International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 12; December 2017; Page No. 8405-8409
DOI: http://dx.doi.org/10.24327/ijcar.2017.8409.1354

Research Articles

BIOLOGICAL SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING BIOSURFACTANT PRODUCING *BACILLUS TEQUILENSIS*

Chandana Vineela K[2]., Hemalatha V[2]., Kalyani D P[2]., Muni Kumar[2] and Hemalatha K.P.J [1]*

[1]Department of Biochemistry, Andhra University, Visakhapatnam - 530 003, Andhra Pradesh, India
[2]Department of Microbiology, Andhra University, Visakhapatnam

A R T I C L E I N F O

Article History:
Received 15th September, 2017
Received in revised form 25th October, 2017
Accepted 23rd November, 2017
Published online 28th December, 2017

Key words:
Silver nanoparticles, *Bacillus tequilensis,* UV-Visible spectrophotometry, SEM-EDX, X-ray diffraction and Fourier transform infrared spectroscopy (FT-IR).

A B S T R A C T

In order to survive in environment containing high levels of metals, organisms adapt evolving mechanisms to cope up with them. These mechanisms may alter the chemical nature of the toxic metal so that it no longer causes toxicity, resulting in the formation of nanoparticles of the metal concerned. Thus nanoparticle formation is the "by-product" of a resistance mechanism against a specific metal, and this can be used as an alternative way of producing them. Nanoparticle*s* have unique thermal, optical, physical, chemical, magnetic and electrical properties compared to their bulk material counterparts. The most important feature of nanoparticles is their surface area to volume aspect ratio, allowing them to interact with other particles easier. In the present study silver nanoparticles are formed in the water-in-oil emulsion phase. Synthesized nanoparticles were characterized using UV-visible spectrophotometer, SEM-EDX, FT-IR and X-ray diffraction methods. The morphology of the nanoparticles is crystalline, more or less spherical and the particle size is 32nm. XRD analysis has given a clear picture indicating the presence crystalline cubic phase of monoclinic silver nanoparticles.

INTRODUCTION

Presently, there is a growing concern at global level to have nontoxic, nonhazardous surface-active agents. Contrary to synthetic surfactants, their biological counterparts or biosurfactants play a primary function, facilitating microbial presence in environment dominated by hydrophilic-hydrophobic interfaces. Recently, the surfactant mediated synthesis of nanoparticles is emerging as a potential method for the stabilization of nanoparticles. Although the chemical and physical processes are potentially useful in the synthesis of nanoparticles, the size-controlled synthesis is still remaining as a challenge in material science (Reddy *et al*., 2009). The size and shape are critical factors that decide the biological activity and specificity. Surfactants are emerging as potential stabilizing agents, however, the synthetic surfactants are not economically viable as well as they are not environmentally friendly. Therefore, the biosurfactants are emerging as a green alternate for the synthesis and stabilization of nanoparticles. Biosurfactants can be used for high-performance nanomaterial production, since they easily form a variety of liquid crystals in aqueous solutions. A glycolipid biosurfactant was evaluated for its effect on the synthesis/stabilization of nanozirconia particles (Biswas and Raichur, 2008).

Corresponding author:* **Hemalatha K.P.J
Department of Biochemistry, Andhra University, Visakhapatnam - 530 003, Andhra Pradesh, India

Although the biosurfactant-mediated processes are highly effective, the processes are not cost-effective, since most of the available efforts are based on the synthetic/commercial surfactants. Considering the need of greener bioprocess and novel enhancers for the synthesis using microbial processes, biosurfactants and/or biosurfactant producing microbes are emerging as an alternate source of rapid synthesis of nanoparticles (Xie *et al.,* 2006; Kasture *et al.,* 2008; Reddy *et al.,* 2009). A micro-emulsion technique using oil-water-surfactant mixture was shown to be a promising approach for nanoparticles synthesis (Xie *et al.,* 2006). Although chemical surfactants are highly promising, these chemicals could be toxic to the environment. Recently, the focus on biosurfactant mediated processes is steeply increasing due to their potential implications on the synthesis of silver nanoparticles (Palanisamy and Raichur, 2009; Reddy *et al.,* 2009). Xie *et al.,* (2006) reported that rhamnolipid biosurfactant could be used as a stabilized agent for silver nanoparticles. The present study, revealed the possibilities' of using glycolipid and lipopeptide mediated synthesis of silver nanoparticles, which would be effective and advantageous over chemical surfactants.

