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Exploring the bacterial based silver nanoparticle for their possible application as disinfectants

Neetu Sharma [1], Kanika Phutela [2], Abhishek Goel [2], Sajeev Soni [3], Navneet Batra [2]

[1] Department of Microbiology, GGDSD College, Chandigarh, India

[2] Department of Biotechnology, GGDSD College, Chandigarh, India

[3] Department of Chemistry, GGDSD College, Chandigarh, India

*corresponding author e-mail address: ***batranavneet@gmail.com***

ABSTRACT

The research on biological synthesized nanoparticles and its applications in all fields ranging from pharmaceutical to electronics have opened new avenues in this area. The present study was aimed at the biological synthesis of silver nanoparticles using bacterial isolate from metal contaminated sites. The preliminary characterization of silver nanoparticles (AgNPs) was carried out using U.V-Visible spectroscopy. The antibacterial activities of the nanoparticles were checked against some pathogenic strains viz. *Aeromonas liquefaciens* and *E. coli*. Clear zone of inhibitions against test strains confirmed good antimicrobial activity of nano-suspensions. The present studies involve the bacterial growth study, minimum inhibitory concentration (MIC), and cytoplasmic leakage analysis followed by study of the effect of nanoparticles on water purification. The obtained results concluded that Ag-NPs exhibit excellent bactericidal effect towards test trains and hold promising potential towards purification of water sample.

Keywords: *silver nanoparticles, antimicrobial, characterization, applications.*

1. INTRODUCTION

The term nanoparticle is used to describe a particle with a size range of 1nm to 100 nm, at least in all the three possible dimensions. Nanotechnology is a rapid growing field with significant applications in various areas [1]. The metal nanoparticles were reported to synthesize using various physical and chemical approaches including photochemical oxidation, aerosol technologies, use of laser technology, microwave assisted production etc. The above-mentioned techniques mainly suffered from a major disadvantage of being expensive and highly toxic to the environment due to the use of hazardous chemicals and extreme conditions of temperature and pH. At present, different types of nanomaterials are being produced using silver, magnesium oxide, aluminum, titanium dioxide, zinc oxide and gold [2]. Bio-nanotechnology has been investigated recently as an alternative to chemical and physical ones. This process benefits from the development of clean, non-toxic and environmentally acceptable procedures which involves organisms ranging from bacteria to fungi and even plants [3]. More and more studies are being focused on microbial synthesis methods due to the potential of microbes to transform the oxidation state of metals when grown in metal containing solutions. Biological synthesis of nanoparticles can be done by using bacteria, fungi, plant extracts and yeast. Either intracellular or extracellular method can be opted for microbial synthesis. Extracellular method of synthesis requires a simple downstream processing while intracellular methods have additional steps like ultrasound treatment or reaction with suitable detergents to release the synthesized nanoparticles [4]. These nanoparticles are widely used in various fields such as optical devices, bactericidal, electronic sensor technology, biological labeling and treatment of some cancers and biomedical applications [5]. Nanomembranes and nanopowder are having wide applications in detection of heavy metals and other biological contaminants in water sample. Further their water purification potential is being explored in the form of nanotubes, nanofibers and zeolites due to their high surface to volume ratio as compare to other available methods [6]. Biological synthesis of silver nanoparticles using metal tolerant microbes is an important field of research as it takes place at ambient temperature, pH, time and pressure and moreover the shape and size can also be controlled in nanoparticles synthesis by microbial route [4]. Silver nanoparticles are mainly of great interest because of its applications in therapeutics, water purifications, catalysis and an antimicrobial agent.

