Egypt. Acad. J. Biolog. Sci., 11(1): 47-57 (2019)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Biosynthesis, Characterization and Cytotoxicity of Selenium Nanoparticles Using *Bacillus tropicus* Ism 2

Omnia M. Abdallah [1*], Khaled Z. EL-Baghdady [2], Mostafa M.H. Khalil[3], Mervat I. El Borhamy [4,5], and Gamal Meligi [3]

1- Department of Microbiology, Faculty of Dentistry, Misr International University, Cairo, Egypt
2*-Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt
3- Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt
4- Department of Microbiology, Faculty of Pharmacy, Misr International University, Cairo, Egypt
5- International Medical Center, Clinical Microbiology Laboratory, Cairo, Egypt
E.Mail: Omniamohamed990@gmail.com

ARTICLE INFO
Article History
Received: 4/10/2019
Accepted:26/10/2019

Keywords:
Selenium nanoparticles, DLS, TEM, Cytotoxicity, *Bacillus tropicus* Ism 2.

ABSTRACT

Green biosynthesis of selenium nanoparticles (SeNPs) using *Bacillus tropicus* Ism 2 (MK332444) isolated from soil polluted with industrial wastewater near the Ismailia Canal, Egypt, was carried out. SeNPs were biogenically synthesized using bacterial pellets mixed with 1 mM Na_2SeO_3. Characterization of biosynthesized SeNPs was carried out by UV-Vis spectrum where SeNPs solution exhibited characteristic peak at 270 nm. Dynamic Light Scattering (DLS) analysis and High-Resolution Transmission Electron Microscope (HRTEM) showed spherical shaped SeNPs with size ranged from 60 to 125 nm with PDI= 0.544 and zeta potential value of -30.6. Biologically synthesized SeNPs showed no antibacterial activity against both Gram-positive and Gram-negative bacteria. Cytotoxicity of SeNPs was studied against Huh-7 human liver cell line and showed low cytotoxicity with IC_{50} value of 367±5.5 µg/ml. In conclusion in this work *Bacillus tropicus* Ism 2 locally isolated bacterium showed the capability to biosynthesize SeNPs with spherical shape and low cytotoxicity.

INTRODUCTION

Selenium (Se) represents an essential trace element that is found in seleniferous soils, meat, vegetables, grains and yeast (Valdiglesias *et al.*, 2010). Different plants absorb Se with different amounts, such as; wheat absorbs high amounts soil, Broccoli stores Se when they are grown in sodium selenate soil (Husen and Siddiqi, 2014). Garlic shows high amounts of Se accumulations and has been used as chemoprotective agent (Ezhuthupurakkal *et al.*, 2017; Vyas and Rana, 2018).

Selenium participates in the biosynthesis of different proteins and enzymes in human body, so it has nutritional value by inhibiting cell death, sustaining cell cycle progression and providing optimum immune response (Forootanfar *et al.*, 2014; Kora and Rastogi, 2016). It acts as antioxidant and anticancer agent with protective effect against the oxidation of DNA by preventing free radicals from invading and damaging the cells (Estevez *et al*., 2014; Riaz and Mehmood, 2012; Srivastava and Kowshik, 2016). Selenium nanoparticles have shown outstanding bioavailability and low toxicity than organic or inorganic with selenium compounds (Kumar *et al*., 2015; Menon *et al*., 2018; Yanhua *et al.,* 2016). They have great biological functions and they represent the optimum substitute for different seleno-compounds (Menon *et al.,* 2018; Yanhua *et al.,* 2016; Zhang *et al*., 2015). Many studies have suggested that SeNPs induce normal growth and reduce cancerous cells growth by selective and substantial Se accumulation that causes high levels of Reactive Oxygen Species (ROS) in cancer cells, but not in normal cells (Kumar *et al.*, 2015). SeNPs has low cytotoxicity and act as excellent nutritional supplement due to its continuous release of Se after ingestion and can be applied to enhance wound healing process in nanomedicine applications (Skalickova *et al.* 2016; Ramya *et al.* 2015).

Various methods have been used to synthesize SeNPs. Physical and chemical methods use high temperatures and pressure, as well as toxic chemicals. These routes have limited the biomedical applications of the synthesized SeNPs. Therefore biological pathway represents the eco-friendly, clean, cheap and nontoxic alternative for the chemical and physical methods.

Many biological synthesis routes have been reported using plants such as *Vitis vinifera* (Sharma *et al*., 2014), *Allium sativum* (Ezhuthupurakka *et al.,* 2017), *Lycium barbarum* (Zhang *et al*., 2018), or bacteria such as *Enterococcus faecalis* (Shoeibi and Mashreghi, 2017), *Acinetobacter* sp. (Wadhwani *et al.,* 2017), *Agrobacterium* sp. (Bajaj *et al*., 2012), and fungi such as *Aspergillus oryzae* (Mosallam *et al.,* 2018) and *Mariannaea* sp. (Zhang *et al.,* 2019). Biogenic synthesis pathway makes SeNPs available for usage in many biomedical applications as it has no side effects (Alagesan *et al*., 2019; Song *et al*., 2017; Wadhwani *et al*., 2016).

