Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 135 – 140

MICROBIAL PRODUCTION OF SILVER NANOPARTICLES

KANNAN NATARAJAN*, SUBBALAXMI SELVARAJ ,
V. RAMACHANDRA MURTY
Department of Biotechnology, Manipal Institute of Technology, Manipal University, Manipal, India-576104.

The biological process with the ability to study the shape of particles produced would therefore be a limelight of modern nanotechnology. The bacterial strain *Escherichia coli*, used for the biosynthesis of silver nanoparticles were investigated. These silver nanoparticles were characterized by means of UV–vis spectroscopy, particle size analyzer. The nanoparticles exhibited maximum absorbance at 400 nm in UV–vis spectroscopy corresponding to the plasmon resonance of silver nanoparticles.

(Received January 25, 2010; February 24, 2010)

Keywords: Extracellular, Nanoparticles, Biological process, silver ion

1. Introduction

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 process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions [1], biolabelling, antimicrobial agent, electrical batteries [2] and optical receptors [3-4]. Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Of course the precipitation of nanoparticles in external environment of a cell, it shows the extracellular activity of organism. Extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis, the fungi being extremely good candidates for extracellular process and also environmental friendly. There are few reports published in literature on the biosynthesis of silver nanoparticles using fungal as source [5]. The use of bacterial strain in the bio-manufacturing process has the advantage that ease of handling than the fungal sources [6-9].

In this present work, microbial production of silver nanoparticles was investigated using the bacterial strain *Escherichia coli.* This research work implies the different medium composition for production of silver nanoparticles and characterization of particles by UV- visible spectrometer and laser diffraction particle size analyzer. To our knowledge, extracellular synthesis of Ag particles by bacterial strain E. coli with two different basal medium compositions has not been reported so far.

*Corresponding author**:** biomatrix.kannan@gmail.com

2. Materials and method

2.1 Source of microorganism

The bacterial strain *Escherichia coli*, was obtained from the microbial tissue collection centre, Chandigarh, India. The obtained pure culture was maintained in nutrient broth agar medium (HiMedia, Mumbai, India) slant at 27°C as well as subcultured from time to time to regulate its viability in the microbiology laboratory (Department of Biotechnology, Manipal Institute of Technology, Manipal University, Manipal, India) during the study period.

2.2 Production of biomass

The bacterial strain *Escherichia coli* were cultured, to produce the biomass for biosynthesis in two different liquid broth namely nutrient broth medium and LB medium. The culture flasks were incubated on an orbital shaker at 27°C and agitated at 220 rpm. The biomass was harvested after 24 hours of growth and centrifuged at 12000 rpm for 10 minutes. The supernatant material was collected for the further reaction to synthesis of nanoparticles.

2.3 Synthesis of silver nanopaarticles

The bacterial strain *Escherichia coli* were cultured, to produce the biomass for biosynthesis in two different liquid broth namely nutrient broth medium and LB medium. The sample was added separately to the reaction vessel containing silver nitrate (AgNO3) at a concentration of 10^{-3} (1% v/v) and control (without the silvernitrate, only biomass) was also run along with the experimental condition. The reaction between this supernatant and Ag+ ions was carried out in bright conditions for 24 hours.

2.4 Characterization of silver nanoparticles.

The bioreduction of the Ag+ ions in the solution was monitored and sample of 2ml was withdrawn at different time intervals and the absorbance was measured at a resolution of 1 nm using UV–visible spectrophotometer (PHARMASPEC UV–1700 series, SHIMADZU CORPORATION Ltd., KYOTO, JAPAN) with samples in quartz cuvette.

2.5 Particle sizing measurements

Particle size analyzing experiments were carried out by means of laser diffractometry (laser particle size analyzer); using an extremely compact optical bench, the CILAS 1064 integrates 2 sequenced laser sources pointed at 0ºand 45º. Measurements were taken in the range between 0.04 up to 500 µm. Through the software, the distribution curve is represented by 100 classes above mentioned range.

