

Combined Efficacy of Antibiotics and Biosynthesised Silver Nanoparticles from *Streptomyces Albaduncus*

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Abstract: Nanotechnology is an important field of modern research dealing with the design, synthesis, and manipulation of particles. Antibiotic resistant pathogens pose an enormous threat to the treatment of wide range of serious infections. Antibiotics in combination with biosynthesized silver nanoparticles minimize the antibiotic doses to cure the dreaded diseases. Actinomycetes are prolific producers of antibiotic and yield over two-third of naturally occurring antibiotics. In this study, Silver nanoparticles were bio-synthesized by extracellular method using *Streptomyces albaduncus* sp [MTCC-924], a member of actinomycetes. The produced silver nanoparticles were confirmed using Visible UV Spectrophotometer (UV) and characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning electron microscope (SEM) and Atomic force microscopy (AFM). The UV Spectrophotometer revealed the formation of silver nanoparticles by yielding silver plasmon absorption maxima at 440 nm and SEM micrograph indicates the uniform spherical particles at the size range of 40 to 60 nm. The FTIR confirmed the presence of proteins as the stabilizing agent surrounding the silver nanoparticle. Atomic force microscopy (AFM) showed the particle height, average roughness of the particle. The biosynthesized silver nanoparticles from *Streptomyces albaduncus* (MTCC-924) were also evaluated for their synergistic effect with antibiotics against the MTCC pathogens. The antibacterial efficacy of various antibiotics was found to be enhanced in the combination of silver nanoparticles.

Keywords: *Streptomyces albaduncus* sp -924, Synthesis of silver nanoparticles, UV-Vis Spectrophotometer, FTIR, FESEM, AFM, Antimicrobial activity

Introduction:

Nanoscience and nanotechnology is an emerging field, which involves in the synthesis, application of nanoscale materials, and structures usually in the range of 1 to 100 nm(1). AgNPs are considered attractive building blocks for nanomaterial architectures(2). Emerging multidrug resistant (MDR) bacteria has raised a demand for the urgent need to identify novel antimicrobial agents(3). Silver has been used since Roman times as a disinfectant because of its well-known antimicrobial properties.

However, advances in generating AgNPs have revived the use of silver as a powerful bactericide(4). Silver nanoparticles have a great number of applications, e.g., in nonlinear optics, spectrally selective coating for solar energy absorption, bio labelling, intercalation materials for electrical batteries, high-sensitivity bimolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and microelectronics(5-7). Silver nanoparticles are also being used as an enhanced substrate in surface enhanced Raman spectroscopy (SERS) for enzyme immunoassay(8). It was well documented that silver nanoparticle production could be possible using the cell mass of certain bacteria, fungi, and yeast strains, either extracellularly or intracellularly(9,10). The advantage of adapting biosynthesis of AgNPs is the simplicity of extracellular synthesis and downstream processing(11,12). Actinomycetes-mediated green chemistry— approach to the synthesis of nanoparticles has many advantages(13). The aim of this study is to bio synthesize silver nano

particle from *Streptomyces albaduncus* sp -924, a member of actinomycetes obtained from MTCC, Chandigarh, India by extracellular method and confirmation using UV Spectrophotometer. Characterization study was performed using SEM and FTIR and AFM. The antimicrobial activity of the produced Silver nanoparticles was checked against MTCC pathogens in combination with antibiotics.

Materials and Methodology

The actinomycetes culture of *Streptomyces albaduncus* (MTCC-924) was obtained from Microbial Type Culture Collection, Chandigarh, India. The obtained species was inoculated in *Streptomyces* media (Glucose 4.0g, Yeast Extract 4.0g, Malt Extract 10.0g, CaCO₃ 2.0g, Agar 12.0g, Distilled Water 1.0L, pH -7.2 which acts as growth media for its growth and proliferation. *Streptomyces albaduncus* (MTCC924) was grown in Yeast malt broth at 37°C for 7 days. Then the culture was subcultured in petriplates at room temperature using 2% malt extract and 0.5% yeast extract and used for biosynthesis of nanoparticles.