MATERIALS AND METHODS

Isolation of Bacteria:

Soil samples were collected from soil polluted with industrial wastewater near the Ismailia Canal (at 53 kilometer) in Egypt, along one km in sterile bags. For selective isolation of selenium tolerant bacteria, tenfold serial dilution was carried out and aliquots of 100 µL were inoculated on nutrient agar plates supplemented with 5 mM sodium selenite (Na_2SeO_3) (Sciencelab.com, inc., Texas, USA). Plates were incubated at 35ºC for 24 hr. After incubation, the colonies with red color were selected as indication for selenium reduction capability (Fernández-Llamosas *et al*., 2016; Srivastava and Kowshik, 2016).

Biosynthesis of Selenium Nanoparticles (SeNPs):

Bacterial isolates (100 µl) (10^8 CFU) were inoculated in Erlenmeyer flasks (250 ml) containing 100 ml of nutrient broth medium (Oxoid Ltd, England), then incubated in a rotary shaking incubator at 35°C and 180 rpm for 24 hr. Bacterial cultures were left to grow until reach 0.9-1.1 at OD_{600} nm then centrifuged at 7000 *xg* for 20 minutes. The bacterial pellets were collected and washed two times with sterile double distilled water. Pellets were mixed with 1 mM Na_2SeO_3 final concentration in 250 ml Erlenmeyer flask and incubated for 24 hr at 180 rpm and 35°C. Sodium selenite (1 mM) and bacterial pellets without sodium selenite were served as controls and kept under the same conditions. After incubation, SeNPs were extracted from bacterial pellets by autoclaving and the resulted solution containing SeNPs was centrifuged at 15000 rpm for 30 minutes, and then washed thrice

with sterile distilled water (Fesharaki *et al.*, 2010)**.**

Characterization of Nanoparticles and Nanocomposite:

UV-Vis Spectrometry:

SeNPs synthesized was fully scanned to detect the presence of selenium nanoparticles specific peaks; using Evolution 201 Scan UV-Visible spectrophotometer (Thermoscientific). The optical absorbance spectra were all collected in equal intervals of wavelength (0.1 nm), with light wavelength ranges from 200 to 500 nm for SeNPS. The samples were diluted in ratio 1:10 by double distilled water, which has been used as blank (Alagesan *et al*., 2019).

Dynamic light Scattering (DLS):

SeNPs particle sizes, PDI (polydispersity index) and zeta potential were measured by dynamic light scattering (DLS) technique using (DLS, Zetasizer Ver. 6.32, Malvern, UK) at the (Egyptian Petroleum Research Institute) (Kora and Rastogi, 2017).

High-Resolution Transmission Electron Microscope (HRTEM):

SeNPs shape and size were investigated by HRTEM model JEOL 2100 Japan electron microscope operating at an accelerating voltage of 200 KV (Egyptian Petroleum Research Institute). Samples for high-resolution TEM analysis were prepared by placing 2 drops of nanoparticles solutions on carbon-coated copper TEM grids. The TEM films were allowed to stand for 2 minutes, solution excess was removed and the grid was dried then examined at different magnifications (Khalil *et al*., 2012).

Molecular Identification of Bacterial Isolate by 16S rRNA Gene :

A single colony from an overnight bacterial culture was picked, eluted in 50 µl sterile distilled water and boiled for 5 min in thermocycler. PCR was performed using Thermoscientific Mastermix according to manufacturer instruction. The universal primer pair PA (5'-AGAGTTTGATCCTGGCTCAG-3'), PH (5'-ACGGCTACCTTGTTACGACT-3') amplifying 1600 bp of 16S rRNA gene were used (Edwards *et al*., 1983). PCR reaction was performed in 25 µl using, 1 µl of DNA template, 12.5 µl of PCR master mix, 10 µM of each primer. PCR was performed using thermal cycler (Applied biosystem 2720). The PCR protocol was as follows: initial denaturation at 94°C for 5 min. then, 25 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 7 min. The PCR product was analyzed using electrophoresis on 1% TAE agarose gel and visualized using ethidium bromide dye (0.5 µg/ml). Finally, the product Gel was inspected using GelDoc. Ingenius 3.

Sequencing of 16S rRNA Genes:

PCR products were purified using Qiagen extraction kit then the DNA was subjected to sequencing. Sequencing was performed at The Animal Health Research Institute, El Dokki, Egypt using by automated fluorescence dye terminator sequencing method (DYEynamic ET Terminator Cycle Sequencing Kit, Amersham Pharmacia Biotech. ABI 3130). The nucleotide sequences were compared to that in database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast) pairwise alignment was carried out by ClustalW in BioEdit (7.2) software.

