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Isolation of bacteria from metal contaminated environment and its application in synthesis of nanosilver

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Abstract

Synthesis of nanosilver (NS) using microbes has acquired insightful interest as they deserve potential to synthesize noble metal nanoparticles of various shape and size. In the present study, NS was synthesized by a bacterial strain isolated from heavily metal contaminated soil.

Phenotypic identification showed that isolated strain may be from the category of *Bacillus* sp. Treatment of this isolated bacteria with 1 mM AgNO₃, showed to have its ability to form NS extracellularly at elevated temperature within 5 minutes. Synthesis of visual observation inferred synthesis of NS, further confirmation by UV-Visible absorption was done which gave surface plasmon characteristic peak at 450 nm. Subsequently, characterization of NS was done by transmission electron microscopy which revealed the size of NS was in the range of 50-100 nm. Present study was a preliminary study to reflect the possibility of isolation of bacteria from heavily metal contaminated soil and its subsequent application in the synthesis of NS which revealed the fulfillment of the objective.

Keywords: Silver nanoparticle, biosynthesis, metal contaminated soil

Introduction

Nanotechnology when applied in life sciences, it is called as nano-biotechnology. Nanobiotechnology can be efficiently used in the drug delivery and diagnostic purpose [2]. Silver and gold nanoparticles are the mostly studied noble metal nanoparticles by many researchers who have proved the safety, efficiency and use of these nanoparticles (NP) in biomedical as well as bioresearch applications [3]. It is needed to maximize now the therapeutic effects mutually with a minimization of the undesired secondary ones, if any. The application of noble metal nanoparticles as drug nanocarriers presents two major advantages, first, they are able to transport several therapeutic molecules adsorbed on their exterior surface and second, owing to the presence of Localized Surface Plasmon Resonances, an enhancement of the spectroscopic signals of the molecules carried used to monitor them in their traverse throughout the body or to specific tissues or organ [1]. Moreover, NPs in its sole form are also capable to show its therapeutic activity and organ targeting potential. Historically, substances such as silver salts and elemental silver have been used in health for healing wounds [10, 11], however, in the new era of antibiotics, use of silver as an antimicrobial was reduced drastically [13, 12]. The NS can be categorized as a broad spectrum [14] and aims a broad range of targets in the micro-organisms. Moreover, it was found that, microbes could not develop resistance against NS, as broad range of mutations is required to protect them.

Its complex process to study comparative growth pattern of microbes between controlled laboratory conditions and natural environment like natural soil or water environments, where always enhanced levels of complexities are encountered [8]. These complexities arise from a number of factors, including, different types of microenvironments, and a limited nutrient status, for which, different consortia of microorganisms are competing [5]. Moreover, metabolic process bacteria needed for metal reduction has not been understood in detail, but rapid advances are expected in this area as now imminent availability of entire sequences of genome for metal-reducing bacteria, as well as genomic and proteomic tools [8]. Consistent and continuous microbial interactions with the environment rich of various metals can be greatly expected to have potential to reduce the metals.

Microbes may use metal by two ways, either, as electron acceptor, or to detoxify it. Furthermore, it is becoming apparent that microbial metal reduction aids in the remediation of environments and waste streams contaminated with metals and certain organics [9].

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In the present study, area identified to collect the soil samples was industrial area of Satara, Maharashtra (Maharashtra Industrial Development Corporation). Isolated colonies were preliminarily identified by phenotypic method. Colonies were screened for the ability to reduce silver ions. Based on phenotypic investigations, strain which have ability to synthesize silver nanoparticles may belong to *Bacillus* sp. The reduction process of silver ions was preliminarily checked by visual inspection as well as by measuring its UV-visible absorption. Transmission electron microscopy revealed the synthesis of silver nanoparticles with size range of 50-100 nm^[4]. Objective of present work was isolation of metal reducing bacteria and its preliminary identification was fulfilled. Isolated strain subsequently studied to demonstrate its potential to reduce silver ions.