Silver Nanoparticle Synthesis

For the synthesis of silver nanoparticles, the spores of *Streptomyces albaduncus* was inoculated in MYGP media (Malt extract-3.0 g, Yeast extract-3.0 g, Glucose-10.0 g, Peptone-5.0 g, Distilled water-1000 ml, pH-7.2) in Erlenmeyer flasks incubated at 25°C on a shaker (150 rpm) for 72 hours. The biomass was filtered using Whatman filter paper (No.1) and washed extensively with distilled water to remove any medium components. Fresh and clean biomass was taken in the Erlenmeyer flasks containing 100cm³ of milli-Q deionized water. The flasks were agitated at the same conditions as described above and again the biomass was filtered through Whatman filter paper. For the synthesis of silver nanoparticles extracellularly 1mM final concentration of silver nitrate AgNO₃, was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25°C in dark for 72 h under static and at 200 rpm and the silver nanoparticle production monitored. Control (without the silver ion, only culture) was also run along with the experimental flask.

Characterization of Silver Nanoparticles

The bioreduction of the Ag⁺ ions in the solution was monitored by changes in color. Periodically, aliquots of the reaction solution were removed and the absorptions were measured in a UV-Vis spectrophotometer between 350-750nm range of the reaction medium. The dried silver nanoparticles were subjected to FTIR analysis. The samples were scanned using infrared in range of 4000- 500 cm⁻¹ using FTIR. Scanning electron microscope (SEM) was used to obtain the surface image and the size of the microbially synthesized silver nanoparticle. EDAX shows the element of the nanoparticles. An atomic force microscopy (AFM) was performed to identify the topological appearance, and the size of the biosynthesized silver nanoparticles.

Antibacterial Activity of the Agnps

The antimicrobial activity of the microbiologically synthesized AgNPs was tested against pathogenic organisms obtained from MTCC, Chandigarh, India. The organisms used were gram positive bacteria *Staphylococcus aureus*, *Micrococcus luteus*, *Streptococcus mutans* and gram negative bacteria *Escherichia coli*, *Pseudomonas sp.*, and *Klebsiella pneumonia*. The combined formulation of silver nanoparticles with standard antibiotic discs such as Ampicillin, Azithromycin, Cefotaxime, Erythromycin, Ofloxacin, P/T were used to find out the synergistic effect against the above pathogens. The zone of inhibition was measured after overnight incubation at 37°C.

Assessment of Increase in Fold Area

Increase in fold area was assessed by calculating the mean surface area of the inhibition zone generated by an antibiotic alone and in combination with silver nanoparticles (19). The fold increase area was calculated by the equation, Fold increase (%) = (b-a)/a*100 where a and b refer to the zones of inhibition for antibiotic alone and antibiotic with silver nanoparticles.

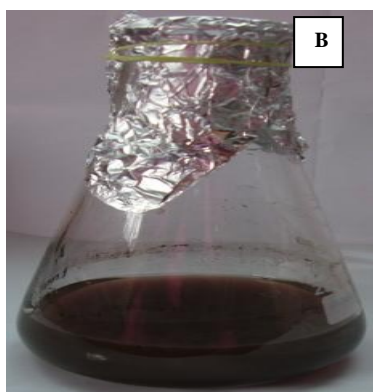
Results and Discussion

Silver Nanoparticle Synthesis

The *Streptomyces albaduncus* (MTCC- 924) was obtained from the Microbial Type Culture Collection (MTCC) Chandigarh, India and utilized in the present study for the biosynthesis of silver Nanoparticles. Silver nanoparticles were biologically synthesized from the culture supernatant *Streptomyces albaduncus* (Fig 1). The Erlenmeyer flasks with the cell filtrate of streptomycetes were a pale yellow color (Fig 2A) before the addition of Ag⁺ ions and this change to a brownish color (Fig 2B) on completion of the reaction with Ag⁺ ions for 28 h.