Synthesis of AgNPs has attracted a lot of attention due to their unusual optical, photoelectrochemical and electronic properties. The general methods of synthesizing AgNPs is by physical method, which yields low amounts of product and generates lots of heat and the chemical protocols generally applied for synthesis of AgNPs, suffer from one or the other limitations like high cost, use of toxic chemicals, etc. Many microorganisms such as bacteria, yeasts, fungi and actinomycetes have been used in synthesis of metal nanoparticles. Microbial synthesis of metal nanoparticles takes place either intracellularly or extracellularly. Additional downstream processing steps like ultrasonication, cell disruption by chemicals to release AgNPs are required in case of intracellular synthesis of AgNPs. On the other hand extracellular biosynthesis is cheap and AgNPs can be separated easily which favours large-scale production of AgNPs to explore its potential applications. Due to this, many studies were focussed on extracellular methods for the synthesis of metal nanoparticles. The approach on using culture supernatants from different bacteria, yeasts, fungi and actinomycetes for the synthesis of silver nanoparticles is well documented. Biosurfactants, which derived from microbial origin, have bulky and complicated structures, higher biodegradability, lower toxicity, and excellent antiviral activities. So biosurfactant as a "green" stabilizer is one of the best candidates. It is believed that biosurfactants will be increasingly attractive as multifunctional materials for the new century.

In the present study the possibility of synthesizing silver nanoparticles in water-in-oil microemulsion stabilized by a low-cost biosurfactant explored. The silver nanoparticles obtained are characterized by UV-Vis absorption spectrum, SEM-EDS, FTIR analysis and XRD.

MATERIALS AND METHODS

Reagents and chemicals

Pure and analytical grade chemicals were used in all experiments including synthesis of silver nanoparticles and media preparation for the growth of bacterial cells. Nutrient Agar, Peptone, Beef extract, Agar-agar, Nutrient broth were purchased from Himedia, Mumbai, India.

Microorganism

The bacteria, *Bacillus tequilensis* (MTCC- 12730), used for the synthesis of silver nanoparticles was isolated from oil contaminated soil. The bacterial culture was maintained on Nutrient agar slant and stored in freezing (-20^{0}C) temperature.

Extracellular synthesis of silver nanoparticles (Ag-NP$_s$)

The bacterial strain *Bacillus tequilensis* (MTCC- 12730) was inoculated into the nutrient broth. Then it was incubated for 24hrs in orbital shaker at 160rpm. After incubation it was centrifuged at 5,000rpm for 20min. The pellet was discarded and supernatant was used for nanoparticles synthesis. One millimolar $AgNO_3$ was added to the supernatant. The control was maintained without addition of $AgNO_3$. The bioreduction of silver ions was monitored at regular intervals by sampling aliquots (2ml) of the reaction mixture. UV-visible spectra of these sample aliquots were recorded as a function of time of reaction from 200-800nm on UV-visible double beam spectrophotometer (Kumar *et al.,* 2011).

UV-Spectrophotometer analysis

The synthesized silver nanoparticles were characterized through UV-Visible spectrophotometer (shimadzu) range of absorbance from 200-800nm. This result indicates that the nano-scale silver can be synthesized in reverse micelles using the low-cost biosurfactant as stabilizer. Decrease in the intensity is due to a change in the free electron density.

FT-IR analysis

The prepared Silver nanoparticles were then subjected to FT-IR spectroscopy measurements. It was used to identify the possible functional groups of biomolecules responsible for the reduction and capping of the nanoparticles. FT-IR analysis was carried for the reduction of silver ions with the spectral range of 400-4000 cm-1 using FT-IR Spectrometer, Shimadzu, Japan.

