(IJIRSE) International Journal of Innovative Research in Science & Engineering
ISSN (Online) 2347-3207

Aspergillus Foetidus Mediated Biosynthesis of CdS Nano Particles and Its Characterization

Tina Nasrin, Swarup Roy, Tapan Kumar Das*
Department of Biochemistry and Biophysics, University of Kalyani,
Kalyani 741235(WB), India
* tapndas@hotmail.com

Abstract—**The $CdSO_4$ salt containing *Aspergillus foetidus* cell free filtrate shows a peak at 407 nm upon UV-Vis scan and fluorescence maxima at 515 nm due to the formation of CdS nanoparticles. The synthesized nanoparticles were further characterized precisely by zeta potential measurements, dynamic light scattering, FT-IR, atomic force microscopy, high resolution transmission electron microscopy, energy dispersive X-ray and X-ray diffraction studies, which are able to characterized the stable CdS nano structure of ~20-30 nm.**

***Keywords: Aspergillus sp*;CdS nano particles; Cadmium tolerance; Biosynthesis**

I. INTRODUCTION

A numbers of bioactive nanoparticles such as, Au, Ag and Cu have been reported so far [1-3]. Microorganism mediated nano particles synthesis in turn produces various inorganic materials like amorphous silica, magnetite, gypsum, and calcium carbonate layers both in intra or extracellular atmosphere that makes them eco-friendly nano factories [4,5]. It has also been reported that *Klebsiella pneumoniae* exposed to Cd^{2+} containing growth media produced 20-200 nm cadmium sulfide (CdS) on the cell surface and intracellular CdS nano crystals formed when *E. coli* is incubated with cadmium chloride and sodium sulphide solution [6,7]. Although, yeast like eukaryotes are mostly explored in biosynthesis of semiconductor nano particles but reports are also available on the exposure of *Candida glabrata* to Cd^{2+} able to produce intracellular CdS nano crystals [8,9]. Furthermore, Kowshik *et al* demonstrated that *Schizosaccharomyces pombe* synthesized intra cellular CdS quantum dots which exhibit ideal diode characteristics [10]. It has been also reported that *Fusarium sp*. is used for extracellular biosynthesis of CdS nano particles using cadmium sulphate salt as a reactant but exposure of fungal biomass of *Fusarium sp*. to the cadmium nitrate salt can't synthesize CdS nano particles, which indicate that secreted sulfate reductase enzymes somehow associated with the biosynthesis mechanism [11]. Some fungal strains of *Aspergillus sp*. showing metal tolerance activity have ability of bioaccumulation of metal ions. Compared with other microorganisms, *Aspergillus sp*. has been known to secrete much higher amount of bioactive substances, which made them more suitable for extracellular biosynthesis of nanoparticles [12,13].

Our recent research demonstrated the *Aspergillus foetidus* mediated biosynthesis of silver nanoparticles [2] but there is no current research reports are available on *Aspergillus foetidus* mediated biosynthesis of CdS nano particles. In view of the above we have undertaken the current research, which will be a new approach of economic as well as eco-friendly process for biosynthesis of CdS nanoparticles.

II. MATERIALS AND METHODS

Materials

All chemicals were analytical grade and purchased from Sigma-Aldrich (MO,USA).

Methods

Growth Media

The Czapek–Dox (CD) media containing [KH_2PO_4 (1 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g), KCl (0.5 g), $FeSO_4$ (0.01 g), $ZnSO_4$ (0.01 g), Glucose (40 g)]/litter and pH-8 was adjusted before autoclave. 2% Agar was added to solidify the media.

Cadmium tolerance study for Aspergillus foetidus

Aspergillus foetidus (MTCC8876) was tested for tolerance by adding 0.25, 0.50, 1.00, 1.50 and 2 mM of $CdSO_4$ to the CD-Agar media and incubated at 30°C for 7 days. The growth diameter was monitored by measuring the culture from the point of inoculation. Tolerance of the *Aspergillus foetidus* was studied by determination of minimum inhibitory concentration (MIC).

