JOURNAL OF PURE AND APPLIED MICROBIOLOGY, August 2014. Vol. **8**(4), p. 3171-3177

Synthesis, Characterization and Antimicrobial Attributes of Gold Nanoparticles Mediated by NADH-Dependent Reductase of *Streptomyces sp.* DBZ-39

Bi Bi Zainab Mazhari and Dayanand Agsar*

A-DBT Research Laboratory, Department of Microbiology,
Gulbarga University, Gulbarga - 585 106, Karnataka, India.

(Received: 20 February 2014; accepted: 16 April 2014)

Production of NADH-dependent nitrate reductase mediated extracellular gold nanoparticles from *Streptomyces sp.* DBZ-39, for their distinct characteristic properties and antimicrobial activities was the major objective of the present investigation. Defined size and distinct gold nanoparticles, obtained from biological sources in general and microbial sources in particular reveal spectacular biomedical applications. *Streptomyces sp.* DBZ-39 was employed as an efficient isolate for the synthesis of extracellular gold nanoparticles. The detection of amino acid residues, namely tyrosine and tryptophan in the culture broth of *Streptomyces sp.* DBZ-39 by UV-visible spectroscopy, confirms the role of NADH-dependent reductase in the synthesis of extracellular gold nanoparticles. Scanning and Transmission Electron Micrographs revealed the characteristic properties of extracellular gold nanoparticles in a controlled size of 24nm. X-ray diffraction analysis exhibits crystal phases of gold nanoparticles. An antiviral property of gold nanoparticles employing bacteriophages is a significant attribute and reported for the first time. The antibacterial activity of gold nanoparticles was encouraging against gram negative bacterial pathogens *E. coli* and *Pseudomonas aeruginosa,* which can be explored further for their efficacy.

Key words: Streptomyces, NADH-dependent reductase, Gold nanoparticles, Antiviral, Antibacterial.

Nanotechnology and Nanoscience are useful in developing entirely new approaches for increasing the bioavailability and advancing the biological tools and refining their applications. Nanoparticles offer unique approach to control wide variety of biological and medical process which has a successful impact on medicine and biology (West and Halas, 2000). The increased demand of gold in many application leads to the growing need for cost effective as well as to implement green chemistry in the development of gold nanoparticles (Tikariha *et al*., 2012). The use of toxic chemicals in nanoparticles synthesis limits their application (Musarrat *et al.*, 2011). Therefore, there is a need for development of clean biocompatible, non hazardous and eco friendly methods for the synthesis.

Novel and green approach method for the synthesis of gold nanoparticles was the use of microbial cell. The biological agents like bacteria, fungi, actinomycetes, yeast, algae and plants were used as a wide range of resources for the synthesis (Sastry *et al.*, 2003 and Ahmad *et al.*, 2003). Among microorganisms, especially actinomycetes are very potential for the synthesis of several nanoparticles with much improved controlled size, shape and composition. This biological system secretes large amount of enzymes which are capable of hydrolyzing metal ions. The reduction of aurium chloride ($AuCl_4$) metal takes place by the reaction

* To whom all correspondence should be addressed.
Tel.: +91-8472 263297
Fax: 08472-263205 & 206; Mobile: +91 9448213398
Email: dayanandagsar@gmil.com;
mbzainab@gmail.com

of specific NADH-dependent reductase enzyme present in the organisms. In actinomycetes, the reduction of metal ions occurs on the surface mycelia along with cytoplasmic membrane leading to the formation of nanoparticles (Das and Marsili, 2010).

Gold in similar way as that of silver does not negatively affect the human body when used in appropriate concentrations. Gold nanoparticles have been employed in biomedicine since recently because of their several attractive properties (Utkarsha *et al.,* 2014).Although, synthesis of gold nanoparticles was mediated by several bacteria (Pankaj *et al.,* 2013 and Abirami *et al.,* 2013), fungi (Kupryashina *et al.,* 2013 and Honary *et al.,* 2013), few actinomycetes (Fatemeh *et al.,* 2014 and Balagurunathan *et al.,* 2011) and yeasts (Nair *et al.,* 2013 and Ryan *et al*., 2011), synthesis of gold nanoparticles with defined dimensions and distinct monodispersity is a challenging one. The most infectious pathogens such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and several others were affected by reaction of metal nanoparticles (Prema and Thangapandiyan, 2013). The antibacterial property of the metal nanoparticles has been explored extensively but the antiviral properties of nanoparticles remain an undeveloped area. The disease caused by viruses present challenging problems and previous reports reveal antiviral activity of only certain chemical agents, such as iodine and chloride dioxide against viral strains, bacteriophages and polioviruses (Ravishankar and Jamuna. 2011). Recent research concluded that, silver nanoparticles have most killing effect on bacteriophages (Narasimha *et al.,* 2012). In the present investigation, we reported on extracellular synthesis of gold nanoparticles by *Streptomyces sp.* and their characterization. Further, antiviral and antibacterial attributes of gold nanoparticles were also assessed and discussed.

