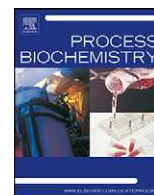
Process Biochemistry 44 (2009) 939–943

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



Short communication

Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain

Nikhil S. Shaligram [a], Mahesh Bule [a], Rahul Bhambure [a], Rekha S. Singhal [a,*], Sudheer Kumar Singh [b,1], George Szakacs [c], Ashok Pandey [b]

[a] Food Engineering and Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India
[b] Biotechnology Division, National Institute for Interdisciplinary Science and Technology, Trivandrum 695 019, India
[c] Department of Agricultural Chemical Technology, Technical University of Budapest, 111 Budapest, Geller ter 4, Hungary

ARTICLE INFO

Article history:
Received 3 December 2008
Received in revised form 10 April 2009
Accepted 15 April 2009

Keywords:
Silver nanoparticles
Penicillium brevicompactum WA 2315
Extracellular synthesis
Reduction
Nanomaterial
Electron microscopy

ABSTRACT

In the present study, an eco-friendly process for the synthesis of nanomaterials using a fungus, *Penicillium brevicompactum* WA 2315 has been attempted. The fungus has been previously utilized for compactin production. Supernatant of seed culture was used for the biosynthesis of silver nanoparticles. The aqueous silver ions were reduced to silver metal nanoparticles when treated with the fungal supernatant. After 72 h of treatment, silver nanoparticles obtained were in the range of 23–105 nm as obtained from TEM. The nanoparticles were characterized by UV, FTIR, SEM, TEM and XRD. The use of supernatant of the seed media of the said fungus opens up the exciting possibility of rational strategy of biosynthesis of nanomaterials.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Nanotechnology deals with the synthesis and stabilization of various nanoparticles. Currently, there is a constant need to develop eco-friendly processes for the synthesis of nanoparticles. The focus for this synthesis has shifted from physical and chemical processes towards 'green' chemistry and bioprocesses [1]. Some examples of nanoparticle formation by organisms are magnetotactic bacteria synthesizing magnetite nanoparticles [2] and S-layer bacteria producing gypsum and calcium carbonate layers [3]. Proteins and other biomacromolecules control the nucleation and growth of these inorganic structures [4,5]. Silver nanoparticles are an effective antimicrobial agent against various pathogenic microorganisms, and various chemical and biochemical methods are being explored for its production [6]. Various microbes are known to reduce metal ions to metals [7–9]. The resistance conferred by bacteria to silver is determined by the 'sil' gene in plasmids [10]. The reduction of silver ions by several *Fusarium oxysporum* strains has been attributed to a nitrate-dependent reductase and a shuttle quinone extracellular process [11]. Active metal transformation processes require viable microbes which enzymatically catalyze the alteration of the metal. The microorganisms probably play a role in providing a multitude of nucleation centers and establish conditions for obtaining highly disperse nanoparticle systems. They slow down or entirely prevent aggregation by immobilizing the particles, and providing a viscous medium [12]. In the remediation of toxic metals, microorganisms such as bacteria and fungi are employed. Such microorganisms have recently been recognized as possible eco-friendly nanofactories [13]. Many investigators have reported biosynthesis of silver [14–19] and gold [20] nanoparticles from microbial sources.

This study demonstrates the extracellular synthesis of stable silver nanoparticles using the fungus, *Penicillium brevicompactum* WA 2315. We have used this fungus previously to study fermentative production of compactin in submerged and solid state fermentation [21–23], and the supernatant of the seed media obtained after separating the cells has been used for the synthesis of silver nanoparticles.

The novelty of the work lies in the fact that we have used the biomass for production of an industrially useful metabolite, compactin. The same seed culture was utilized for nanoparticles biosynthesis. This approach gave silver nanoparticles as a co-product that is as useful as the metabolite for which the culture was utilized. It is industrially attractive, feasible and commercially possible. To the best of our knowledge, this microorganism (*P. brevicompactum* WA 2315) has never been used for nanoparticles biosynthesis.

* Corresponding author. Tel.: +91 022 2414 5616; fax: +91 022 2414 5414.
E-mail addresses: rekha@udct.org, rsinghal7@rediffmail.com (R.S. Singhal).
[1] Present address: Microbiology Division, Central Drug Research Institute (CDRI), Chattar Manzil Palace, Post Box No. 173, Lucknow 226 001, India.