Biomass and cell free filtrate preparation

Biomass and cell free filtrate were prepared as described previously [2] with some modifications. Briefly, Erlenmeyer flask containing 250 ml of CD broth media was inoculated with 4 ml of spore suspention (10^{11}conidia/litre) and incubated for 96 h at 30ºC with a gentle shaking at 150 revolution per minute (r.p.m.) The harvested myceilal biomass was washed several times by autoclaved double distilled water then 10 g of freshly prepared mycelial biomass was suspended in 100 ml of autoclaved milli–Q water (Millipore, USA) was kept at 30 ºC for 72 h with gentle shaking at 150 r.p.m. Mycelial biomass suspention was precipitated upon centrifugation at 5000 r.p.m. for 20 min and cell free filtrate was collected for present studies.

Synthesis of CdS nano particles

50 ml cell free filtrate was taken in a conical flask and cadmium sulphate salt ($3CdSO_4.8H_2O$) was added to it in such a way that the overall concentration made to 1mM and then kept the reaction mixture in incubator shaker at 130 r.p.m. at 30ºC.Furthermore three different experiments were also performedin same reaction condition instead of adding cadmium sulphate salt, where first set was performed with cadmium chloride and sodium sulphate salts (1:1), second with cadmium nitrate and final set with cadmium chloride salt.

Spectro-photometric and fluorometric studies of synthesized nanoparticles

1ml sample was taken in a quartz cuvette and absorbance was measured using UV-visible spectrophotometer by scanning the sample in λ200 nm to λ700 nm wavelength range.According to the spectra, we estimate the band gap of the biosynthesized CdS nanoparticles using Einstein's energy equation, $E_n=hc/\lambda$, where, E_n, energy band gap of the nanoparticles, c, speed of light, h, Planck's constant and λ is the cut off wave-length obtained from the absorption spectra. Furthermore, fluorescence property of the synthesized CdS nano particle was measured by Cary-Eclipse fluorescence spectrometer (Agilent Technologies, USA) with λEx=407 nm and λEm=430 nm.

DLS and Zeta potential measurement of CdS nanoparticles

The aqueous suspension of the synthesized CdS nanoparticles were filtered through a 0.22 µm filter-syringe and the size of the distributed CdS nanoparticles were measured by using the principle of dynamic light scattering (DLS) technique made in a Malvern Zetasizer Nano series compact scattering spectrometer.

FT-IR study for protein bound CdS nanoparticles

The prepared cell filtrate was freeze-dried in lypholizer and potassium bromide was added to the lyophilized sample (100:1). The FT-IR spectrum of the prepared sample was recorded in a PerkinElmer FT-IR in the range of 4000–450 cm^{-1} at a resolution of 4 cm^{-1}.

AFM analysis

Solution of the synthesized CdS nanoparticles was filtered through 0.22 µm syringe-filter and 100µl of the sample was taken for atomic force microscopy (AFM) analysis. The filtered sample was placed on clean cover slip and kept under vacuum desiccation for overnight before loading them onto a specimen holder. The slides were then scanned in the AFM (model dinnova by Veeco) and data was analyzed by Nano drive software.

XRD analysis

X-ray diffraction analysis was performed with a desktop X-ray diffractometer, MiniFlexII by Rigaku, Japan, using CuKα (α=1.5418Å) radiation by solid state detector, where supplied current was 15 mA and X-ray was generated at 35KV. The diffracted intensities were recorded from 20 to 70° 2θ angles scanning. The average particle size (d) was estimated using the Debye−Scherrer equation, $d=0.9\lambda/\beta\cos\theta$, where d, is the crystalline size; λ, wavelength of the CuKα; θ, angle between the incident beam and reflecting lattice plane and β, full width a half maxima (FWHM) of the diffraction peak.

HRTEM & EDAX analysis

To examine the morphology of synthesized nanoparticles,high-resolution transmission electron microscopy (Model Tecnai TF20 G2 ST, FEI, Netherlands) was performed with an electron kinetic energy of 200 kV. In that connection, a drop of synthesized CdS nanoparticles suspension was placed on the carbon coated copper grid and placed under vacuum desiccation for overnight before loading them onto a specimen holder. Including HRTEM study, simultaneously energy dispersive X-ray study was also performed in the other interface of the instrument to obtain the elemental compositions of the synthesized particles.

Statistical analysis

Data were analyzed by unpaired *t* test and analysis of variance followed by the test of least significant differences for comparison within and between the groups [14].

