Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2014, 6 (1):175-181
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Biosynthesis of silver and selenium nanoparticles by *Bacillus* sp. JAPSK2 and evaluation of antimicrobial activity

Nidhi Singh, Prasenjit Saha, Karthik Rajkumar and Jayanthi Abraham*

Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India

---

ABSTRACT

The main aim of the present work was to evaluate the antibacterial activity of selenium and silver biogenic nanoparticles and further characterize them. The bacterial colony isolated from coal mine sample was found to reduce selenium and silver ions to their elemental forms respectively. Under experimental conditions, the isolated bacterium was capable of synthesising these nanoparticles which was indicated by the change in the colour of the medium to red for selenium and brown for silver. The effectiveness of these nanoparticles were tested against four clinical pathogens Escherichia coli, Klebsiella sp., Pseudomonas sp. and Staphylococcus aureus. The nanoparticles synthesised were then characterized by X-Ray diffraction analysis, Atomic Force Microscopy, UV-Vis analysis and Transmission Electron Microscopy. The sizes of these nanoparticles were also calibrated.

Keywords: Biogenic nanoparticles, UV-Vis Microscopy, Atomic Force Microscopy, X-Ray Diffraction, Transmission Electron Microscopy

---

INTRODUCTION

Nanoparticles have been used widely in various fields and the nanoparticles synthesized by the chemical processes are toxic in nature hence there is a growing need to develop environment friendly, cost effective and conveniently reproducible methods of nanoparticle synthesis. Moreover, nanoparticles possess increased surface area and therefore increasing the area of interaction with the pathogenic bacteria. They also are more likely to enter the bacterial surfaces than micron particles due to smaller size, exerting stronger effects on bacterial targets [1]. The microbes are diverse in nature, which can be exploited usefully for the synthesis and harvesting of nanoparticles. These microorganisms when confronted with high concentrations of metal ions (like silver), they reduce them to their elemental state. The enzyme nitrate reductase reduces silver ion to metallic silver. NADH dependant nitrate reductase enzyme is proven to be an important factor in the biosynthesis of metal nanoparticles, reducing silver metal ions to elemental silver nanoparticles. The possible mode of action can be the activity of nitrate reductase enzyme upon silver ion when it is taken up by the cell and converting it to elemental silver [2]. Biological production of nanoparticles by leaf extracts, microorganisms or by enzymes has proven to be environment friendly [3]. Many biosynthesised metal nanoparticles like silver [4], gold [5], zinc oxide [6], etc. are being used as antimicrobial compounds.

The effect of nanoparticles depends on the size which in turn depends on concentration, reaction temperature and pH. Nanoparticle synthesis is also facilitated at pH conditions lower than 10. Selenium nanoparticles have role in bioremediation and toxic waste removal also [7]. In a recent study, biosynthesis of selenium nanoparticles by *Klebsiella pneumoniae* was found to be stable even after wet heat sterilization process [8].

In this research work, the nanoparticles were synthesised by bacteria isolated from a coal mine soil sample and their antimicrobial capacities were evaluated. Furthermore they were characterized using UV-Vis analysis, X-Ray Diffraction studies, Atomic Force Microscopy and Transmission Electron Microscopy.

MATERIALS AND METHODS

Collection of Soil Sample

The soil sample was collected from coal mine sample from Singareni coal fields (Andhra Pradesh) under sterile conditions and after collection it was transported to laboratory for further studies. The soil sample was serially diluted onto nutrient agar plates and incubated for 24 h. The isolate was characterised both physiologically and biochemically followed by 16S rRNA sequencing. The gene sequence thus obtained was searched in BLAST and the phylogenetic tree was constructed using neighbour joining method. The sequence was submitted to NCBI and the accession number was obtained.

Collection of clinical pathogens

Four test microorganisms were collected from Microbial Biotechnology Laboratory, VIT University, Vellore, India. *Escherichia coli, Klebsiella* sp., *Staphylococcus aureus,* and *Pseudomonas* sp. were used for testing antibacterial activity.

