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International Journal of Pure & Applied Bioscience

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (2):** 39-47 (2014)

Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Combining Biological Silver Nanoparticles with Antiseptic Agent and their Antimicrobial Activity

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ABSTRACT

Nanoparticles biosynthesis and its applications is a rapidly developing field. The biological silver nanoparticles (AgNPs) are being used as inhibitory and antimicrobial agents. In the current investigation, synthesis of silver nanoparticles by bacterial strain isolated from soil sample contaminated with crude petroleum oil was studied. Molecular identification of the bacterial isolate showed it as a strain of Sphingobacterium mizutaii. The biosynthesized nanoparticles characterized by the UV-Vis spectroscopy which exhibited a broad absorption spectrum band around 420 nm. Transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) analysis confirmed the formation of varying size silver particles in the range from 4.5-31.6 nm. The Interaction between silver nanoparticles and proteins were characterized by Fourier transform infrared (FTIR) spectroscopy. The combination effects of AgNPs with the antiseptic agent (Dettol) was then studied. The antimicrobial effect of AgNPs- Dettol combination increased significantely in case of E. coli, K. pneumoniae, B. subtilis and Candida krusei.

Keywords: *Sphingobacterium mizutaii, silver nanoparticles, antiseptic agent.*

INTRODUCTION

Nanotechnology involving synthesis and applications of nanomaterials is a rapidly developing field with significant applications in various areas[1]. The silver nanoparticles is mainly important due to its applications in biomolecular detection, therapeutics, catalysis and also as antimicrobial agents[2,3,4]. Primarly silver nanoparticles are considered as an alternative to silver ions which obtained from silver nitrate, that were used as antimicrobial agents. Silver nitrate solution known since ancient times was directly used for treatments and of burns, wounds and several bacterial infections during Second World War[5].

Microbial biosynthesis of nanoparticles is eco-friendly and alternative to chemical and physical methods, since it takes place at relatively ambient temperature and pressure[6,7,8]. Microbial synthesis of metallic nanoparticles can be produced either intracellularly or extracellularly[9,10,11]. Many microorganisms interact with metal ions reducing them into metallic nanoparticles[12]. Extracelluar synthesis of nanoparticles is cheap and simpler to synthesize, so many studies were focused on it for the synthesis of metal nanoparticles[1]. Many studies using bacterial culture supernatants like *Bacillus megaterium*; *Pseudomonas proteolytica*; *Pseudomonas meridian*; *Pseudomonas aeruginosa; Arthrobacter kerguelensis*; *Bacillus indicus*; etc. were proven to form extracellular nanoparticles very effectively[13,14,15]. It has been also found that spores and microcapsules of *Bacillus athrophaeus* able to synthesize silver nanoparticles[16].

The metallic nanoparticles are most promising as they have ability as an inhibitor and antibacterial activities due to their large surface area to volume ratio[17]. Silver nanoparticles are used as antimicrobial agents in most of the public places such as elevators and railway stations in China and also, they are used in surgically implanted catheters to reduce the infections caused during surgery and are proposed due to its anti-fungal, anti-inflammatory, anti-angiogenic and anti- permeability effects[5]. Many bacteria have

developed resistance to antibiotics, so there is a need for alternative antibacterial substance[18]. The advantage of AgNPs have an important over conventional antibiotics in that it has ability to kill all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it[19].
This study is the first report of biological synthesis of silver nanoparticles using bacterium *Sphingobacterium mizutaii* and was designed to compare the *in vitro* antimicrobial effect of silver nanoparticles with one of common local antiseptics such as dettol.

MATERIALS AND METHODS

Isolation and molecular identification of using bacterium

Bacterium was isolated from soil contaminated with crude petroleum oil sample, Egypt. The soil sample was suspended in nutrient broth throughout serial dilution then plated onto nutrient agar for 24 h. The colonies were counted, then picked out and purified by streaking on nutrient agar plates.

