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Rapid biosynthesis, Characterization and Antimicrobial Effects of Silver Nanoparticles from microorganism *serratia marcescens*

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Abstract

Biosynthesised nanoparticles have wider applications in medical and pharmaceutical fields. In our study aims to synthesis silver nanoparticles using biological source *Serratia marcescens.* The nano particle synthesis was optimized with different pH of the culture medium and concentration of metal salts that affected the nanoparticles formation during synthesis. Culture medium with different pH showed variation in the synthesis of nanoparticles. The absorbance of each sample is recorded and a graph was plotted between pH and absorbance at 420nm. Initially on increasing the pH the nanoparticle synthesis increased gradually up to pH 8. Hence the maximum synthesis of silver nanoparticles was observed at pH 8. The samples with 0.1M silver nitrate concentration showed dark brown colour when compared to the samples with concentration of 0.2M and 0.3M. The bacterial biomass was exposed to silver nitrate the metal ions are reduced to nanoparticles. The observed colour change provided the evidence for nitrogen reductase mediated bio reduction of silver salts to produce nanoparticles. The synthesized AgNps were characterized using UV-visible spectrum, FTIR, AFM. The antibacterial activity of synthesised silver nanoparticles is determined using different Gram negative and Gram positive bacteria. The observed zone of inhibition was measured against *Escherichia coli* 12 mm, *staphylococcus aureus* 10 mm, *pseudomonas aeruginosa* 9 mm. The positively charged AgNps exhibit a greater affinity toward the negatively charged bacterial cells resulting in the microbicidal activity.

Keywords: Silver nanoparticle, *Serratia marcesens*, Anti microbial activity, Nitrate reductase, Microbial synthesis.

Introduction

Nanoparticles are microscopic particles in the nanometer range of between 1-100 nm. A wide variety of metal nanoparticles have been found to be produced by prokaryotic and eukaryotic organisms including several bacterial and fungal species, when exposed to solutions containing metal salts. These types of biosynthesised nanoparticles have wider applications in medical and pharmaceutical fields. Silver nanoparticles are synthesized using bacteria by the following mechanism. The bio reduction of silver metal particles may occur via an active reductase enzyme process where H_2 is the electron donor and positively charged metal species act as the electron acceptors becoming reduced to a neutral metal nanoparticle. Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite. The bacteria having this enzyme produce silver nanoparticles. Silver nitrate in aqueous solution gives nitrate and silver ions. (1, 2). The genus *Serratia* of the family *enterobacteriaceae*, contain approximately 13 described DNA related species. Among those the well-known one is the species *serratia marcescens* is its capability to produce lactic acid via oxidative and fermentative metabolism. *Serratia marcescens* also reduce nitrate to nitrite which makes it suitable to synthesis silver and cadmium nano particles using silver nitrate and cadmium nitrate. The extra cellular synthesis of silver nanoparticles and its interaction with protein moiety was performed using an environmental isolate *Serratia marcescens (3)*. Hence in the present study is to synthesis and characterize silver nanoparticles using bacterial isolate *Serratia marcescens* obtained from K.A.P Viswanathan medical college and to study its antimicrobial activity.

Materials and methods

Silver nitrate (Qualigens fine chemicals), Nutrient broth, Nutrient agar, MacConkey agar (Himedia laboratories) and all other chemicals and reagents used were of analytical grade.

Isolation of *serratia marcescens*

The strain of *Serratia* species was bought from K.A.P.Viswanathan Government Medical College, Tiruchirappalli, Tamilnadu. The streak plate technique was used to isolate *S.marcescens* on the surface of MacConkey agar plate. MacConkey agar medium is selective medium for the growth of *Serratia* species. MacConkey agar media 100 ml (55.0g/ L) was prepared and sterilized. After attained the temperature 45-50^{o}C the agar media was poured into petriplates and the bacterial strain was streaked into the media. The media was incubated under 37^{o}C for 2 days. (4, 5, 6, 7)