Antibacterial Activity of SeNPs:

The antibacterial activity of the biosynthesized SeNPs was measured against bacteria provided by the International Medical Centre, Microbiology laboratory, Cairo. *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Klebsiella pneumoniae* (*K. pneumonia*) and *Escherichia coli* (*E. coli*). SeNPs antibacterial activities were tested using well agar diffusion method. Powder form SeNPs was prepared by solution centrifugation for 20 minutes at 20000 *xg*. The supernatant was decanted and the pellet was further freeze-dried by lyophilization (Edwards model RV5, England). SeNPs powder form was suspended in sterile deionized water at concentrations ranged from 20 to 100µg/ml (Nguyen *et al*., 2017). Muller Hinton agar plates were surface inoculated with 10^6

CFU/ml of 24 hr bacterial culture. Using 8 mm sterile cork borer, wells were made and filled SeNPs. Sodium selenite (1 mM) acted as positive control, and as negative controls, Bacterial lysate supernatant and water were used. Plates were left in refrigerator for overnight diffusion, and then incubated for 24 hr at 37°C; finally, average inhibition zone diameters were measured.

Cytotoxicity (MTT- Assay):

Cytotoxic effect of synthesized was evaluated using MTT (3- (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Using Huh-7 human liver cells were seeded at 10^6 cells/ well in a 96-well plate. The plate was incubated at 37°C under 5% CO_2 atmosphere for 24 h. SeNPs sample was diluted 12 times (2 fold dilutions) (25000 µg/ml to 12.20 µg/ml). After incubation at 37°C under 5% CO_2 atmosphere for 24 hr; 50 µl MTT (5 mg/ml) was added and incubated for 6 h. formazan colored product was dissolved in 100 µl DMSO (0.1%) (Sigma–Aldrich, St Louis, MO). IC_{50} values (the inhibitory concentration that causes 50% cell death) and viability percentage of cells was calculated using Microplate Reader (ELx808™ Absorbance, Biotek, USA) by measuring MTT-treated cells absorbance at 570 nm (Hajji *et al.* 2017). Untreated cells acted as control with 100% viability and cell viability percentage was calculated using the following equation:

% of Viability = (Absorbance of sample / Absorbance of control cells) x 100

MTT assay was done thrice where n = 3. Using Mean values and standard errors (±SD) were carried out with Microsoft Office Excel 2017. Statistical significance analyses were performed by variance analysis using Anova, SPP software. $P < 0.05$ was considered statistically significant.

RESULTS

Isolation of Se Tolerant Bacterial Isolate:

Among 40 bacterial isolates only two bacterial (given code Ism 1 and Ism 2) showed different color on nutrient agar supplemented with 5 mM Na_2SeO_3, where isolates showed red colored colonies as Preliminary indication biosynthesis of SeNPs.

Biosynthesis and Characterization of SeNPs:

Bacterial pellets of Ism 1 isolate showed faint red color after 24 h, while Ism 2 after 7 h incubation with 1 mM Na_2SeO_3 showed change in color from colorless to red (Figure 1) and there was no further change in color intensity till 24 h incubation. Control samples (bacterial pellets without Na_2SeO_3 and Na_2SeO_3 solution alone) showed no change in color. UV-Vis measurements of bacterial isolate Ism 1 showed no characteristic peak for SeNPs. While Ism 2 isolate showed maximum peak at 270 nm, which is considered characteristic for SeNPs synthesis (Figure 2). SeNPs synthesized by bacterial isolate Ism 2 was further characterized and used in upcoming experiments.

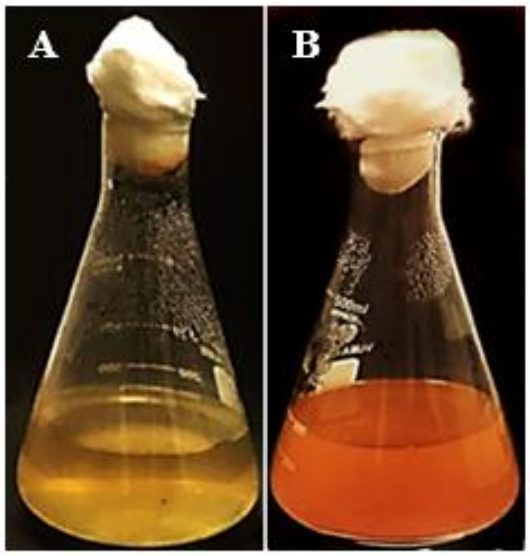

Fig. 1: Visual change in color during SeNPs biosynthesis by bacterial isolate Ism 2, (A): control and (B) SeNPs solution.