3. Results and discussion

Bionanoscience system works in the prominent culture and physicochemical conditions to synthesis inorganic materials. Biological method of synthesis of silver nanoparticles exhibit strong absorption of electromagnetic waves in the visible range due to their optical resonant property, called Surface Plasmon Resonance (SPR), occurs due to its collective oscillation of conduction electrons, combined with the incident light [10]. The SPR is highly influenced by shape and size of the nanoparticles [11]. Likewise, the microorganisms has the metal-microbe interaction to produce inorganic metal ions, and have several applications in biotechnological fields, includes bioremediation, biomineralization, bioleaching and microbial corrosion.

3.1 Effect of media on silver nanoparticle synthesis

The bacterial strain of E. coli was inoculated in to different basal medium such as LB and Nutrient Broth medium. Both the medium showed the same amount of biomass production shown in Fig. 1. Although the growth in NB and LB medium resulted same amount of biomass, but the synthesis of silver nanoparticles was maximum in LB medium than the nutrient broth (data not shown). The primary conformation of synthesis of nanoparticles in the medium was characterized by the changes in color from yellowish white to brown. Addition of Ag^{+} ions to the supernatant and pellet culture, samples showed the results as color formation to brown, the color intensity increased with period of incubation due to the reduction in Ag^{0} (Fig. 2a and 2b). Control (without silver nitrate) showed no color formation in the culture when incubated for the same period and condition. In the supernatant culture color changes depends on incubation period, but the pellet culture takes long lag period to formation of color. Synthesis of silver nanoparticles also depends on incubation period of the culture[12].

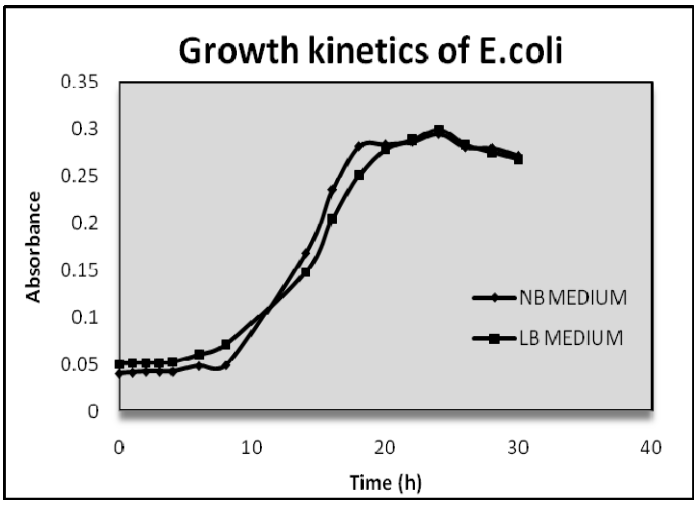

Fig. 1 Growth profile of E. coli in the basal liquid NB and LB medium

3.2 Characterization of silver nanoparticles

The appearance of brown color evident that the formation silver nanoparticles in the reaction mixture and the efficient reduction of the Ag ions occurs extracellularly than the intracellular (pellet material) culture. The knowledge about the reduction of silver ions and formation of silver nanoparticles were still not clear, but believe that protein molecules and enzyme, includes nitrate reductase enzyme act as good regulating agent in silver nanoparticles synthesis. The formed color solution allowed to measure the absorbance against distinct wave length to conform the formation of silver nanoparticles. The corresponding UV/vis absorption spectra are shown in Fig. 3. The control solution (without silver nitrate solution) shows no evidence of absorption in the range 300 to 900 nm. The samples were exposed to the silver nitrate solution shows the wide spectrum range aroung 390 to 410 nm. The presence of the broad resonance indicates the aggregation of the silver nanoparticles in the solution. [13] Suggested that at 370 nm corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 390 nm due to excitation of longitudinal plasmon vibrations. The spectrum with bands in this range has been associated with the surface plasmon resonance of nano-sized silver metal, confirming the occurrence of silver nanoparticles in the culture solution after exposure to UV light.