Materials and methods

Isolation of silver-resistant bacteria:

Soil samples were collected from heavy metal contaminated areas of Satara, India, especially industrial area where forging industries and metal waste dumping are located since long period. Soil beneath (0.5 ft deep) to heavy metal waste dumping area and area around forging in industry were used to collect the soil sample. Borer used to collect samples^[6] in polythene bags. Samples then diluted serially in sterile 0.85% NaCl (10⁻⁷)^[7, 20] and plated onto nutrient agar^[18], incubated at 36°C for 48 hrs. After incubation colonies were studied for shape and size of colony. Former includes round, irregular, filamentous and rhizoid (root-like). Later includes, large to tiny colonies, less than 1mm = punctiform (pin-point). Colonies were also studied for edge/margin. Observations were also noted for chromo genesis (color of colonies, pigmentation) viz. white, buff, red, purple, etc. opacity of colony was also observed like transparency (clear), opaque (non transparency), translucent (almost clear, but distorted vision, Other observations were also noted for elevation, surface, and consistency or texture^[19].

Colonies grown were subcultured on nutrient agar containing 1 mM filter-sterilized AgNO₃ and incubated at 36°C for 48 h and the plates were observed for bacterial growth. Colonies developed were subcultured to obtain pure culture and from this one of the strains B2 was selected randomly to explore its potential to form silver nanoparticles.

Synthesis of NS

Inoculation of strain isolated was done in 100 ml sterile liquid broth {(g/l) KH₂PO₄, 7.0; K₂HPO₄, 2.0; MgSO₄·7H₂O, 0.1; (NH₄)₂SO₄, 1.0; yeast extract, 0.6; and glucose, 10.0.}^[22] and incubated in a rotating shaker at 200 rpm for 72 h RT. Culture was centrifuged for harvesting of biomass and supernatant. Both were employed for the intra and extracellular synthesis of NS.

Extracellular synthesis was carried out by mixing supernatant with 1 Mm filter-sterilized AgNO₃ solution^[15]. For extracellular synthesis, biomass was washed thrice by suspending in double distilled water and subsequently 10 gm of it was dispersed in 100 ml of double distilled water and kept on agitator for 72 h. After incubation, filtrate was obtained by passing through Whatman filter paper. Equal quantity of 1 mM solution of AgNO₃ was mixed with 50 ml of filtrate and agitated interminantly for 72h. Heat-killed sample was also incubated along with test samples as control. 1ml of sample during this period was withdrawn periodically and analyzed spectroscopically.

Both reaction mixtures were incubated on rotating shaker (200 rpm) at RT for 72 h. periodically, both reaction mixtures were observed for NS synthesis^[16].

UV-Vis spectral analysis

UV-Visible spectral analysis was done using UV-visible spectrophotometer (Shimadzu, PharmaSpecUV-1700, Japan). 1 ml of sample was withdrawn at different time intervals, absorbance of which was measured at 437nm^[21].

Transmission Electron Microscopy

Transmission electron micrograph was used to analyze the size and shape of nanoparticles. Micrographs were obtained using a Philips EM 208 from Bombay IIT (TEM operating at 200 kV).

Results and Discussions

Isolation of the bacterial strain

Four colonies were different single colonies from Gram positive and negative bacterial divisions were isolated, respectively.

Extracellular production of NS

After incubation of the bacterial culture supernatants with silver nitrate solution at a final concentration of 1 mmol, the colors of the supernatants of 11 different bacterial colonies changed from yellow to dark brown in contrast to the negative controls which indicated the ability of NS production. Four isolates with the darkest color were identified. Extracellular production was confirmed by recording Surface Plasmon Resonance at 435nm spectrophotometric ally. Although, reduction process was confirmed by spectroscopic method, identification of the substance responsible for reduction of silver ions is equally important. To identify detailed biochemical process and number of all metabolites involved in metabolic process of microorganism is beyond the scope, rather preliminary identification was done by analyzing aqueous solution which was in contact with biomass for 72 h before addition of AgNO₃^[23]. Enzymes and proteins have difference characteristic features as size, shape, net charge, stationary phase used, and binding capacity, each one of these characteristic components can be purified using chromatographic methods especially column chromatography^[24], so in present study, filtrate was subjected to column chromatography and different elutes (each of volume 5ml) were collected and reducing capacity of each elute was determined. Total 50 ml filtrate was subjected to column chromatography, 10 elutes were obtained out of which elute 4 has shown maximum reducing capacity. This observation clearly implies that the reducing agent was present extracellularly in the filtrate and involved in the process of reduction of silver ions.

