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ORIGINAL ARTICLE

Biosynthesis of silver nanoparticles using *Bacillus subitilis* and its antibacterial activity

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ABSTRACT

Background: *Silver nanoparticles (AgNPs) have recently been extensively investigated because of their superior physical, chemical, and biological characteristics, and their superiority is primarily due to the size, shape, composition, crystallinity, and structure of AgNPs compared to their bulk forms.* ***Objective:*** *The current study aimed to investigate the antibacterial activity of silver nanoparticles (AgNPs) synthesized intracellular by using standard strain Bacillus subitilis ATCC 6633 against reference strains Escherichia coli ATCC 2592 and Staphylococcus aureus ATCC 29737.* ***Results:*** *The synthesized AgNPs showed potent antibacterial activities against the two tested bacterial strains with inhibition zones ranged from 42 -52mm and MIC 27.2 µg / ml. The silver nanoparticles were characterized with particle size ≃ 100 nm and zeta potential -19. There was deformation in both tested strains upon treatment with AgNPs which was observed by Scanning Electron Microscopy (SEM).* ***Conclusion:*** *The results indicated that AgNPs could be used as an effective antibacterial agent.*

INTRODUCTION

Broad spectrum antimicrobial activities of AgNPs have enabled their applications in a large variety of consumer disinfecting medical products such as water treatment systems, dental materials, medical textiles, wound dressing, medical devices coating, and others[1]. AgNP synthesis using physical and chemical methods is costly, has adverse environmental impacts and requires high energy. AgNPs biosynthesis is considered a safe and an economic alternative way[2].

Nanostructured mineral crystals and metallic nanoparticles are known to be formed by many microbes with properties similar to chemically produced nanomaterials, thus exercising firm control over the form, size and composition of the nanomaterials. In the field of biogenesis, prokaryotic bacteria have been found to be superior compared to other micro-organisms[3].

Biosynthesis of silver nanoparticles using *Bacillus subitilis* among inorganic antimicrobial agents, silver has been used most commonly to combat infections since ancient times. The antimicrobial effects of silver and silver compounds have been extensively studied [4-9]. Remarkable results on the bactericidal behavior of (AgNPs) were identified in a recent literature survey[10,11]. AgNPs is found to exhibit strong antimicrobial activity against multidrug resistant *E. coli* and *S. aureus* [12]. AgNPs' antibacterial activity against gram-negative *E. coli* dependent on the shape of the nanoparticles [13].

My research deals with synthesis of AgNPs using *B. subtilis* and its antimicrobial activity against *E. coli* and *S. aureus*.

METHODOLOGY

Bacteria

B. subitilis ATCC 6633, *E. coli* ATCC 2592 and *S. aureus* ATCC 29737 are reference strains were kindly provided from Microbiology Department, Egyptian drug authority, former National Organization for Drug Control and Research (NODCAR), Cairo, Egypt.

Synthesis of AgNPs

The *B. subtilis* was grown in Luria-Bertani broth (LB) at 37° C and 150 rpm for 36 hours; then the culture supernatant was obtained by centrifugation at 10,000 rpm for 30 min. To synthesis AgNPs, culture supernatant was treated with 1mM $AgNO_3$ for 5 days in incubator shaker 200 rpm at 37^0C. The supernatant without addition of AgNO3 was saved as a control. The bio-reduction was observed by optical color change from pale yellow to brown.

Agar well diffusion assay

The technique of Agar well diffusion was used to assess the antibacterial activity of synthesized AgNPs against *E. coli and S. aureus*[14]. A volume of 50 µl of each synthesized AgNPs, supernatant without addition of AgNO3 and 1 mM AgNO3 (control) incubation at 37^oC for 24 h., diameters of inhibition zone were measured. This was done in triplicate.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

96-well microtitre plates were used for microtitre dilution assay under aseptic conditions[15]. Synthesized AgNPs ranged from 3.4 -108.8 µg/mL were used to determine MIC and MBC. The plates were incubated for 24 hours at 37^0 C. MIC is the lowest concentration of nanoparticles which show no turbidity was observed. Wells with no visual growth were sub cultured to determine MBCs. The study has been performed in triplicates.