Molecular identification

DNA extraction, purification, amplification and gelelectrophoresis were performed as described previously by Mohamed et al.[20]. PCR product was sequenced in both direction by Solgent company (South Korea) using universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (CGGCTACCTTGTTACGACTT). The bacterial 16S rDNA sequences obtained were then aligned with known 16S rDNA sequences in Genbank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/), and percent homology scores were generated to identify bacteria. After obtaining the DNA sequences homology search was performed using Blast program to find the sequences producing significant alignment with the obtained sequences. A phylogenetic tree was constructed with MEGA version 4.0 using a neighbor-joining algorithm, plus the Jukes-Cantor distance estimation method with bootstrap analyses for 1000 replicates was performed. The sequencing of the gene coding for 16s rDNA gene for the strain has been deposited in the GenBank nucleotide sequence databases (NCBI) under Accession number KF709696 and the strain was also deposited in the culture collection of Assiut University Mycological Centre, Assiut, Egypt as AUMC b-161.

Preparation of supernatants

Biologically synthesis of silver nanoparticles:

Sphingobacterium mizutaii was grown in LB broth (Tryptone 10 g/L, Yeast extract 10 g/L and NaCl 5 g/L) and incubated in an orbital shaker (80 rpm) for 24 hours at 35°C. Then, the culture was centrifuged at 8000 rpm for 15 min and the cell free supernatant was used for the biosynthesis of AgNPs. Aqueous silver nitrate solution (1 mM) was mixed carefully with 50 ml of cell supernatant in 250 ml conical flask and agitated in shaker (80 rpm) at room temperature. Control without the $AgNO_3$ (cell free supernatant) was also kept at the same conditions.

Characterization of silver nanoparticles:

UV-visible spectroscopy analysis

The optical properties of silver nanoparticles were carried out on Ultraviolet-Visible Spectroscopy (UV-Vis) (Perkin-Elmer lambda 750 spectrophotometer) at a resolution of 1 nm and scanning the spectra between 300-900 nm. A strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range due to the surface plasmon resonance. The stability of stored biologically synthesized silver nanoparticles was also performed by UV-Visible spectral analysis.

Energy Dispersive X-ray (EDX) Spectra

To investigate the elemental composition of the sample, JEOL JSM-5400 L.V. scanning electron microscope (SEM) equipped with a Tractor Northern 5200 energy dispersive X-ray (EDX) analysis system was used. Thin film of the silver nanoparticles was prepared on holder, then allow to dry by putting it under a mercury lamp.

Transmission Electron Microscope (TEM)

For transmission electron microscope imaging, samples were prepared by placing a drop aqueous solution of synthesized silver nanoparticles on a negative carbon coated copper grids and dried in air. The shape

and size of the silver nanoparticles were analyzed by TEM micrographs using the JEOL TEM 100 CXII (Electron Microscope Unit, Assiut University, Egypt).

Fourier Transform Infrared Spectroscopy (FTIR)

To investigate the functional groups possible binding sites with nanoparticles. The solution of AgNPs was centrifuged at 9000 rpm for 20 min. The pellet was washed with ethanol for three times and then air dried to obtain dried powder. FTIR spectra was obtained by KBr pellets methods operated on FTIR spectrophotometer (IR470-SHIMADZU-Japan). The FT-IR spectra over the scanning range of 400-4000 cm^{-1} were obtained with the resolution of 2 cm^{-1}**.**

Antimicrobial activity test for synthesized silver nanoparticles

After characterization of silver nanoparticles, their antimicrobial effect was checked by disc diffusion method (Boyce 1984) on some common pathogens of human. The test microorganisms *Escherichia coli* ATCC8739, *Bacillus subtilus* ATCC 6633, *Staphylococcus aureus* BM14, *Enterococcus sp.* BM15, *Klebsiella pneumonia* BM12, *Candida albicans*, *Candida glabrata* AUMC8175 and *Candida krusei* AUMC9211. Nutrient agar and Sabouraud's media were used for growing the test bacteria and *Candida*. The antimicrobial spectrum of the synthesized silver nanoparticles was determined by the disc diffusion method on plates seeded with the tested bacteria or fungi. Overnight cultures of the bacterial and *Candida* were prepared in nutrient broth or sabouraud broth and diluted to $1x10^5$ CFU/ml, 200 µl were stirred well in nutrient or sabouraud agar plates. After solidification, three sterile paper disks were placed on the surface of each agar plate and were impregnated with 10 µl of the silver nanoparticles or Dettol or mixture from silver nanoparticles and Dettol. The plates were then incubated at 30ºC for 24 hours, then the diameters of inhibition zones were measured to estimate their inhibitory effects.

