ORIGINAL RESEARCH PAPER

Microbiology

Biological Synthesis of Silver Nanoparticles and Its Antimicrobial Activity

KEYWORDS:

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ABSTRACT

In present work silver were nanoparticles (AgNps) were synthesized by using Bacillus subtilis sp. Biomass of Bacillus subtilis were added into the reaction vessel in which 100 ml of silver nitrate (1 mM) solution. The silver nanoparticles were characterized by Transmission Electron Microscopy (TEM), UV-Visible Spectroscopy (UV). Antibacterial activity was studied against the human pathogenic microorganism such as C. albicans NCIM 715, K. pneumoniae NCIM 5082, S. aureus NCIM 5084. The nanoparticles demonstrated antimicrobial activity against all of these pathogenic microorganisms. These results indicated that the biosynthesized silver nanoparticles showed excellent antimicrobial effect against bacterial pathogens.

INTRODUCTION

Currently, the development of a biological methodology for the synthesis of nanoparticles as well as for their characterization and applications on medical and environmental platforms are important aspects of nanotechnology. Research on nanoparticles is currently an area of intense scientific interest due to its wide range of applications (Abdulrahman, Krajczewski, Aleksandrowska, & Kudelski, 2015; Park, Lee, and Lee, 2016; Taylor, Coulombe, et al., 2013; Yahyaei et al., 2016). In spite of being the size of the ultra- fine particles individual molecules are usually not referred to as nanoparticles (Hewakuruppu et al., 2013). Nanoparticles form a bridge between bulk materials and atomic/molecular structures. Nanoparticles do not need to have constant physical properties, they may vary (Taylor, Otanicar, et al., 2013).

From a medical perspective, the development of resistance mechanisms against antibiotics by pathogenic microorganisms has been a subject of major concern.10 These resistance mechanisms, due to several enzymatic and genetic mutations in the pathogens that cause infectious diseases, has encouraged researchers to design new antimicrobial agents against pathogens to control infections. (Sibanda and Okoh 2007). Therefore, there is always a need to develop new alternatives to control pathogenic organisms. Metal nanoparticles are an effective way to control many pathogenic and antibiotic-resistant microorganisms (Kollef et. al., 2011). Nanoparticles are applicable in diverse areas such as medicine, diagnostic agents, drug and gene delivery, electronics, cosmetics, coatings, biosensors, imaging, and environmental remediation. Among the many metal nanoparticles, silver nanoparticles have been intensely studied because of the distinct properties of their optical behavior, conductivity, chemical stability, and catalytic activity (Rai et. al., 2012)

The present research was carried out to explore a biological method for the synthesis of silver nanoparticles and to apply these nanoparticles in controlling pathogenic microorganisms. Silver nanoparticles were biosynthesized by Bacillus subtilis. These nanoparticles were tested against different pathogenic microorganisms for their antibacterial efficacy.

MATERIALS AND METHODS

Microorganisms

Microorganisms selected for this study was Bacillus subtilis Sp, C. albicans NCIM 715, K. pneumoniae NCIM 5082, S. aureus NCIM 5084. which was obtained from NCCL, Pune and was maintained on nutrient agar.

Synthesis of AgNps

The Bacillus subtilis was grown in 250-mL Erlenmeyer flasks containing 100 ml Nutrient broth at 37° C and 150 rpm for 24 hours. After incubation, biomass was separated by centrifugation and washed with sterile distilled water to remove the traces of media components, and, challenged with $AgNO_3$ solution (1 mM). Incubate the solution for 48 hr and after that centrifuged the solution and separate out the biomass and supernatant solution. Characterization of Ag-NPs:

After 48 hours of incubation of the above mixture, the preliminary detection of Ag-NPs was carried out by visual observation of color change of the cell filtrate. These samples were later subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Shimadzu 1650 PC) and scanning the spectra between 430 nm at the resolution of 1 nm. Transmission electron microscopy (TEM) was used to record the micrograph images of synthesized Ag-NPs.

Agar well diffusion assay to evaluate antibacterial effects

Agar well diffusion method was used to evaluate in vitro antibacterial activity of biosynthesized silver nanoparticles against Escherichia coli, Staphylococcus aureus, Pseudomonas aeureginosa, and Proteus vulgaris on Nutrient agar media. Bacterial cell filtrate used for the synthesis of silver nanoparticles was used as negative control. These plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured

RESULTS AND DISCUSSION

In the present study, Bacillus subtilis was successfully used for the synthesis of AgNPs. After treatment of Bacillus subtilis with 1mM $AgNO_3$ colour change was observed in the reaction mixture from light -green to dark- brown (Figure 1). It takes about 5-10 minutes to complete the reaction with the 30 sec exposure to sunlight. The change in colour indicated the formation of AgNPs which occurs due to excitation of surface plasma resonance in metal nanoparticles

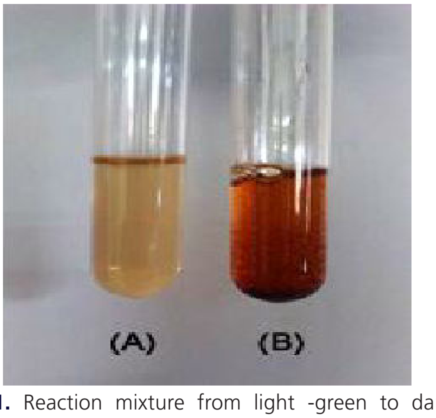

Fig. 1. Reaction mixture from light -green to dark- brown indicating synthesis of AgNPs

Absorption measurement was carried out using UV-Visible spectrophotometer at a resolution of 1 nm. The UV- Visible spectra of synthesized AgNPs showed absorption peak at 448 nm which is specific for AgNPs. These results showed resemblance with many previous studies. Jain et al. 2010 showed the absorbance peak at 450 nm for AgNPs synthesized using spore crystal of Bacillus thurenginesis. The results obtained ensure the existence of AgNPs in the solution.

