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Ecofriendly and cost effective synthesis of silver nanoparticles by *Bacillus subtilis* NCIM 2010

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ABSTRACT

An economic and simple process of green silver nanoparticles synthesis was attempted by Bacillus subtilis NCIM 2010. The cell suspension of Bacillus subtilis NCIM 2010 prepared in sterile distilled water and utilised as mediator of silver nanoparticles fabrication at room temperature. The synthesis of particles occurs during incubation. The aqueous silver ions present in sterile distilled water getting reduced to their respective nanoparticles after 48 h of treatment at pH 7.0 and 30°C. The structural characteristics of nanoparticles were revealed by various analytical procedures. Peak obtained at 430 nm in UV visible spectroscopic analysis confirms the presence of particles which is specified for its surface Plasmon resonance, Synthesized particles are in spherical form and having practical size ranging from 7 to 40 nm with average 17 nm and showed crystal nature. TEM analysis revealed surface anaionic charge on synthesized nano particles. These nanoparticles have characteristic broad spectrum antibacterial properties for different Gram negative and positive bacteria. However, there is a quite resistance showed by selective Gram-negative microorganisms.

KEYWORDS

Nanoparticles, Bacillus, Antibacterial, Silver nanoparticles.

1. INTRODUCTION

Nanobiotechnology gained lots of attention in current decade because of its predictable influence in different fields like power, pharmaceutical, electrical devices and spacecrafts industry. From last decade this area of science increased its demand exceptionally around the globe [1]. Amongst all metalonanoparticles, AgNPs had various essential applications; because of its antibacterial and disinfecting properties and well known ever since Roman empire era. Though, advancement in synthesizing silver nanoparticles is increased because of its bactericidal properties [2] AgNPs shares one of the significant inputs at the era of bio-labelling, sensor technologies, microbicidal components and in filtration technologies. The silver nanoparticles also having potential to be safe utilized in water purification plants, in pesticide denaturation as well as in humans it acted as a potent agent for iradicating different disease causing virulent microbial pathogen. At present, the exploration of silver nanoparticle in antimicrobial treatment fact has gain attention because of increase in multiple drugs amongst microorganism [3].

Various physicochemical methodologies like electro-chemical, ultra sonication assisted, photo-chemical, reverse micelles, emission, etc. where been reported for fabrication of nanoparticles [4] but, these techniques has limitation of expensiveness and secondary pollution due to utilization of chemicals for synthesis and would exert toxicity to the natural flora-fauna and other biological entities of natural ecosystems and also to the environment [5] As biological or green synthesis of nanoparticles consumes different prokaryotes and eukaryotes organallies and cells and reported for having a good quality for have inorganic nanomaterial's fabrication by intracellularly or extra-cellularly with their biomineralization process of [6]. Microorganism assisted fabrication of silver nanoparticles is recognised as a renowned and hopeful method for exploitation of various kinds and properties bearing silver nanomaterials [7]

Manufacture of nano size metallic AgNPs with diverse structural properties and sizes by various route were been already documented the simple method for the AgNPs synthesis concerning a reduction of silver salts and these will be carried out by physical method involves reduction of an ionic salt in an suitable media with surfactant using various reducing agents, such as sodium borohydride [8] hydrazine hydrate, [9] sodium citrate, (Pillai et al., 2004)and ascorbic acid [10] there are different type of microorganism which were used for synthesis of AgNPs including bacteria, Fungi, actinomycets and fungi [11]. In this context we studied the synthesis of AgNPs by *Bacillus subtilis* NCIM 2010 its characterization and its antibacterial activities.

2. EXPERIMENTAL SECTION

2.1. Materials

Microorganism used- *Bacillus subtilis* NCIM 2010 was got from National Collection of Industrial Microorganisms (NCIM - Pune) India. Obtained pure strain repeatedly transferred grown and maintained on Nutrient agar at 4°C during study *B. cereus* NCIM 2703; *S. aureus* NCIM 2654; *P. earuginosa* NCIM 5032; *P. vulgaris* NCIM 2813 and *S. typhi* NCIM 2501 were used for analysis of antibacterial activity of AgNPs, were grown on nutrient agar and maintained on same medium at 4 °C until their use.