Fig 1: Culture growth of *Streptomyces albaduncus*



(A) Before treatment

(B) After treatment of silver nitrate

Fig 2.Synthesis of silver nanoparticles from *Streptomyces albaduncus* (MTCC924)

The appearance of a yellowish-brown color in the silver nitrate treated flask was a clear indication of the formation of silver nanoparticles in the reaction mixture(13) due to the reduction of metal ions and formation of surface plasmon resonance, whereas no color change was observed in either the culture supernatant without silver nitrate.

Uv-Visible Spectroscopic Analysis

In this study, AgNPs were successfully synthesized in the culture supernatant *Streptomyces* sp. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by a UV-vis spectrophotometer(3). In the UV-visible spectrum (Fig-3), a strong peak was observed at 440 nm, and the surface plasmon resonance (SPR) confirmed successful formation of AgNps.

Fourier Transform Infrared Spectroscopy (Ftir)

FTIR spectral analysis showed array of absorbance bands in 500 cm⁻¹ to 4000 cm⁻¹. The silver nanoparticles synthesized using *Streptomyces albaduncus* showed strong bands (Fig 4). The FTIR spectrum analysis of AgNPs showed intense absorption bands at 3273.20, 2960.73, 1610.56, 1384.89, 1234.44, 1064.71 and 788.89cm⁻¹ respectively. The intense broad absorbance peak at 3273.20 (O-H stretch) is the characteristic

of the of the H bonded functional group in alcohols and phenolic compounds. The band at 2960.73 and 2922.16 cm^{-1} (C–H stretch) can be assigned to the alkanes group. The intense medium absorbance at 1610.56 cm^{-1} (N–H bend) is the characteristic of the amine group. The intense medium absorbance at 1384.89 cm^{-1} (C–H bend) is the characteristic of the alkanes group. The intense broad absorbance at 1064.71 cm^{-1} (C–N stretch) is the characteristic of the amine group.

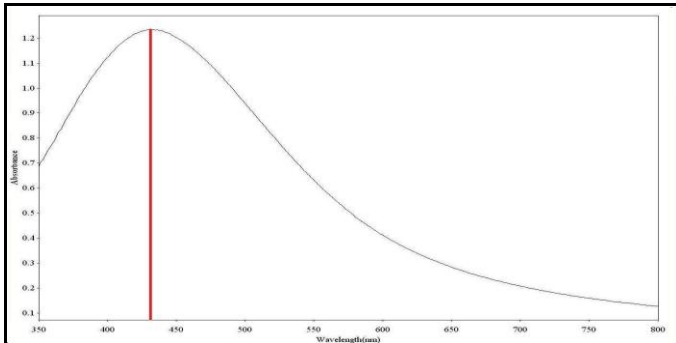


Fig. 3: Uv–Vis spectrum of silver nanoparticles synthesized from *Streptomyces albaduncus* (MTCC924)

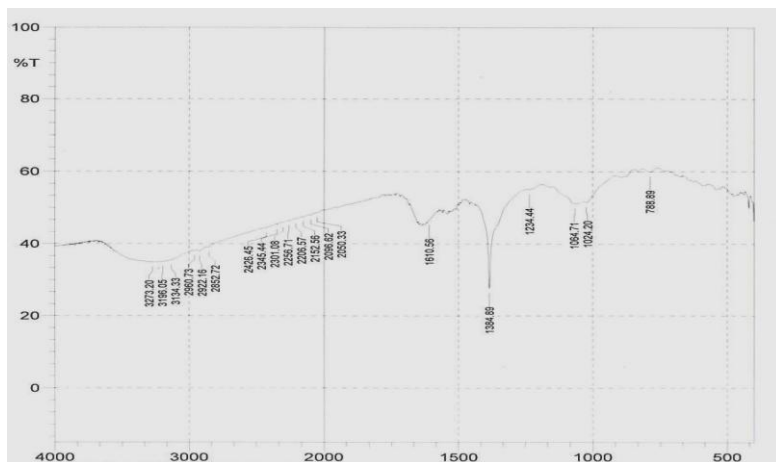
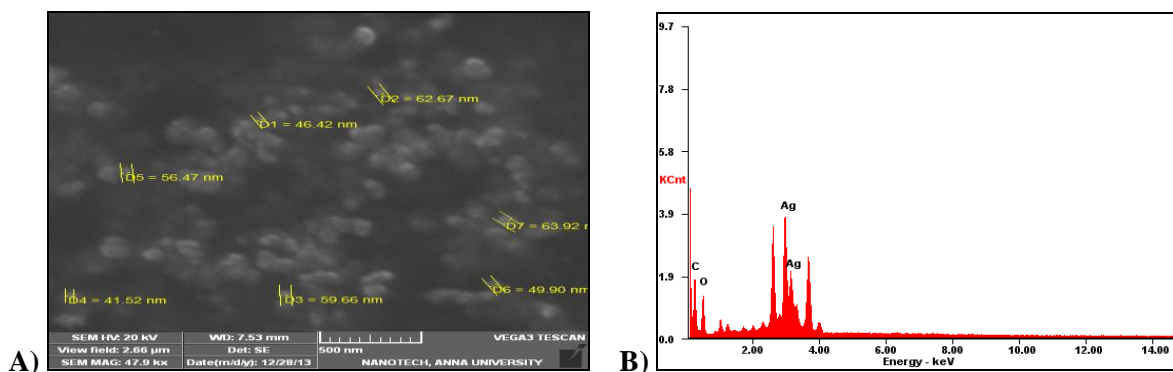