2. EXPERIMENTAL SECTION

2.1. Isolation and characterization of the isolates. Under sterile conditions, soil samples were collected using standard enrichment procedures from different locations such as iron and steel industry; metal welding area situated in local industrial area, India and were used for screening and selection of bacterial isolates having potential to transform silver nitrate. Individual colony of bacteria which varied in shape and color was picked and purified by streaking on nutrient agar plates supplemented with 1mM $AgNO_3$ and incubated for 24 hours. After incubation, single colony from nutrient agar plate was picked by sterilized loop and further streaked on fresh nutrient agar plates for obtaining a pure culture of the isolate. The morphological and biochemical characterization of isolates was carried out in accordance with the Bergey's manual of systematic bacteriology. Morphological studies, gram staining

and various biochemical tests viz. IMVIC, starch hydrolysis test, lipid hydrolysis test, caseine hydrolysis test, catalase test, urease test, carbohydrate fermentation test and hydrogen sulfide test were carried out for the identification of isolates under study.

2.2. Biological synthesis of Silver Nanoparticles. The biosynthesis of silver nanoparticles was carried as per the method described by Hinal and Shabib [7]. The bacterial isolate was inoculated into sterile nutrient broth and was incubated at 27°C at 220 rpm. After incubation, broth was centrifuged at 12000 rpm for 10 minutes. The supernatant was collected and used further for transformation of metal. Equal volume of 1mM $AgNO_3$ was added to that supernatant in dark condition and incubated for 24 hours. Along with this control flask containing only silver nitrate in distilled water was also incubated to check any secondary reaction. After incubation, there was a change in the color of test sample containing bacterial isolate and silver nitrate solution from light yellow to dark brown. The solution was further centrifuged at 14000 rpm for 10 minutes for harvesting silver nanoparticles. Supernatent was discarded and pellets were collected and dried in oven for 24 hours at 600˚C and stored at 4˚ C till further use.

2.3. Characterization of nanoparticles. The formation of silver nanoparticles was characterized using UV-VIS spectroscopy based on its characteristic Plasmon resonance. The UV- VIS spectra of AgNPs were recorded immediately after synthesis [8]. The optical absorption spectra of produced silver nanoparticles were analysed using Shimadzu UV-Vis spectrophotometer at wavelength between 200 to 700 nm. Transmission electron microscopy (TEM) was carried out using Hitachi (H-7500) TEM facility available at Panjab University, Chandigarh, India.

2.4. Effects of silver nanoparticles on growth of strains. The effect of silver nanoparticles on growth and adaptive response mechanisms were studied as per method given by Losasso et al. [9]. Cell growth was monitored using optical density measurement at different time intervals after adding silver nitrate in the early exponential growth phase. The relative growth rates were plotted against the silver nitrate concentrations. The flasks having varying concentration of silver nanoparticles ranging from 0.1 mM, 0.5 mM, 1.0 mM, 2.0 mM and 3.0 mM were inoculated with 1% of overnight grown culture of the isolate under study.

2.5. Agar well diffusion assay. The antimicrobial activity of silver nanoparticle was measured as described by Nidhi et al. [10]. The varying concentration of silver nanoparticles in range of 0.1mM to 2.0 mM were used to evaluate antimicrobial activity against test strains *E. coli* (MTCC no. 44) and *Aeromonas liquefaciens* (MTCC no. 2654) on Mueller Hinton Agar plates by agar well diffusion method [11]. Overnight grown cultures of selected test strains were spread by using sterile spreader on agar plates. The Minimum Inhibitory Concentration (MIC) for all test bacterial strains was determined.

2.6. Effect of contact time on disinfection. In the time dependent treatment of bacterial strains, sample collected from pond was treated with silver nanoparticles of concentration 1mM. Treated bacterial samples were homogenously spread on nutrient agar plates with time interval of 1 minute followed by incubation at 37^oC overnight.

