Synthesized nanoparticles were subjected to minimum inhibitory studies and 40 µl gave significant results compared to other concentrations. The biosynthesized silver nanoparticles by *Streptomyces* sp. showed better in killing pathogens that might be due to the synergistic effect. Thus, opens new platform for other application oriented studies.

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Introduction

It is an inevitable truth that human activities are disturbing the traditional ecosystem leading to different types of pollution indeed. Soil is one of the detrimental sources of heavy metal pollution and hence results in the starting phase of disturbing microbial flora continuing the damage of natural cycle such as nitrogen and potassium fixation, assimilation and degradation of organic residues in releasing nutrients to the soil (Raja and Prabakarana, 2011). Generally, a number of *Streptomyces* sp. is relatively low while compared to other soil microorganisms but the role of *Streptomyces* sp. is portentous and involved in the degradation, nitrogen fixation and the inhibition of various pathogens (Hanan *et al.,* 2016). Eventually, *actinomycetes* are most predominantly used in antibiotic production technology.

Silver nanoparticles are increasingly popular among other metal nanoparticles due to their minimum toxic level, cost-effective and antimicrobial potential. Consequently, there are different methods available in synthesizing nanoparticles such as chemical, physical and biological. Biological synthesizing of nanoparticles is cheap, eco-friendly and also has a synergistic effecton the selected biological source and silver nanoparticles (Sneha *et al.,* 2018; Mohamedin *et al.,* 2015). Hence, the current study aimed to evaluate biosynthesis of *Streptomyces* sp. bactericidal silver nanoparticles against antibiotic-resistant bacteria.

Materials and methods

Soil samples were collected from Muthupet Mangrove forest (Latitude of 10°46'N Longitude of 79.51'E), Tamilnadu, India in sterile airlock polythene bags and transported to the laboratory according to a previously described method. Collected samples were stored at 4°C until do the further use. The collected soil samples were subjected to pre-treatment of dry heat at 56°C for 10 minutes in order to increase the number of mycelium-forming actinomycetes relative to the non-actinomycetal heterotrophic microbial flora. After that one gram dried soil samples were added to 10 ml sterile water and further diluted up to 10^{-6} dilution in sterile water. 0.1 ml of each diluted sample was inoculated by spreading with a sterile glass rod on *Streptomyces sp*. isolation agar medium separately. The media were supplemented with antibiotics of cycloheximide (40 µg/ml), nystatin (30 µg/ml) and nalidixic acid (10 µg/ml) after autoclave to inhibit the fungal and non-filamentous bacterial growth. The inoculated plates were incubated at 30°C for 7 to 9 days or until appearance of colonies with a tough leathery texture, dry or folded appearance and branching filaments with or without aerial mycelia.

The screening of antimicrobial producing *Streptomyces* sp. was selected and its antimicrobial spectrum was tested against the pathogenic bacteria. The selected isolates was inoculated into Starch casein broth, and shaken at 28-30°C at 250 rpm for seven-ten days. After incubation, the staling substance is centrifuged. The supernatant was collected and added equal volumes of ethyl acetate (1:1 v/v) then shaken vigorously for 1h. The solvent phase was separated from the aqueous phase by using a separating funnel and subjected to a rotary vacuum evaporator at a water bath (60°C at 100 r/min) to get residue particles. The dried residue was weighed and dissolved with DMSO (Anupama Sapkota *et al.,* 2020). The single isolate was selected based on the highest zone of inhibition and maximum inhibition against test isolates.

The selected fresh single isolate culture of *Streptomyces* sp. (ISB1) was inoculated into 100ml of SCM broth. The inoculated flask was incubated in the orbital shaker (150 rev min1) at 26°C for 14 days. After the incubation period, the culture was centrifuged at 1000rpm for 15 min and the supernatant was combined with 2 mM of $AgNO_3$ (1: 1, v/v) as well as incubated at 26°C in a shaker for 3 days in the dark (Railean-Plugaru Viorica *et al.,* 2021). Along with this control sample (2mM silver nitrate solution) was kept without any inoculation to check the role of *actinomycetes* in nanoparticle preparation. The reduction of silver nitrate into silver nanoparticles was identified with the colour change from light yellow to brown. The reduction of silver

nanoparticles was confirmed by various characterization techniques.