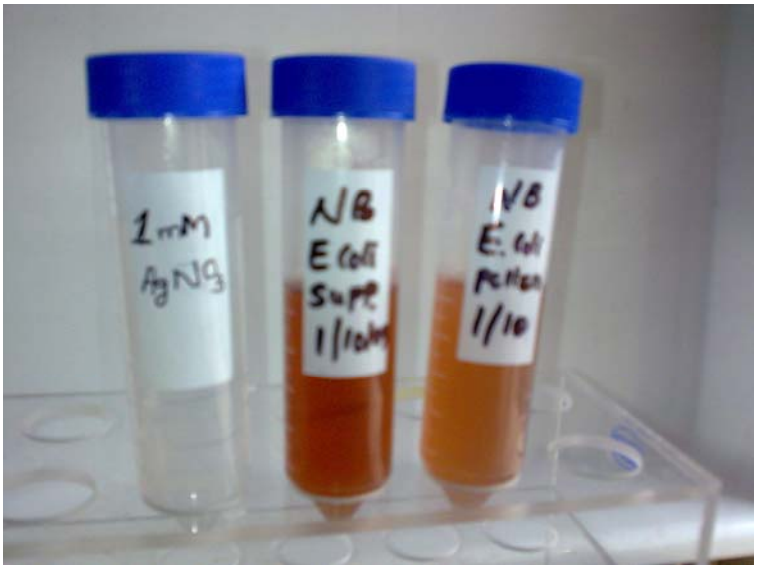

Fig. 2a Solutions of silver nitrate (1 mM) before (left) and after (right) exposure to the culture supernatant and pellet of E. coli in NB medium. The cells or samples were collected and were incubated with 1×10−3 M silver nitrate solution.

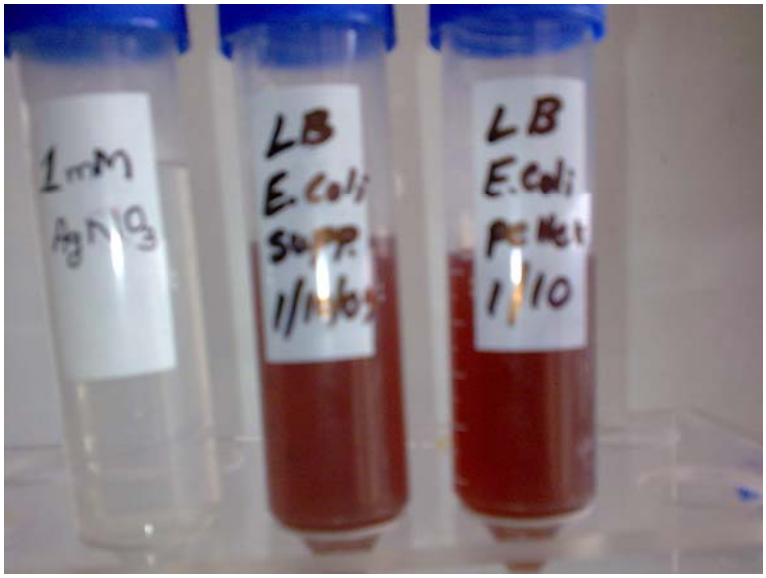

Fig. 2b Solutions of silver nitrate (1 mM) before (left) and after (right) exposure to the culture supernatant and pellet of E. coli in LB medium. The cells or samples were collected and were incubated with 1×10−3 M silver nitrate solution.