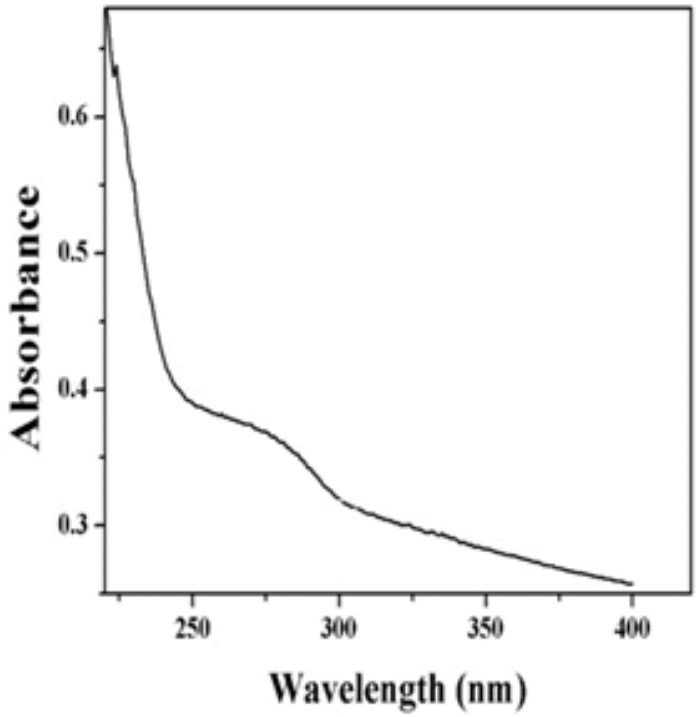

Fig. 2: UV-Vis spectrum of SeNPs biosynthesized by bacterial isolate Ism 2

DLS analysis of SeNPs synthesized by bacterial isolate Ism 2 showed that SeNPs have particles size ranged from 60 to 125 nm (Figure 3) and showed narrow particle size distribution where 25% were less than 60 nm, 50% less than 70 nm, 75% less than 80 nm, and 90% less than 90 nm. SeNPs showed PDI value of 0.544 and zeta potential value of -30.6 (Figure 4).

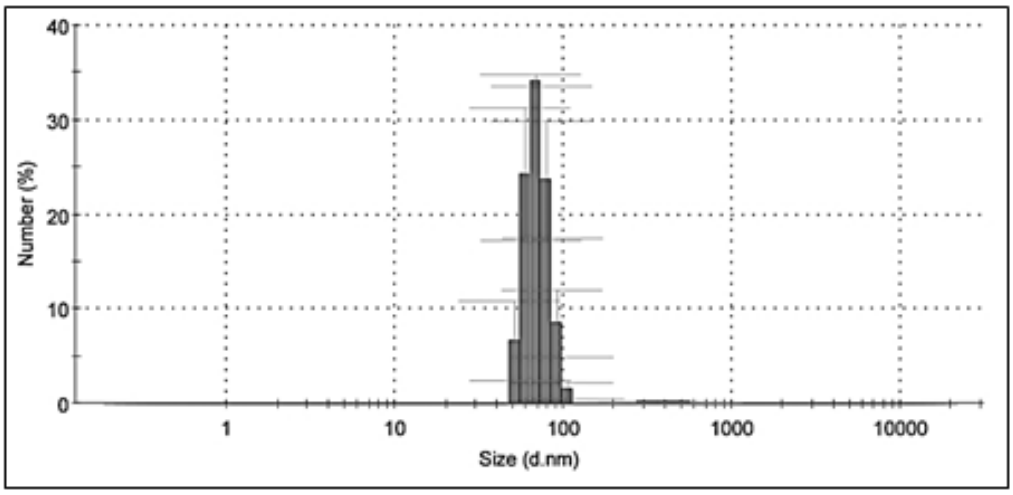

Fig. 3: DLS analysis of biosynthesized SeNPs by bacterial isolate Ism 2

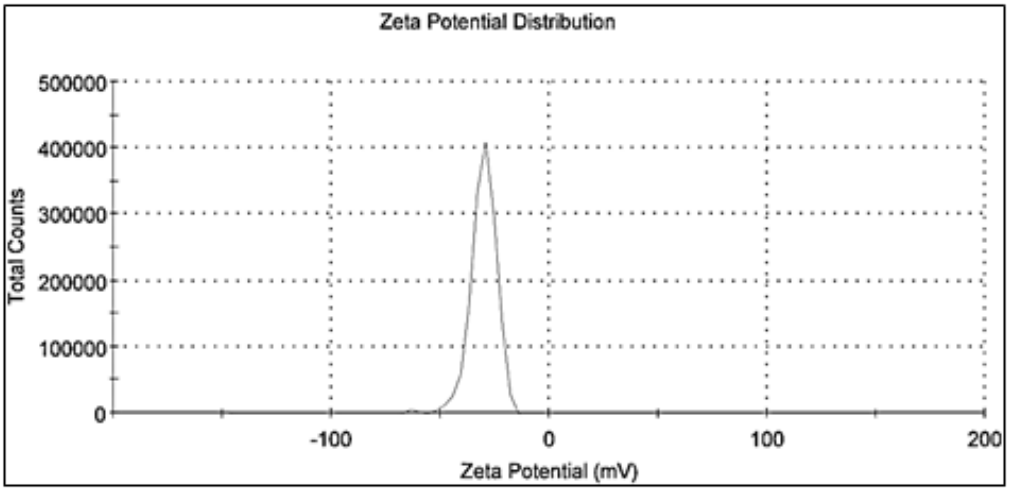

Fig. 4: Zeta Potential analysis of biosynthesized SeNPs by bacterial isolate Ism 2

High-resolution transmission electron microscopy micrographs of biogenically synthesized SeNPs using bacterial isolate Ism 2 showed nanoparticles size ranged from 125 to 60 nm, where nanoparticles shape was spherical with an average size less than 125 nm (Figure 5).