Biosynthesis of silver nanoparticles

For extracellular synthesis of silver nanoparticles, the isolated organism was first incubated in Luria Broth at 37^0 C for 36 h and at 150 rpm. After incubation period, the culture was centrifuged at 5000 rpm for 10 min and the supernatant was collected. 50 mL of 1 mM silver nitrate ($AgNO_3$) was treated with 50 mL of supernatant solution in a 250 mL Erlenmeyer flask. The whole mixture was kept in shaker at 40^0C for 5 d and maintained in the dark. Control experiment was conducted with uninoculated media, to check for the role of bacteria in the synthesis of nanoparticles [9].

Biosynthesis of selenium nanoparticles

A uniform inoculum was prepared by aseptically transferring a loopful of culture from a nutrient agar (NA) plate to 100 mL of sterile Luria Broth and growing the culture (inoculum). Luria broth at pH 7.2 was prepared, sterilized, and supplemented with a 200 mg/l Se^{+4} solution (equal to 559.19 mg of selenium chloride) [8]. 1% (v/v) of the inoculum was added to selenium containing Luria broth, and the culture flask was incubated at 37 °C for 24 h [10]. A control flask containing Luria broth without selenium chloride was inoculated with bacterial isolate and incubated under the same conditions. The appearance of red colour in culture flask suggested the formation of elemental selenium.

Characterization of selenium and silver nanoparticles

For UV-Vis spectroscopic analysis of the nanoparticles, 2mL of cell filtrate containing nanoparticles after 5 d of incubation was taken in a cuvette and the absorbance was recorded by UV Vis spectroscopy between 200 to 800nm. The absorbance at which the peak was formed was noted.

For X-Ray Diffraction Analysis, the filtrate containing silver nanoparticles were dried and powdered and then analysed using Powder X-Ray Diffractometer. The 2θ peaks were noted to confirm the presence of the nanoparticles. The instrument used for the analysis was Model-D8 Advanced, BRUKER, Germany.

After the biosynthesis of selenium and silver nanoparticles, the samples were further characterized by Atomic Force Microscopy (AFM) analysis. They were centrifuged at 4722 × g for 10 min. And the acquired cell pellet was gently washed twice with deionised-distilled water and re-suspended in it. A drop of culture was placed on a slide and a thin smear was made. The smear was air-dried and the slide was scanned and observed under Atomic Force Microscope (Model- Nanosurf easyscan 2 AFM, Switzerland). The size of silver nanoparticles was also calibrated in nm.

Antibacterial assay of nanoparticles

After the silver and selenium nanoparticles were characterized, their antimicrobial activity were tested against the clinical pathogens i.e. *Escherichia coli, Staphylococcus aureus, Pseudomonas* sp. and *Klebsiella* sp.

RESULTS AND DISCUSSION

The bacterial isolate tested Gram positive and exhibited motility. The isolate showed positive result for oxidase, urease, indole and Triple sugar iron test and negative for methyl red, Voges Proskauer, citrate and catalase tests. It

can ferment dextrose, sucrose but not lactose. The 16S r RNA of the strain was sequenced and the gene sequence was 100% similar to the strain *Bacillus* sp. and hence designated as *Bacillus* sp. JAPSK2. The phylogenetic tree is given in fig. 1.