MATERIALS AND METHODS

Synthesis of gold nanoparticles

An isolate of *Streptomyces sp*. DBZ-39 obtained from limestone quarry soil earlier (Bi Bi Zainab *et al.,* 2014) in our A-DBT (Actinomycetes-Diversity and Bioprocess Technology) research laboratory was employed for the synthesis of extracellular gold nanoparticles, as per the standard protocol prescribed by Ahmad *et al.,* (2003) and Shahverdi *et al.,* (2007). A loopfull of three days old culture of *Streptomyces sp*. DBZ-39 was inoculated into starch casein broth containing Starch-1g, casein-0.003g, KH_2PO_4- 2.0g, KNO_3-2.0g, NaCl-2.0g, $MgSO_4$-0.002g, $FeSO_4$-0.001g, $CaCo_3$-0.001 and incubated at 40 ^oC for 5 days on rotatory shaker (200rpm). After incubation, the broth culture was centrifuged at 8000rpm.The biomass obtained was suspended in $AuCl_4$ solution and kept for incubation at 37 ^oC on shaker (200rpm) for three days. The gold nanoparticles synthesized in the solution were confirmed by the development of deep purple color as visual observation and UV-visible absorption spectrum in the range of 500-550nm.

NADH-dependent reductase assay

NADH-dependent reductase activity in the culture broth of *Streptomyces sp*. DBZ-39 was determined as per the procedure prescribed by Saifuddin *et al.,* (2009) and Nelson D *et al.,* 2005). A three days old culture broth of Streptomyces DBZ-39 was obtained in 100ml Starch Casein broth incubated at 40 ^oC. Culture broth was poured into two sets of tubes containing three each. One set of tubes were kept in boiling water to denature the enzyme present in the broth culture. NADH was added immediately after cooling the set of test tubes and also to another set of test tubes. Both sets of tubes were incubated in dark for 60 min at room temperature. After incubation, NEED (n-Napthyl Ethleyene dichloride) was added to stop the reaction in the broth of both sets. Absorbance was measured at 540nm using UV-vis spectrophotometer. The activity of enzyme was determined based on the increase in the color within 60 min of incubation. The presence of tryptophan and tyrosine residues in the culture broth was determined spectrophotometrically (Saifuddin *et al.,* 2009).

Characterization of gold nanoparticles

The gold nanoparticles were characterized to determine their size, dispersion and structure. The gold nano solution was subjected to Scanning electron microscopy, Transmission electron microscopy and X-ray diffraction analysis as per the standard protocols of Vigneshwaran *et al.,* (2007), Krishnaraj *et al.,* (2009) and Kwoshik *et al.,* (2003) respectively.

Antimicrobial activities

Antiviral activity of gold nano solution was determined against bacteriophages, based on the number of plaques formed as per the standard protocol prescribed by N. Beaudoin *et al*, (2007). *E. coli* isolated from sewage was inoculated on Tryptone Glucose Yeast Extract (g/L Tryptone; 10, Glucose; 10, Yeast extract; 1, NaCl; 8) agar by spread plate to obtain the formation of lawn after incubating overnight at 37 ^{0}C. Equal ratios of phage solution obtained from sewage sample and gold nano solution, in the range of 10 to 80µl, with an increment of 10 was inoculated on to the lawn of *E. coli* and incubated at 37 ^{o}C for 24hrs. After incubation, the plates were observed for the formation of number of plaques (Guttman *et al.,* 2005).