SEM-EDX

Morphology and particle size of silver nanoparticles were determined by SEM analysis. The SEM analysis was established by using Scanning Electron Microscope (SEM) Jeol Asia PTE Ltd, Japan with 1mm resolution at 20 kv with 20 mm Oxford, UK, EDS detector. The elemental composition in the reaction mixture was determined by EDX analysis.

X-Ray Diffraction

The crystalline structure of the silver nanoparticles was determined by X-Ray diffraction analysis using X-Ray Diffraction Unit (XRD) Pan Alytical, X-Pert pro, Netherlands operating at 40 kV with 2 sec time interval at room temperature.

RESULTS

The selected *Bacillus tequilensis* isolated from oil contaminated soil was screened by using different methods. It is positive for oil displacement and Drop collapse method and emulsification index (E_{24}). The strain was grown under favourable cultural conditions for increasing the yield. The results were shown in Table1.

Table 1 preliminary screening of biosurfactant producing *Bacillus* tequilensis strain

S.No	Screening tests	Positive/Negative
1.	Heamolytic test	β- heamolysis
2.	Drop collapse test	+
3.	Oil displacement test	+
4.	Emulsification index (E_{24})	+

Synthesis of Silver Nanoparticles (Visual Inspection)

During the biosynthesis, formation of nanoparticles is indicated by the change in colour of the mixture. After 72 hrs of reaction, the reaction mixture changes its colour from light to dark colour indicating the synthesis of silver nanoparticles due to the reduction of Ag^+ ions and due to the excitation of Surface plasmon vibration in metal nanoparticles (Fig.1).

Fig 1 Digital photograph of visible color changes, after adding 1mM $AgNO_3$

Characterization of Silver Nanoparticles

UV-Visible Spectral Analysis

UV-Visible absorption spectrum is the preliminary characterization to know the optical property of synthesized nanoparticles. The result obtained from UV-Visible spectroscopy analysis of the nanoparticles sample is presented in Fig.2. UV-visible spectra of the formed Silver Nanoparticles dispersed in water exhibited the maximum absorption peaks at about 400 nm.

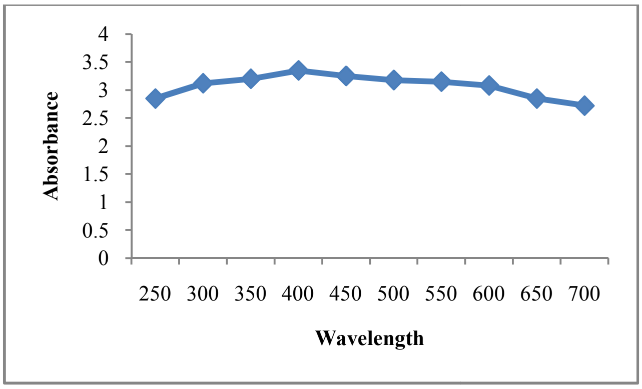

Fig 2 UV-visible spectra of silver nanoparticles

FT-IR analysis

FT-IR spectra of biosynthesized Silver nanoparticles were recorded to identify the capping and efficient stabilization of metal nanoparticles. The FT-IR spectroscopy of synthesized silver nanoparticles is shown in Fig.3. For Silver nanoparticles, peak values at 2953.14, 2924.21, 2853.61, 2724.57, 1614.49, 1074.40 and 720.44cm-1 were observed. In the region 3000-2700 cm-1 several C-H stretching bands of CH_2 and CH_3 groups were also observed. The band seen at 1600-1650 cm -1 is characteristic of -C=O carbonyl groups and -C=C- stretching.