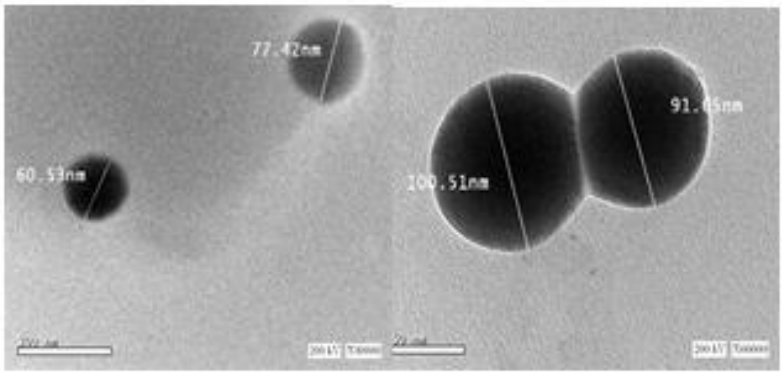

Fig. 5: HRTEM micrograph of SeNPs biosynthesized by Ism 2

Molecular Identification of Bacterial Isolate Using 16S rRNA:

Bacteria isolate Ism 2 PCR and partial sequence analysis showed that the isolate was identified as *Bacillus tropicus* (99% similarity) and was submitted to *GenBank* under accession number MK332444.

Antibacterial Activity of SeNPs:

Different concentrations (20-100 µg/ml) of SeNPs synthesized by *Bacillus tropicus* Ism 2 showed no antibacterial activity against both Gram-positive and Gram-negative bacteria exhibited by no inhibition zones against all tested bacteria.

Cytotoxicity (MTT- Assay):

MTT assay was used to investigate biosynthesized SeNPs at different concentrations, where the first three concentrations of SeNPs showed the same significant effect on cells, but as the concentration of SeNPs decreased as the cell viability % increase in significant amounts till reaching around the IC_{50} value at 367±5.5 µg/ml that caused cell growth inhibition by 50%. This high IC_{50} value for biosynthesized SeNPs indicates very low cytotoxic activity against Huh-7 cell line (Figure 6).

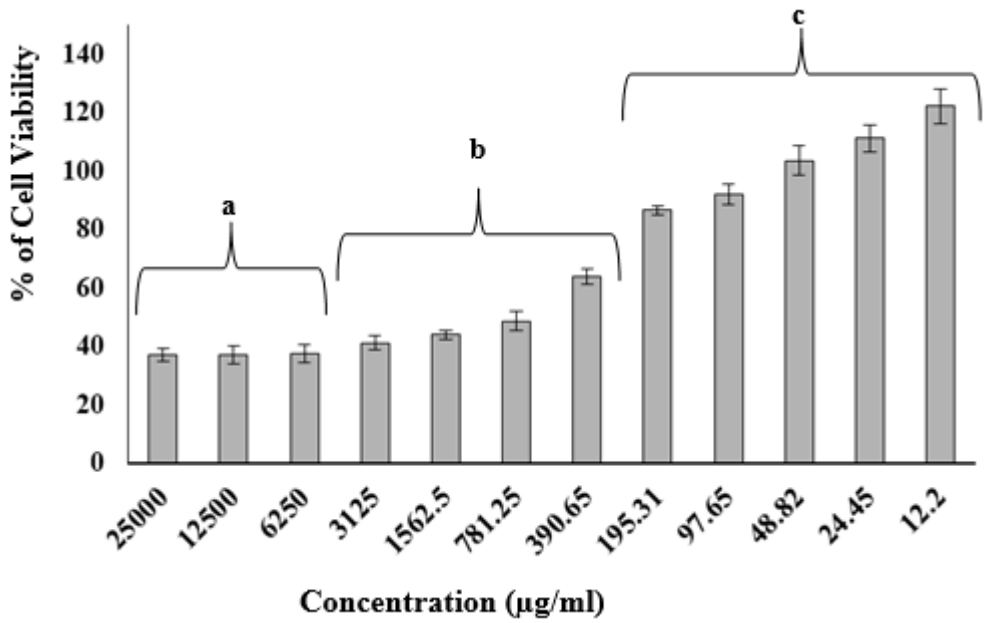

Fig. 6: Effect of SeNPs on cell viability percentage of Huh-7 cells. Results represented as mean ± SD (a, b, c groups are considered statistically significant values, $p<0.05$; compared to control).