The antibacterial property of gold nanoparticles was examined by following agar well diffusion method (Grace and Pandian, 2007 and Madigan *et al.,* 2005) - against bacterial pathogens namely *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* obtained from clinical samples of local hospital. A uniform lawn of bacterial pathogens was prepared on nutrient agar. About 6 mm diameter, wells at equidistance were made on the lawn of agar plates. The solutions of gold nanoparticles was prepared at different concentrations of 20, 40, 60, 80 and 100 µl and were tested for their antibacterial activity in terms of zone of inhibition formed. 50 µl of each test gold nano solution was added into every well and incubated at 37 ^{o}C for 24 to 48 hrs to observe zone of inhibition.

RESULTS AND DISCUSSION

Actinomycetes are known to produce highly diverse bioactive compounds or molecules (Berdy 2005). Streptomyces is a predominant genus among actinomycetes for the synthesis of variety bioactive compounds. In the present investigation, an attempt was made to synthesize extracellular gold nanoparticles by *Streptomyces sp.* DBZ-39. Figure 1A reveals the presence of gold nanoparticles in aurium chloride solution. The development of deep purple color exhibits (Ahmad *et al.,* 2003) the presence of extracellular gold nanoparticles, when compared to the test solution,

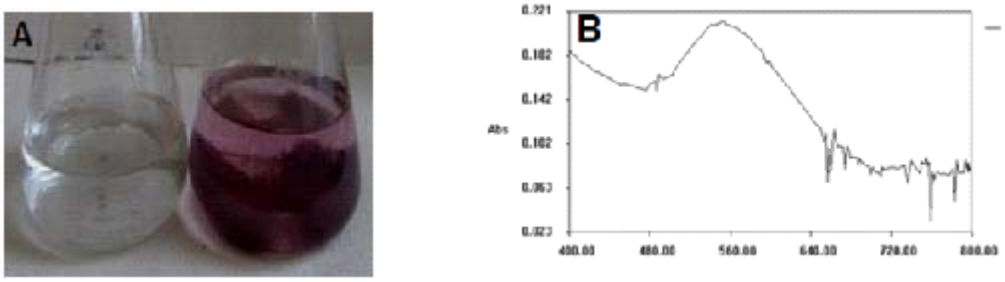

Fig. 1. Visual observation (A) and UV- visible absorption spectrum (B) of gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39

Table 1. Antibacterial activities of gold nanoparticles against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*

Concentration of gold nano-particles (µl)	Antibiotic property of gold nanoparticles (Zone of inhibition in mm)			
	E. coli	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Staphylococcus aureus*
20	-	-	-	-
40	+	+	+	+
60	++	+	+	++
80	+++	+++	+	++
100	++	+	+	++

-: no zone of inhibition +: 1 to 10mm; ++: 11 to 20mm; +++: 21 to 30mm

which is colorless. A higher absorption peak (Figure 1B) in UV-visible range of 500-550nm confirms the synthesis of extracellular gold nanoparticles by *Streptomyces sp.* DBZ-39.

NADH-dependent reductase mediates the release of gold nanoparticles in aurium chloride solution (Saifuddin *et al.*, 2009). Accumulation and reduction of gold by NADH-dependent reductase was monitored with visual observation for the development of deep purple color. NADH-dependent reductase is an enzyme constituting specific amino acid residues, especially tyrosine and tryptophan in the catalytic domain, which mediates the release of gold nanoparticles in aurium chloride solution (Fatemeh *et al.*, 2010). UV-visible absorption spectra (Figure 2) at 220 and 270nm reveal the presence of tyrosine and tryptophan residues respectively in the culture broth of *Streptomyces sp.* DBZ-39. This reveals that, *Streptomyces sp.* DBZ-39 has produced NADH-dependent reductase enzyme, which is mainly responsible to mediate the synthesis of extracellular gold nanoparticles in aurium chloride solution.

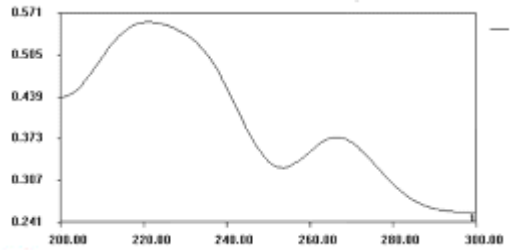

Fig. 2. UV-visible absorption spectra of tyrosine and tryptophan residues in the culture broth of *Streptomyces sp.* DBZ-39