2.2. Synthesis of Silver nanoparticle

Growth of *Bacillus subtilis* NCIM 2010 was obtained by inoculating and grow organism in 250-mL conical having 100 mL nutrient broth at 30°C and 110 rpm on rotary shaking incubator for 24 h. completely incubated broth having cell density of 5×10^7 cells / mL was then centrifuged at 4000 X g for 10 min to obtain cell biomass. Collected biomass was then washed with sterile distilled water to make this biomass resudual component free and re-suspended gain same volume of (100 mL) in sterile distilled water with 1 mM $AgNO_3$, and allowed to incubate at 25°C after incubation cell free extracts were characterized by UV-visible spectrophotometry.

2.3. Analysis of AgNPs and its Characterizations

a. UV/visible spectroscopic investigation

The depiction of the silver nanoparticles was done by UV/Visible spectroscopy using a Hitachi U-2800 double beam spectrophotometer. The wavelength spectra of cell free extracts was taken starting from wavelength 300nm to 800nm and observed for the appearance of the peak in given wavelength. For determination of the maximum synthesis of AgNPs same experiment was done after interval of 6h from the inoculation point.

b. X-ray Diffraction studies

The purified crystalled powder of metal AgNPs was examined by X –ray diffraction using Philips analytical – PW3710, X ray diffractometer with a target CrKα (α = 2.28 Ao) the generator was operated at 40 Kv with a 25mA current. The scanning range (2θ) was 10o to 100 o.

c. TEM

The morphological characters of AgNPs were obtained monitoring silver nanoparticles for TEM. The analysis, of an aliquot of aqueous solution of silver nanoparticles was locate on copper grid which is carbon coated, sample is allowed to dry. Scanning of this grid done by with a Philips model (M200) Transmission electron microscope monitored at a current from 20 - 200 kV with 2- 4 Ao resolution.

d. Effect of pH and Temperature on AgNPs synthesis

To study various pH and Temperatures impact on AgNPs synthesis, AgNPs synthesis by microorganism was checked at diverse temperatures like 15, 20, 25, 30 35, 40 and 45oC and from pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10 by monitoring an absorbance at 430 nm.

e. Antibacterial activity of AgNPs

Antimicrobial activity of AgNPs was tested against Gram negative bacterial strains, by agar well diffusion assay [12]. Inhibitory zones were noted after incubation.

f. Statistical analysis

Values obtained are mean of three or additional factors. Examination of the variants done by all data at $P< 0.05$ using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

Aqueous solution of 1mM $AgNO_3$ supplemented with cell biomass which caused development of AgNPs within 12h due to reductive ability microorganisms on silver ions was visibly detected through colour change as colourless to greenish and greenish to yellowish brown. The extent of AgNPs synthesis was checked with UV/Visible spectroscopic technique by evaluating distinguished absorption peak was observed near about 430nm (**Figure-1**).

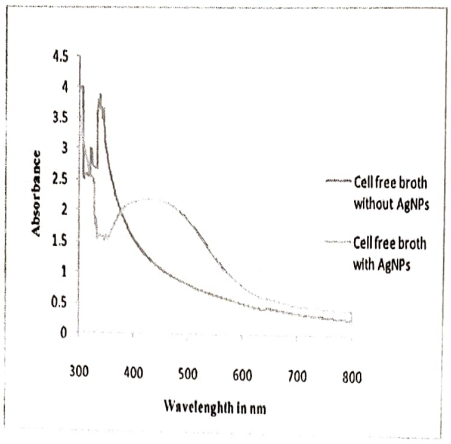

Figure-1. spectroscopic analysis of AgNPs synthesized by *Bacillus subtilis* NCIM 2010.

