Volume-4, Issue-5, May-2015 • ISSN No 2277 - 8160

Research Paper | Microbiology

Biosynthesis of Silver Nanoparticles (AgNPs) Using Bacteria Isolated From Heavy Metal Contaminated Soil Sample

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ABSTRACT

Recently the biological synthesis of nanoparticles and its therapeutic use has been increased. The uses of microorganisms such as bacteria, fungi and Actinomycetes as well as materials of animals and plant origin are reported to be used for the biosynthesis of nanoparticles. In present study an attempt has been made for the synthesis of silver nanoparticles using bacterial species isolated from heavy metal (Ag) contaminated soil. All the isolates were tested for Extracellular synthesis of Nanoparticle. The nanoparticles synthesized were screened adopting silver ion reduction test and was monitored by UV-Visible spectroscopy. The nanoparticles exhibited λ max in the range of 420-450nm, corresponding to the Plasmon resonance of silver nanoparticle. Amongst total 26 isolates, only 5 isolates viz., E.coli, Klebsiella, Enterobacter, Proteus, Pseudomonas, Species showed synthesis of Ag nanoparticle this indicates the potential of soil bacteria to reduce the silver salts. The present study indicates the possibilities of bacterial transformation method for the synthesis of Silver nanoparticle as an effective and economical method over chemical synthesis.

KEYWORDS : Silver Contaminated Soil, Bacterial Transformation, Silver Nanoparticles (AgNPs)

Introduction

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht et al., 2006). These nanomaterials are already having an impact on health care. At present, different types of metal nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate, and silver. Classically the nanoparticles are produced by physical and chemical method. However these methods are not only costly and non ecofriendly, but also lead to toxic effects. Scientists are looking forward to synthesize cost effective, nontoxic nanoparticles that also will be ecofriendly (Naveen et al., 2010). One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and environmentally acceptable "green chemistry" procedures involving organisms ranging from bacteria to fungi and even plants (Bhattacharya et al., 2005 and Sastry et al., 2003). A number of microorganisms have been found to be capable of synthesizing intra or extra cellular inorganic Nano composites (Navin et al., 2011). Biological production systems are of special interest due to their effectiveness and flexibility (Nithya et al., 2011). Studies using culture supernatants of bacteria like Pseudomonas proteolytica, Pseudomonas meridiana, Arthrobacter kerguelensis, Bacillus indicus, etc., were also proven its property to form extracellular nanoparticles very effectively (Shivaji et al., 2011). Studies on reduction of Ag+ ions to AgNPs by Staphylococcus aureus also highlight the potential of extracellular method of nanoparticle formation (Nanda and Saravanan, 2009). Silver nanoparticles are undoubtedly the most widely used nanomaterials among all. Silver nanoparticles are used in textile industries, water treatment, sunscreen lotions and as antimicrobial agents (Naveen et al., 2010). The review inferred the constraints in existing methods to synthesize the nanoparticles as wells enlights the necessity of lucrative alternative method for the synthesis of the nanoparticles. Hence, in the present study the work with the focus of attention on bacterial transformation of metals to its nanoparticles, have been carried out. Soil bacteria especially from heavy metal (Ag) contaminated area were isolated & screened for its potential to transform (AgNPs).

Material and Method

Isolation and Identification of Ag-resistant bacteria

Soil samples were collected from different silver contaminated areas viz; jewellery processing shop, Ag plating industries and sarafa market yard of Washim, Akola & Buldhana Districts of Maharashtra, respectively and further used as the natural source for Ag resistant bacteria. The samples were serially diluted in sterile 0.8 % NaCl and then plated onto nutrient agar plates (Babu and Gunasekaran, 2009). The colonies obtained were further subcultured on nutrient agar supplemented with 1 mM concentration of filter-sterilized $AgNO_3$ and further incubated at 37^{0}C for 48 hrs. The cultured colonies were considered as Ag-resistant strains. These colonies were further identified and confirmed by Bergeys manual of systematic bacteriology. The identified bacterial cultures were further screened for its potential to synthesize silver nanoparticles.

Synthesis and Characterization of silver nanoparticles

For silver nanoparticle biosynthesis studies, the selected bacterial isolates were inoculated in to 250-ml Erlenmeyer flask containing 100 ml sterile nutrient broth. The cultured flasks were incubated in rotary shaker at 200 rpm at 37^{0}C for 48 h. Further, the enriched cultures were centrifuged at 5^{o}C on 12,000 rpm for 10 min. The supernatant was used for studying extracellular production of silver nanoparticles by mixing it with filter-sterilized AgNO3 solution at 1 mM concentration. The reaction mixture was incubated on rotary shaker (200 rpm) at 37^{o}C for a period of 72 hrs in the presence of light. The Heat-killed samples with $AgNO_3$ were also incubated along with experimental samples as control. Visual observation for colour reaction was conducted periodically to check for the nanoparticles formation. The synthesized silver nanoparticle containing samples were subjected to absorption analysis at 200–700 nm range using UV–Vis spectrophotometer (Hitachi U5100) and their optical characteristics were analysed (Das et al., 2013).