III. RESULTS AND DISCUSSION

Study of cadmium tolerance

The tolerance level performed upon treatment of different concentration of $CdSO_4$ Salt (0.25mM, 0.50mM, 1mM, 1.50mM, 2mM respectively) to the CDA growth media. The result showed that the radial growth of the *Aspergillus foetidus* gradually decreases with increase in salt concentration but as days go on, the growth increases in each set of experiment and upon 1mM $CdSO_4$ treatment *Aspergillus foetidus* grown in healthy population (Fig. 1). We are most interested on 1mM treatment because of later that concentration was considered for synthesis of CdS nanoparticles.

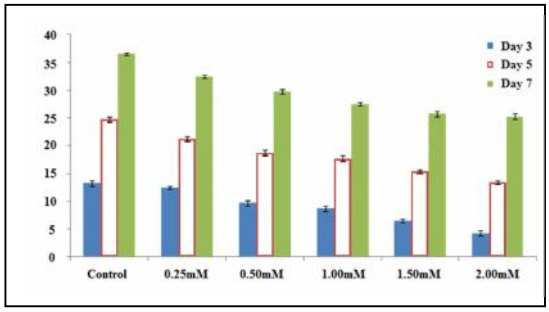

Fig 1 Cadmium tolerance study of *Aspergillus foetidus.* The blue column represents at day 3, red border at day 5 and green at day 7. Data are mean ± SE (n = 4); P<0.001 compared to the control value.

UV-VIS Spectroscopy and fluorescence studies of the nanoparticles

After adding $CdSO_4$ in to the cell free filtrate, straw yellow colour of the cell free filtrate was converted to bright yellow colour. But it is very difficult to distinguish cell filtrate colour and $CdSO_4$ treated cell filtrate by visual appearance(data not shown).

The absorbance peak of control sample was appeared at 274 nm, most probably due to the presence of protein (Fig. 2A). On the other hand, 1 mM $CdSO_4$ treated cell free filtrate showed an absorbance peak at 407 nm (Fig. 2B) but this characteristic absorbance peak was not observed when sample was untreated (Fig. 2A), which is predicted to be due the presence of the CdS nanoparticles in the cell filtrate.Interestingly, a fluorescence maxima at 515 nm was observed with λEx=407 nm and λEm=430nm (Fig. 2C).Such absorbance and fluorescence peak was also observed when cadmium chloride and sodium sulphate salts (1:1) were taken as reactant instead of cadmium sulphate. But when cadmium nitrate or cadmium chloride were taken as reactant separately in the cell filtrate does not showed any absorbance peak at 407 nm or fluorescence maxima at 515 nm (data not shown). From which it may conclude that $S^{=}$ anion of the CdS nano particle obviously comes from the $SO_4^{=}$ but not from the cysteine residue of the cellular proteins or any other sources. Therefore, most predictably extra-cellular sulfate reductase enzyme but not the nitrate reductase is involved in this reaction mechanism.

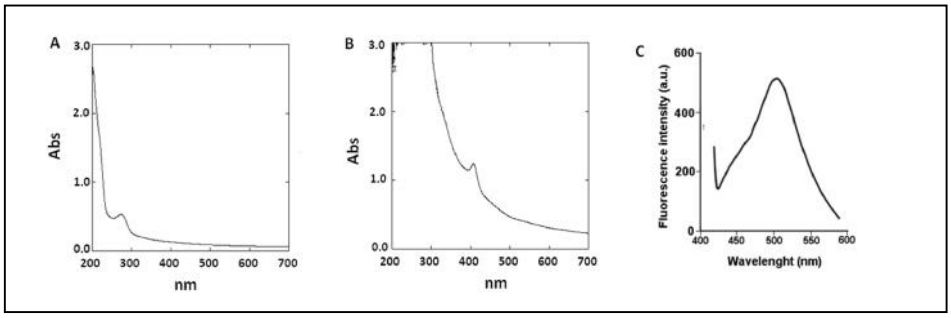

Fig 2 Spectrometric study of CdS nanoparticles. (A) Spectro-photometric scans of cell free filtrate without treatment of $CdSO_4$ salt. (B) Spectro-photometric scans of cell free filtrate treated with $CdSO_4$ salt. (C) Spectro-fluorometric study of cell free filtrate containing $CdSO_4$ salt.