DISCUSSION

In this study, we have used nutrient agar medium supplemented with 5 mM Na_2SeO_3 as selective media for selenium tolerant bacteria. Bacterial isolates showed red colored colonies on the selective media, this could indicate accumulation of selenium within bacterial cells and indicates its ability to reduce sodium selenite. A similar observation was reported by Srivastava and Kowshik, (2016) using *Idiomarina* sp. PR58-8 and Zare *et al*. (2013) using soil isolated *Aspergillus terreus*. The bacterial isolate Ism 1 and Ism 2 were able to form SeNPs after incubation with sodium selenite. UV-Vis spectroscopy analysis showed no characteristic peaks for Se by Ism 1 indicating that Se reduction may have not reached the nanosize (Shoeibi and Mashreghi, 2017). Bacterial isolate Ism 2 showed SeNPs surface plasmon response (SPR) characteristic peak at 270 nm,

indicating the capability of Ism 2 to synthesize SeNPs. Ism 2 bacterial isolate was identified using 16S rRNA as *Bacillus tropicus* Ism 2 and submitted under accession number MK332444. *Bacillus tropicus* Ism 2 was able to synthesis intracellular SeNPs, similar results were obtained by using *Klebsiella pneumonia*, *Pseudomonas alcaliphila, Idiomarina* sp. PR58-8, *Bacillus licheniformis* JS2, *Bacillus mycoides* SelTE01, *Bacillus* sp. MSh-1 to synthesize SeNPs intracellular (Fesharaki *et al*., 2010; Forootanfar *et al.*, 2014; Piacenza *et al*., 2017; Sonkusre and Cameotra, 2017; Srivastava and Kowshik, 2016; Zhang *et al.*, 2011). Other studies recorded extracellular synthesis of SeNPs using *Bacillus subtilis*, *Bacillus cereus, Escherichia coli* ATCC 35218, *Enterococcus faecalis, Klebsiella pneumonia* (Dhanjal and Cameotra, 2010; Kora and Rastogi, 2017; Sasidharan and Balakrishnaraja, 2014; Shoeibi and Mashreghi, 2017) *Bacillus tropicus* Ism 2 biosynthesized SeNPs nanospheres with particle size range of 60 to 125 nm. This nanoparticle size range is similar to that synthesized by *Enterobacter* sp. and *Escherichia coli* ATCC (Kora and Rastogi, 2017; Mollania *et al*., 2016). However, these particles size was smaller than that synthesized by *Bacillus cereus* (Dhanjal and Cameotra, 2010), *Klebsiella pneumoniae* (Fesharaki *et al*., 2010), *pseudomonas alcaliphila* (Zhang *et al*., 2011). In our work, SeNPs biosynthesized showed PDI=0.544 and low zeta potential value of -30.6, indicating SeNPs stability and high polydispersity of negatively charged unlikely to aggregates. Similar results were showed by Mollania *et al*. (2016) using *Enterobacter* sp. and Cremonini *et al*. (2016) using *Bacillus mycoides* SelTE01 indicating that negatively charged nanoparticles tend to have long prevalence in blood giving these particles biomedical application prospect.

The antibacterial activity of SeNPs is controversial. SeNPs synthesized by *Bacillus tropicus* exhibited no antibacterial activities against both Gram-positive and Gram-negative bacteria. These results were similar to other studies carried out against *Pseudomonas aeruginosa, Staphylococcus aureus* and *staphylococcus epidermidis* showing that SeNPs had very low to no antibacterial activity (Cremonini *et al.,* 2018; Khiralla and El-Deeb, 2015; Nguyen *et al*., 2017). Similar to our conclusion, other studies showed that SeNPs failed to cause antifungal against *Aspergillus niger* and *Candida albicans* and acted as growth inducers to *Aspergillus niger* (Kazempour *et al*., 2013). However, some studies showed that SeNPs have antibacterial activities against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Pseudomonas aeruginosa* and *E. coli* (Zonaro *et al*., 2015). Results obtained in this work was congruent with previous work indicating that SeNPs antibacterial activity can be influenced by many factors such as size of SeNPs, concentration, type of bacterial and fungal strain (Cremonini *et al*., 2016).

In our study, SeNPs was studied for their effect on Huh-7 cell viability percentage and recorded high IC_{50} value at 367±5.5 µg/ml, indicating low cytotoxicity. Lower IC_{50} values and higher cytotoxicity were recorded against fibroblasts (Ramos and Webster, 2012) and HeLa cell line (Srivastava and Kowshik, 2016). Biogenically synthesized SeNPs has lower cytotoxicty, higher bioavailability with higher efficacy in treatment usage than organic and inorganic selenite (Liu *et al*., 2015). At size range of 5–200 nm, SeNPs can be used in many antioxidant seleno enzymes assays (Kora and Rastogi, 2017) and act as chemopreventive agent with nutritional value and wound healing applications (Skalickova *et al.* 2016; Ramya *et al.* 2015; Forootanfar *et al*., 2014; Zhang *et al*., 2008).