Phenotyping identification

Phenotyping identification was done and may infer that, the colony grown on solid medium containing AgNO₃ may be the *species* of *Bacillus*. Phenotyping identification of bacterial isolates with the ability of AgNPs production was done according to the microscopic properties of them. After that, the characters of the isolates were compared with those listed in Bergey's Manual of Systematic Bacteriology^[17] to identify strains preliminarily. Three isolates were screened, which were able to grow on the medium containing AgNO₃. The characteristics of the isolates were given in table no. 1

Table 1: Colony characteristics of isolates

Code	Opacity	Size	Shape	Margin	Color	Elevation	Gram stain	Arrangement	Shape
B1	Transparent	3	Round	Entire	White	Raised	+ve	Pairs	Coccobacilli
B2	Translucent	2	Irregular	Filamentous	White	Flat	-ve	chains	Coccobacilli
B3	Opaque	2	Round	Entire	White	Raised	+ve	Pairs	Rods
B4	Opaque	2	Rhizoid	Undulate	White	Flat	-ve	Pairs	Cocci

UV-Vis spectral analysis

The colour modification determined for the extracellular synthesis was additionally confirmed by UV-Visible spectral analysis. Silver nanoparticles are better-known to possess an intense absorption peak in UV absorption spectra because of its surface plasmon resonance. For the culture supernatant of B2, an absorption peak was determined at 435 nm and is a sign of formation of NS. Peak was recorded at a regular interval of time up to 72h. After 72h, no appreciable change was observed for SPR of NS. For intracellular production, peak was noted at 435nm, however, it was not intense and no appreciable change was noted over the time period up to 72h. The results were shown in figure 1. The color modification

and UV absorption data analysis therefore confirms the reduction of AgNO₃ to NS by the culture supernatant of B2. In the current study, a microorganism strain isolated from heavy metal contaminated soil samples was found to possess resistance to nitrate. Very apparently, the chosen eubacteria strain showed the power to synthesize NS by each extracellular and intracellular synthesis mechanisms. Metabolic pathway, responsible for the production of NS by bacillus is beyond the scope of present investigation, however, Isolated species may be bacillus, which can be supported by the observation made by Thomas *et al* [25], who reported bacillus may synthesize NS extra as well as intracellularly.