Fig. 4: FTIR analysis of Silver Nanoparticles synthesized from *Streptomyces albaduncus* (MTCC924)

Scanning Electron Microscope (Sem)

The dried silver nanoparticles were obtained by centrifugation at 10000 rpm for 20 mts. The size and shape of the silver nanoparticles biosynthesized was studied by SEM (Fig 5a). EDAX image shows the presence of silver (Fig 5b) in the silver nanoparticles solution.



Figures 5(A)–5(B) show the SEM and EDAX image of the biosynthesized silver nanoparticles

Particle Size Analysis

Particle size analysis shows that, when scanning from 1 nm, the particles count is very low and its gradually reached the higher value at 65 nm and again it gradually decreased. So this indicates that the maximum nanoparticles in the range of 40 to 65 nm and only very few particles are present below and above this range (Fig 6).

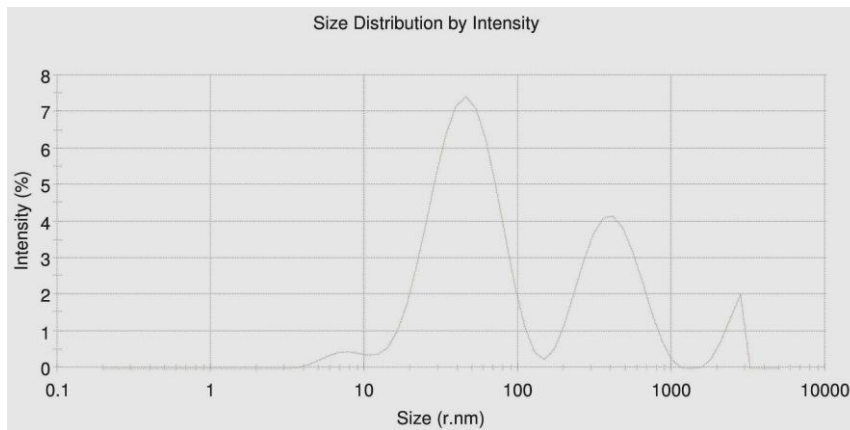


Fig.6 Particle size of the silver nanoparticles synthesized by *Streptomyces albaduncus*

Atomic Force Microscopy

The particle size and average roughness were further characterized by AFM .Three dimensional image showed the particle height, average roughness (Fig 7.A). Two dimensional image (Fig 7.B) showed the agglomeration of the particle. Nanoparticles size was spherical and poly dispersed whose range was in between 40 to 60 nm.