DLS and FTIR study of synthesized nano particles

The data of Dynamic light scattering (DLS) supported that the average size of the synthesized nanoparticles are 125 nm with 0.200 PDI value and the obtained single peak indicated that the quality of the synthesized CdS nano particles are good but size distribution pattern is poly dispersive(Fig. 3A).

Proteins are IR active and it has been well known that protein-nanoparticles interactions can occur either through free amino groups or cysteine residues in proteins and also via the electrostatic attraction of negatively charged carboxylate groups. The two bands observed at 1416 and 1121 cm^{-1} can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively, which are shifted from the control values (Fig. 3B, C).

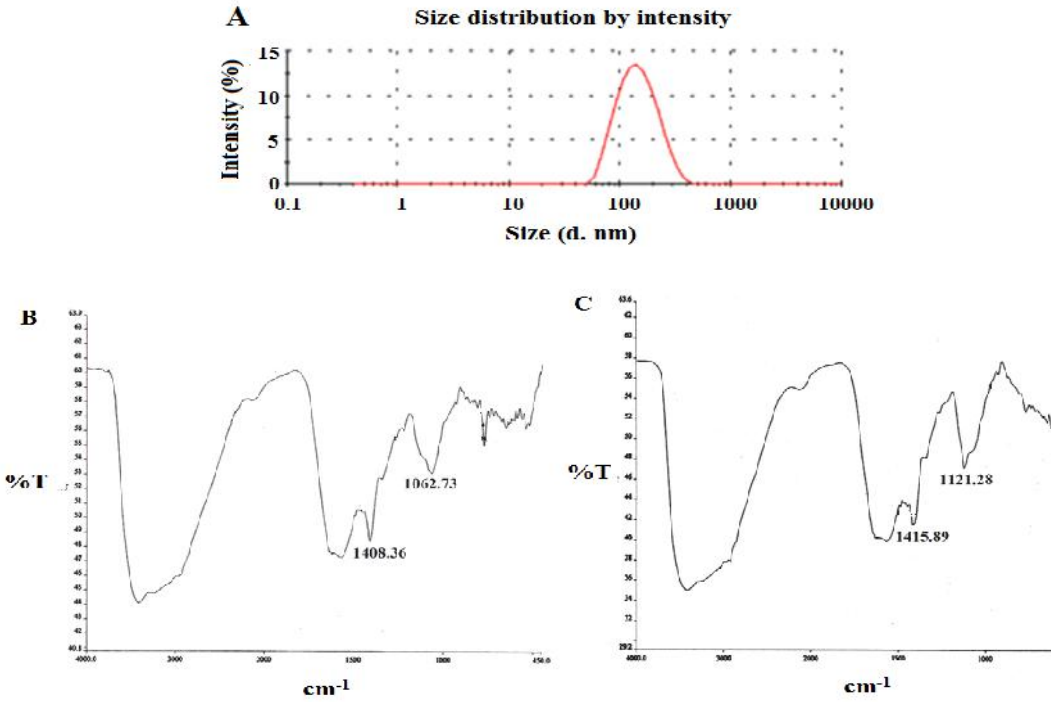

Fig 3 DLS and FT-IR studies. (A) The dynamic light scattering (DLS) histogram of aqueous suspension of the synthesized CdS nanoparticles. (B) The FT-IR spectrum of the cell free filtrates. (C) The FT-IR spectrum of the cell free filtrate treated with $CdSO_4$.

This observation strongly supports the binding of proteins with CdS nanoparticles which may be the possible reason of their stabilization. Zeta potential value is also in agreement with the stabilization of synthesized nano CdS. The value of zeta potential ~(⁻16 mV) (data not shown) also demonstrated that there is a repulsion force acts among the nano particles to inhibit the tendency of the particles to assemble together.