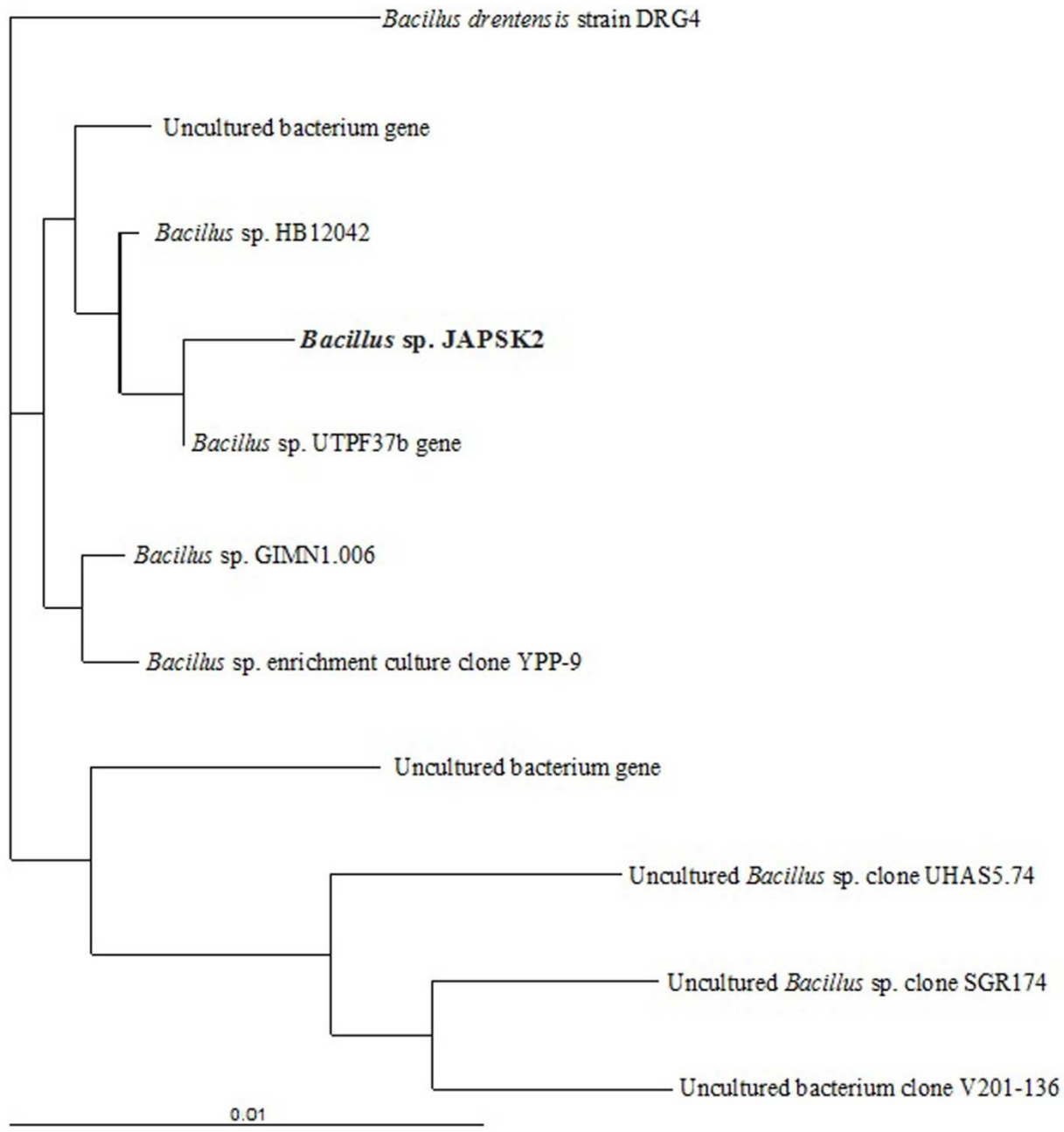

Fig. 1. Phylogenetic tree of *Bacillus* sp JAPSK2

Characterization of silver nanoparticles

The silver nanoparticles synthesised biogenically were characterized by techniques like UV-Vis spectrum, Atomic Force Microscopy and X-Ray Diffraction.

UV-Vis studies: UV-Vis spectral analysis revealed a peak at 393nm at range between 300 to 600nm. This represents the formation of biogenic silver nanoparticles which were formed by reduction of silver ion to elemental silver in the medium (Fig. 2).