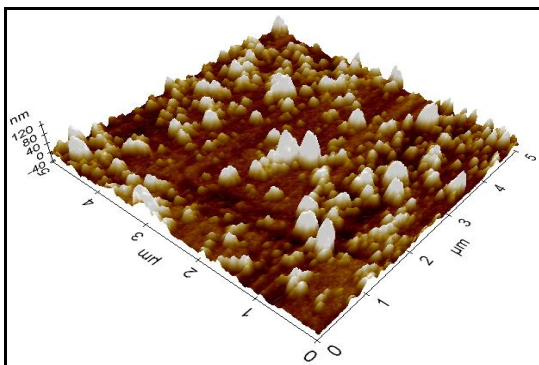


Fig 7.a: 3D picture of Atomic Force microscopy (AFM) of silver nanoparticles synthesized from *Streptomyces albaduncus* (MTCC 924)

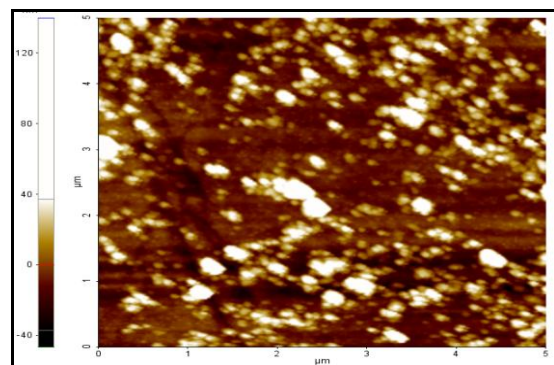


Fig 7.b: 2 D picture of Atomic Force Microscopy of silver Nanoparticles synthesized from *Streptomyces albaduncus* (MTCC 924)

Antibacterial Activity of the Agnps

The biologically synthesized AgNPs inhibited different pathogenic microorganisms. According to(14), the mechanism behind the bactericidal effect of the silver nanoparticles against bacteria is not well known.. It is believed that DNA loses its replication ability and cellular proteins become inactivated upon silver ion treatment(15,16). Furthermore, higher concentrations of Ag⁺ ions have been shown to interact with cytoplasmic components and nucleic acids(17, 18). The antibacterial activities of Ampicillin, Azithromycin, Cefotaxime, Erythromycin, Ofloxacin, P/T increased in the presence of AgNPs against the test pathogens (Fig -8).The synergistic effect of silver nanoparticles represents the highest percentage of increase in inhibition, which was found against Erythromycin (15mcg/disk), followed by Azithromycin (15mcg/disk), Ampicillin, (10mcg/disk), Cefotaxime (30mcg/disk) Ofloxacin (5mcg/disk) and P/T against all test pathogens as shown in Table 1&2.

