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Investigating the Antibacterial Properties of Silver Nanoparticles Acquired using Streptomyces strain AK3 from Riverbank Soil

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The soil sample was acquired from a heavily metal polluted site on the Tapi River in Surat, Gujarat, India, diluted serially, and dispersed over an actinomycetes isolation medium. Isolates were cultured in 100 ml of starch-casein broth at 300 C for 72 hours in an incubator with shaking. The cell-free filtrate was added to a final solution of 1 mM silver nitrate, which was then dried at 2500 C. Using a spectrophotometer, silver nanoparticles were quantified, data on size distribution and zeta potential were acquired from Malvern, and the 16S rRNA gene was amplified in a PCR mixture. As a result of the addition of silver nitrate to the S. atacamensis strain AK3 filtrate, the reducers altered the broth's color from yellow to light brown. The highest absorbance was measured at 420 nm, and the 0.25 polydispersity index was below the agglomeration threshold. The TEM indicated their spherical to ellipsoidal shape and 20 nm size. The NJ approach to sequence alignment revealed that the strain was 99.42% similar to S. atacamensis C60. Zones of inhibition of S. epidermidis, A. baumanni, N. gonorrhoeae, and L. monocytogenes were found to be 18±1 mm, 19±1 mm, 20±1 mm, and 14±1 mm respectively, at 35 μg/ml AgNPs, proving the efficiency of AgNPs synthesized by the strain.

Keywords: Antibacterial activity; Silver nanoparticles; TEM; UV-Vis spectra; Zeta potential.

At various times, one or more medications were administered to the pathogenic bacterial species. Due to the natural selection of resistant strains each time, none of these therapies were very effective; hence, the scientific community is using a variety of strategies to address this problem.[1-4] Due to their unique physical features, nanoparticles have been considered as a potential agent against pathogenic microorganisms[5,6]. Compared to gold, zinc, iron, etc., silver nanoparticles demonstrate greater efficiency[7]. Nanoparticles of silver smaller than 10 nm exhibit deleterious effects on human cells[8,9]. By altering the procedure temperature, the size of the silver nanoparticles may be modified. This is because silver nanoparticles are stable at high temperatures and the nucleation rate constant varies with temperature[10]. Optimizing the concentration of silver nanoparticles will limit their electrical conductivity and prevent the formation of big crystals[11,12]. Biosynthesized silver nanoparticles have more clinically effective uses than chemically produced ones. Moreover, hazardous substances may be absorbed by silver nanoparticles during chemical production[13,14]. Silver nanoparticles

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synthesised by actinomycetes are both mild on human cells and inexpensive to produce[15-17]. The optimal conditions for the biosynthesis of silver nanoparticles should be determined using statistical models[18]. Actinomycetes are dual-natured, soil-dwelling bacteria that exhibit traits of both bacteria and fungus[19-21]. Work has been done on the ability of actinomycetes to make secondary metabolites that can be used to treat patients[22,23]. The fabrication of silver nanoparticles by Streptomyces inhabiting industrially polluted areas, as well as their therapeutic applications, has rarely been described[24,25]. Biosynthesis of silver nanoparticles by Streptomyces inhabiting an industrially contaminated location; Tapi river, Surat, Gujarat; and their medical uses in humans are the focus of this work.

MATERIALS AND METHODS

Soil collection and isolation of actinomycetes

The soil sample was obtained from the heavy metal contaminated location on the Tapi river in Surat, Gujarat, India, diluted serially, and disseminated over actinomycetes isolation medium (HI media M490) enriched with 25 ìg/ml cycloheximide, 50 ìg nystatin, and 25 ìg nalidixic acid. The pH of the media was set at 6.9[26,27]. After 1.5 weeks of incubation at 30^0 C, actinomycetes had successfully developed. Isolates were stored in 50% (V/V) glycerol at -40^0 C and sub-cultured for later use.

Silver nanoparticles synthesizing ability-based selection of isolate

The isolates were grown in 100 ml of Starch casein broth (SCB), (S7968-25B Molecular grade) at 30^0C for 72 hours in the shaking incubator so that mycelia can clump as several small balls. Each SCB was filtered with a bacteriological filter and washed with distilled water to remove any impurities then, a 10-gm wet cluster of actinomycetes was suspended in 100 ml distilled water followed by shaken incubation at 30^0C for 72 hours. Mycelia in distilled water were centrifuged, and the cell-free filtrate was added to a final solution of 1 mM silver nitrate (RANKEM AR) and 0.5 mM sulphur-containing amino acid[28]. The color of the added cell-free filtrate turned pale brown on the third day of incubation, and then the cell-free filtrate was heated at 250^0C to dry. This dried substance containing nanoparticles of silver was thoroughly combined with 100 ml of distillated water[29].