To determine maximum nanoparticles synthesis absorbance were recorded at the function of time with a definite time interval and the optimum absorbance was observed after 48 h and afterwards it remains constant (**Figure-2**).

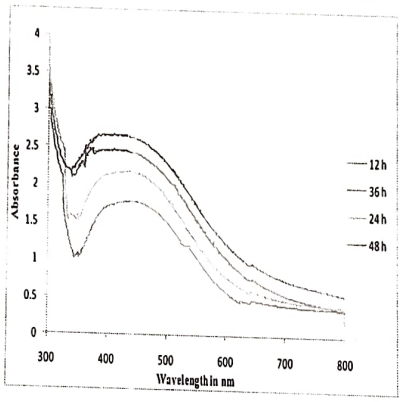

Fig.2

Figure-2. Synthesis of silver nanoparticles at different time interval of incubation.

Previous studies suggested that many bacteria having ability to produced AgNPs but duration of synthesis and its structural forms were different from each other such as *B. lechiniformis* has ability to synthesize the nanoparticles maximum in stationary phase [13]. *Bacillus Sp* produces Ag nanoparticles in their periplasmic space of the cell [8]. described production of AgNPs by yeast strain MKY3 in logarithmic phase [14]. elaborated the AgNPs synthesis with utilising spore crystals mixture of *Bacillus thurengenesis*. Other reports suggested that some bacteria synthesize nanoparticles extracellularly. *Pseudomonas stutzeri* intracellular synthesizing particle size approximately 200 nm [11,15], whereas AgNPs Synthesized by *Morganella sp.* were produced extracellularly and having size 20–30 nm [16], similarly silver and gold nanoparticles synthesis was carried out intracellularly by *Lactobacillus* strains [17] *Lectonemaboryanum* (Cyanobacteria) synthesizes Ag nanoparticles Intracellulary 1–10 nm [18].

The AgNPs exhibit an absorption spectral peak at 430nm at 12 h of reaction, in initial state after 6h medium starts to change the colour from transparent to green and after 9h it changes the colour from green to brown. The absorbance detected at 430nm is a typical SPR - band (surface plasmon resonance) of AgNPs and its observed so due to the excitation of longitudinal plasmon vibrations in silver nanoparticles [17, 19]. (**Figure-3**) shows forms of X-ray diffraction for the AgNPs. results display the incidence of diffraction peaks diffraction of face center cubic silver in the hole spectrum of 2θ value ranging from 10 to 80.

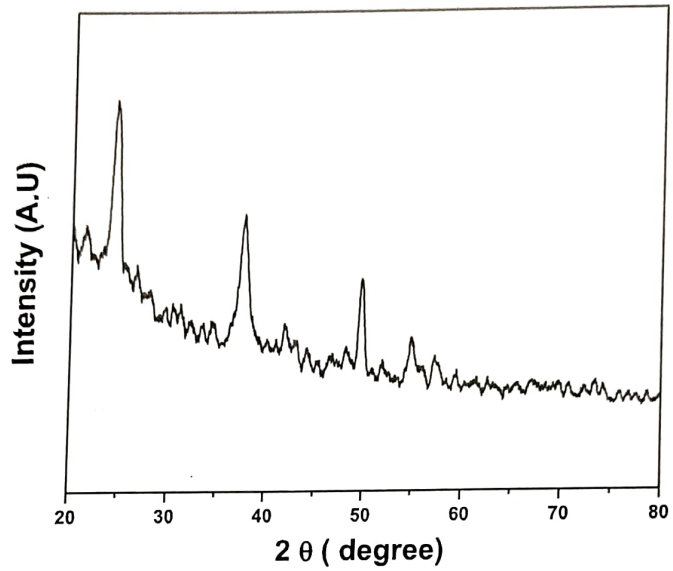

Figure 3. XRD evaluations of AgNPs synthesized by *Bacillus subtilis* NCIM 2010.