AFM and XRD analysis

Atomic Force Microscopy (AFM) is a useful tool for surface topography of nanostructures. The two dimensional and three dimensional images from the Atomic Force Microscopy of biosynthesized CdS nanoparticles by *Aspergillus foetidus* shows the particles which are spherical in shape under optimized condition for the production of CdS nanoparticles (Fig. 4A, B). The size distribution pattern of the synthesized nanoparticles within the Region of interest (ROI) was represented in the histogram of figure 4C. The distribution pattern demonstrated that the synthesized particles are poly-dispersive in nature. The ROI within the figure 4A of mean area 0.019μm^2 shows mean diameter of the particles is 130 nm (Fig. 4C) which is nearly match to the DLS study result (Fig. 3A).
XRD patterns also confirmed the formation of crystalline phase of CdS nanoparticles in the extracellular environment of the *Aspergillus foetidus*. The XRD pattern exhibited diffraction peaks (2θ angel) at 23.42°, 27.41°, 29.27°, 36.91°, 42.31°, 48.84° and 52.38° (Fig. 4D) corresponding to (100), (002), (101), (102), (110), (103) and (112) planes of hexagonal phase CdS (JCPDS No.41−1049), respectively. The average particle size of the synthesized CdS nanoparticles estimated from the Debye−Scherrer equation was found to be ~30 nm having average surface area of 41.15 m^2/g.

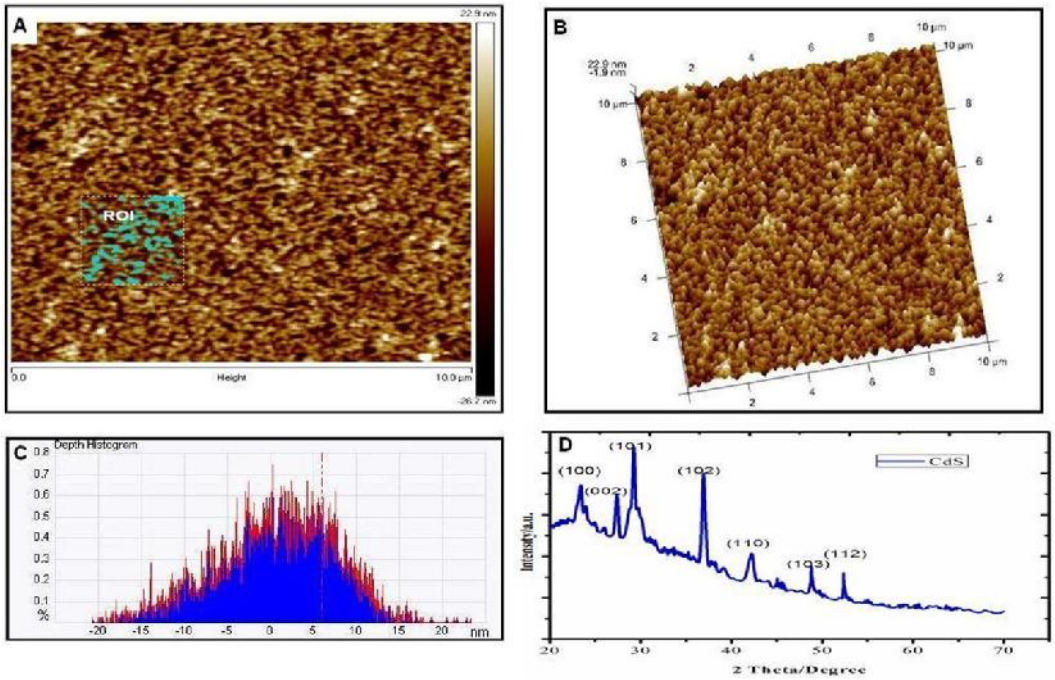

Fig 4 AFM and XRD studies. (A) Two dimensional AFM images of synthesized CdS nanoparticles. (B) The three dimensional AFM images. (C) Histogram of nanoparticle distribution within ROI of figure A. (D) Representative X-ray diffraction patterns of CdS nano particles (a.u.= arbitrary units).

HRTEM and EDAX analysis

A typical HRTEM image of synthesized CdS nanoparticles is displayed in Figure 5A and the figure shows that the formation of roughly spherical poly-dispersed nanoparticles are in the average size range of ~20-30 nm. The spectrum of the Energy Dispersive X-ray (EDX) studies showed multiple peaks very near to the Y-axis for cadmium and sulfur in the sample (Fig. 5B). The elementary composition of cadmium by weight% is 75.57, atomic% is 46.87 and sulfur by weight% is 24.42, atomic% is 53.12, therefore data of the elemental analysis also in support with the formation of CdS nanoparticles. The peaks observed for Cu in the figure 5B is for the grid composition.