The growth of isolates and production of AgNPs are briefly shown in the flowchart below.

Soil samples '! Serial dilution '! Spreading over the actinomycetes isolation media supplemented with 25 ìg/ml of cycloheximide, 50 ìg/ml of nystatin, and 25 ìg/ml of nalidixic acid '! Isolates transferred to Starch casein broth '! Filtration of SCB '! Suspension of cell filtrates in distilled water '! Centrifugation '! Addition of $AgNO_3$ to cell free filtrates '! Incubation '! Colour observation

Quantification and structural properties of synthesized silver nanoparticles

The quantification of silver nanoparticles was done with the spectrophotometer [UV-VIS 1700, Range- (190nm-1100nm), Wavelength accuracy- ±0.3, and Shimadzu made]. The size distribution and the zeta potential reports were obtained using Malvern. The Characterization of silver nanoparticles was accomplished with Transmission electron microscope (Accelerating voltage- 80 to 200 kV, Joel.).

16S rRNA gene sequencing of actinomycetes isolate

The 16S rRNA gene was amplified in 100il PCR mixture [54.3ng DNA, 0.5mM of each dNTP, 400ng of each primer (5'GGATGAGCCCGCGGCCTA3' and 5'CGGTGTGTACAAGGCCCGGGAAC3'), 3.2mM $MgCl_2$, and 1il 3U Taq DNA polymerase]. In the PCR machine, the amplification was accelerated for 30 cycles and two hours. Each cycle lasted one minute for denature, one minute for anneal, and two minutes for extension. The amplified DNA was sequenced using an ABI3130 genetic analyser, sequence homology was determined using BLAST, and a phylogenetic tree was constructed using the bootstrap[30].

Treatment of human pathogens with various concentrations of Silver nanoparticles

At doses of 7 mcg/ml, 8 mcg/ml, 9 mcg/ml, 10 mcg/ml, 11 mcg/ml, and 12 mcg/ml, silver nanoparticles were examined for *Neisseria gonorrhoeae* ATCC49226 and *Acinetobacter baumannii* ATCC19606. AgNPs concentrations of 10 mcg/ml, 15 mcg/ml, 20 mcg/ml, 25 mcg/ml, 30 mcg/ml, 35 mcg/ml, and 40 mcg/ml were used

to treat *Listeria monocytogenes* ATCC13932 and *Staphylococcus epidermidis* ATCC12228.

RESULTS

Selection of actinomycetes isolate for synthesis of the silver nanoparticles

After 48 hours, the addition of silver nitrate to the cell free-filtrate caused the reducers of *Streptomyces atacamensis* strain AK3 to change the colour of the broth from yellow to pale brown. UV-VIS spectrophotometer, Zeta sizer, and TEM were used to examine the characteristics of silver nanoparticles. The maximum absorption was recorded at 420nm, confirming the presence of silver nanoparticles shown in Figure 1.

It was observed that the 0.25 polydispersity index (PDI) was lower than the agglomeration value. The zeta potential of silver nanoparticles was determined to be -14 mV, indicating the slight aggregation of silver nanoparticles as shown in Figure 2.