1359-5113/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2009.04.009

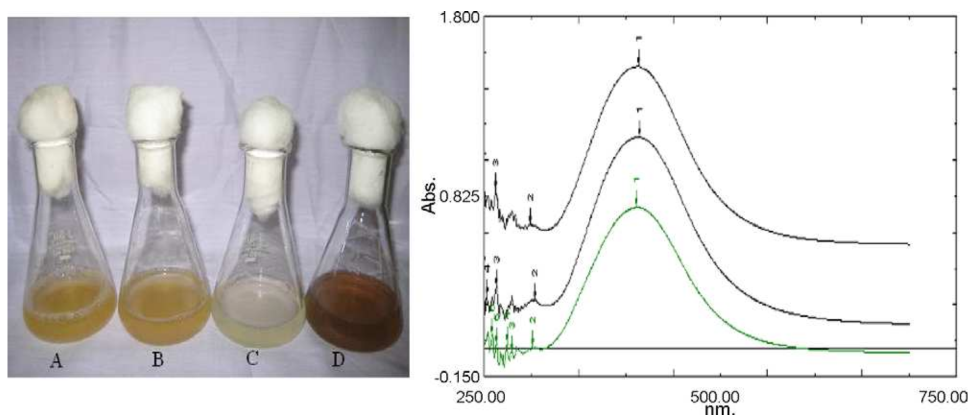


Fig. 1. (1) A: Seed medium, B: seed medium + silver nitrate, C: aqueous extract of fungal biomass, and D: aqueous extract with silver nitrate (formation of nanoparticles, dark brown colour) and (2) UV spectrum of reaction mixture of 1 mM silver nitrate and aqueous filtrate of *P. brevicompactum* WA 2315. The curves are recorded after a period of 24 h. Spectra recorded as a function of time for a 1 mM aqueous solution of silver nitrate that was reacted with culture supernatants of *P. brevicompactum* WA 2315. After the incubation period, the culture was centrifuged at 6000 rpm and its supernatants used to reduce the silver nitrate solution.

2. Materials and methods

2.1. Preparation

The fungus culture, *P. brevicompactum* WA 2315, was obtained from the culture collection of Technical University of Budapest, Hungary. All chemicals used were of analytical grade. *P. brevicompactum* WA 2315 was grown in seed medium containing (g/l) glucose 20, glycerol 30, peptone 8, $NaNO_3$ 2, $MgSO_4$ 1 and soyabean meal 20, the pH was adjusted to 6.5 ± 0.2 with 2N H_3PO_4. The flasks were incubated in the incubator shaker at 180 rpm at 25 °C. After 3 days of incubation, the mycelium was separated by centrifugation (6000 rpm) and washed thrice with deionized water. The washed mycelia were suspended in deionized water and kept on rotary shaker for 96 h at 180 rpm. The aqueous extract obtained after separation of mycelial suspension was challenged with 100 mL of 1 mM silver nitrate solution (prepared in deionized water) and incubated in shaker at 180 rpm in dark conditions at 25 °C. Simultaneously, a positive control of incubating the fungal supernatant with deionized water was maintained under same conditions.

2.2. Characterization of nanoparticles

The biosynthesis of silver nanoparticle by *P. brevicompactum* WA 2315 was supervised visually. The absorption spectra of the reaction mixture of silver nitrate and aqueous extract were analyzed by withdrawing 5 mL aliquots on UV visible 1650 PC Shimadzu double beam spectrophotometer (France), from 250 nm to 750 nm, at an interval of 24 h for 72 h. The reaction mixture containing silver nanoparticles was freeze-dried and used for further characterization. A laboratory scale vacuum freeze dryer (Ref-Vac Consultancy, India) was used for this study. It consists of cylindrical stainless steel drying chamber, a condenser capable of operating at −35 °C and a vacuum pump (Crompton Greaves, India). Sample was frozen in an external deep freezer (Blue Star, India) at −23 °C for 6 h. Meanwhile, freeze dryer was started and the temperature of the condenser was allowed to reach at its minimum level of −35 °C. Thereafter, the frozen sample was placed in the drying chamber and subsequently started the vacuum pump and the heater. The temperature of the sample and pressure inside the chamber were set at 25 °C and 30 Pa, respectively. The scanning electron microscopy (SEM) analysis of freeze-dried sample was performed by mounting nanoparticles on specimen stubs with double-sided adhesive tape and coated with platinum in a sputter coater and examined under JEOL 6386® SEM (Japan) at 10 kV. For transmission electron microscopy (TEM), a drop of aqueous solution containing the silver nanomaterials was placed on the carbon coated copper grids and dried under infrared lamp. Micrographs were obtained using a Philips® CM 200 operating at 200 kV (USA).