REFERENCES

Alagesan, V. and Venugopal, S. (2019) Green Synthesis of Selenium Nanoparticle Using Leaves Extract of *Withania somnifera* and Its Biological Applications and Photocatalytic Activities. BioNanoSci., 9:105-116.

Cremonini, E.; Boaretti, M.; Vandecandelaere, I.; Zonaro, E.; Coenye, T.; Lleo, M.M; Lampis, S. and Vallini, G (2018). Biogenic selenium nanoparticles synthesized by *Stenotrophomonas maltophilia* SeITE02 loose antibacterial and antibiofilm efficacy as a result of the progressive alteration of their organic coating layer. Microb. Biotechnol., 11(6): 1037-1047.

Cremonini, E.; Zonaro, E.; Donini, M.; Lampis, S.; Boaretti, M.; Dusi, S.; Vallini, G. (2016). Biogenic selenium nanoparticles: characterization, antimicrobial activity and effects on human dendritic cells and fibroblasts. Microb. Biotechnol., 9(6): 758-771.

Dhanjal, S., and Cameotra, S.S. (2010). Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil, Microb. Cell Fact., 9: 1–11.

Edwards A, Civitello A, Hammond HA, Caskey CT (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49: 746-756.

Estevez, H.; Garcia-Lidon, J.C.; Luque-Garcia, J.L. and Camara, C. (2014). Effects of chitosan-stabilized selenium nanoparticles on cell proliferation, apoptosis and cell cycle pattern in HepG2 cells: Comparison with other selenospecies. Colloids Surf. B. Biointerfaces.,122: 184-193.

Ezhuthupurakkal, P.B.; Polaki, L.R.; Suyavaran, A.; Subastri, A.; Sujatha, V. and Thirunavukkarasu, C. (2017). Selenium nanoparticles synthesized in aqueous extract of *Allium sativum* perturbs the structural integrity of Calf thymus DNA through intercalation and groove binding. Mater. Sci. Eng. C., 74: 597-608.

Fernández-Llamosas, H.; Castro, L.; Blázquez, M.L.; Díaz, E. and Carmona, M. (2016). Biosynthesis of selenium nanoparticles by *Azoarcus* sp. CIB. Microb. Cell Fact., 15(1): 109.

Fesharaki, P.J.; Nazari, P.; Shakibaie, M.; Rezaie, S.; Banoee, M.; Abdollahi, M. and Shahverdi, A.R. (2010). Biosynthesis of selenium nanoparticles using *Klebsiella pneumoniae* and their recovery by a simple sterilization process. Brazilian J. Microbiol., 41(2): 461-466.

Forootanfar, H.; Adeli-Sardou, M.; Nikkhoo, M.; Mehrabani, M.; Amir-Heidari, B.; Shahverdi, A.R. and Shakibaie, M. (2014). Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide. J. Trace Elem. Med. Biol., 28(1): 75-79.

Husen, A. and Siddiqi, K.S. (2014). Plants and microbes assisted selenium nanoparticles: Characterization and application. J. Nanobiotechnol. 12:1-28

Kazempour, Z.B.; Yazdi, M.H.; Rafii, F. and Shahverdi, A.R. (2013). Sub-inhibitory concentration of biogenic selenium nanoparticles lacks post antifungal effect for *Aspergillus niger* and *Candida albicans* and stimulates the growth of *Aspergillus niger*. Iran. J. Microbiol., 5(1): 81-85.

Khalil, M.M.H.; Ismail, E.H. and El-Magdoub, F. (2012). Biosynthesis of Au nanoparticles using olive leaf extract. 1st Nano Updates. Arab. J. Chem. 5(4): 431-437.

Khiralla, G.M. and El-Deeb, B.A. (2015). Antimicrobial and antibiofilm effects of selenium nanoparticles on some foodborne pathogens. Food Sci. Technol., 63(2), 1001–1007.

Kora, A.J. and Rastogi, L. (2016). Biomimetic synthesis of selenium nanoparticles by *Pseudomonas aeruginosa* ATCC 27853: An approach for conversion of selenite. J. Environ. Manage., 181: 231-236.

Kora, A.J. and Rastogi, L. (2017). Bacteriogenic synthesis of selenium nanoparticles by *Escherichia coli* ATCC 35218 and its structural characterisation. Nanobiotechnology, 11(2): 179–184.

Kumar, S.; Tomar, M.S. and Acharya, A.

(2015). Carboxylic group-induced synthesis and characterization of selenium nanoparticles and its anti-tumor potential on Dalton's lymphoma cells. Colloids Surf. B. Biointerfaces., 126: 546-552.

Liu, T.; Zeng, L.; Jiang, W.; Fu, Y.; Zheng, W. and Chen, T. (2015). Rational design of cancer-targeted selenium nanoparticles to antagonize multidrug resistance in cancer cells. Nanomedicine: Nanotechnol. Biol. Med., 11(4): 947–958.