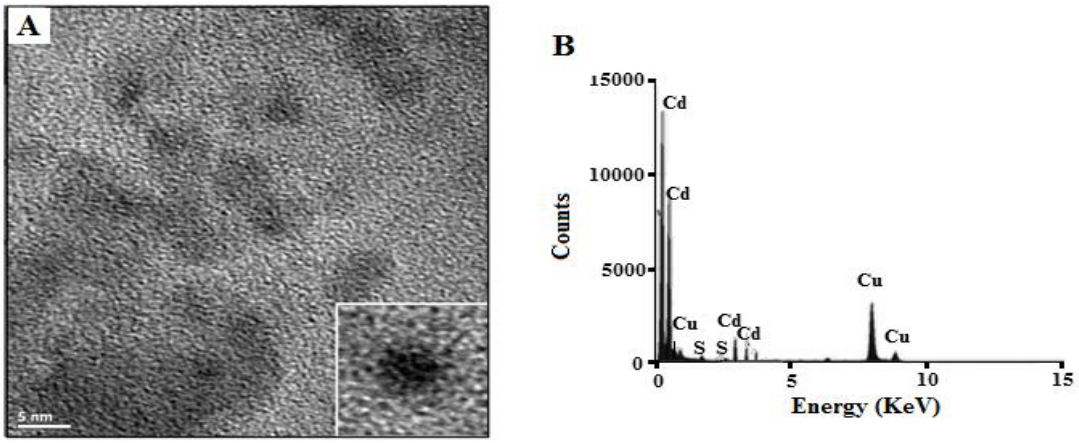

Fig 5 HRTEM and EDAX studies. (A) Characterization and size determination of CdS nanoparticles by HRTEM. Inset represents zoom view. (B) Energy dispersive X-ray study to obtain the elemental compositions of CdS nano.

IV. CONLUSION

Aspergillus foetidus has shown the potential of biogenic production of CdS nanoparticles from cadmium sulphate salt due to extracellular reduction having size range of ~20-30 nm hexagonal crystalline structure. The probable mechanism for the synthesis of CdS nano particle involves the enzyme sulfate reductases. It presents a convenient and eco-friendly approach for the biosynthesis of stable CdS nano particles compared to the other approaches for biosynthesis of CdS nanoparticles. The antimicrobial mechanism of the conventional antimicrobial agents depends on the specific binding with surface and/or metabolic components of the microorganisms [15,16]. Various microorganisms have evolved drug resistance over many generations [17]. Therefore, nanoparticles as antibacterial agents are nowadays took place in research interest [18,19]. This has a distinct advantage over than conventional antimicrobial agents. The most important disadvantage of the conventional antimicrobial agents is multidrug resistance property of microorganisms [20]. Therefore, an alternative way to overcome the drug resistance of various microorganisms is very much essential. Investigations on antimicrobial activities of the synthesized CdS nanoparticles are in progress. Furthermore in future an attempt would be taken to study of degradation of different carcinogenic dyes used in different industries as colouring agent by the synthesized CdS nanoparticles.

ACKNOWLEDGEMENTS

All financial credit of the present work goes to Maulana Azad national fellowship (UGC, New Delhi) to Tina Nasrin and partly supported by DST-purse programme of University of Kalyani. The authors would like to thank for the technical supports to the Department of Chemistry,Department of Physics, University of Kalyani and S. N. Bose National Centre for Basic Sciences, Kolkata.