To determine Fourier transform infra-red (FTIR) pattern of the sample fungal filtrate containing the silver nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FTIR Spectrum BX (Wellesley, MA, USA).

The freeze-dried reaction mixture embedded with the silver nanoparticles was used for X-ray diffraction (XRD) analysis. XRD patterns were recorded on X'Pert Pro, PANalytical, USA operating at 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.54 Å). The diffracted intensities were recorded from 5° to 120° 2θ angles.

3. Results and discussion

In this work, the fungus *P. brevicompactum* WA 2315 was used for the synthesis of stable silver nanoparticles. To verify the synthesis of silver nanoparticles, the flasks containing aqueous filtrate (without silver nitrate solution) as positive control, and pure silver nitrate solution (without the aqueous filtrate) as negative control were monitored visually. The fungal aqueous filtrate incubated with deionized water (positive control) and the silver nitrate solution (negative control) was observed to retain its original colour, and the silver nitrate treated supernatant turned dark brown after 72 h due to the deposition of silver nanoparticles (Fig. 1(1)). The colour observed was mainly due to the surface plasmon resonance of deposited silver nanoparticles [24]. In case of negative control, no change in colour was observed even after 72 h.

Fig. 1(2) shows the UV–visible spectra of fungal supernatant treated with the silver nitrate solutions. A characteristic surface plasmon absorption band at 420 nm was observed after 24 h and the maximum intensity was attained after 72 h. After 72 h of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions. Apart from this, an absorption peak was also observed in the UV region corresponding to 280 nm. The peak at 280 nm could be attributed due to the tryptophan and tyrosine residues present in the protein [25].

The scanning electron micrograph of the silver nanoparticles synthesized after treatment of 1 mM silver nitrate solution with aqueous filtrate for 72 h is shown in Fig. 2, which clearly shows the surface deposited silver nanoparticles. Silver nanoparticles have been characterized using SEM by various investigators [26,27].

Transmission electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. A representative TEM image recorded from the silver nanoparticles solution with the particle size distribution profile of 31 nanoparticles is shown in Fig. 3. The biosynthesized silver nanoparticles were previously characterized using TEM by several investigators [14,28]. The analysis of data obtained from transmission electron micrograph showed the average size of nanoparticles to be 58.35 ± 17.88 nm.

Fig. 4 shows the FTIR spectrum of the freeze-dried powder of silver nanoparticles formed after 72 h of incubation with the fungus. The amide linkages between amino acid residues in proteins give rise to the well-known signatures in the infrared region of the electromagnetic spectrum. The bands seen at 3356 cm^{-1} and 2922 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, respectively; while their corresponding bending vibrations were seen at 1622 cm^{-1} and 1527 cm^{-1}, respectively. The two bands observed at 1412 cm^{-1} and 1029 cm^{-1} can be assigned to the C–N stretching vibrations of aromatic and aliphatic amines, respectively. The overall observation confirms the presence of protein in the samples

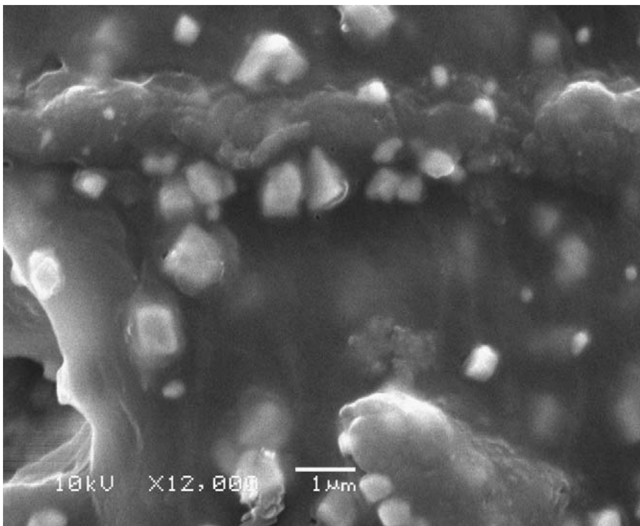


Fig. 2. Scanning electron micrograph of the freeze-dried silver nanoparticles synthesized after 72 h incubation of aqueous filtrate of *P. brevicompactum* WA 2315 with 1 mM silver nitrate.

of silver nanoparticles. Earlier FTIR studies were carried out to analyze presence of proteins in the biosynthesized nanoparticles [14,28]. It is reported that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins [29,30], and thereby possibly stabilize the silver nanoparticles.