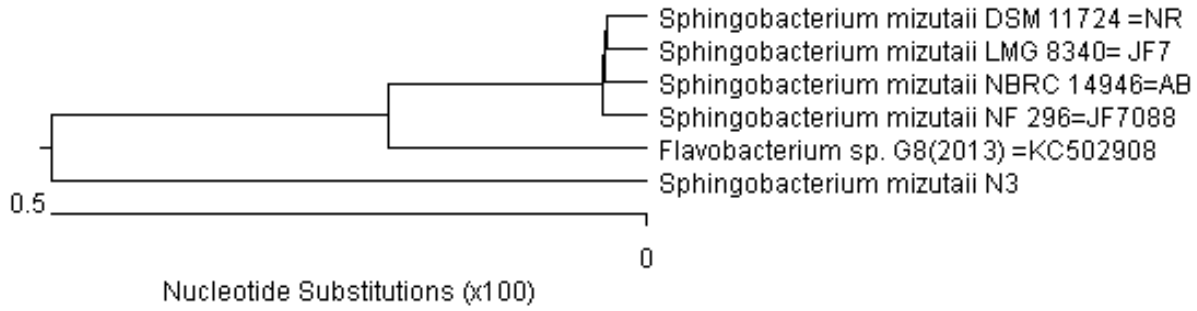
Statistical analysis

It was performed by using the SPSS 10 Software. Paired-Sample *t*-tests were carried out to determine whether there was a significant diffrences between mixture of AgNPs and dettol. The values of *P* value< 0.05 were considered significant.

Results

The using bacterium *Sphingobacterium mizutaii* was found to have ability to form silver nanoparticles as observed by change in the colour of the reaction. The selected bacterium was subjected to molecular identification by partial sequencing of the 16s rDNA gene (approx. 1100bp) and the result demonstrate 99% homology to *Sphingobacterium mizutaii* (Fig. 1). Furthermore the nucleotide sequence was submitted to GenBank in NCBI with the accession number KF709696.

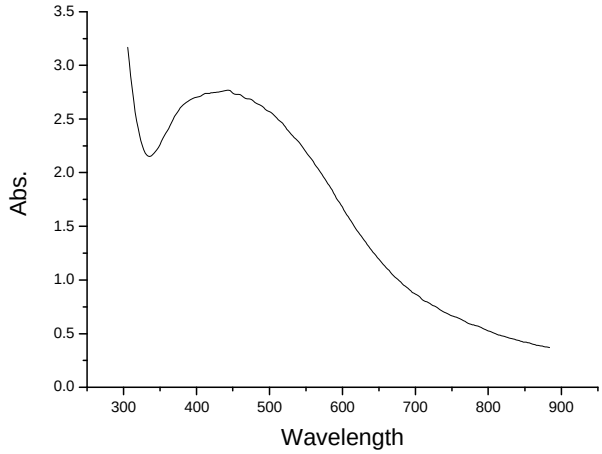
Fig. 1: The phylogenetic homology tree based on multiple sequence alignments of the 16s rDNA of the *Sphingobacterium mizutaii* reference to international isolates



The synthesis of silver nanoparticles by the culture supernatants and bacterial biomass of *Sphingobacterium mizutaii* were separately investigated through the observation of colour change in the presence of 1mM $AgNO_3$. Rapid appearance of a yellowish – brown colour in the reaction vessels suggested the formation of colloidal silver nanoparticles. The colour change was observed for samples containing both biomass and supernatant, further experiments were carried out using only cell free supernatant.

The UV-Visible absorption spectra of the synthezied nanoparticles were measured in the range 300-900 nm (Fig.2) using a double beam UV-Vis spectrophotometer, showing a strong broad absorption band located between 405-450 nm for silver nanoparticles prepared by the tested bacterium. The silver nanoparticles band remained around 420 nm revealed that the particles were well dispersed without aggregation.

Fig. 2: UV-Vis spectrum of silver nanoparticles produced by *Sphingobacterium mizutaii* culture supernatant



The EDX (Energy Dispersive Absorption Spectroscopy photograph) analysis is performed and represented in Fig. 3 to investigate the elemental composition of silver nanoparticles. The EDX analysis exhibits various intense peaks associated with Ag atoms, confirming the formation of silver nanoparticles. Peaks for Cu and C are from the grid used and the peaks for S, P and N correspond to the proteins or enzymes capping over the AgNPs.