2.7. Antimicrobial studies. Antibacterial action of silver nanomaterials was studied using Colony Forming Unit (CFU) and Most Probable Number (MPN) [12]. In CFU detection experiment samples (pond water) treated with different concentrations of silver nitrate ranging from 0.2 to 1 mM silver nanoparticles were spread on nutrient agar plates and incubated at 37°C for 24 h, and the number of CFU/ml was counted. In MPN method variable sample volume (10ml, 1ml, 0.1ml) of the treated pond water sample was transferred to lactose fermentation broth incubated at 37°C. After 24 hours, each tube was swirled and examined for gas production and growth. The number of positive tubes for each dilution was noted and compared with the MPN index chart to calculate MPN/100 ml of total coliforms.

2.8. Cytoplasmic leakage analysis. The nanoparticles treated cells were mixed with Phosphate buffer solution (PBS) buffer (pH-7.4) and incubated at 37°C in a rotatory shaker (120 rpm) in the presence of 0.1% SDS. The two controls were also tested containing *E. coli* cells and SDS treated *E.coli* cells. The amount of nucleic acid released into suspension was analysed by measuring the absorbance by DPA method and the concentration of protein released in the sample of the silver nanoparticle treated cell was estimated by Lowry method [13].

3. RESULTS SECTION

3.1. Characterization of isolates. The ten isolates based on the difference in their morphological characteristics were selected and screened for their metal reduction potential using silver nitrate. The isolate showing maximum growth was further used for experimental studies and were entitled as SB1. A combination of comprehensive biochemical tests was carried out to obtain the general identification of the isolate. The observations of the biochemical characterization studies are outlined in Table 1.

Table 1. Biochemical characterization of Strain SB1.

Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Urease test	Catalase Test	Carbohydrate Fermentation	H_2S Test
Positive	Negative	Negative	Negative	Negative	Positive	Negative

In the current study, results of various tests viz. Indole, Voges Proskauer, Citrate utilization and urease indicated that the strain SB1 was similar to the Bacillus sp. which was reported to carry out extracellular synthesis of nanoparticles [14].

3.2. Synthesis and characterization of nanoparticles. The isolate under study was exploited for its biotransformation potential of converting silver nitrate to metallic silver for nanoparticle formation. The extracellular reduction of the silver nitrate solution was observed with the supernatant obtained from

overnight grown culture of bacterial isolates. In a similar study done by Sintubin et al [15] silver nanoparticles were produced by lactic acid bacteria. The color change (figure 1) that was observed was further confirmed by UV-Vis spectral analysis. These nanoparticles gave an absorption peak at 420 nm that is indication of formation of AgNP's. This confirmed the reduction of silver nitrate to silver nanoparticles by the culture supernatant of SB1. In a similar study, Iranani et al [8] synthesized AgNP's and got absorption maximum between the range of 420-475nm.

The size and morphology of the biosynthesized silver nanoparticles were analyzed by Transmission electron microscopy (TEM) using Hitachi (H-7500) TEM facility at Panjab University, Chandigarh, India at 160 KV with the magnification of x30 K. From the micrographs after analysis indicated the presence of spherical particles with the average size 20-28 nm in the bioproduct. The TEM images clearly showed the formation of separated nanoparticles.

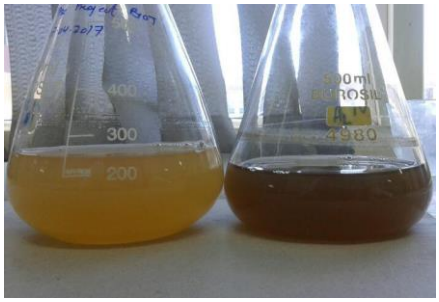


Figure 1. Control and Test flask showing color change due to bioreduction of silver nanoparticles.

3.3. Effect of Silver Nitrate on Microbial Growth. The growth pattern of bacterial isolate SB1 was monitored at different concentrations of silver nitrate. Increasing concentration of silver nitrate (0.5mM, 1 mM, 2 mM, 3mM), decreased the growth of microbes and the growth was inhibited at 3mM concentration.