The probable mechanism for the nanoparticles biosynthesis and role of proteins during the same has been reported by Kalimuthu et al. [26] and Ahmad et al. [8]. These reports suggested the probable role of NADH-dependent nitrate reductase in the reduction of silver ion to metallic silver. The reduction may occur by means of the electrons from NADH where the NADH-dependent reductase can act as a carrier.

Balaji et al. [14] reported FTIR spectroscopic studies on silver nanoparticles obtained from the fungus, *Cladosporium cladosporioides*. Their study confirmed that the carbonyl groups from the amino acid residues and peptides of proteins have strong ability to bind silver. The proteins could possibly form a coat covering the metal nanoparticles to prevent their agglomeration and aid in its stabilization in the medium. Hence, the biological molecules could possibly function in the formation and stabilization of the silver nanoparticles in aqueous medium. Gole et al. [29] reported that proteins can bind to silver

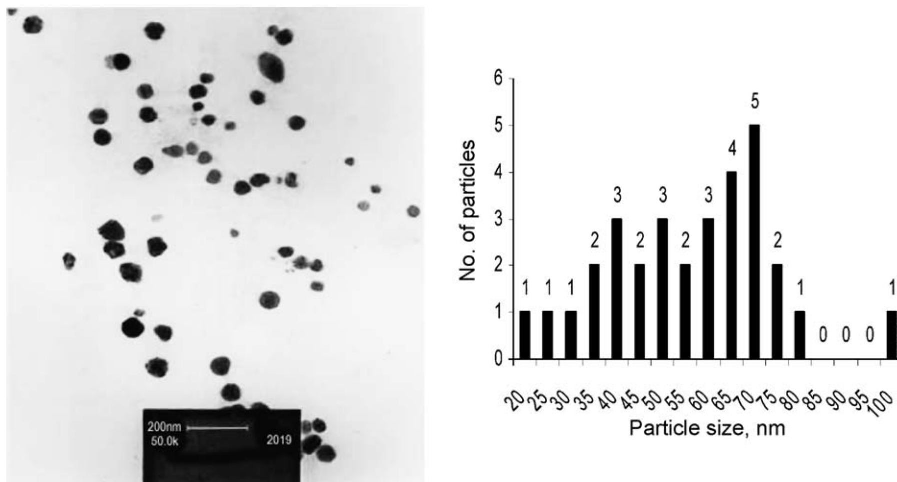


Fig. 3. Transmission electron micrograph (left picture) recorded from a region of a drop-coated film of silver nitrate solution treated with the culture supernatant of *P. brevicompactum* WA 2315 for 72 h (scale bars correspond to 200 nm). The particle size distribution histogram obtained is shown on the right.

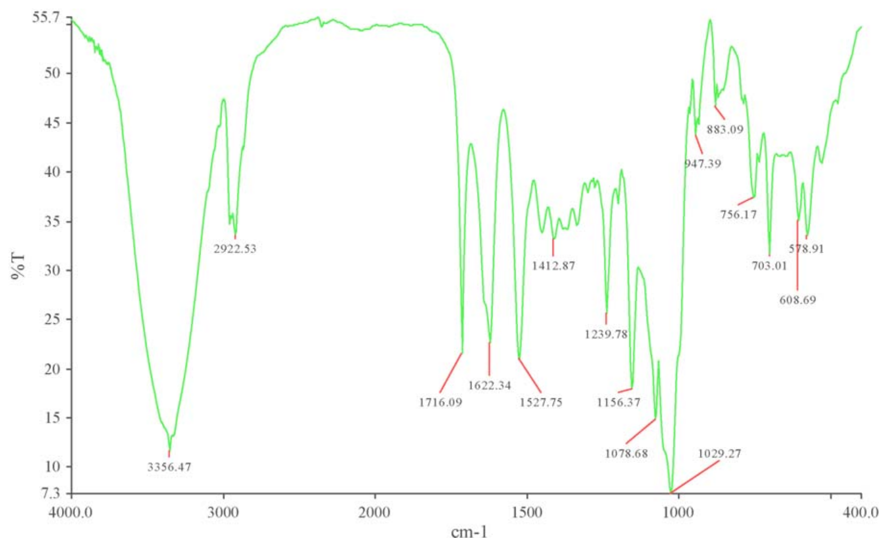


Fig. 4. FTIR spectra of freeze-dried powder of silver nanoparticles formed after 72 h of incubation of the culture supernatant of *P. brevicompactum* WA 2315 treated with silver nitrate (1 mM) solution.