Gold nanoparticles synthesized by biological sources have been investigated extensively in recent years because of their potential applications. Gold nanoparticles exhibit intriguing properties than metallic gold which imply their tremendous biomedical applications (Utkarsha *et al.*, 2014). Highly regulated composition including size, shape and dispersion of gold nanoparticles would exhibit varied biological applications. Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD)

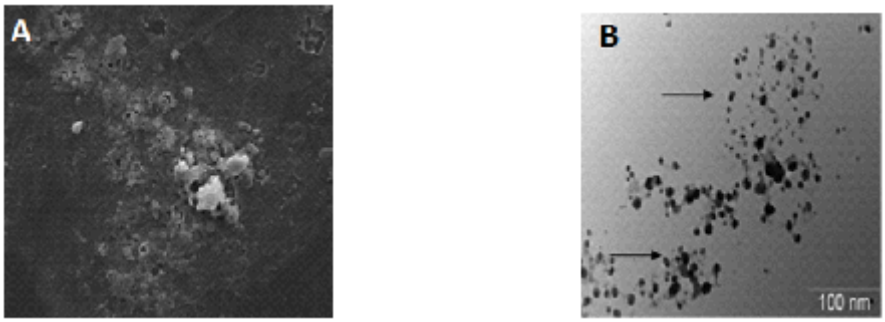

Fig. 3. Scanning (A) and Transmission (B) electron micrographs of gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39

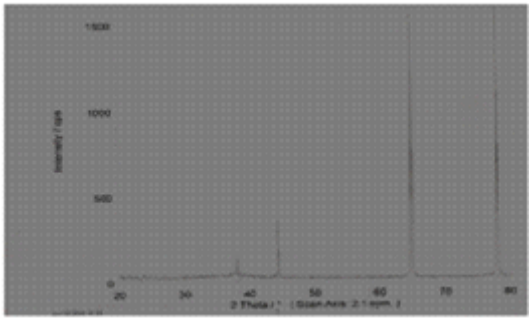

Fig. 4. X-ray diffraction analysis of gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39

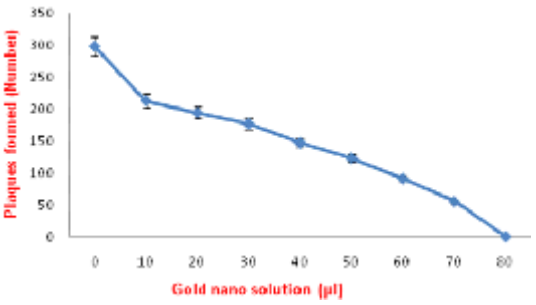

Fig. 5. Antiviral activity of gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39

are the important analytical techniques generally employed to understand size, shape and dispersion as characteristic properties of gold nanoparticles. In the present investigation, gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39 were characterized by SEM, TEM and XRD analysis. Scanning (Figure 3A) and Transmission (Figure 3B) electron micrographs of gold nanoparticles reveals an average size of about 24nm which are dispersed uniformly. X-ray diffraction pattern of the gold nanoparticles with two major characteristic peaks (Fig. 4) at the range of 2 theta correspondingly illustrates the crystal phases of gold nanoparticles.

Antimicrobial properties of silver nanoparticles, although are well recorded, the antimicrobial properties of gold nanoparticles are poorly understood. It appears that, no earlier reports are available pertaining to the antiviral activity of gold nanoparticles against bacteriophages. However, role of gold nanoparticles in the development of bacteriophages based biosensors for the detection of food borne pathogens has been reported (Amit *et al.*, 2013). In the present study, an attempt was made to understand both antiviral and antibacterial attributes of gold nanoparticles synthesized by *Streptomyces sp.* DBZ-39. Antiviral properties of gold nanoparticles at different concentration, from 10 to 80µl were examined (Fig. 5) against bacteriophages, in terms of the number of plaques formed on the medium. Antiviral property of gold nanoparticles was increased along the increase in the concentrations of gold nanoparticles, which was indicated by decrease in the number of plaques formed on the medium. The total inhibition of viral multiplication was recorded at 80 µl concentration of gold nanoparticles, revealing hundred percent antiviral activity. Similarly, antibacterial properties of gold nanoparticles were also examined. Antibacterial activity of silver nanoparticles was well recorded by several researchers. However, antibacterial activity of gold nanoparticles is relatively less studied. In the present study, effect of gold nanoparticles on three gram negative and one gram positive bacterial pathogen was reported. Different concentrations of gold nano solutions were employed to understand the efficacy of gold nanoparticles against bacterial pathogens. Table 1 reveals the effect of gold nanoparticles on bacterial pathogens at different concentrations. Gold nano solution with a concentration of 80 µl showed maximum activity against almost all pathogens. However, greater effect of gold nanoparticles was on *E. coli* and *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*. The least effect was observed on *Salmonella typhi*. Several other reports were also revealed the similar observations.