Biological synthesis of metal nanoparticles has direct contact with the effect of different stages in microbial growth. The maximum biomass was obtained in mid exponential phase (starting range of stationary phase) of culture and also the maximum synthesis of nanoparticles obtained in the same incubation period (Fig. 4). [12] Reported that the maximum synthesis of nanoparticles obtained when the culture in stationary phase, our investigation maintained the same results. At the same time our results be in agreement with the [14], showed that the synthesis of maximum cadmium sulfide during the stationary phase. These results are supported by previous studies suggesting that the formation silver nanoparticle has maximum activity at stationary phase of Bacillus licheniformis [15]. The present experimental results explain that cells in exponential phase (maximum reproduction of microorganism) produce maximum activity of enzymes involved in aggregation and reduction of silver ions to produce silver nanoparticles. Make an account to the investigated results provides the idea about the produced silver nanoparticles, the characteristic of

microbial growth exposed with silver nitrate solution explain that the produced silver nanoparticles are growth associated product.

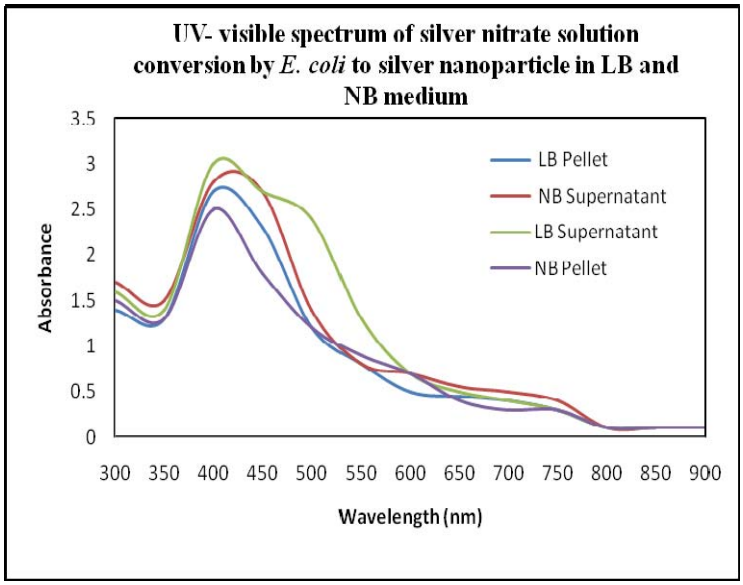

Fig. 3 UV–vis absorption spectra of silver nanoparticles synthesized by E.coli culture. The absorption spectrum of silver nanoparticles exhibited a strong broad peak at 400 nm and observation of such a band is assigned to surface Plasmon resonance of the particles.

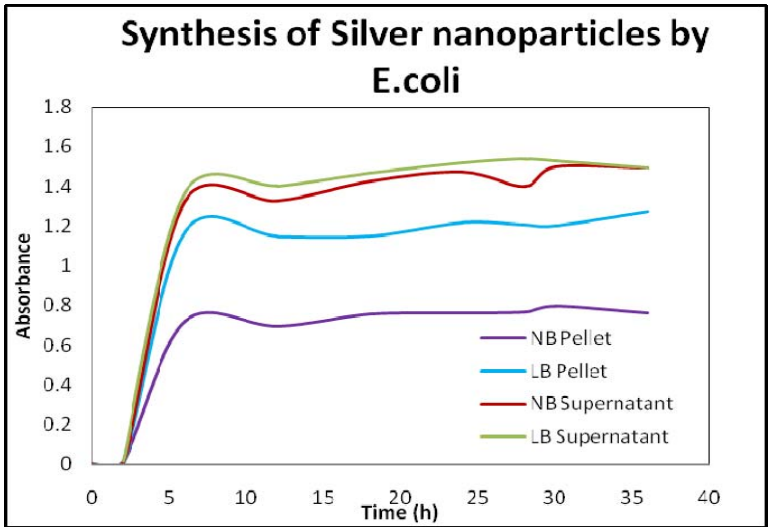

Fig. 4 Kinetic profile of synthesized silver nanoparticles using NB and LB medium by E.coli

3.3 Particle size analyzer

Laser diffraction particle size analyzer provides the detail about the particle nature, such as monodispersed, didispersed and polydispersed. Our investigation revealed that nanoparticles are in polydispersed mixture, with the various sizes range from 40 to 60 nanometers (data not shown). Our result has similarity with [16] reported that produced nanoparticles size varies nearly 100 nm and also the solution possesses polydispersed nanoparticles [17]. The particle size ranges from 35 to 45 nm in different cell growth stages and various metal incubation conditions. The future work concentrates on control the particle size by changing the culture composition and physicochemical properties.