Methods

Nutrient broth was used for the growth of *S.marcescens*. Nutrient broth 100ml (21g /L) was prepared and sterilized. The culture flask was then incubated for 24hrs in an incubator at 37^{o}C. After the incubation the bacterial growth was identified by taking OD in spectrophotometer. The inoculum was adjusted to 1Mc Farland standard. Nutrient broth 300 ml was prepared (13g/L) and sterilised. Five conical flasks containing 50ml of broth taken and their pH was adjusted from 5 to 9 using sodium

hydroxide solution. After pH adjustment equal volume of 1 McFarland turbidity standard inoculum was added to each conical flask of varying pH. Then these conical flasks were incubated in shaker at 37^{o}C and 120 rpm for 24hrs. Different concentration of $AgNO_3$ solution 0.1M, 0.2M, and 0.3M was prepared. The weight of $AgNO_3$ for the preparation of different concentrations is calculated by using the formula W=neL. The synthesis of nanoparticles is initially confirmed by visual inspection. (8, 9)

Characterization

A synthesized nanoparticle was characterised using UV visible spectroscopy, FTIR and AFM. The absorption spectrum of the nanoparticles is recorded 300-600nm. FTIR spectrum analysis was performed in order to find the type of protein bonding (7). The morphological characterization of the synthesized bionanoparticles were studied using Atomic force Microscopy. Initially the sample was placed in ultrasonicator. The sample preparations for the AFM studies was done by dissolving a bionanoparticles samples with mica and spin coating the sample using apex instruments spin coater at a maximum speed of 9000 rpm. The sample was then dried for 30 Minutes. Then the morphological features were observed.

Results and discussion

Isolation of *S.marcescens*

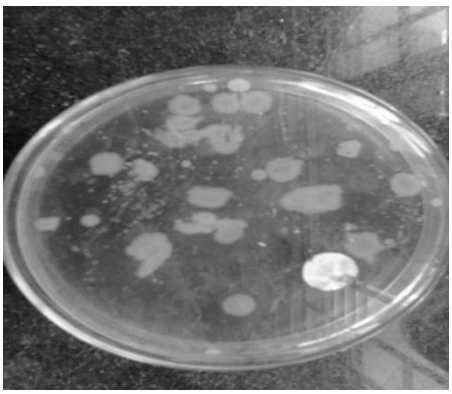

Fig.1. Isolation of *S.marcescens*

The strain of *Serratia* species was grown in MacConkey agar media. *S.marcescens* forms typical reddish colonies that clearly distinguishable from other colonies in the media was isolated (4). The image of typical red colonies formed by the species is given in Fig.1.

Characterisation of nanoparticles by Visual inspection

The primary confirmation of synthesis of silver nanoparticles from *S.marcescens* was monitored visually by the colour change of the culture medium from light-yellow to brown shown in figure 2. The samples showed colour change; colour formation is due the reduction of Ag^{+} to Ag^{o}. Control showed no colour change when incubated for the period under same condition.

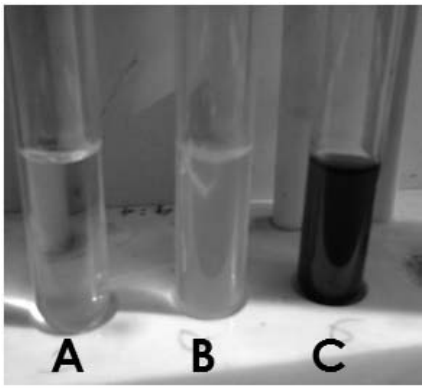

Fig.2. Biosynthesis of silver nanoparticles by *S.marcescens* -visible observation

- $AgNO_3$ solution without supernatant
- Supernatant without AgNO3
- Supernatant with $AgNO_3$

The samples with 0.1M silver nitrate concentration showed dark brown colour when compared to the samples with other concentration. This may because at higher concentration of $AgNO_3$ solution may produce toxic or inhibitory effect on the microorganism under study (9). The sample at pH 8 with 0.1M concentration of $AgNO_3$ showed deep dark brown colour which may indicates synthesis of silver nanoparticles. The results are tabulated below in table 1.