CONCLUSIONS

Streptomyces sp. DBZ-39 was found to be an efficient isolate for the extracellular synthesis of gold nanoparticles. Controlled size nanoparticles of about 24nm were synthesized by *Streptomyces sp.* DBZ-39, which were confirmed and characterized by visual observation, UV spectroscopy, SEM, TEM and XRD analysis. The mediation of NADH- dependent reductase for the synthesis of extracellular gold nanoparticles was well recorded with better yield and stable nature. Exploration of the antiviral activity of gold nanoparticles employing bacteriophages for the first time proved to be significant and quite potential. Antibacterial activity of gold nanoparticles reveals to be encouraging against *E. coli* and *Pseudomonas aeruginosa.* Synthesis of gold nanoparticles with defined composition and greater stability for biomedical and pharmaceutical applications is the future prospectus.

ACKNOWLEDGEMENTS

First author is grateful to Department of Science and Technology, New Delhi, India for having awarded with INSPIRE (Innovation in Science Pursuit for Inspired Research) Fellowship (IF-110367).

REFERENCES

1. West, J.L., Halas, N.J. Applications of nanotechnology to biotechnology. *Curr. Opin. Biotech.*, 2000; **11**:215.
2. Tikariha, S., Singh, S., Banerjee, S., Vidyarthi, A.S. Biosynthesis of gold nanoparticles, scope and application: A Review. *International Journal of Pharmaceuticals Sciences and Research*., 2012 **3**:6.
3. Musarrat, J., Sourabh, D., Raj, B. Singh., Quaiser,

S., Abdul, A. Aziz., Al-Khedhairy. Microbially synthesized nanoparticles: Scope and applications. *Springer-Verlag Berlin Heidelberg: Germany*., 2011.