Fig. 3: EDX spectrum of AgNPs produced by *Sphingobacterium mizutaii* culture supernatant

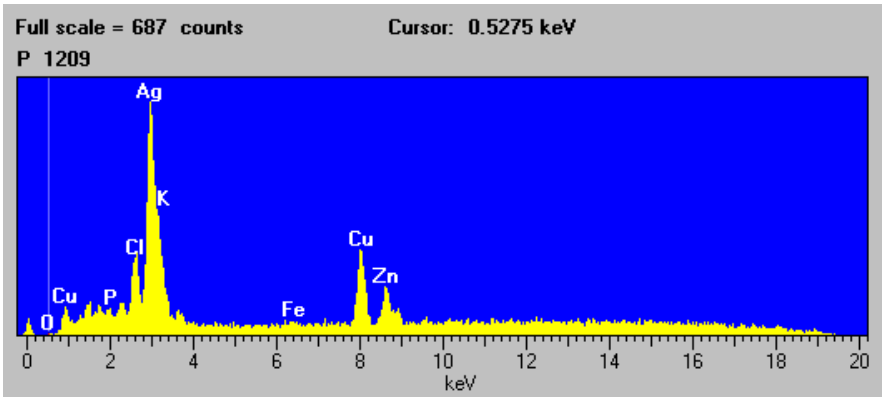
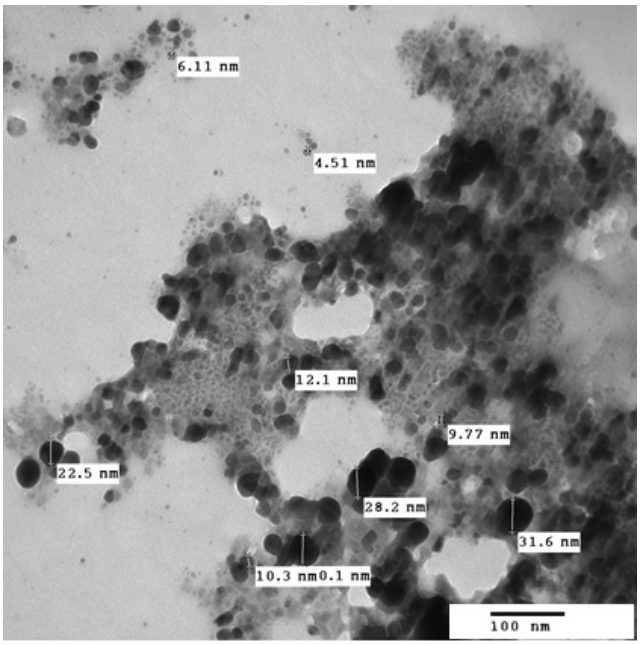


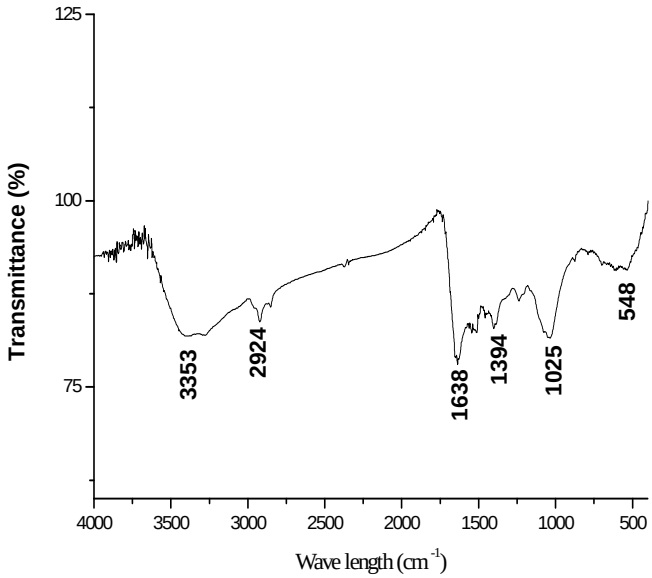
Fig.4 shows the TEM images of silver nanoparticles synthesized using *Sphingobacterium mizutaii*. The particle size ranges in 4.5-31.6 nm and posses an average size of 18 nm.