Silver nanoparticles solution was centrifuged at 1000rpm for 15 minutes and the supernatant was taken further. Then, the solution was subjected to UV-visible spectrophotometer for the preliminary confirmation, SEM (Zeiss EVO 18 at a voltage of 20kV) and TEM for the size and morphology determination and EDX to determine the quantity of silver nanoparticles. Silver nanoparticle solutions supernatant are used to check the antibacterial properties by agar well diffusion method.

Results and discussion

In this study, 6 soil samples were taken and from the 10 different strains of *Streptomyces* sp. were isolated based on the microscopic and macroscopic observations. The identified isolates were then subjected to primary screening, almost all the isolates showed antibacterial activity against pathogens but in that *Streptomyces* sp. (ISB1) gave the most promising results of coverage of broad spectrum of bacterial range from gram-positive and gram-negative bacteria compared to the other isolates of *Streptomyces* (Table 1). Hence, *Streptomyces* sp. (ISB1) has been taken for further studies. The adaptation of microorganisms in such harmful conditions is challenging and portrays the ability to produce resistant structures such as spore and the ability to survive among other harmful pathogens (Gurung *et al,* 2009). *Streptomyces* sp. dry residues four concentrations such as 10, 20, 30 and 40 µg (Fig. 1) were analysed antibacterial activity by well method. The most prominent zone of inhibition and broad-spectrum antibacterial activity was observed at 40 µg compared to other concentrations as well as with control sample DMOS. A similar type of study was carried out with extracted pigments of *Streptomyces sp*. inhibitory growth of *Escherichia coli* and except yellow pigment showed inhibitory effect against gram-negative bacteria (Parmar *et al.,* 2016; Tandale *et. al.,* 2018). Microorganism has different morphology according to gram positive and gram negative strains as well as reason behind the different sensitivity. Gram-positive bacteria are not effectively permeable due to an outer peptidoglycan layer whereas Gram-negative bacteria have an outer polysaccharide membrane making the cell wall impermeable to the lipophilic solute. Thus, the reason for antimicrobial producing *Streptomyces* sp. shows selective permeability of killing pathogens (Shirling and Gottlieb, 1996). Different environmental conditions influence the growth and antimicrobial activity of *Streptomyces* sp. (Boroujeni *et al.,* 2012). Hence such parameter analysis gives the better result to standardize the growth *Streptomyces* sp. to fight against broad-spectrum of bacteria, fungi and virus.

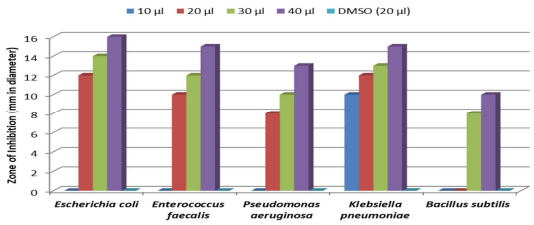

Fig. 1. Antibacterial activity of *Streptomyces* sp. (ISB1) producing antibacterial substance

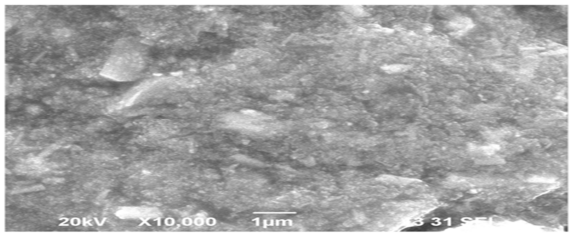

Fig. 2. SEM observation of AgNPs Synthesized using *Streptomyces* sp. (ISB1)

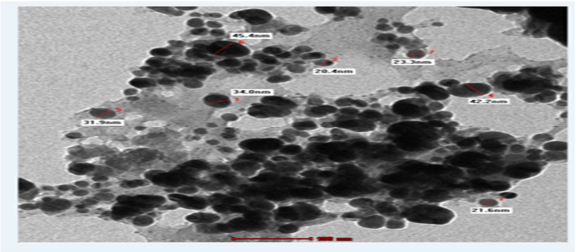

Fig. 3. TEM observation of AgNPs Synthesized using *Streptomyces* sp. (ISB1)

The main study was to determine the ability of *Streptomyces* sp. (ISB1) in the synthesis of silver nanoparticles. *Streptomyces* sp. (ISB1) supernatant was added to silver nitrate solution note for the colour change.