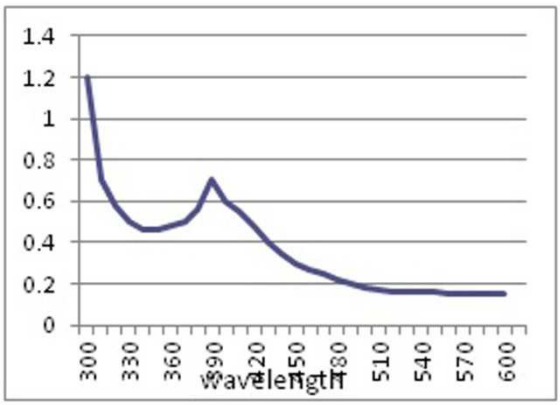

Fig. 2. UV-Vis analysis of Silver nanoparticles synthesised by *Bacillus* sp. JAPSK2 showing peak at 393nm

X-Ray Diffraction analysis confirmed the presence of silver ions in the medium by observing the pattern of peaks after the analysis. The studies showed a characteristic peak at 2θ value of 31.002, 46.761, 56.700 and 75.488 (Fig. 3).

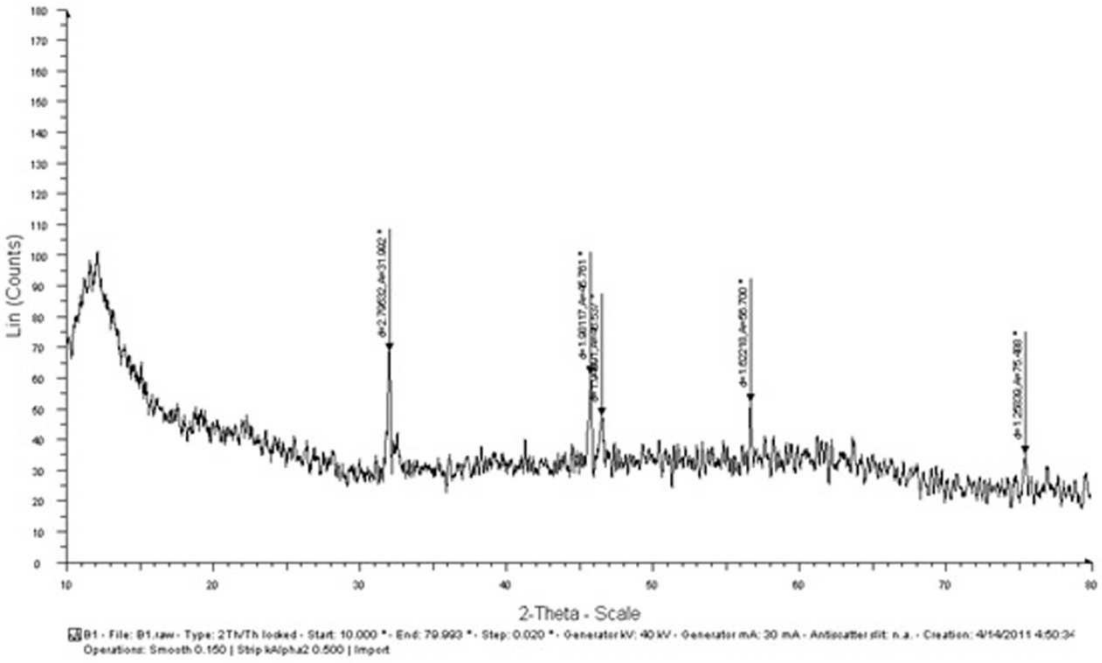

Fig. 3. X-Ray Diffraction Studies of Silver nanoparticles by *Bacillus* sp. JAPSK2

Atomic Force Microscopy showed the presence of silver nanoparticles clusters in the medium. The size of silver nanoparticles synthesised by bacteria isolated from coal mine soil sample was also calculated and was observed to be 69.9 nm (Fig. 4).