4. Conclusions

We have demonstrated a simple biotechnological process for the intracellular and extracellular synthesis of silver nanoparticles using this bacterial strain. The formation of nanoparticles using different basal medium was investigated; the LB medium has the ability to maximize the synthesis of silver nanoparticles. The characterization of silver ion exposed to microbial strain and the reduction of silver nitrate to silver nanoparticles was confirmed by UV-visible spectrophotometer. The synthesis of maximum silver nanoparticles and maximum production of biomass occur in the stationary phase of incubation period. It reveals that the particles formed from the distinct basal medium are associated with growth. The extracellular formation of silver nanoparticles would be more expressed than intracellular formation. Additionally, the extracellular synthesis would make the process easier for downstream processing. Furthermore investigation to understand the morphological and structural studies to predict the shape of nanoparticles in future experiments. Addition to this study is the application of antimicrobial activity of produced silver nanoparticles.

References

[1] A. Kumar, S., Mandal, P.R. Selvakannan, R. Parischa, A.B. Mandale, M. Sastry Langmuir, **19,** 6277 (2003).
[2] G. Peto, G.L. Molnar, Z. Paszti, O. Geszti, A. Beck, L. Guczi, Materials Science and Engineering: C, **19**, 95 (2002).
[3] A. Krolikowska, A. Kudelski, A. Michota, J. Bukowska, Surface Science, **532**, 227 (2003).
[4] T. Klaus-Joerger, R. Joerger, E. Olsson, C. G . Granqvist, Trends in Biotechnology, **19**, 15 (2001)
[5] M. I. Husseiny, M. A. El-Aziz, Y. Badr, M. A. Mahmoud, Spectrochimca Acta Part A: Molecular and Biomolecular Spectroscopy **67,** 1003 (2007).
[6] J.R. Morons, J. L. Elechiguerra, A. Camacho- Bragado, X. Gao, H. M. Lara Yacaman, Journal of Nanobiotechnology **3,** 6 (2005).
[7] T. Beveridge, R. Murray, Journal of Bacteriology **141,** 876 (1980).
[8] D. Mandal, M. Bolander, D. Mukhopadhay, G.Sarkar, and Mukherjee, P., Applied Microbiology Biotechnology, **69,** 458 (2006).
[9] S. Mandal, S. Phadtare, M. Sastry, Current Applied Physics, **5,** 118 (2005).
[10] U. Kreibig, Vollmer, M., Optical Properties of Metal Clusters. New York: Springer, 1995.
[11] Link, S., M. A. *El*-Sayed, Annual Review of Physical Chemistry, **54,** 331 (2003).
[12] G. Sangiliyandi, K. Kalimuthu, Ramanathan, V., Venkataraman, D., Sureshbabu, R., Jeyaraj, M., Nellaiah, H. and Soo, H. E., Colloids and Surfaces B: Biointerfaces, **74**, 328 (2009).
[13] Shankar, S., Ahmad, A. and Sastry, M., Biotechnology Progress, **19(6),** 1627 (2003)
[14] Sweeney, S.F., Woehrle, G.H. and Hutchison, J.E., Journal of American Chemical Society, **128,** 3190 (2006).
[15] Kalimuthu, K., Suresh Babu, R., Venkataraman, D., Mohd, Bilal. And Gurunathan, S., Colloids and Surfaces B: Biointerfaces, **65,** 150 (2008).
[16] Sadowski, Z., Maliszewska, I. H., Grochowalska, B., Polowczyk, I. and Kozlecki, T., Materials Science-Poland, **26(2),** 419 (2008).
[17] Haefeli, C., Franklin, C. Hardy, K., Journal of Bacteriology, **158,** 389 (1984).