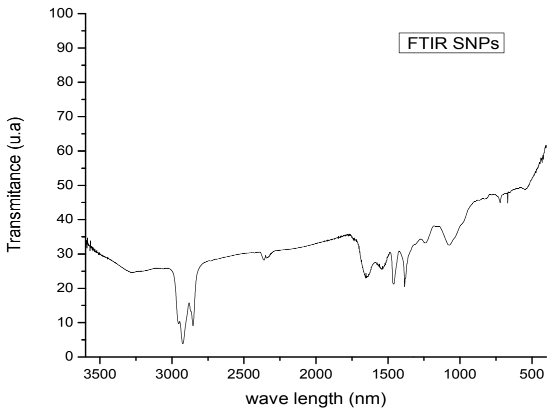

Fig 3 FTIR analysis of Silver nanoparticles

SEM-EDX

SEM image revealed that the synthesized silver nanoparticles were aggregated as irregular rhombic shapes and 32nm in size (Fig-3). Dispersive X-ray Spectroscope (EDX) analysis showed the presence of elemental silver signal in the sample (Fig-4).

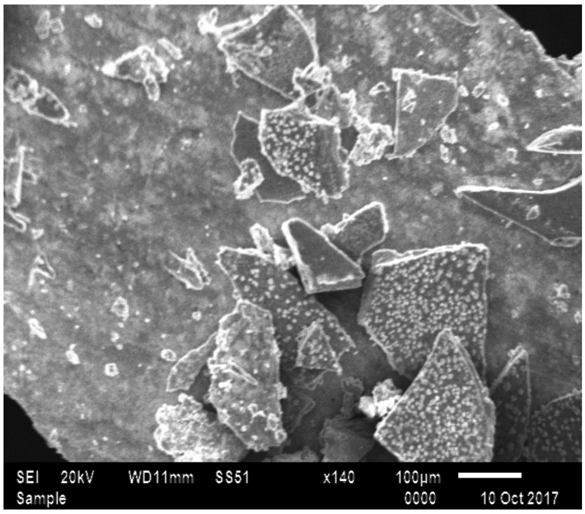

Fig 4 SEM image of silver nanoparticles

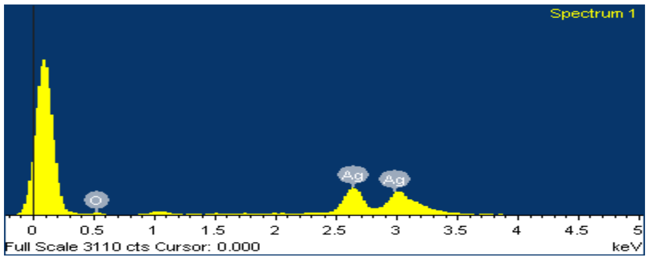

Fig 5 EDX image of silver nanoparticle

XRD analysis

The phase identification and crystalline structure of the nanoparticles were characterized by X-ray diffraction. The X-ray diffraction pattern obtained for the Silver nanoparticles synthesized using *Bacillus sps.,* is shown in Fig. 4. The synthesized particles when subjected to XRD analysis, given a clear picture on the presence crystalline cubic phase of monoclinic silver nanoparticles exhibiting 2θ values 33.3°, 46.1°, 54.7°, 57.3°, 67.3°, 74.4°, 76.3° and 85.6° In addition, the peak observed at 2θ value of 27.7°, might be due to the presence of trace amount of hollow silver nanoparticles. Above all, it is encouraging to note that the 2θ values of the synthesized silver nanoparticles are also matched with Joint Committee for Powder Diffraction Standard (JCPDS).