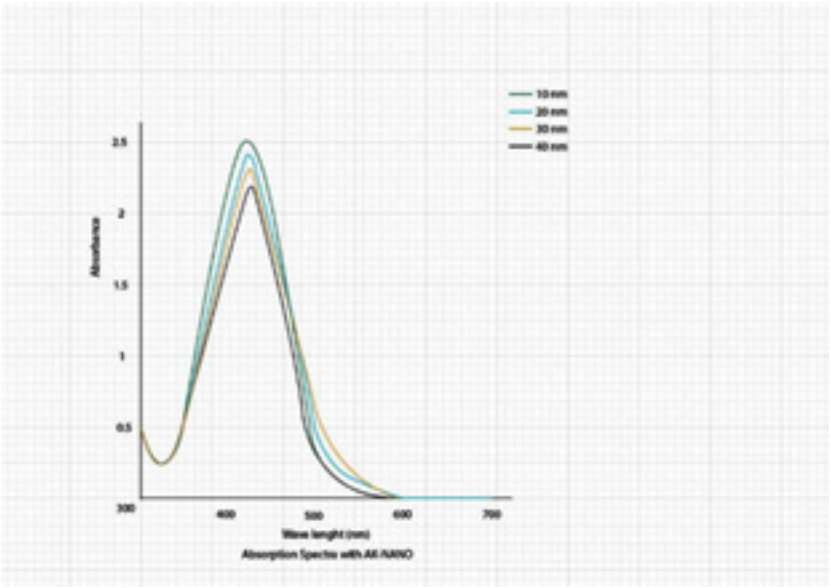

Fig. 1. UV-Vis spectra of sample containing AgNPs

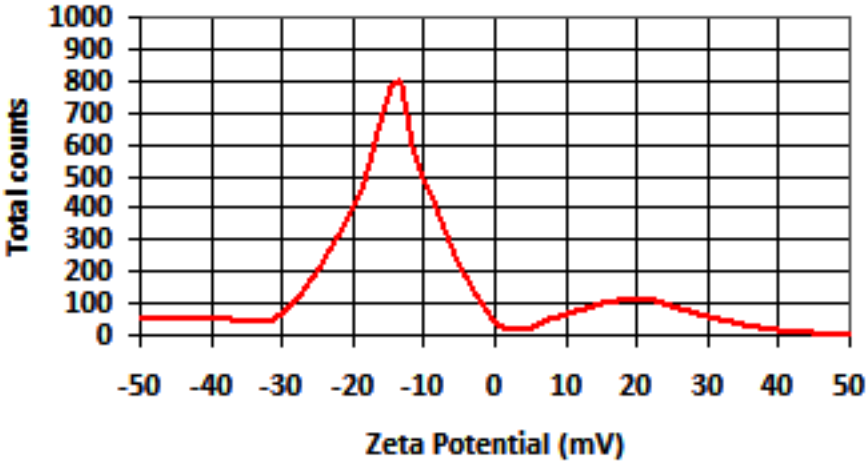

Fig. 2. Zeta potential of silver nanoparticles

The TEM micrograph of silver nanoparticles revealed their spherical to ellipsoidal form and 20 nm size as shown in Figure 3.

Morphological and molecular identification of the actinomycetes isolate

The colonies were wrinkly, slightly curved, and resembled chalk, and the strain thrived in aerobic conditions at 32^0C. The 16s rRNA gene was used to molecularly identify the strain. Sequencing developed an image of a 1040 bp-long gene for research. The NJ technique of sequence alignment discovered that the strain is 99.42% identical to *S. atacamensis* C60. The 16s rRNA gene sequencing data for this strain is accessible from the NCBI gene bank with the accession number MT626067. Figure 4 depicts the band of 16S rRNA gene of the strain.

A comparative analysis of strain-produced AgNPs and widely recognized antibiotics

As indicated in Table No. 1 and Figure 5, in a comparison of the efficacy of synthesized silver nanoparticles and antibiotics against some human microbiological diseases, synthesized silver nanoparticles delivered the expected results.

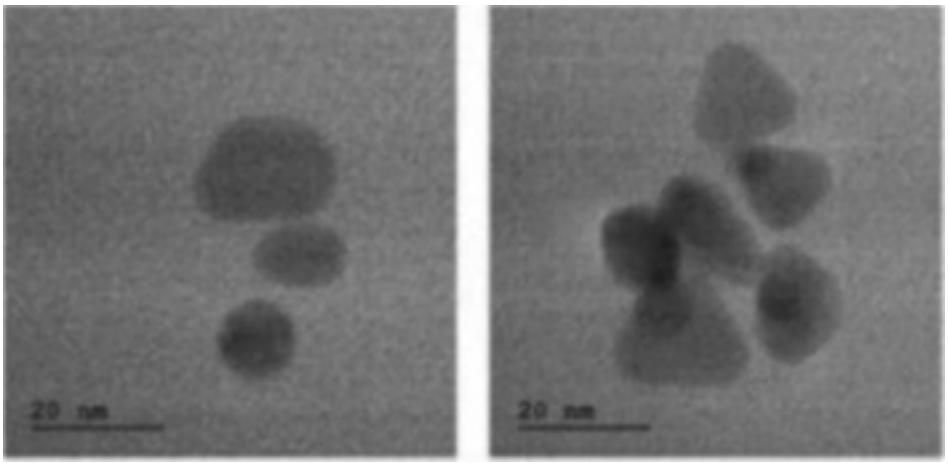

Fig. 3. TEM images of obtained AgNPs

The relative effects of different silver nanoparticle concentrations on *N. gonorrhoeae, A. baumannii, L. monocytogenes,* and *S. epidermidis* are as follows:

With data fetched from Figures 6 & 7, Regression, P value, ANOVA, and Coefficient of

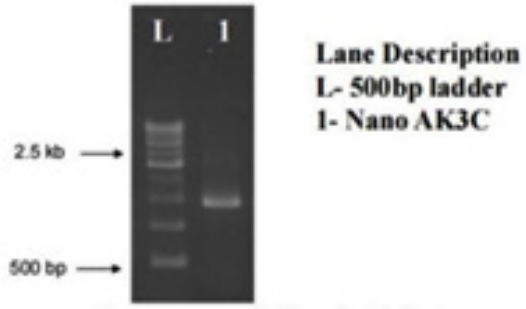

Fig. 4. Band of strain's rRNA gene

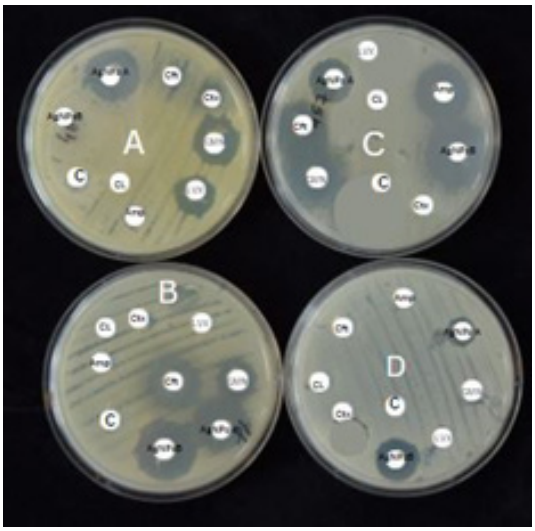

Fig. 5. (A) *S. epidermidis*, (B) *A. baumannii*, (C) *N. gonorrhoeae*, (D) *L. monocytogenes* with zones of inhibition created by AgNPs A, AgNPs B, Ampicillin, Cefotaxime, Cefoxitin, Chloramphenicol, Gentamycin, Levofloxacin, and Control.

determination as shown in Table-2 incline to use the optimum concentration of AgNPs .

DISCUSSION

The physical and chemical processes used to create silver nanoparticles are ecologically toxic and expensive. In the last decade, microorganisms have been harnessed extensively in the fabrication of silver nanoparticles[31-33]. The production medium for the biogenesis of silver nanoparticles may be optimised to reduce production costs, with temperature, pH, and $AgNO_3$ concentration serving as three of its most important parameters[34]. Compared to typical biomolecules, silver nanoparticles are much more resistant to harsh manufacturing circumstances[35]. Actinomycetes are gaining prominence as sources for the synthesis of AgNPs, and this is the first report on the synthesis of AgNPs using *S. atacamensis* living in the industrially contaminated Tapti river bank for the treatment of selected human diseases in the present study[36,37]. Surface area, shape, and size of silver nanoparticles are adjustable with media-involved variables and play a crucial function in defining the level of AgNPs' efficiency[38]. As the thickness of bacterial peptidoglycan grows, the effectiveness of AgNPs diminishes[39]. *N. gonorrhoeae* and *A. baumannii* were shown to be more susceptible to the synthesised silver nanoparticles than *S. epidermidis* and *L. monocytogenes* in the present research. The acceleration of diameter of