REFERENCES

[1] A. Annamalai, V. L. Christina, D. Sudha, M. Kalpana, and P. T. Lakshmi,“Green synthesis, characterization and antimicrobial activity of Au NPs using *Euphorbia hirta L*. leaf extract”,Colloids Surf B Biointerfaces,vol.108,pp.60-65,2013.
[2] S. Roy, T. Mukherjee, S. Chakraborty, and T. K. Das,“Biosynthesis, characterisation& antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* MTCC8876”, Digest J Nanomats Biost,vol.8,pp.197- 205,2013.
[3] K. Giannousi, K. Lafazanis, J. Arvanitidis, A. Pantazaki, and C. Dendrinou-Samara ,“Hydrothermal synthesis of copper based nanoparticles: Antimicrobial screening and interaction with DNA”, J Inorg Biochem ,vol.133,pp. 24-32,2014.
[4] R. K. Mehra,and D. R. Winge,“Metal ion resistance in fungi: molecular mechanisms and their regulated expression”, J Cell Biochem,vol.45,pp.30-40,1991.
[5] D. Mandal, M. E. Bolander, D. Mukhopadhyay, G. Sarkar, and P. Mukherjee,“The use of microorganisms for the formation of metal nanoparticles and their application”, Appl Microbiol Biotechnol,vol.69,pp.485- 492,2006.
[6] J. D. Holmes, D. J. Richardson, S. Saed, R. Evans-Gowing, D. A. Russell, and J. R. Sodeau,“Cadmium-specific formation of metal sulfide 'Q-particles' by Klebsiella pneumonia”, Microbiology,vol.143,pp.2521- 2530,1997.
[7] R. Y. Sweeney, C. Mao, X. Gao, J. L. Burt, A. M. Belcher, G. Georgiou, and B. L. Iverson,“Bacterial biosynthesis of cadmium sulfide nanocrystals”, Chem Biol,vol. 11,pp.1553- 1559, 2004.
[8] C. T. Dameron, R. N. Reese, R. K. Mehra, A. R. Korran, P.J. Carroll, M.L.Steigerwald, L. E. Brus,and D. R. Winge,“Biosynthesis of cadmium sulphide quantum semi conductor crystallites”, Nature ,vol.338,pp. 596-597,1989.
[9] M. Kowshik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, S. K. Kulkarni, and K. M. Paknikar,“Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3”, Nanotechnology,vol. 14,pp. 95-100, 2003.
[10] M. Kowshik, N. Deshmukh, W. Vogel, J. Urban, S. K. Kulkarni, and K. M. Paknikar,“Microbial synthesis of semiconductor CdS nanoparticles, their characterization, and their use in the fabrication of an ideal diode”,Biotechnol Bioeng,vol. 78,pp.583-588,2002.
[11] A. Ahmad, P. Mukherjee, D. Mandal, S. Senapati, M. I. Khan, R. Kumar, and M. Sastry,“Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium oxysporum*”, J Am Chem Soc,vol.124,pp.12108-12109,2002.
[12] P. Mohanpuria**,** N. K. Rana, and S. K. Yadav,“Biosynthesis of nanoparticles: technological concepts and future applications” J Nanopart Res , vol.10,pp.507-517, 2008.
[13] K. B. Narayanan,and N. Sakthivel,“Biological synthesis of metal nanoparticles by microbes”, Adv Colloid Interface Sci,vol.156, pp.1-13, 2010.
[14] W. W. Daniell, Biostatistics. In: Daniel WW (ed) A foundation for analysis in the health sciences (estimation). Wiley (NY) 1978, pp.121-157.
[15] E. J. Prenner, R. N. Lewis, and R. N. McElhaney, “The interaction of the antimicrobial peptide gramicidin S with lipid bilayer model and biological membranes”, Biochim Biophys Acta, vol. 1462, pp. 201-221,1999.
[16] R. P. Lange, H. H. Locher, P. C. Wyss, and R. L. Then, “The targets of currently used antibacterial agents: lessons for drug discovery”, Curr Pharm Des, vol. 13, pp. 3140-3154, 2007.
[17] O. Nolte, “Antimicrobial resistance in the 21st century: a multifaceted challenge”, Protein Pept Lett, vol. 2, pp. 330-335, 2014.
[18] M. Veerapandian, and K. Yun, “Functionalization of biomolecules on nanoparticles: specialized for antibacterial applications”, Appl Microbiol Biotechnol, vol. 90, pp.1655-1667, 2011.
[19] M. J. Hajipour, K.M. Fromm, A. A. Ashkarran, D. Jimenez de Aberasturi, I. R. de Larramendi, T. Rojo, V. Serpooshan, W. J. Parak, and M. Mahmoudi, “Antibacterial properties of nanoparticles” ,Trends Biotechnol, vol. 30, pp.499-511, 2012.
[20] M. Bassetti, M. Merelli, C. Temperoni, and A. Astilean, “New antibiotics for bad bugs: where are we?”, Ann Clin Microbiol Antimicrob, vol. 12, pp.22, 2013.