The average dimensions of crystalline AgNPs assessed by FWHM is (111) peak calculated by the Scherrer formula is 9.7 nm. On the scale of nanometer, maximum of face centered cubic (fcc) arranged metallic structures included silver were getting nucleated and grown within twinned and multiplied twinned AgNPs with their surfaces bounded by the minimum energic level (111) facets [20].

From TEM results (**Figure-4a**) it was exploited that AgNPs were mono-dispersed and round shaped. The particle size variation in size was seen in TEM micrograph (**Figure-4b**). The synthesized AgNPs were found to have size in between of 7–40 nm and average 17 nm.

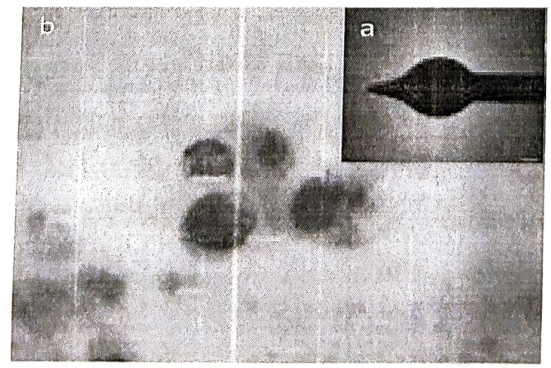

Figure-4. TEM analysis of AgNPs a) Inset: Selected area electron diffraction outline of AgNPs. b) TEM image of the AgNPs synthesize by *Bacillus subtilis* NCIM 2010 Scale bar corresponds to 50 nm.

From optimization studies it was revealed that *Bacillus subtilis* NCIM 2010 synthesize silver nanoparticles optimally at 7 pH and 30°C. whereas best gold agumetation by microorganisms was normally occured in the 2–6 pH [11, 15] and variations in the pH has affected on the size of silver nanoparticles as shown in figure (**Figure-5**)

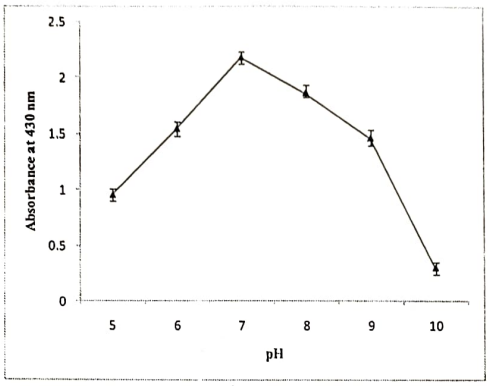

Figure-5. Synthesis of AgNPs at different pH.

and similar results are also observed in case of temperature optimization where the optimum temperature of AgNPs synthesis was 30°C and for other temperature it was decreasing (**Figure-6**).

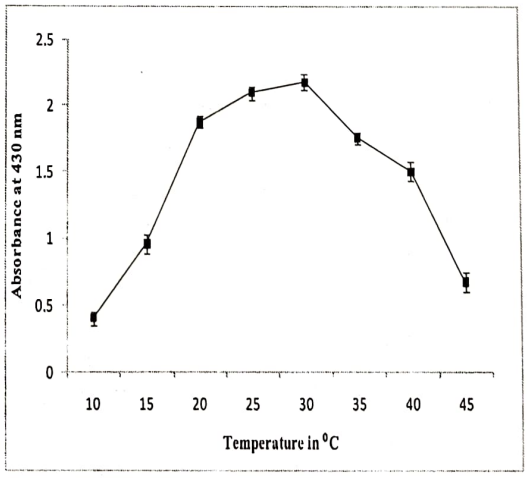

Figure-6. Synthesis of AgNPs at different Temperature.

It was revealed by previous studies that the silver nano particles possess antibacterial activity against of microorganisms selected from the ATCC strain collection and various another microorganism [21]. The antagonistic pattern of AgNPs on microbial cells showed to retard the DNA replication and translation processes so could impact on synthesis of some essential cellular and ribosomal proteins because of AgNPs action [22].

