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Substrate Dependant Microbial Synthesis of Silver Nanoparticle and its Application as Antimicrobial Agent

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Microbial synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and microbial biotechnology. In this present study, synthesis of silver nanoparticles (AgNPs) has been demonstrated using a metabolically versatile actinobacteria *Rhodococcus* sp. by reducing aqueous silver nitrate. The AgNPs were characterized by Ultraviolet–Visible (UV – vis) Spectrometer, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Dynamic Light Scattering (DLS), Selected Area Diffraction Pattern (SAED) and Transmission Electron Microscopy (TEM). The TEM showed spherical particles with an average size of 10 nm. The SAED pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face centered cubic (fcc) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature.

Keywords: Biosynthesis, Silver Nanoparticles, Rhodococcus sp., Biomaterials, Biotransformation, Substrate Variation.

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1. INTRODUCTION

The intense light emission properties of noble metals (gold, silver, etc.) nanoparticles have caught a lot of attention. In variety of biological techniques of nanoparticle synthesis, microbial synthesis would help circumvent many of the detrimental features by enabling synthesis at mild pH, pressure and temperature and at a substantially lower cost. Biosynthesis of metal and metal alloy nanoparticles by bacteria, actinomycetes, fungi, yeasts and viruses have been reported [1]. AgNPs have been used mostly for the synthesis of stable dispersions, which are useful in areas such as photography, catalysis, biological labeling, photonics, optoelectronics, surface-enhanced Raman scattering (SERS) detection and antibacterial activity [2, 3]. Several plant extracts such as alfalfa, aloe vera, amla, Capsicum annuum, geranium, coriandrum, tea, and neem were used for green synthesis of AgNPs [4-11]. There are re-ports on use of microorganisms such as Bacillus koriensis, Bacillus subtilis, etc. and fungi such as Aspergillus fumigatus, Fusarium oxysporum, etc. for the biosynthesis of AgNPs [12-14]. The culture supernatant of Pseudomonas aeruginosa was also used for green synthesis of AgNPs [15]. The versatile genus Rhodococcus are suitable industrial microorganisms not only for biotransformation but also for biodegradation of many organic compounds as they are able to uptake and metabolize hydrophobic compounds, and persist in adverse conditions.

In the present paper, green biological route for the synthesis of silver nanoparticles of different sizes with variation of the substrate using *Rhodococcus* sp. is demonstrated. The biosynthesis method is optimized for room temperature and pH 7. The nanoparticles have been characterized by UV–vis spectroscopy, SEM-EDAX, DLS, zeta potential, TEM and XRD analysis. The antimicrobial activity of the AgNPs was also observed on different pathogenic Gram positive and Gram negative organisms.

2. MATERIALS AND METHODS

2.1 Synthesis of AgNPs

Rhodococcus NCIM 2891 was maintained on Glucose Yeast extract Malt extract (GYEME) agar medium at 4°C. The bacterium was inoculated in two 500 ml Erlenmeyer flasks containing sterile M9 medium [sodium acetate (0.45 %) as carbon source] without NaCl in double distilled water having pH 7.0 prior to autoclaving, and incubated in dark on orbital shaker (130 rpm) for 24 h at room temperature and used as the inocula for further experiments. The effect of the $AgNO_3$ was studied with variation from 1 mM to 10 mM with sodium acetate as substrate. Then the organism was grown on different substrates as glucose, sucrose, fructose, mannose, mannitol and sodium acetate and incubated for 24 h at 30°c. After the incubation period aqueous solution of $AgNO_3$ (Lobachemie Pvt. Ltd. of GR grade) at a final concentration 1m M was added to each flask with culture and to the other flask containing supernatant obtained by centrifuging at 8000 rpm for 10 min. These flasks were further incubated in dark for 18 h at room temperature under agitation (130 rpm). The formation of the AgNPs was monitored by UV – vis spectroscopy using Shimadzu (Model No-UV 1800) double beam UV–vis spectrophotometer.