Characterization of selenium nanoparticles

After incubation of the isolated strain in Luria Broth for 24h in presence of selenite ions, the colour of the medium turned red due to the reduction of selenite to elemental selenium whereas no difference was noted in the control. The microbe has thus reduced Se^{+4} to elemental selenium (Se^{0}).

The culture containing these nanoparticles was autoclaved (wet heat sterilization process for recovering selenium nanoparticles) and was characterized by UV-Vis spectroscopic analysis between wavelength of 200 to 800nm. The peak was seen at around 222nm which represents the presence of selenium nanoparticles formed by the reduction of selenite ion to elemental selenium (Fig. 5).

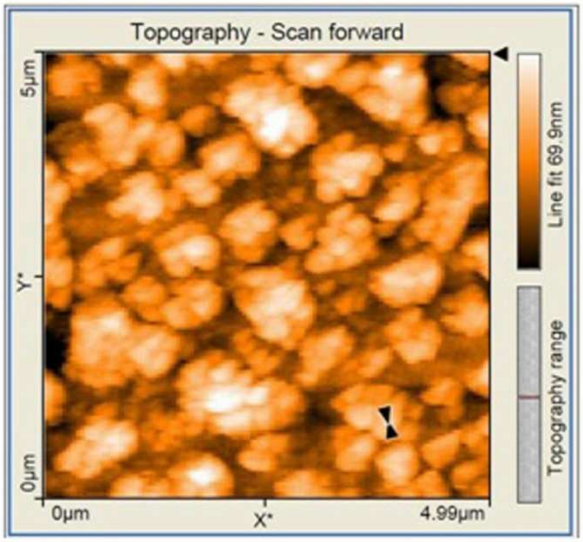

Fig. 4. Atomic Force Microscopis analysis of Silver nanoparticles

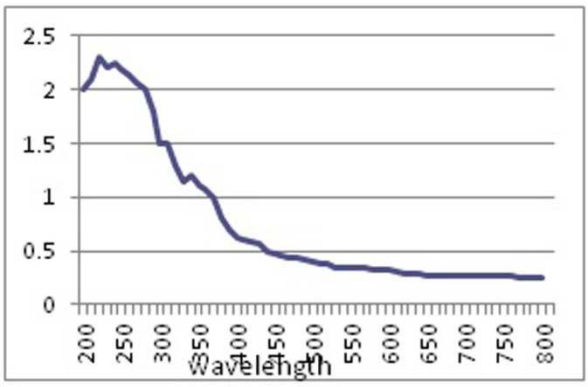

Fig. 5. UV-Vis analysis of Selenium nanoparticles synthesised by *Bacillus* sp. JAPSK2 showing peak at 222nm