Menon, S.S.D.; Santhiya, R.; Rajeshkumar, S. and Venkat K. S. (2018). Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf. B. Biointerfaces.,170 (2018): 280-292

Mollania, N.; Tayebee, R. and Narenji-Sani, F. (2016). An environmentally benign method for the biosynthesis of stable selenium nanoparticles. Res. Chem. Intermed., 42(5): 4253–4271.

Nguyen, T.H.D.; Vardhanabhuti, B.; Lin, M. and Mustapha, A. (2017). Antibacterial properties of selenium nanoparticles and their toxicity to Caco-2 cells. Food Control., 77: 17-24.

Piacenza, E.; Presentato, A.; Zonaro, E.; Lemire, J.A.; Demeter, M.; Vallini, G. and Lampis, S. (2017). Antimicrobial activity of biogenically produced spherical Se-nanomaterials embedded in organic material against *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains on hydroxyapatite-coated surfaces. Microb. Biotechnol., 10(4): 804–818.

Ramos, J.F. and Webster, T.J. (2012). Cytotoxicity of selenium nanoparticles in rat dermal fibroblasts. Int. J. Nanomedicine., 7: 3907–3914.

Ramyaa, S., Shanmugasundarama, T. and Balagurunathan, R. (2015). Biomedical potential of actinobacterially synthesized selenium nanoparticles with special reference to anti-biofilm, anti-oxidant, wound healing, cytotoxic and anti-viral activities. J. Trace Elem. Med. Biol., 32: 30-39.

Riaz, M. and Mehmood, K.T. (2012). Selenium in human health and disease: A review. J. Postgrad. Med. Inst., 14(7):120-133.

Sasidharan, S. and Balakrishnaraja, R. (2014). Comparison Studies on the Synthesis of Selenium Nanoparticles by Various Micro-organisms. Int. J. Pure App. Biosci., 2(1): 112-117.

Shoeibi, S. and Mashreghi, M. (2017). Biosynthesis of selenium nanoparticles using *Enterococcus faecalis* and evaluation of their antibacterial activities. J. Trace Elem. Med. Biol.,39: 135-139.

Skalickova, S.; Milosavljevic, V.; Cihalova, K.; Horky, P.; Richtera, L. and Vojtech A. (2016). Selenium nanoparticles as a nutritional supplement. Nutrition.,33: 83-90.

Song, D.; Li, X.; Cheng, Y.; Xiao, X.; Lu, Z. and Wang, Y. (2017). Aerobic biogenesis of selenium nanoparticles by *Enterobacter cloacae* Z0206 as a consequence of fumarate reductase mediated selenite reduction. Nature; scientific reports., 7: 3239- 3249.

Sonkusre, P. and Cameotra, S.S. (2017). Biogenic selenium nanoparticles induce ROS-mediated necroptosis in PC-3 cancer cells through TNF activation. J. Nanobiotechnol., 15(1): 1–12.

Srivastava, P. and Kowshik, M. (2016). Anti-neoplastic selenium nanoparticles from *Idiomarina* sp. PR58-8. Enzyme Microb. Technol., 95: 192-200.

Valdiglesias, V.; Pásaro, E.; Méndez, J. and Laffon, B. (2010). In vitro evaluation of selenium genotoxic, cytotoxic, and protective effects: A review. *Arch. Toxicol*., 84:337-351.

Vyas, J. and Rana, S. (2018). Synthesis of selenium nanoparticles using *Allium sativum* extract and analysis of their antimicrobial property against gram positive bacteria. *Pharm. Innov. J.*, *7*(9): 262–266.

Wadhwani, S.A.; Shedbalkar, U.U.; Singh,

R. and Chopade, B.A. (2016). Biogenic selenium nanoparticles : current status and future prospects.

Yanhua, W.; Hao, H.; Li, Y. and Zhang, S. (2016). Selenium-substituted hydroxyapatite nanoparticles and their in vivo antitumor effect on hepatocellular carcinoma. Colloids and Surf. B. Biointerf., 140: 297-306.

Zare, B., Babaie, S., Setayesh, N., Shahverdi, A. R., & Shahverdi, A. (2013). Isolation and characterization of a fungus for extracellular synthesis of small selenium nanoparticles Extracellular synthesis of selenium nanoparticles using fungi. Nanomed J, 1(1), 13–19.

Zhang, C.; Zhai, X.; Zhao, G.; Ren, F. and Leng, X. (2015). Synthesis, characterization, and controlled release of selenium nanoparticles stabilized by chitosan of different molecular weights. Carb. Polym., 134: 158–166.

Zhang, J.; Wang, X. and Xu, T.T. (2008). Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: Comparison with se-methylselenocysteine in mice. Toxicological Sciences, 101(1): 22-31.

Zhang, W.; Chen, Z.; Liu, H.; Zhang, L.; Gao, P. and Li, D. (2011). Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*. Colloids and Surf. B. Biointerf., 88(1): 196–201.

Zonaro, E.; Lampis, S.; Turner, R.J.; Junaid, S. and Vallini, G. (2015). Biogenic selenium and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms. Front Microbiol., 6(5), 1–11.