Table.1. Characterisation of silver nanoparticles by the colour change

pH	Concentration of $AgNO_3$ (M)		
	0.1	0.2	0.3
5	_	_	_
6	++	+	+
7	+	+	+
8	+++	+	+
9	++	+	+

(-) No colour change
(+) Light brown
(++) Dark brown
(+++) Deep dark brown

But the sample prepared using cadmium nitrate solution for the synthesis of cadmium nanoparticles doesn't show yellow precipitate. Hence there is no formation of cadmium nanoparticles. So for further analysis the sample cadmium nitrate preparation was avoided.

Effect of pH on the synthesis of nanoparticles using *S.marcescens*

In figure 3 showed variation in the synthesis of nanoparticles with different pH. The absorbance of each sample is recorded at 420nm wavelength and a graph is plotted between pH and Absorbance at 420nm. Initially on increasing the pH the nanoparticle synthesis also increased gradually, and then it starts decrease once reached the pH 8.Hence the maximum synthesis of silver nanoparticles was observed at pH 8. (10)

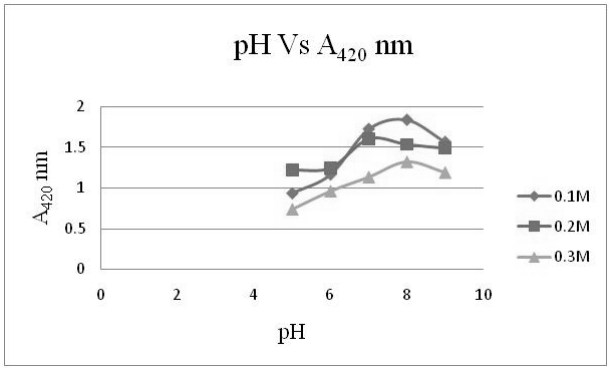

Fig.3. Effect of pH on the synthesis of nanoparticles by *S.marcescens*

From the graph it was found that, the supernatant with 0.1M silver nitrate solution produced more silver nanoparticles than 0.2M and 0.3M. Hence 0.1M concentration of silver is more prominent for silver nanoparticles synthesis.

Characterisation of silver nanoparticles using UV spectrophotometer

UV-Vis absorption spectroscopy is one of the important tools which has been routinely utilised in nanomaterial characterisation. Colour transitions arise during the bioreduction of metal salts to metals, thus leading to corresponding changes in the ability to absorb light in the UV or visible region of the electromagnetic spectrum. The absorption spectrum of the samples with 0.1M $AgNO_3$ concentration was recorded using UV spectrophotometer at 200-700nm. Empty broth is used as blank.

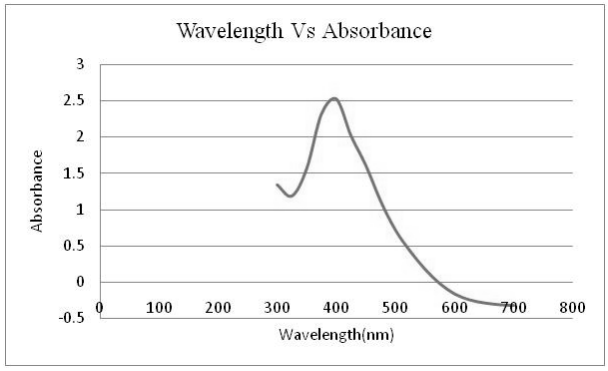

Fig.4. The absorbance spectrum of silver nanoparticles synthesised at pH 8 and 0.1M $AgNO_3$ concentration by *S.marcescens*

In figure 4, showed a strong, broad peak, located at 390nm in UV absorption spectrum, was observed for synthesised AgNPs using the culture supernatant of pH 8. Observation of this peak assigned to surface plasmon is well documented for various metal nanoparticles with sizes ranging from 2 to 100nm (11). The control solution shows no evidence of absorption in the range of 300-700nm.