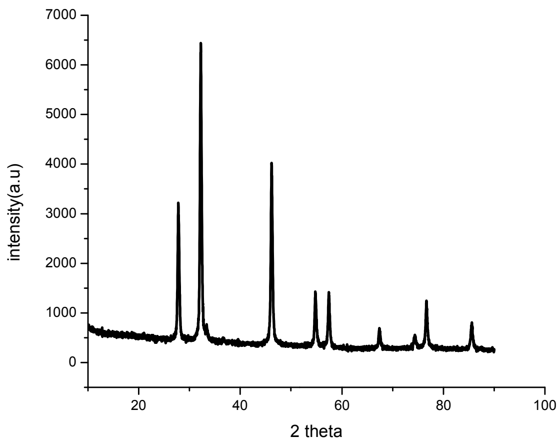

Fig 6 XRD analysis of silver nanoparticles

DISCUSSION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. The synthesis of silver nanomaterials/nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis provides a wide range of environmentally acceptable methodology with low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles have many applications including catalysts in chemical reactions, biolabelling, antimicrobial agents, electrical batteries and optical receptors.

The selected *Bacillus tequilensis* grown in optimized condition, reduced the surface tension and showed 78% emulsification capacity. In the present study culture supernatant was used for silver nanoparticles synthesis.

Recently, several researchers exploited biosurfactant producing extracts showing the synthesis of Ag nanoparticles (Charles Farias *et al*., 2014). Kumar and Mamidyala (2011) reported extracellular synthesis of silver nanoparticles. The reduction of the silver ions was due to the enzyme nitrate reductase. They suggested that the nanoparticles were stabilized by rhamnolipid present in the culture supernatant.

Another unidentified glycolipid produced by a sponge-associated marine *Brevibacterium casei* MSA19 was reported by Kiran *et al.*, (2010) as a suitable stabilizer in Ag nanoparticles biosynthesis under solid-state fermentation. Similar to other observations it was reported that nanoparticles produced by Rhamnolipid were stable for two months and the biosurfactant acted as stabilization agent and prevented the formation of aggregates.

The characteristics of the synthesized silver nanoparticles were studied using UV-Visible spectrophotometer, FT-IR and XRD analysis. The reduction of silver nanoparticle was monitored by measuring the UV-Visible spectrum. UV-visible spectra of the formed silver nanoparticles dispersed in water exhibited the maximum absorption peak at about 400 nm. Similar absorption maximum of silver nanoparticles was reported by Yingwei Xie *et al.,* (2006) where they synthesized silver nanoparticles using $NaBH_4$ as the reduing agent. The surface plasmon absorption in the metal oxide nanoparticles is due to the collective oscillation of the free conduction band electrons which are excited by the incident electromagnetic radiation. This type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter. Surface Plasmon absorption band with a maximum at 400nm indicates the formation of silver nanoparticles (Petit *et al.,* 1990; Barnickel *et al.,* 1990; Huang *et al.,* 1997; Kapoor, 1998; Ji *et al.,* 2007). The FT-IR analysis results were compared with Ida Maragatham and Govindammal (2014) and Bhavna *et al*.,(2015). They reported the peaks of 3000-2700 cm-1 several C-H stretching bands of CH_2 and CH_3 groups. The band seen at 1600-1650 cm -1 is characteristic of -C=O carbonyl groups and -C=C- stretching. The results were also compared with Jeevan *et al.,* (2012) and Anarkali *et al.,* (2012).

The synthesized particles when subjected to XRD analysis, showed the presence of crystalline cubic phase of monoclinic silver nanoparticles exhibiting 2θ values 33.3°, 46.1°, 54.7°, 57.3°, 67.3°, 74.4°, 76.3° and 85.6° which are closely matched with the values of monoclinic phase silver nanoparticles reported by Xiyun Zhao *et al.,*(2015). Above all, it is hopeful to note that the 2θ values of the synthesized silver nanoparticles are also matched with Joint Committee for Powder Diffraction Standard (JCPDS).

CONCLUSION

The present work demonstrates a simple eco-friendly method for synthesis of silver nanoparticles by microemulsion technique. Silver nanoparticles were successfully synthesized using the biosurfactant from *Bacillus tequilensis*. The synthesized nanoparticles were found to be irregular rhombic in shape with uniform distribution. This may provide a new example of synthesizing inorganic nanoparticles by the use of biosurfactant as "green" template. It is potential for using biosurfactant in the preparation and stabilization of monodisperse silver nanoparticles. The characteristics of the synthesized silver nanoparticles were studied using UV-Visible spectrophotometer, SEM-EDX, FT-IR and XRD.