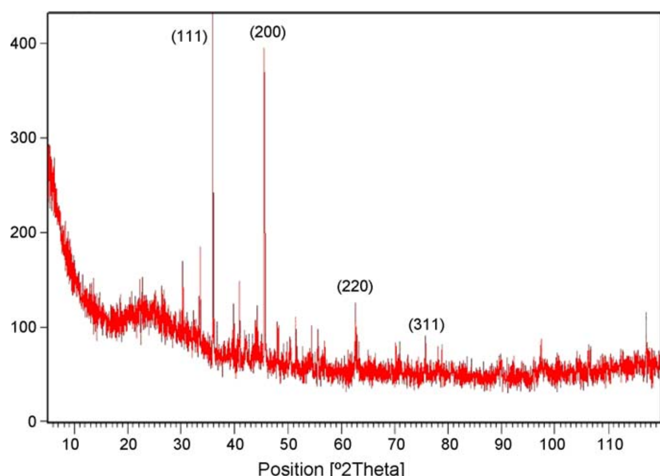


Fig. 5. XRD spectra of freeze-dried powder of silver nanoparticles formed after 72 h of incubation of the culture supernatant of *P. brevicompactum* WA 2315 treated with silver nitrate (1 mM) solution.

nanoparticles either through free amine groups in the proteins and possibly play a role in stabilization of the silver nanoparticles by surface-bound proteins.

X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles (Fig. 5). The presence of peaks at 2θ values of 35.91°, 45.54°, 62.68° and 75.75° correspond to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of silver, respectively. Thus the XRD spectrum confirmed the presence of silver nanoparticles. The values agree well with those reported for silver (face centric cubic) by Joint Committee on Powder Diffraction Standards File No. 04-0783. Silver nanoparticles have been characterized using XRD by various investigators [14,28]. The size of silver nanoparticles can also be determined by laser diffraction [31,32].

The extracellular synthesis of nanoparticles by the fungal system is advantageous in homogenous catalysis and non-linear optics. This is impossible when nanoparticles are bound to the cell biomass. This process of production of nanoparticles is free from any toxic chemicals or solvents. It is eco-friendly and also lends itself readily for large scale production. Monodispersed nanoparticles can be biosynthesized by varying reaction parameters such as pH and temperature [33]. A combinatorial synthesis approach by using a combination of fungal culture supernatant and microwave irradiation in water can be used as one of the approach for monodisperse nanoparticles biosynthesis [34].

4. Conclusions

In the present study nanoparticles of 58.35 ± 17.88 nm were synthesized extracellularly by using *P. brevicompactum* WA 2315, as confirmed by SEM and TEM. These nanoparticles showed characteristic absorption peak at 420 nm in UV spectra. The possibility of protein as a stabilizing material in silver nanoparticles is revealed by FTIR analysis. The crystalline structure of silver nanoparticles was confirmed by XRD.

Acknowledgements

We are thankful to TEQIP authorities for funding this project, to Institute of Chemical Technology, University of Mumbai. We acknowledge the support extended by Sophisticated Analytical Instrument Facility, IIT, Mumbai for analyzing the samples by TEM and XRD.