Fig. 4: TEM images of silver nanoparticles (AgNPs) synthesized using *Sphingobacterium mizutaii* culture supernatant



FTIR measurement was carried out to identify the biomolecules for capping and stabilization of the metal nanoparticles synthesized by *Sphingobacterium* culture supernatant. The FTIR spectrum of silver nanoparticles in Fig. 5 showed absorption bands at 3353, 2924, 1638, 1394, 1025 and 548 cm^{-1}. The band at 3353 cm^{-1} may be corresponds to O-H stretching H-bonded functional group in alcohols and phenolic compounds. The peak at 2924 cm^{-1} corresponds to O-H streches carboxylic acids. The assignment at 1638 cm^{-1} corresponds to N-H bend primary amines. The peak at 1394 cm^{-1} corresponds to C-N stretching of aromatic amine group and bands obsorved at 1025 and 548 cm^{-1} corresponds to ether and esters.

Fig. 5: FTIR spectrum of silver nanoparticles (AgNPs) synthesized using *Sphingobacterium mizutaii* culture supernatant



The antimicrobial activity of biosynthesized silver nanoparticles showed different inhibitory effect on different human pathogenic bacteria and *Candida*. The results in Table 1 revealed that biosynthesized silver nanoparticles showed antibacterial and antifungal activity with varying magnitudes. The inhibitory effect of nanoparticles increased significantely in the presence of Dettol against all tested microorganisms. The antimicrobial effect of Dettol was increased significantely in the case of *E. coli, K. pneumoniae, B. subtilis and Candida krusei.*

Table 1: Paired-sample *t*-test of the antimicrobial activity of silver nanoparticles, Dettol and their combination on different microorganisms

Microorganism	AgNPs and Dettol ≠ AgNPs	AgNPs and Dettol ≠ Dettol
E. coli ATCC8739	0.030*	0.025*
B. subtilis ATCC6633	0.025*	0.045*
St. aureus BM14	0.023*	0.126
K. pneumoniae BM12	0.030*	0.049*
Enterococcus sp. BM15	0.017*	0.090
Candida albicans	0.018*	0.170
C. krusei AUMC9211	0.016*	0.045*
C. glabrata AUMC8175	0.032*	0.126

* Significant

P value< 0.05 was considered significant

DISCUSSION

Biological synthesized silver nanoparticles have many applications in different fields such as DNA sequencing, biosensor technology, diagonistics, biomedical science and etc[23]. In the current study, to employ an environmental friendly and cost effective manner for synthesis of AgNPs by extracellular synthesis mechanism from *Sphingobacterium mizutaii*. Rapid appearance of a yellowish–brown colour suggested the formation of colloidal silver nanoparticles and due to the excitation of surface Plasmon vibrations[24]. The synthesis process of silver nanoparticles was formed within 10 minutes of silver ion coming in the cell supernatant of *Enterobacterium*[24]. Con and Loan[25] confirmed that the colour of AgNPs solution changed from yellow to dark green when silver concentration increased from 40 to 200 mg per liter.

Formation of silver nanoparticles was confirmed by UV-vis spectroscopy which shown that the band remained around 420 nm revealed that the nanoparticles were well dispersed without aggregation. Obsevation of this beak, assigned to a surface Plasmon, is confirmed for various metal nanoparticles with sizes from 2-100 nm [26].

EDX is reported earlier that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins[27] and via the electrostatic attraction of negatively charged carboxyl groups in enzymes which present in the cell wall of bacteria[28] and therefore, stabilization of the AgNPs by protein is possible.

TEM micrograph confirmed the development of mixed silver nanoparticles sizes. The size of silver nanoparticles produced by *Sphingobacterium* range from 4.5-31 nm that can be used in several applications[29].

FTIR confirmed that the synthesized nanoparticles surrounded by proteins and metabolites and the stability of silver nanoparticles by proteins and enzymes is a clear possibility[15,22].

The previous studies revealed that the alcohols, phenolic, alkanes, alkynes group able to interact with biological synthesized nanoparticles[21,15,22].