4. Sastry, M., Ahmad, A., Khan, M.I., Rajiv, K. Biosynthesis of metal nanoparticles using fungi and actinomycetes. *Current Science*., 2003; **85**: 162-170.
5. Ahmad, A., Senapati, S., Khan, M.I., Kumar, R., Ramani, R., Srinivas, V., Sastry, M. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycetes Rhodococcus sp. *Nanotechnology*., 2003; **14**; 824-828.
6. Das, S.K., Marsili, E. A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect. *Rev Enviromn Sci Biotechnol.*, 2010; **9**: 199–204.
7. Utkarsha, Shedbalkar ., Richa, Singh., Sweety, Wadhwani. , Sharvari, Gaidhani., Chopade, B. A. Microbial synthesis of gold nanoparticles: Current status and future prospects. *Advances in Colloid and Interface Science*., 2014; CIS-01374.
8. Pankaj Kumar Singh., Subir Kundu. Biosynthesis of Gold Nanoparticles Using Bacteria. *Proceedings of the National Academy of Sciences. India Section B: Biological Sciences.*, 2013.
9. Abirami, G., Asmathunisha, N., Kathiresan, K. Biosynthesis Of Gold Nanoparticles By Marine Purple Non Sulphur Bacterium, *Rhodopseudomonas Sp. Indian Streams Research Journal* ., 2013; **3**.
10. Kupryashina , M. A., Vetchinkina, E. P., Burov, A. M., Ponomareva , E. G., Nikitina, V. E. Biosynthesis of Gold Nanoparticles by *Azospirillum brasilense. Microbiology*., 2013; **82**(6), 833–840.
11. Honary, S., Gharaei-Fathabad , E., Barabadi, H., Naghibi, F. Fungus-mediated synthesis of gold nanoparticles: a novel biological approach to nanoparticle synthesis. *J Nanosci Nanotechnol.*, 2013; **13**(2):1427-30.
12. Fatemeh Khadivi Derakhshan., Alireza Dehnad., Mojtaba Salouti. Extracellular Biosynthesis of Gold Nanoparticles by Metal Resistance Bacteria: *Streptomyces griseus. Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*., 2014.
13. Balagurunathan. Radhakrishnan, M., Babu , Rajendran, R., Velmurugan, D. Biosynthesis of gold nanoparticles actinomycetes *Streptomyces viridogens* strain HM10. *Indian Journal of Biochemistry and Biophysics*., 2011; **48**: 331-335.
14. Nair Vinay., Sambre Devashree., Joshi, Swanand., Bankar , Ashok., Ravi, Kumar, Ameeta., Zinjarde, Smita. Yeast-Derived Melanin Mediated Synthesis of Gold Nanoparticles. *Journal of Bionanoscience.*, 2013; **10** : 159-168.
15. Ryan William Stegenga., Shiem Al-Azawi., Debalina, Bandyopadhyay., Krisanu, Bandyopadhyay. Biosynthesis of Gold Nanoparticles by *Saccharomyces cerevisiae*. *The FASEB Journal.*, 2011; **8**: 25:726.
16. Prema, P., Thangapandiyan, S. In-vitro antibacterial activity of gold nanoparticles capped with Polysaccharide stabilizing agents. *International Journal of Pharmacy and Pharmaceutical Sciences*., 2013; **5**(1) : 0975-1491.
17. Ravishankar, V. Rai., Jamuna Bai. A Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens. *Communicating current research and technological advances.*, 2011.
18. Narasimha, G., Habeeb, K., Mohammed, A. Antiviral properties of silver nanoparticles synthesized by *Aspergillus sps. Scholars Research Library Der Pharmacia Letter.*, 2012; **4**(2): 649-651.
19. Bi Bi Zainab Mazhari., Madhusudhan, D.N., Raghavendra, H., Dayanand Agsar., Syed Dastager. Development of bioconjugate from Streptomyces tyrosinase and gold nanoparticles for rapid detection of phenol constituents. *Accepted for publication in International Journal of Experimental Biology, CSIR, India* ., 2014.
20. Shahverdi, A. R., Minaeian, S., Shahverdi, H.R., Jamalifar, H., Nohi, A. A. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach. *Process Biochemistry*., 2007;. **42**: 919-923.
21. Saifuddin, N., Wong, C.W., Nur, A.A., Yasumira. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *E-Journal of Chemistry*., 2009; **6**: 61-70.
22. Nelson, D., Priscyla, M.D.D., Oswaldo, A.L., Gabriel, D.H.I., Elisa, E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *Journal of Nanobiotechnology*., 2005; **3**(8): 1477-3155.
23. Vigneshwaran, N., Ashtapure, N.M., Varadarajan, P.V., Nachane, R.P., Paralikar, K.M., Balasubramanyam , R.H. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus. Materials Letters*., 2007; **614**: 13-1418.

24. Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P.T., Mohan, N. Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and surfaces: Biointerfaces*., 2009; **76**: 50-56 .
25. Kowshik, M., Ashtaputre, S., Kharrazi, S., Vogel, W., Urban, J., Kulkarni, S.K., Paknikar, K.M. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. *Nanotechnology* ., 2003; : 95-100.
26. Rene N. Beaudoin., Danielle R. De Cesaro., Debrah L. Durkee., Susan E. Barbaro. Isolation of a bacteriophage from sewage sludge and characterization of its bacterial host cell. *Rivier Academic Journal*., 2007; **3** :1.
27. Guttman, B., Raya, R., Kutter, E. Basic Phage Biology. In: Bacteriophages: *Biology and Applications.*, 2005; 29-66.
28. Grace, N.A., Pandian, K. Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles - A brief study. *Colloids Surf. A*., 2007; **297**: 63–70.
29. Madigan, M.T., Martinko, J.M., Parker, J. Antibiotics: Isolation and characterization in Brook Biology of Microorganisms, 8th edition. Prentice Hall International Inc. New Jersey, 2005; 440-442.
30. Berdy, J. Bioactive microbial metabolites. A personal view. *J. Antibiotic.*, 2005; **58**: 1-26.
31. Fatemeh Ramezani., Moazzameh Ramezani., Saeid Tlebi. Mechanistic aspects of biosynthesis of nanoparticles by several Microbes. *Nanocon.*, 2010; **10**: 12-14.
32. Amit Singh., Somayyeh Poshtiban., Stephane Evoy. Recent Advances in Bacteriophage based biosensors for food-borne pathogen detection. *Sensors.*, 2013 ; **13**: 1763-1786.