FTIR analysis

To explore the reduction process of $AgNO_3$ by the culture supernatant of *S.marcescens*, FTIR measurements were carried out to identify possible interactions between silver salts and protein molecules. This could account for the reduction of Ag^+ ions and stabilization of AgNPs. The amide linkages between amino acid residues in proteins give rise to the well known signatures in the infrared region of the electromagnetic spectrum.

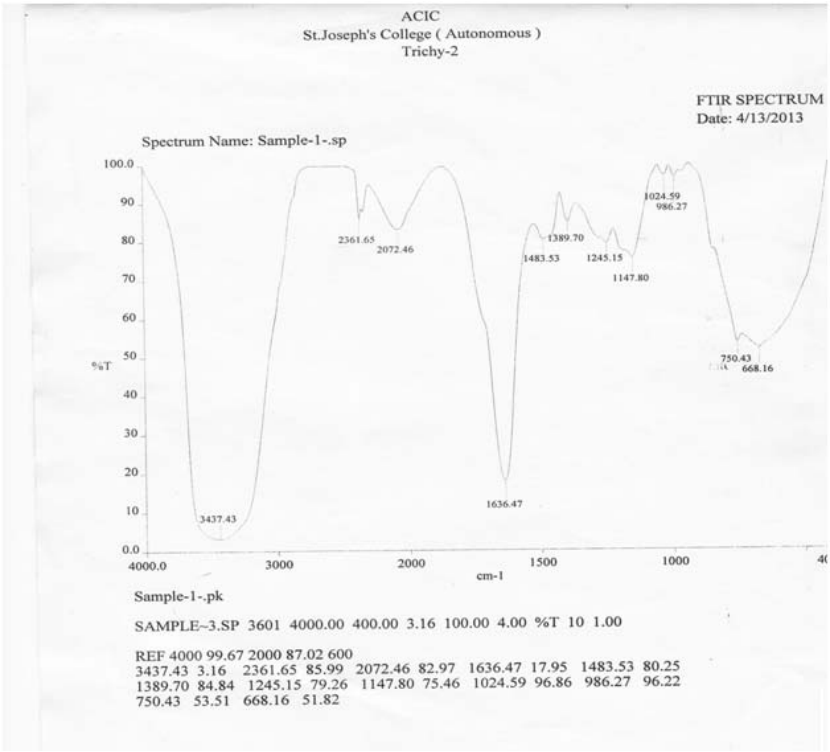

Fig.5. FTIR spectra of silver nanoparticles

The figure 5 explained the stretching and bending vibrations in different regions. The band seen at 3500 and 3400cm^{-1} was assigned to the stretching vibrations of primary and secondary amines. Their corresponding bending vibrations of primary and secondary amines were seen at 1636.47cm^{-1} and 1389.70 cm^{-1}. The peak at 2361.65cm^{-1} corresponds to the aldehydic C-H stretching. The overall observation confirms the presence of protein in the samples of AgNPs. It has been reported earlier that proteins can bind to nanoparticles either through their free amine groups or cysteine residues (10). Therefore the stabilization of synthesis AgNPs by proteins is confirmed.

AFM analysis

Atomic force microscopy was used to analyse the surface morphology and topology of the prepared samples. The images were taken using PARK system AFM XE 100. The samples were measured in the non-contact mode. From the figure 6, 7 & 8 the AFM images of the silver nanoparticle, size at -3.051° angle is 103 nm was recorded.