The antimicrobial effect of biological silver nanoparticles-dettol increased with all using microorganisms. The mechanism of inhibitory effect of silver nanoparticles on the DNA of microorgamisms and inactivate cellular proteins[30]. It was also suggested that silver ions bind to functional proteins, causing protein denaturation[31,32]. Stonimenov et al. [33] showed when *E. coli* was treated with highly reactive metal oxide nanoparticles, a bacterial membrane exhibits a significant increase in permeability and loss of regulating transport through plasma membrane and causing cell death.

It has been reported that ionic silver strongly interacts with thiol group of vital enzymes and inactivates them[34,30]. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions[35].

The active ingredient in Dettol Antiseptic Liquid is 4.8% chloroxylenol which inhibits the growth of a large range of microorganisms. Chloroxylenol destroys the bacteria by inhibiting the production of adenosine triphosphate by causing the disruption of cell membrane potentials, destroying the protein gradient essential for the synthesis of ATP. Presence of silver nanoparticles with dettol accelerate and increase its antimicrobial effect.

In conclusion, the present study represent a reliable and ecofriendly biotechnological process for the extracellular synthesis of silver nanoparticles using cell free bacterial supernatant. Additionally it has been found that the combination of silver nanoparticles with antiseptic agents enhanced their antimicrobial activity and faciliate binding the antimicrobial agents to bacteria and fungi.

REFERENCES

1. Duran N., Priscyla D., Marcato P.D., Alves O., De Souza G. and Esposito E., Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains, *J. Nanobiotech.* **l3:**1–7 (2005)
2. Shrivastava S., Bera T., Singh S.K., Singh G. and Ramachandrarao Dash P., Characterization of antiplatelet properties of silver nanoparticles, *ACS Nano*, **3:**1357–1364 (2009)
3. Sadhasivam S., Shanmugam P. and KyuSik Yun K., Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganism, Coll. Surf. B, **81:**358–362 (2010)
4. Christopher P., Xin H. and Linic S., Visible-light-enhanced catalytic oxidation reactions on plasmonic silver nanostructures, *Nat. Chem.*, **3:**467–472 (2011)
5. Gurunathan S., Kalishwaralal K., Vaidyanathan R., Deepak V., Pandian S.R.K., Muniyandi J.H., Hariharan N. and Eom S.H., Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*, *Coll. Surf. B*, **74:**328–335 (2009)
6. Gade A.K., Bonde P., Ingle A.P., Marcato P.D., Duran N. and Rai M.K., Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles, *J. Biobase Mater Bioenergy*, **2:**243–247 (2008)
7. Mukherjee P., Roy M., Mandal B.P., Dey G.K., Mukherjee P.K., Ghatak J., Tyagi A.K. and Kale S.P., Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*, *Nanotech.* **19:**103–110 (2008)
8. Wei X., Luo M., Li W., Yang L., Liang X., Xu L., Kong P. and Liu H., Synthesis silver nanoparticles by solar irradiation of cell-free *Bacillus amyloliquefaciens* extracts and $AgNO_3$, *Bioresource Technol*. **103:**273-278 (2012)
9. Ahmad A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R. and Sastry M., Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, *Coll. Surf.* B, **28:** 313–318 (2003)
10. Kalishwaralal K., Deepak V., Pandian S.R.K., Kottaisamy M., BarathManiKanth S., Kartikeyan B. and Gurunathan S., Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*, *Coll. Surf. B*, **77:**257–262 (2010)
11. Jain N., Bhargava A., Majumdar S., Tarafdar J.C. and Panwar J. Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective, *Nanoscale*, **3:**635–641 (2011)
12. Prasad K. and Jha A., Biosynthesis of metal and oxide nanoparticles using Lactobacilli from yoghurt and probiotic spore tablets, *Anaytical J. of Biotechnol.*, **5:** 285-291 (2010)
13. Saravanan M., Vemu A.K. and Barik S.K., Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. *Coll. Surf. B*, **88:**325–331 (2011)