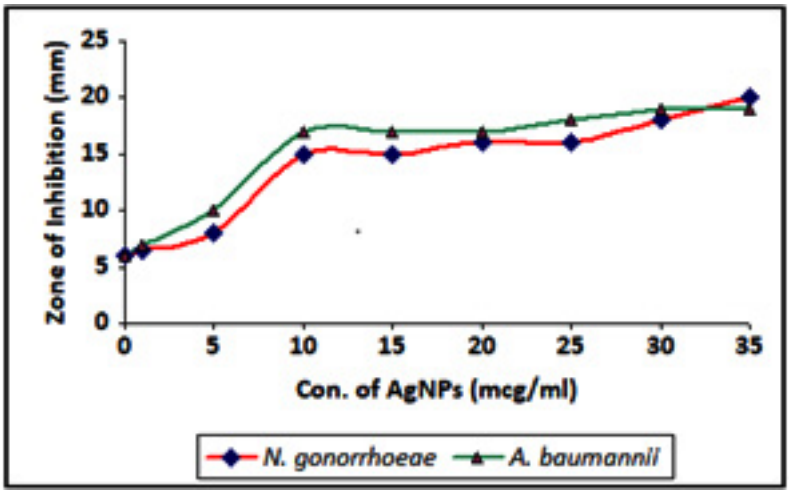

Fig. 6. Effects of various concentrations of *N. gonorrhoeae* and *A. baumannii*

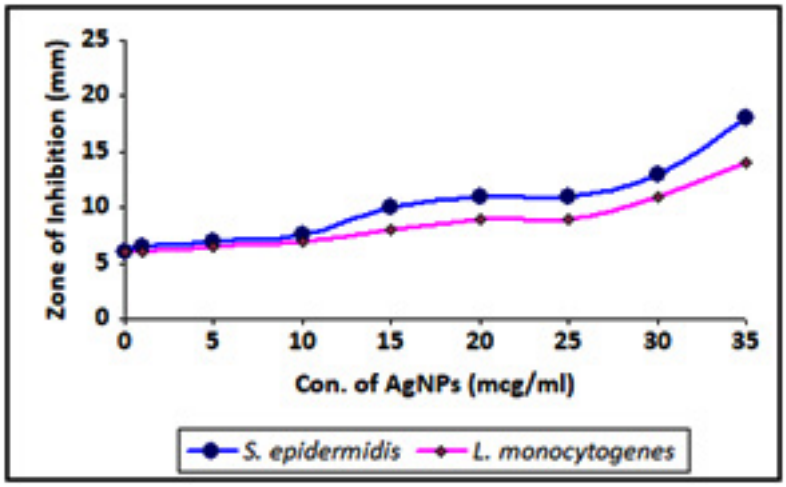

Fig. 7. Effects of various concentrations of AgNPs on *S. epidermidis* and *L. monocytogens*

Table 1. Resistance (R) and sensitivity (S) with zone of inhibition of selected human pathogens to varied quantities of fabricated silver nanoparticles and antibiotics

S. No.	Antibacterial agent name	Antib-acterial agent con.	*S. epider-midis*	*A.baumannii*	*N. Gonorrhoeae*	*L mono-cytogenes*
1	Synthesized AgNPs (A)	10 µg	R	S (17±2 mm)	S (15±1 mm)	R
2	Synthesized AgNPs (B)	35 µg	S (18±1 mm)	S (19±1 mm)	S (20±1 mm)	S (14±1 mm)
3	Ampicillin	10 µg	R	R	S (18±2 mm)	R
4	Cefotaxime	30 µg	R	S (18±0.6 mm)	S (18±1 mm)	R
5	Cefoxitin	30 µg	R	R	R	R
6	Chloramphenicol	30 µg	R	R	R	R
7	Gentamycin	10 µg	S (14±1 mm)	R	S (19±0.8 mm)	R
8	Levofloxacin	05 µg	S (11±1 mm)	R	R	R
9	Control	-	R	R	R	R

Table 2. Regression, *P* value, ANOVA, and Coefficient of Determination

	Pathogen	Regression	*P* value	F	R^2
1	*S. epidermidis*	Y = 5.5 + 0.28X	Â 0.001	64.02	0.9
2	*L. monocytogenes*	Y = 5.4 + 0.2X	Â 0.001	62.12	0.9
3	*N. gonorrhoeae*	Y = 7.40 + 0.38X	Â 0.001	50.28	0.88
4	*A. baumannii*	Y = 8.74 + 0.36X	Â 0.002	24.46	0.78

inhibitory zones grew for optimal concentrations of silver nanoparticles, then decreased at greater concentrations, with the increased concentration of AgNPs inducing agglomeration later. When silver nanoparticles get into a bacterial cell, they cause ROs to be made, which damage the chromatin[40,41]. In contrast to the previous work, the cell-free filtrate on the addition of silver nitrate was found to have the potential to synthesise silver nanoparticles. AgNPs were chosen above TiO_2, ZnO, Fe_3O_4, Au, CuO, MgO, NO, and Al_2O_3 because they have the requisite chemical and physical properties to battle germs[42,43].

CONCLUSION

The synthesis of silver nanoparticles using actinomycetes has been documented; however, this work opens the door for silver nanoparticle synthesis by utilizing a strain of *Streptomyces sp.* isolated from the soil of the Tapi riverbank. The zones of inhibition against human pathogens formed by increasing the concentration of silver nanoparticles did not produce a consistently linear graph, as the acceleration of effectiveness decreased after a particular discrete point, indicating the use of an optimum concentration of AgNPs. This work's strain AK3 combats the challenges of producing silver nanoparticles in an environmentally hazardous manner, antibiotic side effects, and multidrug-resistant human pathogens.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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