In another studies carried out by Pandian et al. [16] similar observations were reported where 5mM concentration of silver nitrate was the minimum inhibitory concentration showed to arrest the growth of *Bacillus licheniformis* while the lower concentrations favoured the synthesis of nanoparticles.

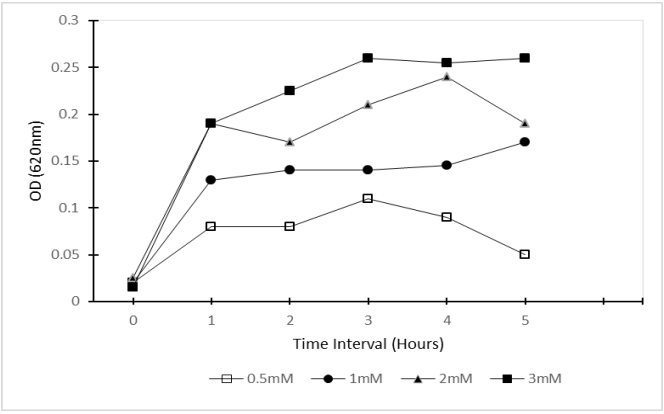
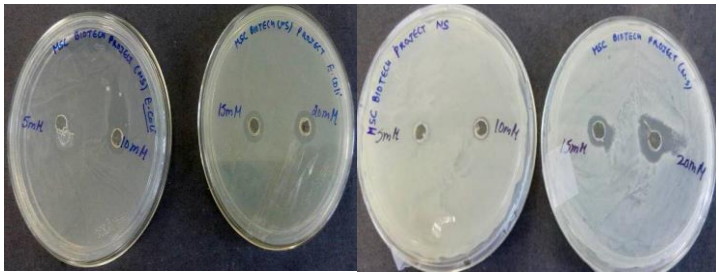


Figure 2. Growth curve of strain SB1 in presence of 0.5, 1, 2 and 3 mM silver nitrate.

Figure 2 depicts that 2mM concentration of silver nitrate is best for maximum yield of nanoparticles as compared to other concentrations. At 0.5 mM lower growth rate was observed while 1mM and 2 mM concentrations have an intermediate effect on the growth of isolate.

3.4. Antimicrobial Activity. The antimicrobial activity of AgNPs was evaluated against pathogenic strains *Escherichia coli* & *Aeromonas liquefaciens* by agar well diffusion method. In case of *E. coli* the minimum inhibitory concentration of AgNPs was 10 mM which produced a inhibition zone of 10 mm. In case of *Aeromonas liquifaciens* the minimum inhibitory concentration of AgNP was 15mM with a inhibitory zone of 11mm. The maximum zone of inhibition was 12 mm at 20 mM against *Escherichia coli* (Fig. 3 a) and minimum zone of inhibition was 11mm and maximum zone of inhibition was 14mm against *Aeromonas liquefaciens* (Fig. 3 b). The interaction of silver nanoparticles with disulphide groups of enzymes leading to disruption of metabolic pathways accountable for the antibacterial activity of nanoparticles [17]. In our earlier studies carried out for nanoparticles synthesised from the bark of *Syzygium cumini* showed the minimum 11 mm and maximum was 25mm inhibition zone at 1 mM concentration against *Aeromonas liquefaciens* [18]. The high bactericidal activity was due to the released silver cations form AgNP's that caused changes in membrane of bacteria leading to increased membrane permeability. Hungund et al [19] concluded that AgNPs from orange showed about 8 mm and 5mm inhibition zone against the test organisms *E. coli* and *S. aureus* respectively. The SEM and TEM studies revealed the formation of pits in the cell walls of *E. coli* resulting in accumulation of nanoparticles in the cell leading to cell death and the rate of inhibition is directly dependent on the concentration of AgNPs [20]. Similarly, in a study by Chandrakanth et al. [21] minimum inhibitory concentration was 20 µg with a 12 mm zone while the maximum inhibitory concentration was 60 µg with 14 mm zone recorded in *E. coli*. In *Staphylococcus aureus* with 11.5 mm and 14.0 mm of zone of inhibition recorded at respective concentrations.