Table 1. Screening of antimicrobial producing *Streptomyces* sp.

Isolated Streptomyces sp.	Pathogenic bacteria				
	Escherichia coli	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Bacillus subtilis*
ISB1	+	+	+	+	+
ISB2	+	-	-	-	-
ISB3	+	+	-	-	+
ISB4	+	-	-	-	+
ISB5	-	-	-	-	+
ISB6	-	-	-	-	+

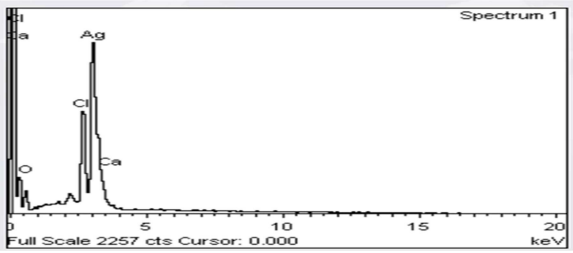

Fig. 4. EDAX analysis of AgNPs Synthesized by cell free supernatant of *Streptomyces* sp. (ISB1)

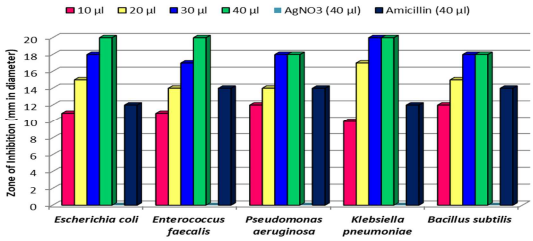

Fig. 5. Mean zone of inhibition (mm) of AgNPs synthesized using *Streptomyces* sp. (ISB1) against bacterial pathogens (well diameter was 6 mm)

After the addition, slowly there was colour change from colourless to pale yellow and at the end of incubation it was yellowish brown in colour. Yellowish brown colour is the clear indicator of silver nitrate reduction to silver nanoparticles. Then, the progress of their action was monitored by UV-Visible spectrophotometer and the maximum peak was obtained at 427 nm. The size and morphology were determined by SEM (Fig. 2) and TEM (Fig. 3), morphology of the nanoparticles are spherical and size ranges from 21-50 nm. The elemental identification and quantitative compositional information was obtained using EDX. From Fig. 4, it confirms the elemental composition as silver along with calcium and oxygen that might be compounds from *Streptomyces* sp. (ISB1). The microbiologically synthesized silver nanoparticles exhibited an excellent antibacterial activity compared with the control sample (silver nitrate and ampicillin) which might be due to the synergistic effect of both silver nanoparticles and *Streptomyces* sp. (ISB1). Similarly, as above 10, 20, 30 and 40 µg same concentrations were taken and results were compared with it. Here, 30 µg itself has given more promising results than the 40 µg of *Streptomyces* sp. silver nanoparticles, itself that proving the viability and strong immunity against these pathogens (Fig. 5).

Silver nanoparticles reduction was indicated with colour change to yellowish brown and this was preliminarily confirmed by UV-Vis spectrophotometer. It used to not only monitor the reaction between supernatant of the *Streptomyces sp.* and silver ions but also for the confirmation of silver nanoparticle production by the maximum absorbance value between 400-450nm (Sastry *et al.,* 2003; Sneha Paul *et al.,* 2015). In our study, it ranges around 427nm confirming the synthesizing of silver nanoparticles. SEM and TEM are used to determine the morphology along with size. The minimum size results in maximum penetration and capacity to invade the microorganism. However, the combined effect of biological synthesis of silver nanoparticles gives promising results in application oriented studies.

Conclusion

A significant need in the field of nanotechnology is the development of a green and reliable method to produce silver nanoparticles compared to other classical methods. *Streptomyces* sp. (ISB1) as such have predominately used in the production of antibiotics. Hence, this has been isolated from the soil sample and used for the reduction of silver nitrate to

silver nanoparticles. However, synthesized silver nanoparticles show synergistic effect and have broad coverage of antibacterial than the *Streptomyces* sp. (ISB1).

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