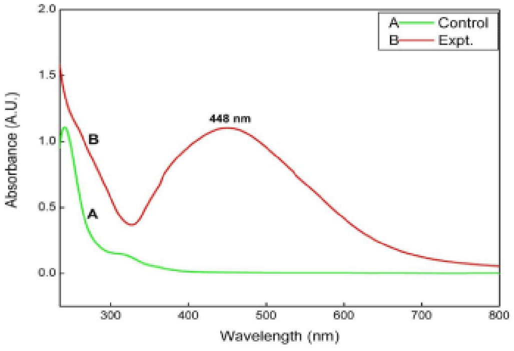

Fig. Uv – Visible spectrophotometer graph of silver nanoparticles synthesized by Bacillus subtilis showed adsorption peak at 430 nm.

Characterization of silver nanoparticles by SEM, TEM and X – ray diffraction pattern generated by TEM:

After Uv – Visible spectrophotometer, further characterization was carried out by scanning electron microscopy, transmission electron microscopy and X – ray diffraction pattern generated by transmission electron microscope. On analysis, Spherical silver nanoparticles were observed and the size of silver nanoparticles synthesized by Bacillus subtilis 38 – 42 nm. The silver nanoparticles were found to be capped by sodium citrate. On X – ray diffraction pattern generated by transmission electron microscope, it was confirmed that the silver nanoparticles are crystalline in nature.

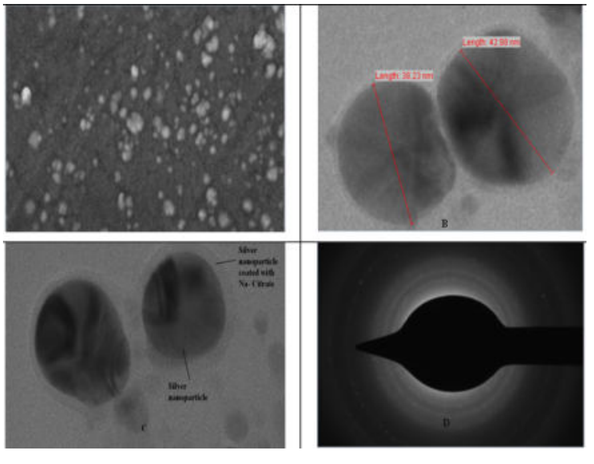

Fig. 1. showing SEM, TEM and XRD images of silver nanoparticles synthesized by Bacillus subtilis (A: SEM images, B and C: TEM images, D: XRD image.)

Antimicrobial activity of silver nanoparticles on standard cultures:

After incubation period, the plates were observed for zone of inhibition. The synthesized silver nanoparticles showed activity against all three standard cultures. However, no activity was shown by sodium citrate.

Table 2. The antimicrobial activity of silver nanoparticles on standard cultures.

Organisms	Zone of inhibition in mm		
	1 mM AgNO3	Na - Citrate	Nanoparticles
C. albicans NCIM 715	12	-	14
K. pneumoniae NCIM 5082	13	-	15
S. aureus NCIM 5084	11	-	18

Figure 2 showing results of antimicrobial activity of silver nanoparticles synthesized by Pseudomonas aeruginosa. (A: Candida albicans NCIM 715, B: Klebsiella pneumoniae NCIM 5082, C: Staphylococcus aureus NCIM 5084, 1: Silver nitrate, 2: Sodium citrate, 3: silver nanoparticles)

In vitro antibacterial activity of synthesized AgNPs from Bacillus substilis evaluated against Candida albicans NCIM 715, Klebsiella pneumoniae NCIM 5082, Staphylococcus aureus NCIM 5084 The results clearly indicate that synthesized AgNPs exhibit significant antibacterial activity against all the test organisms and it was calculated from the zone of inhibition. Thus, synthesized AgNPs were found to have almost similar activity against all the bacteria. In case of S. aureus it showed somewhat higher activity showing zone of inhibition or 18 mm. However, it showed similar zone of inhibition for Klebsiella pneumoniae (15 mm) and Candida albicans (14mm) On the other hand, no zone of inhibition was observed in case of Sodium citrate.

CONCLUSION

The biological synthesis of silver nanoparticles using Bacillus substilis was shown to be rapid and produce particles of fairly uniform size and shape. The synthesized silver nanoparticles were characterized by UV–Vis spectroscopy and confirmed by TEM. The nanoparticles formed by the isolate were found to be stable with size range of 38–42 nm which indicate its potential applications. silver nanoparticles using Bacillus substilis may be use to control a variety of diseases caused by both Gram-negative and Gram-positive bacteria.

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