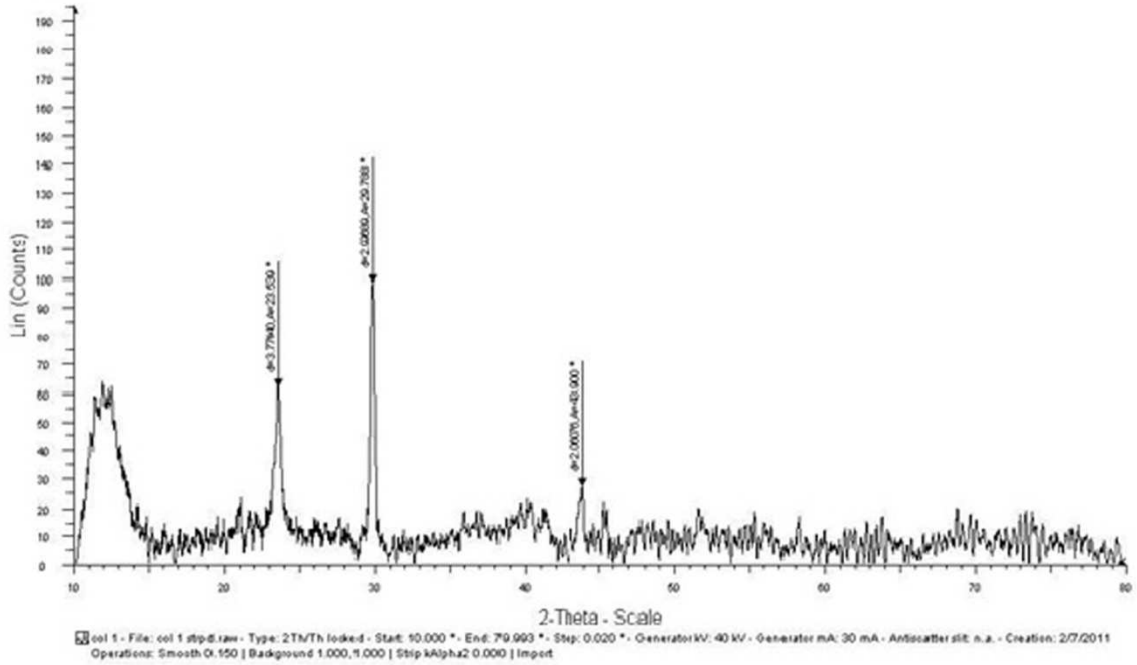

Fig. 6. X-Ray Diffraction Studies of Selenium nanoparticles by *Bacillus* sp. JAPSK2

XRD analysis using Powder X-Ray Diffractometer was done to confirm the presence of nano-crystalline selenium particles. This XRD analysis was mainly performed to confirm the presence of elemental selenium nanoparticles in the medium. The studies showed a characteristic peak at 2θ value of 23.680, 29.788 and 43.9 (Fig. 6). This indicates the presence of selenium nanoparticles reduced from selenite ions present in the medium.

Atomic Force Microscopy results showed the presence of bacterial cells along with selenium nanoparticles dispersed in the medium. This technique was performed to locate the selenium reduced from selenite ions present around the vicinity of microbial cells (Fig. 7). The size of the selenium nanoparticles was also calibrated by this technique. The size of selenium nanoparticles was observed to be 21.9 nm. Thus, these studies indicated the presence of nanoparticles in the medium after incubation. The TEM picture revealed the shape of the selenium nanoparticles to be spherical (Fig. 8).

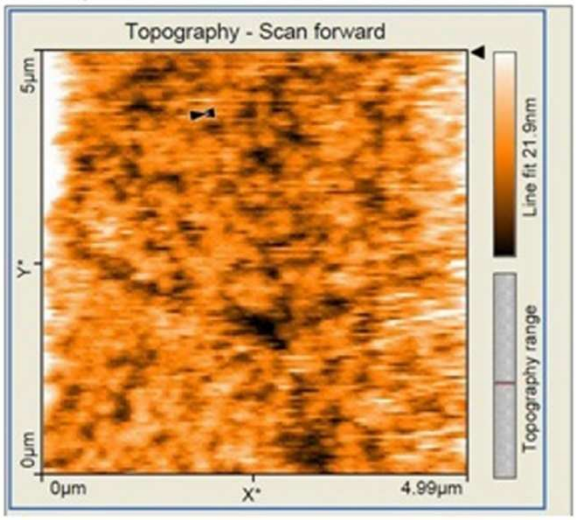

Fig. 7. Atomic Force Microscopis analysis of media containing Selenium nanoparticles