(a) (b)

Figure 3 (a) Antimicrobial activity of AgNps concentration 5- 20 mM against *Escherichia coli*, (b) Antimicrobial activity of AgNPs concentration 5- 20 mM against *Aeromonas liquefaciens.*

3.5. Effect of Contact time on disinfection. In order to check the antimicrobial activity of silver nanoparticles sample of pond water was used. A sample of pond water treated with 1mM concentration of silver nanoparticles was homogeneously spread on nutrient agar plates (1ml on each plate) with time interval of 1 minute. The number of colonies decreased as time increased and hence can be concluded that increase in time at same concentration, there was increased chance of interaction of nanoparticles with bacteria. The number of colonies decreased with increase in contact time in the current treatment. Thus, the culture treated in liquid phase is more effective because of greater chance of bacteria-nanoparticles interaction and the number of colonies decreased as the time increased. In a similar study,

positive results were obtained against *E. coli* with 0.01-1mg/ml of Ag nanoparticles with a contact time of 0-26 hours [22] and 12.5 mg/l of Ag nanoparticles with 2 h contact time against *E.coli* [23].

3.6. Antimicrobial studies. Antimicrobial action of Ag nanomaterials was further evaluated on the basis of Colony forming unit (CFU) and Most Probable Number (MPN). In CFU detection method samples (pond water) treated with silver nanoparticles were spread on nutrient agar plates and after incubation at 37°C for 24 h, the number of CFU/ml was counted. It was found to be 27 CFU / 0.1 ml in control and no colony was observed in the sample plate for 1mM. The CFU method signified that 1mM concentration of silver nanoparticles is effective for microbial inhibition than 0.4 mM concentration of silver nanoparticles. Thus, no colony was found in 1mM concentration plate while 9 colonies were observed in 0.4mM concentration plate. In a similar study done by Harikumar et al [24], the results obtained showed that the percentage of reduction by CFU assay was 95% in case of coliform bacteria and 100% in *E.coli*.

In MPN method 10ml, 1ml, 0.1ml of the treated pond water sample was transferred to lactose fermentation broth incubated at 37°C. After 24 hours, each tube was swirled and examined for gas production. For control tubes the MPN index per 100ml was 1600 and for the sample it was 27. Thus, the result for the MPN showed that there is considerable decrease in number of colonies in the sample treated with Ag nanoparticles as compared to the control. Hence, can be used as an effective disinfectant.

3.7. Cytoplasm Leakage Analysis. In further step to confirm the influence of nanoparticles on the cell wall of bacteria, nucleic acid and protein concentration was evaluated which could be the other indicator of the cell injury as a result of nanoparticle treatment. The DNA concentration was calculated using DPA method. The results depicted below in Table 2 clearly indicated that maximum concentration of DNA 0.08mg/ml was found in sample T4 which contained both nanoparticles and SDS, thus showing their role in cell lysis. In sample T2 the DNA conc (0.04mg/ml) was comparatively higher than in sample T3 (0.02mg/ml) showing that nanoparticles are more effective than SDS and it only helps nanoparticles in cell lysis. The protein concentration was estimated by Lowry et al [13]. As shown in Table 2, maximum concentration of protein 0.40 mg/ml was found in sample T4 that contained both nanoparticles and SDS which resulted in effective action for nanoparticles. In sample T2 (0.32mg/ml).

Table 2. Concentration of DNA and protein in supernatant of treated.