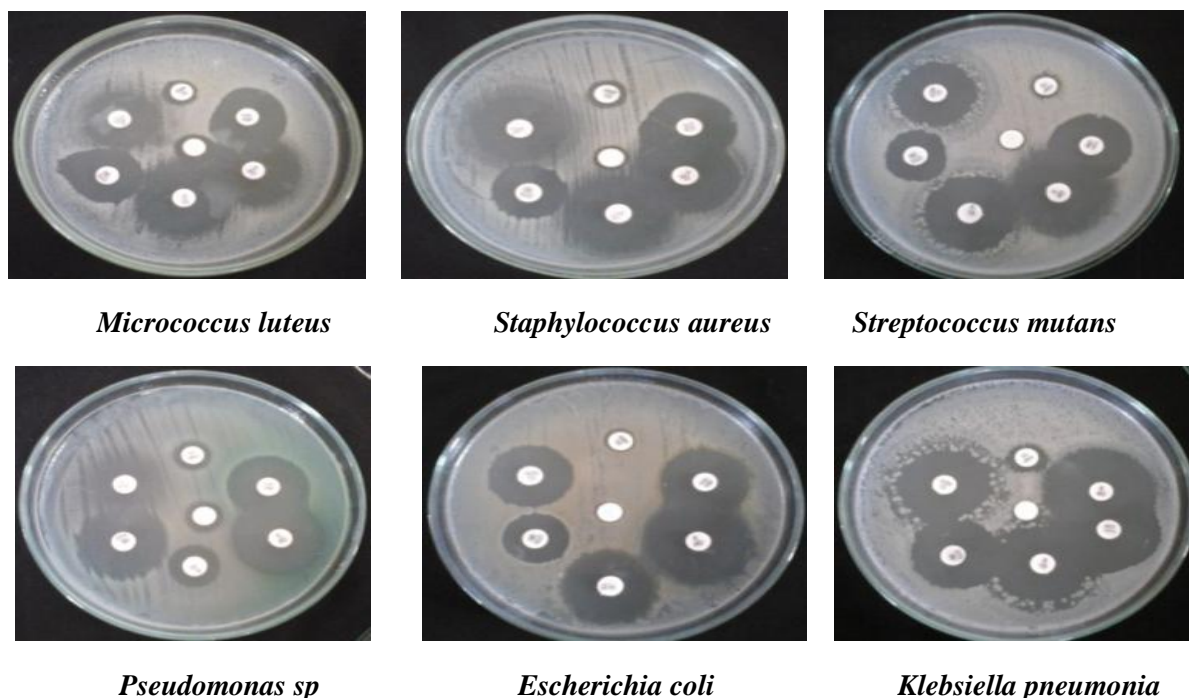


Fig 8: Antibacterial activity of silvernanoparticles Streptomyces albaduncus(MTCC-924) with Antibiotics

Tab:1&2: Synergistic effect of different antibiotics with and without extracellularly biosynthesized AgNPs against pathogens. F.I-Fold Increase $F = ((b-a)/a)*100$

Note: In the absence of bacterial growth inhibition zones, the disc diameter (6 mm) were used to calculate the fold increase.

Si.No	Pathogens	AgNps	Antibiotics Zone of inhibition(mm)								
			Ampicillin			Azithromycin			Cefotaxime		
			Ab	Ab+NPs	F.I %	Ab	Ab+NPs	F.I %	Ab	Ab+NPs	F.I %
1	S.aureus	12	9	10.5	16.7	19	24	26.3	17	20	17.6
2	M.luteus	13	-	10.5	75.0	19	22	15.8	18	21	16.7
3	S.mutans	16	-	9	50.0	18	24	33.3	14	18	28.6
4	Pseudomonas	14	-	11	83.3	12	15	25.0	23	25	8.7
5	E.coli	14	8	9	12.5	18	23	27.8	19	21	10.5
6	K.pneumonia	15	8	12	50.0	19	24	26.3	18	24	33.3

Si.No	Pathogens	AgNps	Antibiotics Zone of inhibition(mm)								
			Erythromycin		F.I %	Ofloxacin		F.I %	P/T		F.I %
			Ab	Ab+NPs		Ab	Ab+NPs		Ab	Ab+NPs	
1	S.aureus	12	21	29	38.0	25	29	16.0	25	26	4.0
2	M.luteus	13	22	29	31.8	25	29	16.0	25	26	4.0
3	S.mutans	16	23	29	26.0	28	30	7.14	23	24	4.3
4	Pseudomonas	14	12	15	25.0	28	29	3.57	27	28	3.7
5	E.coli	14	15	26	73.3	26	30	15.38	25	26	4.0
6	K.pneumonia	15	20	29	45.0	26	32	23.07	28	29	3.5

Conclusion

In this study silver nanoparticles which was biologically synthesized is completely toxic free and it is low cost and ecofriendly compared to chemical synthesis methods. The silver nanoparticles was synthesized extracellularly from *Streptomyces albaduncus* (MTCC-924). The size of the silver nanoparticle obtained was between 40-60 nm. The silver nanoparticle shows good antibacterial activity against MTCC pathogens and its synergetic effect with antibiotics shows its antimicrobial potency. The conjugation of antibiotic with silver nanoparticles would prevent development of resistance by microbes and enhance the antimicrobial property of the antibiotic and minimize the dosage of antibiotics against multi drug resistance pathogens. The side effects caused due to the antibiotics can be minimized up to an extend and at the same time cost effective also. The anti-cancerous activity of the silver nanoparticles will be analyzed in future study to find its biocompatibility with animal and human beings for drug designing.

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