14. Shivaji S., Madhu S. and Singh S., Extracellular synthesise of antibacterial silver nanoparticles using psychrophilic bacteria, *Process Biochem.*, **49:**830–837 (2011)
15. Jeevan P., Ramya K. and Edith Rena A., Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. *Indian J. of Biotechnol.* **11:** 72-76 (2012)
16. Hosseini-Abari A., Emtiazig G. and Ghasemi S.M., Development of an eco-friendly approach for biogensis of silver nanoparticles using spores of *Bacillus athrophaeus, World J. Microbiol. Biotechnol*., doi:201310.1007/ 511274-013-1403-4 (2013)
17. Rai M., Yadov A. and Gade A., Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Advces*, **27:**76-83 (2009)
18. Pal S., Tak Y. and Song J.M., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli, Appl. Enviro. Microbiol.* **73:**1712-1720 (2007)
19. Dameron C.T., Reese R.N., Mehra R.K., Kortan A.R., Carroll P.J., Steigerwald M.L.,Brus L.E. and Winge D.R., Biosynthesis of cadmium sulfide quantum semiconductor crystallites, *Nature*, **38:**596–597 (1989)
20. Mohamed E., Yousef N.M.H. and Farag A., Isolation and molecular identification of polyaromatic hydrocarbons–utilizing bacteria from crude petroleum oil samples, *African J. microbiol. Res.* **6:**7479-7484 (2012)
21. Matsumura Y., Yoshikata K., Kunisaki S. and Tsuchido T., Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate, *Appl. Enviro. Microbiol*., **69:**4278-4281 (2003)
22. Sivalingam P., Antony J.J., Siva D., Achirman S. and Anbarasu K., Mangrove *Streptomyces* sp. BDUKAS10 as nanofactory for fabrication of bactericidal silver nanoparticles, *Coll. Surf. A*, **98:** 12-17 (2012)
23. Jain D., Kachhaeaha S., Jain R., Sirvastava G. and Kothari S.L., Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. *Indian J. Exp. Biol.* **48:**1152-1156 (2010)
24. Shahverdi A.R., Minaeian S., Shahverdi H.R., Janalifar H. and Nohi A., Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria, A novel biol approach, *Process biochem.*, **42:**919-923 (2007)
25. Con T.H. and Loan D.K., Preparation of silver nano-particles and use as a material for water sterilization, *Enviro. Asia*, **4:**62-66 (2011)
26. Sastry M., Patil V. and Sainkar S.R., Electrostatically controlled diffusion of carboxylic acid derivatized silver colloidal particles in thermally evaporated fatty amine films, *J. Phys. Chem. B*, **102:**1404–1410 (1998)
27. Sastry M., Ahmad A., IslamKhan M. and Kumar R., Biological synthesis of metal nanoparticles using fungi and actinomycetes. *Current Sci.*, **85:**162-170 (2003)
28. Vigneshwaran N., Ashtaputre N.M., Varadarajan P.V., Nachane R.P., Paralikar K.M. and Balasubramanya R.H., Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus, Materials Lett.* **61:**1413-1418 (2006)
29. Klaus T., Joerger R., Olsson E. and Granqvist C.G., Silver-based crystalline nanoparticles, microbially fabricated. *Proc. Nat.l Acad. Sci*. USA, **96:**13611-13614 (1999)
30. Feng Q.L., Wu J., Chen G.Q., Cui F.Z., Kim T.N. and Kim J.O., A Mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus. J. Biomed. Materials Res.*, **52:**662-668 (2000)
31. Sondi I. and Matijevic E., Homogeneous precipitation by enzyme catalyzed reactions. II. strontium and barium carbonates, *Chemical of Material*, **15:**1322-1326 (2003)
32. Sondi I. and Salopek-Sondi B., Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, *J. of Coll. and Interface Sci*. **275:**177–182 (2004)

33. Stoimenov P.K., Klinger R.L., Marchin G.L. and Klabunde K.J., Metal oxide nanoparticles as bactericidal agents, *Langmuir,* **18:**6679-6686 (2002)
34. Gupta A., Maynes M. and Silver S., Effects of halides on plasmid mediated silver resistance in *Escherichia coli, Appl. Enviro. Microbiol*., **64:**5042-5045 (1989)
35. Singh M., Singh S., Prasad S. and Gambhir I.S., Nanotechnology in medicine and antibacterial effect of silver nanoparticles, *Digest. J. Nanomaterials and Biostrstructures* **3:**115-122 (2008)
36. Boyce J.M., Reevaluation of the ability of the standardized disk diffusion test to detect methicillin resistant strains of *Staphylococcus aureus, J. Clin. Microbiol*. **19:**813-817 (1984)