Sample	DNA Conc. (mg/ml)	Proteins Conc. (mg/ml)
T_1 (*E.coli*)	0.01	0.26
T_2 (*E.coli* + nanoparticles)	0.04	0.32
T_3 (*E.coli* + SDS)	0.02	0.29
T_4 (*E.coli* + SDS + nanoparticles)	0.08	0.40

the protein concentration was comparatively less while in sample T3 (0.29mg/ml) it was least. Thus, the nanoparticles work efficiently, in creating minute pores by destabilizing the membrane of bacteria and the cells are finally lysed through SDS. In a similar study carried out by Singh et al [25] released protein and DNA contents in the supernatant of NP-treated bacterial cells was profound for different time points (0, 1, 3, 5 and 7 h) as compared with control for all bacteria. There were statistically significant ($P \leq 0.05$) differences in DNA concentration released at different time intervals. Amount released was the highest in *S. dysentriae* followed by those of *V. parahemolyticus* and *S. infestis*. Increase in DNA content in the supernatant incubated up to 5 h was noticed for all the tested bacteria, followed by significant decrease. For protein quantification, increase of protein concentration in the supernatant was seen up to 5 h; beyond this time point there was decrease in the amount of protein content.

4. CONCLUSIONS

In the current study, bacterial isolate was used to synthesize silver nanoparticles by the reduction of silver nitrate to metallic silver. The synthesized nanoparticles were characterized by using UV visible spectrophotometer and the peak was obtained at 410 nm. The morphological and biochemical characterization showed the resemblance of the isolate under study with Bacillus species. The growth curve studies revealed that 2mM concentration of silver nitrate was the most favourable concentration and provided maximum yield of nanoparticles. These nanoparticles were further used for antimicrobial studies and purification of water. There was significant decrease in number of colonies and MPN with increase in contact time of samples treated with different concentrations of silver nanoparticles. The zone of inhibition against *Aeromonas liquifaciens* and *E.coli* with minimum inhibitory concentrations of 15 and 10 mM was observed. Moreover, the silver nanoparticles were also shown to be capable of destabilizing the cell membrane of bacteria resulting in increase in concentration of proteins and nucleic acids. Thus, it is concluded that the biological synthesis of silver nanoparticles is much easier and ecofriendly approach as compared to chemical and physical methods which requires a lot of chemical and physical energy and are expensive and hazardous with potential of environmental and biological risks. The plant based methods suffers from a major drawback of yield and purification and extraction of synthesized nanoparticles. On the other hand, bacterial synthesis methods are simple and extraction steps were easy with minimal chances of contamination. It is concluded from the current studies that antimicrobial potential of nanoparticles holds promising future in various fields like purification of water sample which is the most crucial area for the welfare of mankind.

5. REFERENCES

[1] Duran N., Marcato P.D., Nanobiotechnology perspectives. Role of nanotechnology in the food industry: a review, *Int. J. Food Sci.* Tech., 48(6), 1127-34, **2013**.

[2] Iravani S., Korbekandi H., Mirmohammadi S.V., Zolfaghari B., Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res. Pharma. Sci.* 9(6), 385, **2014.**

[3] Pantidos N., Horsfall L.E., Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *J. Nanomed. Nanotechnol.*, 5(5):1, **2014**.

[4] Narayanan KB., Sakthivel N., Biological synthesis of metal nanoparticles by microbes. *Adv. Colloid Interface Sci.*, 156(1), 1-3, **2010.**

[5] Sarkar S., Guibal E., Quignard F., SenGupta A.K., Polymer-supported metals and metal oxide nanoparticles: synthesis, characterization, and applications. *J. Nanoparticle Res*., 14(2), 715, **2012.**

[6] Ghosh B., Ramamoorthy D., Effects of Silver Nanoparticles on Escherichia Coli and its Implications. *Int. J. Chem. Sci.*, 8(5), 2010.

[7] Gandhi H., Khan S., Biological synthesis of silver nanoparticles and its antibacterial activity, *J. Nanomed. Nanotechnol.* 7 (2), 1-2, **2016**.

[8] Iravani, S. Green synthesis of metal nanoparticles using plants. *Green Chem.*, 13(10), 2638-2650, **2011**.