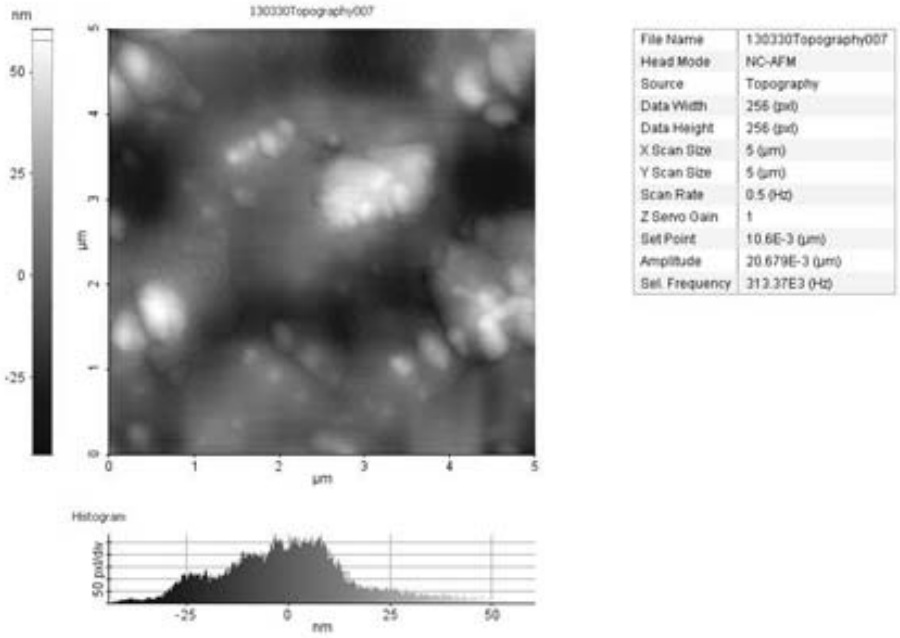

Fig.6. 2D image of silver nanoparticles

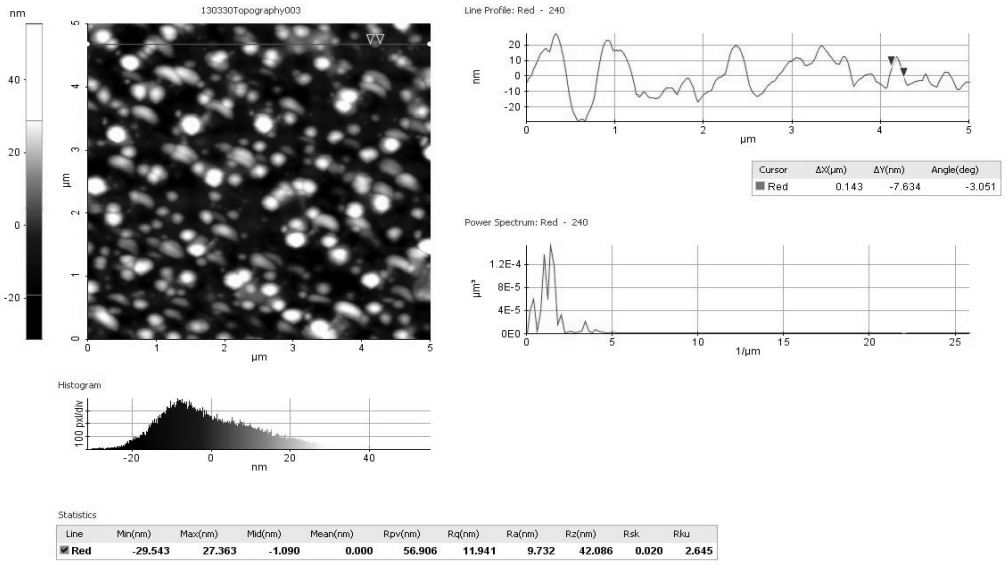

Fig.7. 2D AFM image of silver nanoparticles

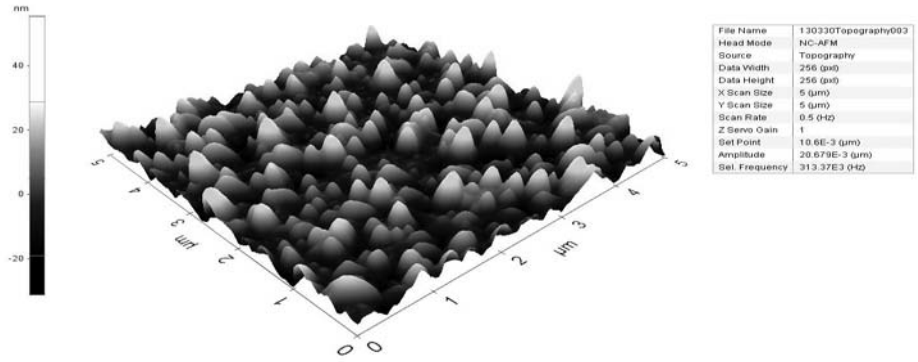

Fig.8. 3D AFM images of silver nanoparticles

Antimicrobial screening of nanoparticles

Silver nanoparticles exhibit antibacterial properties against various types of bacteria. The antibacterial activity of synthesised nanoparticles has been investigated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* which were shown in figure 9.

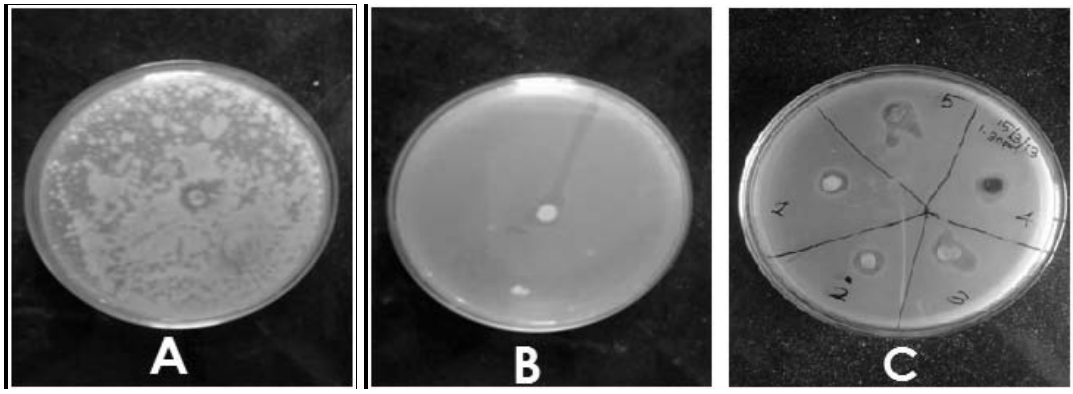

Fig.9. Antimicrobial screening of synthesised nanoparticles (A) *E.coli*, (B) *S.aureus*, (C) *P.aeruginosa*

Table.2. Zone of inhibition

Bacterial culture	Zone of inhibition (in mm)
E.coli	12
S.aureus	10
P.aeruginosa	9

Synthesised microbial nanoparticles of *S.marcescens* showed more inhibitory effect against *E.coli* (12mm zone of inhibition) followed by *S.aureus* (10mm zone of inhibition) and *P.aeruginosa* (9mm zone of inhibition) (12).

Conclusion

Biological synthesis of metal nanoparticles is reliable and ecofriendly method. The study included the synthesis of silver nanoparticles using bacteria namely *S.marcescens* and their antimicrobial activity. The culture supernatant of *S.marcescens* was used to produce nanoparticles extracellularly. The concentration contributing to the maximum synthesis was found to be 0.1M silver nitrate and pH 8 was found to the optimal condition for the synthesis of silver nanoparticles. It was concluded that S.marcescens also has the ability to produce silver nanoparticles. The antimicrobial efficiency of synthesised nanoparticles against certain Gram-negative and Gram-positive bacteria was found. Silver nanoparticle has many positive attributes, such as good conductivity, chemical stability, and catalytic activity. Also the nanoparticle has a good antimicrobial effect and hence it has wide application in antibacterial resistance.

Conflict of Interest:

The author (s) declare (s) that there is no conflict of interests regarding the publication of this article.

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