References

[1] Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Materials Letters 2007;61:1413–8.
[2] Lowenstam HA. Minerals formed by organisms. Science 1981;211:1126–30.
[3] Pum D, Sleytr UB. The application of bacterial S-layers in molecular nanotechnology. Trends Biotechnology 1999;17:8–12.
[4] Cha JN, Shimizu K, Zhou Y, Christiansen SC, Chmelka BF, Stucky GD, et al. Silicate in filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. Proceedings of the National Academy of Sciences USA 1999;96(2):361–5.
[5] Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. Proceedings of the National Academy of Sciences USA 1999;96(24):13611–4.
[6] Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO. Biomimetic synthesis and patterning of silver nanoparticles. Nature Materials 2002;1:169–72.
[7] Chen JC, Lin ZH, Ma XX. Evidence of the production of silver nanoparticles via pretreatment of *Phoma* sp. 3.2883 with silver nitrate. Letters in Applied Microbiology 2003;37(2):105–8.
[8] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B Biointerfaces 2003;28:313–8.
[9] Sastry M, Ahmad A, Islam Khan M, Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Current Science 2003;85(2):162–70.
[10] Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiology Reviews 2003;27(2–3):341–53.
[11] Duran N, Marcato PD, Alves OL, DeSouza GIH, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. Journal of Nanobiotechnology 2005;3:8.
[12] Sun Y, Xia Y. Shape-controlled synthesis of gold and silver nanoparticles. Science 2002;298:2176–9.
[13] Southam G, Beveridge TJ. The in vitro formation of placer gold by bacteria. Geochimica et Cosmochimica Acta 1994;58:4527–30.
[14] Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids and Surfaces B Biointerfaces 2009;68(1):88–92.
[15] Ingle A, Gade A, Pierrat S, Sőnnichsen C, Rai M. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. Current Nanoscience 2008;4(2):141–4.
[16] Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, et al. Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnology Letters 2007;29(3):439–45.
[17] Lengke MF, Fleet ME, Southam G. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. Langmuir 2007;23(5):2694–9.
[18] Mandal D, Bolander ME, Mukhopadhyay D, Sarkar G, Mukherjee P. The use of microorganisms for the formation of metal nanoparticles and their application. Applied Microbiology and Biotechnology 2006;69(5):485–92.
[19] Parikh RY, Singh S, Prasad BLV, Patole MS, Sastry M, Schouche YS. Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from *Morganella* sp.: towards understanding biochemical synthesis mechanism. ChemBioChem 2009;9(9):1415–22.
[20] Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K. A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. Colloids and Surfaces B Biointerfaces 2007;57(1):97–101.
[21] Shaligram NS, Singh SK, Singhal RS, Szakacs G, Pandey A. Compactin production in solid-state fermentation using orthogonal array method by *P. brevicompactum*. Biochemical Engineering Journal 2008;41:295–300.
[22] Shaligram NS, Singh SK, Singhal RS, Szakacs G, Pandey A. Effect of pre-cultural and nutritional parameters on compactin production by solid-state fermentation. Journal of Microbiology and Biotechnology 2009. doi: 10.4014/jmb.0805.324.
[23] Shaligram NS, Singh SK, Singhal RS, Szakacs G, Pandey A. Compactin production studies using *Penicillium brevicompactum* under solid-state fermentation conditions. Applied Biochemistry and Biotechnology 2008. doi: 10.1007/s12010-008-8461-3.
[24] Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 1996;12:788–800.
[25] Bhainsa KC, D'Souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids and Surfaces B Biointerfaces 2006;47:160–4.
[26] Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids and Surfaces B Biointerfaces 2008;65:150–3.
[27] Mouxing FU, Qingbiao LI, Daohua S, Yinghua LU, Ning H, Xu D, et al. Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. Chinese Journal of Chemical Engineering 2006;14(1):114–7.
[28] Kasthuri J, Veerapandian S, Rajendiran N. Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids and Surfaces B Biointerfaces 2009;68:55–60.
[29] Gole A, Dash C, Ramachandran V, Sainkar SR, Mandale AB, Rao M, et al. Pepsin–gold colloid conjugates: preparation, characterization, and enzymatic activity. Langmuir 2001;17:1674–9.
[30] Mandal S, Phadtare S, Sastry M. Interfacing biology with nanoparticles. Current Applied Physics 2005;5:118–27.

[31] Sadowski Z, Maliszewska I, Polowczyk I, Kozlecki T, Grochowalska B. Biosynthesis of colloid-silver particles using microorganisms. Polish Journal of Chemistry 2008;82(1/2):377–82.
[32] Sadowski Z, Maliszewska I. Synthesis of silver nanoparticles using microorganisms. Material Science Poland 2008;26(2):419–24.
[33] Sanghi R, Verma P. Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresource Technology 2009;100:501–4.
[34] Saifuddin N, Wong CW, Nur Yasumira AA. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. E-Journal of Chemistry 2009;6:61–70.