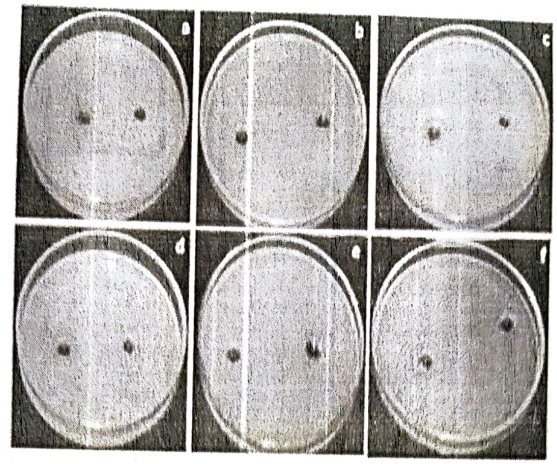

Figure-7. Antimicrobial activity of AgNPs on a) *Bacillus cereus* NCIM 2703 , b) *Staphylococcus aureus* NCIM 2654 , c) *Bacillus subtillis* NCIM 2635 d) *Pseudomonas earuginosa* NCIM 5032 e) *Proteus vulgaris* NCIM 2813 f) *Salmonella typphi NCIM 2654.*

(**Figure-7**) shows the results of antimicrobial activities it is seen that synthesized AgNPs shows the antimicrobial activities counter to some Gram negative and some positive bacteria. Within experimental microorganism zone of inhibition in diameter was observed against *Bacillus cereus* NCIM 2703, *Staphylococcus aureus* NCIM 2654, *Bacillus subtillis* NCIM 2635 and *Pseudomonas earuginosa* NCIM 5032 however *Proteus vulgaris* NCIM 2813 and *Salmonella typhi* NCIM 2654 does not show the zone of inhibition (**Figure-7; Table-1**). The increase in silver resistance in microorganism was observed due to extensive and abandoned use of Ag may result in more emerging bacterial resistant [23]. Though, these bacterial specificity in resistance within microorganism is depend on the possibility of transmission of Ag resistance gene and is lowest among other resistance [24, 25], that is the incidence of frequency of AgNPs is different amongst other organism [24]. Examination of the occurrence of silver resistance is very significant due to plasmid transfer and responsible for inter resistance amongst microorganism [3].

Table-1. Antimicrobial activity of AgNPs synthesized by *Bacillus subtilis* NCIM 2010.

Test microorganism	Zone of inhibition in diameter
B. cereus NCIM 2703	12 mm± 0.5
S. aureus NCIM 2654	13 mm± 0.2
B. subtillis NCIM 2635	16 mm± 0.3
P. earuginosa NCIM 5032	15 mm± 0.4

P. earuginosa NCIM 5032	-
S. typhi NCIM 2501	-

(-) = results not observed

Obtained results are the mean of three sets at (± SD) inhibitory impact of AgNPs shown to be significant from control as *P < 0.05.

4. CONCLUSION

In conclusion the cell suspension prepared in sterile distilled water of the *Bacillus subtilis* NCIM 2010 utilised as green synthesis of AgNPs with $AgNO_3$ at moderate temperature. It was anticipated that reductivity in silver ions is because of microbial production of different macromolecules especially proteins that may be contributed by the nitrate reductase enzyme. The structural characteristics of nanoparticles were revealed by various analytical procedures. The UV/Visible spectral analysis shown to have a spectral peak at about 430 nm, which typicaly observed for AgNPs. The AgNPs formed were round shaped with an average 17 nm particle size with crystal structure and anionic nature confirmed by TEM. The AgNPs seen to have large spectrum of antibacterial activities against various Gram-positive and negative bacteria but there is a resistance that observed in case of selective Gram-negative microorganisms which might be due to the extra chromosomal characteristics of that microorganism.

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