[9] Losasso C., Belluco S., Cibin V., Zavagnin P., Mičetić I., Gallocchio F., Zanella M., Bregoli L., Biancotto G., Ricci A., Antibacterial activity of silver nanoparticles: sensitivity of different *Salmonella* serovars. *Front. Microbiol*., 5. **2014.**

[10] Nidhi S., Prasenjit S., Karthik R., Jayanthi, A., Biosynthesis of silver and selenium nanoparticles by Bacillus sp. JAPSK2 and evaluation of antimicrobial activity. *Der. Pharmacia. Lettr*. 6(1), 175-181, **2014**.

[11] Prasad R., Swamy VS., Antibacterial activity of silver nanoparticles synthesized by bark extract of *Syzygium cumini*. *Journal of Nanoparticles*, 1-6, **2013**.

[12] Zhang H., Application of silver nanoparticles in drinking water purification. University of Rhode Island, **2013**.

[13] Lowry OH., Rosebrough NJ., Farr AL., Randall RJ., Protein estimation by Lowry's method. *J. Biol. Chem.*, 193:265, **1951**.

[14] Vithiya K., Kumar R., Sen S., *Bacillus* sp. mediated extracellular synthesis of silver nanoparticles, *Int. J. Pharm. Sci.*, *6*,525- 525, **2014**.

[15] Sintubin L., De Windt W., Dick J., Mast J., van der Ha D., Verstraete W. and Boon N., Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles, *Appl. Microbiol Biotechnol.*, 84(4),741-749, **2009**.

[16] Deepak V., Kalishwaralal K., Pandian SRK., Gurunathan S.,"An insight into the bacterial biogenesis of silver nanoparticles, industrial production and scale-up." In *Metal nanoparticles in microbiology*, pp. 17-35. Springer Berlin Heidelberg, **2011**.

[17] Yun H., Kim JD., Choi HC., Lee CW., Antibacterial activity of CNT-Ag and GO-Ag nanocomposites against gram-negative and gram-positive bacteria. *Bull. Korean Chem. Soc.*, 34(11),3261-4, **2013.**

[18] Sharma A., Kaur P., Bhattacharya A., Sharma N., Batra N., Biological synthesis and antibacterial activity of silver nanoparticles. *Int. J. Neosci.* 2(2), 13-15, **2015**.

[19] Hungund BS., Dhulappanavar GR., Ayachit NH., Comparative Evaluation of Antibacterial Activity of Silver Nanoparticles Biosynthesized Using Fruit Juices. *J. Nanomed. Nanotechnol.* 6:271, 2015.

[20] Sondi—I., Salopek-Sondi B., Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, *J. Colloid Interface Sci.*, 275(1), 177-182, **2004**.

[21] Chandrakanth R.K., Ashajyothi C., Oli A.K., Prabhurajeshwar C., Potential Bactericidal Effect of Silver Nanoparticles Synthesised from *Enterococcus* Species, *Orient J. Chem.* 30(3), **2014.**

[22] Pal SL., Jana U., Manna PK., Mohanta GP., Manavalan R., Nanoparticle: An overview of preparation and characterization, 2000-2010, **2011**.

[23] Morones JR., Elechiguerra JL., Camacho A., Holt K., Kouri J.B., Ramírez JT., Yacaman MJ., The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10), 2346-53, **2005.**

[24] Harikumar PS., Joseph L., Manjusha CM., Biosynthesis of silver nanoparticles and its application in microbial treatment of drinking water. *Nanosci. Nanotechnol*., 5(1), 23-27, **2011.**

[25] Singh M., Mallick AK., Banerjee M., Kumar R., Loss of outer membrane integrity in Gram-negative bacteria by silver nanoparticles loaded with *Camellia sinensis* leaf phytochemicals plausible mechanism of bacterial cell disintegration. *Bull. Mater. Sci.* 39(7), 1871–1878, **2016.**