2.2 Characterization of AgNPs

The air dried culture mass was scanned on a Philips SEM equipped with an EDAX attachment. The SEM micrograph of the $AgNO_3$ exposed, 2.5 % glutaraldehyde fixed and alcohol dehydrated microorganisms and the corresponding EDAX spectrums were recorded by focus-

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ing on clusters of particles. DLS and Zeta potential measurements of the AgNPs formed were performed using NICOMP™ 380 ZLS (Santa Barbara, California, USA) a particles size distributions in culture broth. The supernatant of the flask showing brown color change was transferred on to a carbon coated copper grid and allowed to dry for TEM. The grid was then scanned using Philips CM200 Model TEM, operating voltage 20–200 kV with resolution 2.4 Å. The XRD pattern of silver nanoparticles drop coated and air dried on the glass substrate was recorded. Lattice parameters were calculated by using high-angle reflections of XRD. Using the Scherrer's equation

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

where $\lambda = 1.5405$ Å wavelength of incident X-ray, θ the corresponding Bragg's diffraction angle and β the full width at half maxima (FWHM) of X-ray diffractions, crystal size of AgNPs was calculated.

2.3 Antimicrobial activity

Antimicrobial activity of AgNPs was observed on *E.coli*, *Enterococcus fecalis*, *Staphylococcus aureus*, *Klebsialla pneumoniae*, *Pseudomonas arugenosa* and *Proteus vulgaris* on the growth cycle with different concentration as 30 µg/ml, 50 µg/ml and 100 µg/ml.

3. RESULTS AND DISCUSSIONS

The change in color from white to brown was observed in the Erlenmeyer flask containing culture after incubation for 18 h which was absent in the flask containing supernatant of the culture. UV–vis spectroscopy was primary used to detect the AgNPs synthesis. The effect of various concentrations of $AgNO_3$ was shown in the Fig.1. The maximum intensity of AgNPs is shown for 1mM and further increase in the concentration caused for the decrease in the intensity. So 1mM $AgNO_3$ was used for further experiments. The Fig. 2 shows the characteristic surface plasmon resonance (SPR) peak at about 405 nm for AgNPs in the culture [16] which was not observed in the supernatant treated with $AgNO_3$.

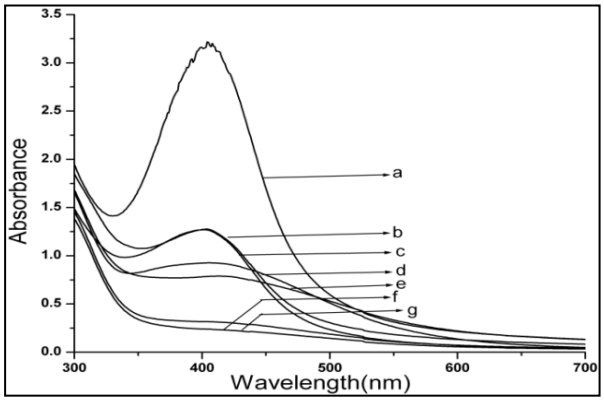

Fig. 1 – UV–vis spectra showing effect of the various concentration of the $AgNO_3$ from 1 mM to 10 mM on the synthesis of the AgNPs

This SPR peak may correspond to spherical AgNPs [17]. The synthesis of AgNPs was observed in the ask containing glucose, mannitol, sucrose, fructose and sodium acetate.

There is no detectable amount of AgNPs were formed when Rhodococcus was grown on maltose and lactose as carbon sources. The SEM image of glutaraldehyde fixed microorganisms is given in Fig. 3a which shows the AgNPs attached to the microorganisms of size around 100 nm. The compositional analysis was done by EDAX and showed characteristic signals of crystalline AgNPs at 3 keV (Fig. 3b) [18]. Fig. 3c is showing DLS of the AgNPs synthesized in medium containing sodium acetate as carbon source.

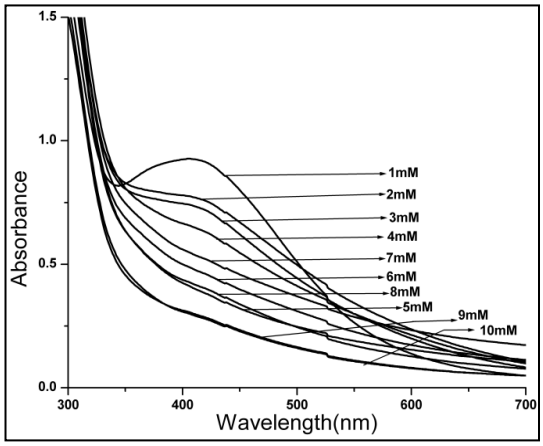

Fig. 2 – UV–vis spectra showing absorption recorded as a function of 1mM aqueous solution of $AgNO_3$ with *Rhodococcus* sp. grown in different carbon source as a) glucose, b) mannitol, c) fructose, d) sodium acetate, e) sucrose, f) maltose and g) lactose.