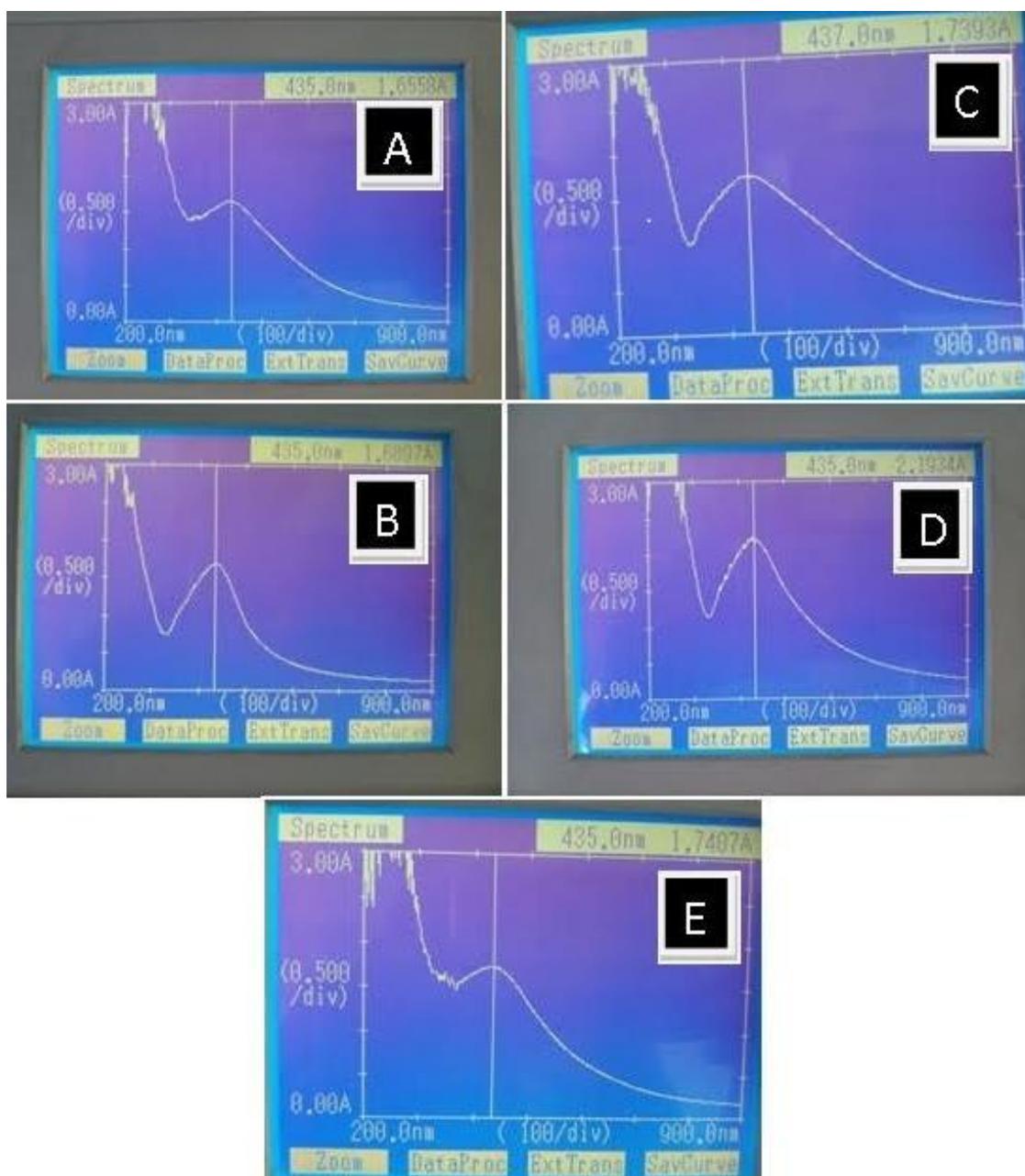


Fig 1: SPR peak for extracellular filtrate A, SPR at day1, B, SPR at day2, C, SPR at day3, D, SPR at day4, E, SPR peak for extracellular filtrate

Transmission Electron Microscopy

Transmission electron microscopy image discovered that the NS generally existed in the spherical and roughly hexagonal. The average particle sizes of NS were estimated to be 7-28 nm. NS synthesized in present investigation ranges in the region of quantum dots. If we tend to think about the 3 dimensional space vectors for a particular nanomaterial and length scales are within the critical regime of 20-50 nm, they will be referred to as 0-dimensional particles or quantum dots, typical examples being spherical (1-10 nm diameter) nanoparticles of Au, Ag, CdS, CdSe etc^[26]. SAED (Selected Area Electron Diffraction) suggested that sample was amorphous (diffuse rings) in nature. Results of which were shown in figure no. 2.

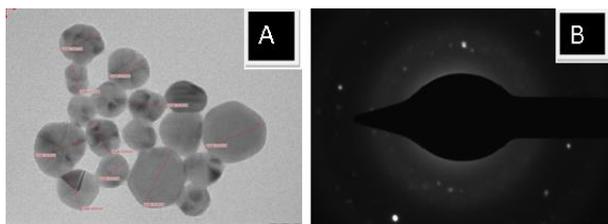


Fig 2: A, Transmission electron micrograph, B, SAED pattern.

Conclusion

Present research was focused around the ability of microorganisms to synthesize NS, which are grown in the highly metal contaminated soil. During the study abundant sampling was carried out for the isolation of microorganisms. It was noticed during study that less number of organisms are actually able to grow in the highly contaminated soil and need laborious work of collection and isolation of microorganisms. In present study, only four microorganisms were isolated and identified preliminarily. These organisms were screened for ability to synthesize NS, out of which only B2 was found to synthesize NS, intracellularly and extracellularly. Intracellular production of NS was noticed insignificant than that of extracellular. UV-Vis study and TEM confirmed the ability of B2 to synthesize NS. It may be confirmed that the soil collected from the industrial area of Satara, Maharashtra, shown the ability to synthesize NS successfully.