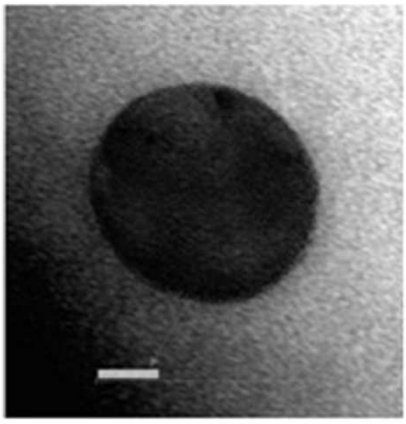

Fig. 8. TEM image of Selenium nanoparticle having size of 191.19 nm

Antimicrobial assay of nanoparticles

The silver nanoparticles synthesised biologically were tested against *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus* and *Pseudomonas* sp. They showed good antimicrobial activity against all the clinical pathogens, though the effect of silver nanoparticles was found to be more pronounced with *Pseudomonas sp.* compared to other pathogens (Table 1).

Table 1. Antimicrobial activity of Ag nanoparticles towards clinical pathogens (in mm)

Colonies		*Escherichia coli*	*Staphylococcus* aureus	*Pseudomonas* sp.	*Klebsiella* sp.
Bacillus sp. JAPSK2 nanoparticles	25 µL	7	7	9	7
	50 µL	8	8	10	8
	75 µL	9	8	11	9
	100µL	9	10	13	9

The antimicrobial activity of selenium nanoparticles were also tested against *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus* and *Pseudomonas* sp. They showed good antimicrobial activity against *Pseudomonas* sp. than *Staphylococcus aureus*, however it failed to show activity against *Escherichia coli* and *Klebsiella* sp. (Table 2).

T

Table 2. Antimicrobial activity of Se nanoparticles against clinical pathogens (in mm)

Colonies		*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas* sp.	*Klebsiella* sp.
Bacillus sp. JAPSK2 nanoparticles	25 µL	-	7	7	-
	50 µL	-	8	9	-
	75 µL	-	9	9	-
	100µL	-	9	11	-

CONCLUSION

Bacillus sp. JAPSK2 reduce selenium and selenium ions to their respective nanoparticles which is confirmed by the change in colour of the medium and by UV-Vis spectroscopy. The sizes were calibrated by AFM and TEM analysis. The biologically synthesised selenium nanoparticles was found to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas* sp. whereas silver nanoparticles could inhibit all the four pathogens to some extent.

REFERENCES

[1] P.A. Tran, T.J. Webstar. *Int. J. Nanomedicine*, **2011**, 6: 1553-1558.
[2] K. Kalimuthu, S.R. Babu, D. Venkataraman, M. Bilal, S. Gurunathan. *Colloids Surfaces B: Biointerfaces*, **2008**, 65: 150-153.
[3] A.G. Ingale, A.N. Chaudhari. *J. Nanomed. Nanotech*., **2013**, 4: 165.
[4] C. Malarkodi, S. Rajeshkumar, K. Paulkumar, G. Gnanajobitha, M. Vanaja, G. Annadurai. *Nanoscience and Nanotechnology: An International Journal*, **2013**, 3: 26-32.
[5] E. Lima, R. Guerra, V. Lara, A. Guzmán. *Chem. Cent. J*., **2012**, 7: 11.
[6] M. Vielkind, I. Kampen, A. Kwade. *Adv. Nanoparticles*, **2013**, 2: 287-293.
[7] K.N. Thakkar, S.S. Mhatre, R.Y. Parikh. *Nanomedicine: NBM*, **2009**, 6: 257-262.
[8] P.J. Fesharaki, P. Nazari, M. Shakibaie, S. Rezaie, M. Banoee, M. Abdollahi, A.R. Shahverdi. *Braz. J. Microbiol*., **2010**, 41: 461-466.
[9] S. Sunker, C.V. Nachiyar, K.R. Namasivayam. *Asian Pac. J. Trop. Biomed*., **2012**, 2: 953-959.
[10] V. Yadav, N. Sharma, R. Prakash, K.K. Raina, L.M. Bharadwaj, N.T. Prakash. *Biotechnol*., **2008**, 7: 299-304.