AgNPs with sodium acetated showed large zeta potential and uniform particles size of 29 nm. The Table. 1 is showing the parameters associated with synthesis and stability.

Table. 1 – Parameters associated with different characteristics of AgNPs.

Sr. No.	Substrate	SPR band (nm)	DLS (nm)	Zeta potential (mV)
1	Glucose	404	23	5.50
2	Mannitol	402	45	28.74
3	Fructose	403	22	11.50
4	Sodium acetate	404	35	45
5	Sucrose	413	38	11.50

The TEM image illustrates that average size of AgNPs is 10 nm, confirming spherical shape (Fig. 4a). SAED spots that corresponded to the (111), (200), (220) and (311) planes of face-centered cubic (fcc) structure of elemental silver are clearly seen in Fig. 4b. The obtained XRD spectrum of silver nanoparticles (data not shown) is matched with JCPDS Card No. 89-3722 which exhibits the characteristic peaks of the silver crystallites observed at 2θ values of 37.8°, 44.1°, 62.9°

and 75.9° corresponding to (111), (200), (220) and (311) of the face centered cubic (fcc) silver nanoparticles, which is in agreement with SAED result. The crystal size of the AgNPs was calculated from Scherer's equation using FWHM value measured for (111) plane of reflection. The crystallite size obtained from XRD is about 15 nm which is in agreement well with the TEM results. AgNPs showed very good antimicrobial activity for all pathogenic microorganisms above 30 μg/ml concentration. There is complete inhibition for 50 μg/ml and 100 μg/ml of AgNPs (Fig. 5). Most of the microorganisms developed resistance after prolong lag phase to the 30 μg/ml AgNPs.

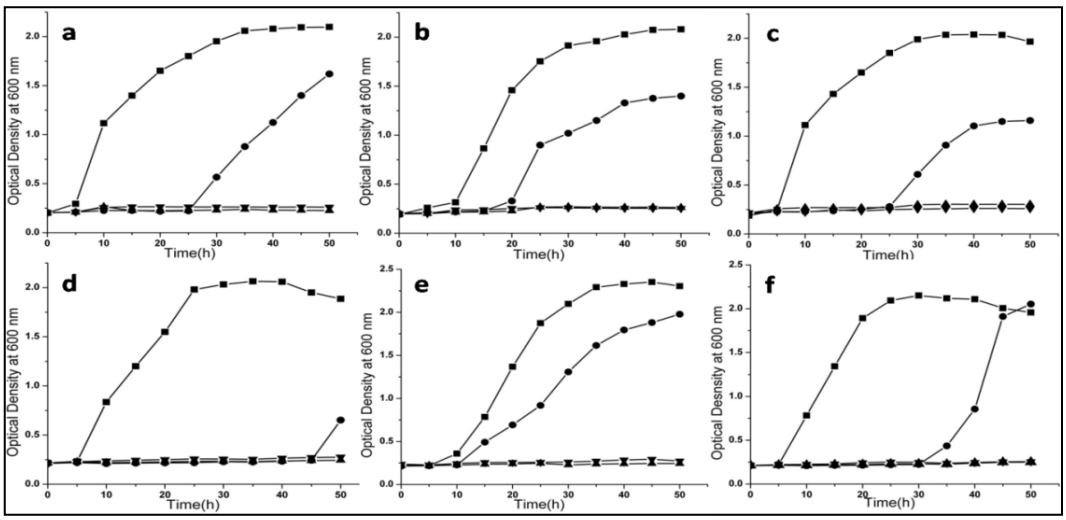

Fig.5. Antimicrobial activity of silver nanoparticles on a) *E.coli*, b) *Enterococcus fecalis*, c)*Staphylococcus aureus*, d) *Klebsialla pneumoniae*, e) *Pseudomonas arugenosa* and f) *Proteus vulgaris* with different concentration as —■— control, —●— 30 μg/ml, —▲— 50 μg/ml, —▼— 100 μg/ml