References

- Retrieved from <http://digital.lscic.es/bitstream/10261/104773/1/495775.pdf>
- Subramanian P, Shanmugam K. Sustainable Biological Systems for Agriculture Emerging Issues in Nanotechnology, Biofertilizers, Wastewater, and Farm Machines. Apple Academic Press, CRC Press, Taylor & Francis, 2018, 159-180.
- Rajasekar A, Janakiraman V, Govindarajan K, Asian J Pharm Clin Res. 2016; 9(5):189-93.
- Das VL, Thomas R, Varghese RT, Soniya EV, Mathew J, Radhakrishnan EK. 3 Biotech. 2014; 4(2):121-6.
- Environmental Microbiology: Ed 2 Ian L. Pepper Charles P. Gerba Terry J Gentry Raina M. Maier, October 13, Academic Press, 2011, 34-54.
- Vacca DJ, Bleam WF, Hickey WJ. Applied and Environmental Microbiology. 2005; 71(7):3797-805.
- Porter JN, Wilhelm JJ, Tresner HD, Ncbi. nlm. nih.gov. 2018 [cited 21 September 2015]. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1057598/pdf/applmicro00329-0052.pdf>
- Lloyd JR. Microbial reduction of metals and radionuclides. FEMS microbiology reviews. 2003; 1, 27(2-3):411-25.
- LovleyS DR. Dissimilatory metal reduction. Annual Reviews in Microbiology. 1993; 47(1):263-90.
- Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Applied and environmental microbiology. 2007; 15, 73(6):1712-20.
- Chudasama B, Vala AK, Andhariya N, Mehta RV, Upadhyay RV. Highly bacterial resistant silver nanoparticles: synthesis and antibacterial activities. Journal of Nanoparticle Research. 2010; 12(5):1677-85.
- Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of applied microbiology. 2012; 11, 2(5):841-52.
- Chen X, Schluesener HJ. Nanosilver: a nanoproduct in medical application. Toxicology letters. 2008; 4, 176(1):1-2.
- Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ *et al* Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2007; 1, 3(1):95-101.
- Deljou A, Goudarzi S. Green extracellular synthesis of the silver nanoparticles using thermophilic *Bacillus* sp. AZ1 and its antimicrobial activity against several human pathogenetic bacteria. Iranian journal of biotechnology. 2016; 14(2):25.
- Kannan N, Selvaraj S, Murty RV. Microbial production of silver nanoparticles. Digest journal of nanomaterials and biostructures. 2010; 5(1):135-40.
- Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA *et al* editors. Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes. Springer Science & Business Media; 2011-28.
- Usha R, Sangeetha T, Palaniswamy M. Screening of polyethylene degrading microorganisms from garbage soil. Libyan agriculture research center journal international. 2011; 2(4):200-4.
- Bisen PS, Debnath M, Prasad GB. Microbes: concepts and applications. John Wiley & Sons. 2012; (2)2:275-286.
- Kumar SV, Karpagambigai S, Rosy PJ, Rajeshkumar S. Controlling of disease causing pathogens using silver nanoparticles synthesized by one step green procedure. Journal of Applied Pharmaceutical Science. 2018; 8(01):142-6.
- Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and biosystems engineering. 2009; 1, 32(1):79.
- Bhainsa KC, D'souza SF. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids and surfaces B: Biointerfaces. 2006; 1, 47(2):160-4.
- Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R *et al* Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and surfaces B: Biointerfaces. 2003; 1, 28(4):313-8.
- Coskun O. Separation techniques: chromatography. Northern clinics of Istanbul. 2016; 3(2):156.
- Thomas R, Janardhanan A, Varghese RT, Soniya EV, Mathew J, Radhakrishnan EK. Antibacterial properties of silver nanoparticles synthesized by marine *Ochrobactrum* sp. Brazilian Journal of Microbiology. 2014; 45(4):1221-7.
- Gu H, Zheng R, Zhang X, Xu B. Facile one-pot synthesis of bifunctional heterodimers of nanoparticles: a conjugate of quantum dot and magnetic nanoparticles. Journal of the American Chemical Society. 2004; 12, 126(18):5664-5.