Result and discussion

In the present study, total 22 bacterial isolates were isolated from soil samples collected from silver contaminated areas. Out of which five bacterial isolates were found to be resistant to AgNO3 and identified by conventional method (Table 1) Viz., E.coli, Klebshella, Enterobacter, Proteus, Pseudomonas Species. Hence, the selected isolates were further subjected to biosynthesis of silver nanoparticles using extracellular component of an enriched culture and were investigated primarily for the observation of colour change test. The results in (Fig 1.) showed the reduction of AgNO3 to silver nanoparticles by the culture supernatant analyzed as compared to control, in which no colour change was recorded. Observation on colour change is a method reported for screening microbial isolates for silver nanoparticle synthesis (Kalimuthu et al., 2008). The excitation of surface Plasmon vibration in the silver nanoparticles was considered as the basis for formation of brown colour. Similar observation was previously reported for the supernatant of Bacillus megaterium, where a pale yellow to brown colour was formed due to the reduction of aqueous silver ions to silver nanoparticles (Saravanan et al., 2011). The samples were fur-

ther subjected to UV–Vis spectral analysis as part of primary confirmation of Silver nanoparticles. The maximum absorption peaks recorded were in the range of 420-450nm for all isolates studied (Fig. 2.) the peaks recorded was in correlation with the peak range reported by reviewed workers. Methods based on UV–Vis spectroscopy have been shown to be an effective technique for the analysis of nanoparticles (Sastry et al., 1998). This indicates the formation of silver nanoparticles and thus confirms the reduction of AgNO3 to silver nanoparticles due to the extracellular component of an enriched culture. Hence, the extracellular component may be considered as the source of enzymes for transformation of nanoparticles.The mechanism behind the extracellular synthesis of nanoparticles using microbes is not fully known. But it is considered that the enzymes like nitrate reductase secreted by microbes help in the bio reduction of metal ions to metal nanoparticles (Duran et al., 2005). This was reported in Bacillus licheniform is where nitrate reductase secreted by the bacteria was found to be responsible for the reduction of Ag ions to nanoparticles (Kalimuthu et al., 2008). Presence of such peak, assigned to a surface plasmon, was also well documented for silver nanoparticles as reported in the case of Neurosporacrassa (Longoria et al., 2011).

Conclusion

In conclusion, the present work demonstrates the potential of metal resistance bacteria to transform silver nanoparticles by extracellular mechanisms. However, further research should focus on optimisation study for large scale production of Silver nanoparticles. Focus may also be given towards the Toxicity studies of Silver nanoparticles on human pathogenic in relation to human physiology which may open a door for new range of antibacterial agents.

Table 1: Characterization of Metal ($AgNO_3$) resistance bacterial isolates.

Table 1.1- Morphological characters

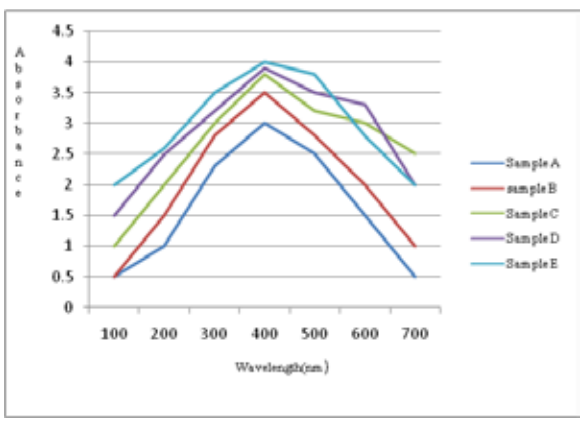
Morphology	Sample A	Sample B	Sample C	Sample D	Sample E
Shape	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Irregular	Entire	Irregular
Elevation	Raised	Slightly Raised	Convex	Raised	Flat
Surface	Smooth	Mucoid	Smooth	Smooth	Smooth
Opacity	Opaque	Translucent	Opaque	Translucent	Translucent
Color	Grayish white	Colorless	Yellow pigmented	Creamy white	Bluish green pigmented

Sr. No	Characters	A	B	C	D	E
1.	Gram Staining	-ve	-ve	-ve	-ve	-ve
2.	Motility	+ve	-ve	+ve	+ve	+ve
3.	Biochemical Test					
3.a	Indole	+ve	-ve	-ve	-ve	-ve
3.b	Methyl Red	+ve	-ve	-ve	+ve	-ve
3.c	VP	-ve	+ve	+ve	-ve	-ve
3.d	Citrate	-ve	+ve	+ve	-ve	+ve
3.e	H_2S	-ve	+ve	-ve	+ve	-ve
4.	Sugar Fermentation					
4.a	Glucose	+ve	+ve	+ve	-ve	+ve
4.b	Lactose	+ve	+ve	+ve	-ve	-ve
4.c	Sucrose	+ve	+ve	+ve	-ve	-ve
5.	Enzyme study					
5.a	Catalase	+ve	+ve	+ve	+ve	+ve
5.b	Oxidase	-ve	-ve	-ve	-ve	+ve
5.c	Urease	-ve	+ve	-ve	+ve	+ve
5.d	Gelatinase	-ve	-ve	-ve	+ve	+ve
Possible Species Sample A -*E.coli spp.* Sample B- *Klebsiella spp.* Sample C- *Enterobacter spp.* Sample D- *Proteus spp.* Sample E- *Pseudomonas spp.*						

Table 1.2 – Biochemical characters

Fig1: Reduction colour test for AgNO3

Fig :2 Absorbance spectra of silver nanoparticles synthesized by bacterial isolates.



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