Acknowledgements

The authors are thankful to Advanced Analytical Laboratory **(AAL),** DST-PURSE Programme, Andhra University for assisting in FTIR, SEM-EDX and XRD analysis for the synthesized silver nanoparticles

Reference

Anarkali, J., Raj, D., Rajathi, K., Sridhar, S. 2012. Biological synthesis of silver nanoparticles by using Mollugo nudicaulis extract and their antibacterial activity. *Arch. Appl. Sci*., 4(3): 1436 1441.

Barnickel, P., Wokaum, A., 1990. Synthesis of metal colloids in inverse microemulsions. *Mol. Phys*. 69, 1-9.

Biswas M., and Raichur A.M., 2008. Electrokinetic and rheological properties of nano zirconia in the presence of rhamnolipid biosurfactant. *J. Am. Ceram. Soc*. 91: 3197- 3201.

Charles B.B. Farias *et al*., 2014 Synthesis of silver nanoparticles using a biosurfactant produced in low-cost medium as stabilizing agent. *Electronic journal of Biotechnology*, 122-125.

Huang, H., Yan, F., Kek, Y., Chew, C., Xu, G., Ji, W., Oh, P., Tang, S., 1997. Langmuir 13, 172.

Jeevan, P., Ramya, K., Rena, A. 2012. Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. *Indian J. Biotechnol.*, 11: 72- 76.

Ji, J.H.; Jung, J.H.; Kim, S.S.; Yoon, J.U.; Park, J.D.; Choi, B.S.; Chung, Y.H.; Kwon, I.H.; Jeong, J.; Han, B.S.; Shin, J.H.; Sung, J.H.; Song, K.S. & Yu, I.J. (2007). Twenty-eightday inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol*, Vol.19, pp.857-871

Kasture, M.B., Patel, P., Prabhune, A.A., Ramana, C.V., Kulkarni, A.A., Prasad, B.L.V., 2008. Synthesis of silver nanoparticles by sophorolipids: effect of temperature and sophorolipid structure on the size of particles. *J. Chem. Sci.* 120, 515-520.

Kumar C.G.,and Mamidyala S.K.,(2011) Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa,* colloids Surf. B Biointerfaces 84, 462-466.

Palanisamy, P., Raichur, A.M., 2009. Synthesis of spherical NiO nanoparticles through a novel biosurfactant mediated emulsion technique. *Mater. Sci. Eng*. C 29, 199-204.

Petit, C., IxonL, Pileni, M.P., 1990. Synthesis of cadmium sulfide in situ in reverse micelles 2. Influence of the interface on the growth of the particles. *J. Phys. Chem.* 94, 1598-1603.

Reddy S.A., Chen C.Y., Baker S.C., Chen C.C., Jean J.S., and Fan C. W., 2009. Synthesis of silver nanoparticles using surfactin: a biosurfactant as stabilizing agent. *Mater.Lett.* 63:1227-1230.

Xie, Y., Ye, R., Liu, H., 2006. Synthesis of silver nanoparticles in reverse micelles stabilized by natural biosurfactant. Coll. Surf. A: Physicochem. *Eng. Aspects* 279, 175-178.

Xiyun Zhao, Jie Zhang, Binsong Wang, Amir Zada, and Muhammad Humayun,(2015). “Biochemical Synthesis of Ag/AgCl Nanoparticles for Visible-Light-Driven Photocatalytic Removal of Colored Dyes,” *Materials*, vol. 8, no. 5, pp. 2043-205.

How to cite this article:

Chandana Vineela K *et al* (2017) 'Biological Synthesis And Characterization of Silver Nanoparticles Using Biosurfactant Producing Bacillus Tequilensis ', *International Journal of Current Advanced Research,* 06(12), pp. 8405-8409.
DOI: http://dx.doi.org/10.24327/ijcar.2017.8409.1354

****