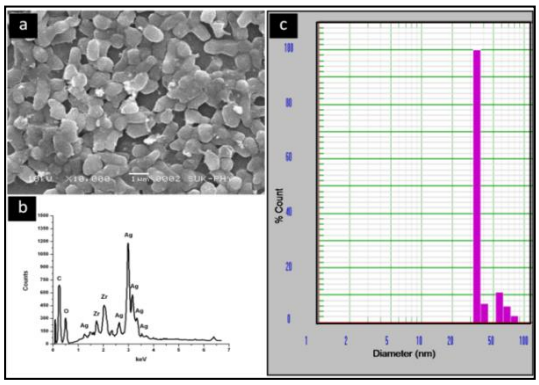

Fig. 3. – (a) SEM image Rhodococcus synthesizing AgNPs incubated for 24 hrs with aqueous $AgNO_3$ (b) EDX spectrum of the biomass showing presence of silver (c) DLS histogram of AgNPs in colloidal solution (number wt NICOMP distribution)

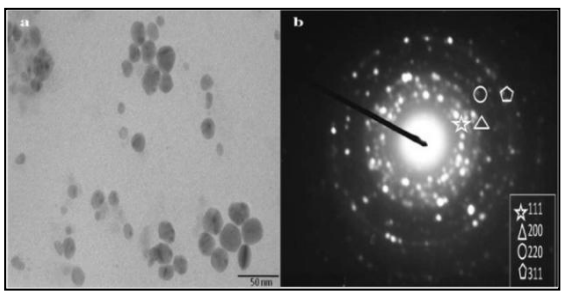

Fig. 4 – (a) TEM image of spherical AgNPs, and (b) SAED showing the characteristic crystal planes of elemental silver

The change in color from white to yellow was not observed in the flask containing culture supernatant exposed to $AgNO_3$, as the synthesis of AgNPs did not occur in the culture supernatant. Hence it is evident that proteins and carbohydrates secreted by organisms or media components present in the culture supernatant did not carry out the transformation of $AgNO_3$ to AgNPs, ruling out the chemical conversions. The color change i.e. AgNPs synthesis was seen in the flask containing culture. This reduction reaction of Ag^+ to Ag° may be carried out by the enzyme systems of the *Rhodococcus* sp. present inside the cell, signifying the biological conversion. The enzyme involved in the synthesis of AgNPs may be α-NADH dependent nitrate reductase which was shown to be present in *F. oxysporum* [19].

In *Bacillus licheniformis*, the nitrate reductase enzyme was found at the membrane, called as respiratory enzyme [20]. The activity of the nitrate reductase enzyme was demonstrated in *Rhodococcus* sp. in the presence of higher nitrate concentration but it was not of respiratory type [21]. *Rhodococcus* was found tolerant to 1mM $AgNO_3$ as SEM image (Fig. 3a) shows the intact microbial cells after 24 h of incubation with $AgNO_3$. The intensity of AgNPs decreased as increase in the $AgNO_3$ concentration. Higher concentration of $AgNO_3$ may inhibit the growth of the microorganism and so to the synthesis of the AgNPs. Thus the synthesis of AgNPs may occur intracellular. The size variation of AgNPs may be due the expression of proteins and carbohydrates for different for the different substrate. These proteins and carbohydrates may not allow the agglomeration and oxidation of the AgNPs in the colloidal solution.

4. CONCLUSIONS

Bacteria are easy to handle and can be manipulated genetically without much difficulty. Considering these advantages, a bacterial system could prove to be an excellent alternative for synthesis of AgNPs. *Rhodococcus* NCIM 2891 can be a good candidate for the synthesis of the AgNPs using silver nitrate of average size 10 nm. The synthesis of different size AgNPs can be done by only altering the substrate for the growth of *Rhodococcus* sp. The efficiency of the antimicrobial drug can be enhanced with addition of these cost effective AgNPs and can reduce the cost of the production of these expensive synthetic drugs.

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