Scanning electron microscopy (SEM)

To observe the morphological modifications of *E. coli and S. aureus* treated with AgNPs at determined MIC, SEM (FE-SEM) (JSM- 5200, JEOL, Japan) was used.

Nanoparticles characterization

Visual change in the solution color from pale yellow to brown was an indication of AgNPs synthesis. Potential measurement on particle size and zeta were performed using Zetasizer Nano ZS Ver. 7.13 (Malvern Instruments Limited, Malvern, MAL1203718 WR, UK).

Statistical analysis

These studies were carried out in triplicates and the findings were shown to be average ±S.D.

RESULTS

The present study evaluated the antibacterial activity of synthesized AgNPs by using *B. subitilis* culture supernatant against *E. coli* (ATCC 2592) *and S. aureus* (ATCC 29737). The results showed potent antibacterial activity against *E. coli* with mean inhibition zone of 52 mm and *S. aureus* with mean inhibition zone of 42 mm (table1). No inhibition zone was observed for *B. subtilis* culture supernatant or 1 mM $AgNO_3$ which act as control.

Table 1: Antimicrobial activity of AgNPs synthesized from *B. subtilis*

Solution	(Average of readings, Zone of inhibition of growth in mm)	
	S. aureus	*E. coli*
1 mM $AgNO_3$	0	0
B. subtilis culture supernatant	0	0
AgNPs	42±0.057	52±0.057

To determine the lowest concentration that fully inhibited visible development, MIC was used. The findings revealed that the MIC was considered to be 27.2 µg /ml, while the MBC was 54.4 µg / ml for the two tested strains.

Upon visual inspection of synthesized AgNPs, there was colour transition from pale yellow to brown which clearly indicates the formation of AgNPs in the reaction mixture. Synthesized AgNPs were characterized by particle size ≃ 100 nm (fig. 1) and Z- potential -19 (mV), which indicates a good stability.

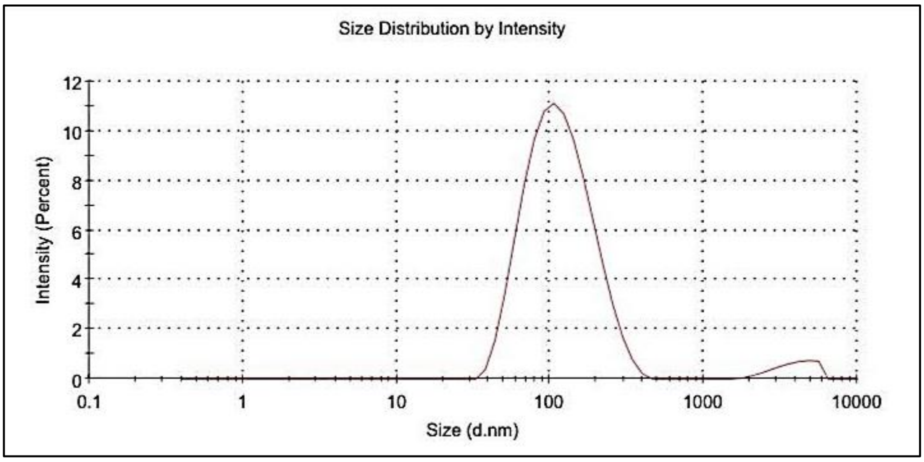

Fig. 1: Particle size of synthesized AgNPs

SEM has observed the morphological changes in bacterial cells (fig.2). *S. aureus*, control cells were grape-shaped. While the AgNPs treated cells showing complete deformation. In *E. coli* cells, rod-shaped community cells were observed; while the treated ones showed sever cell elongation which can be detected as complete cell damage.

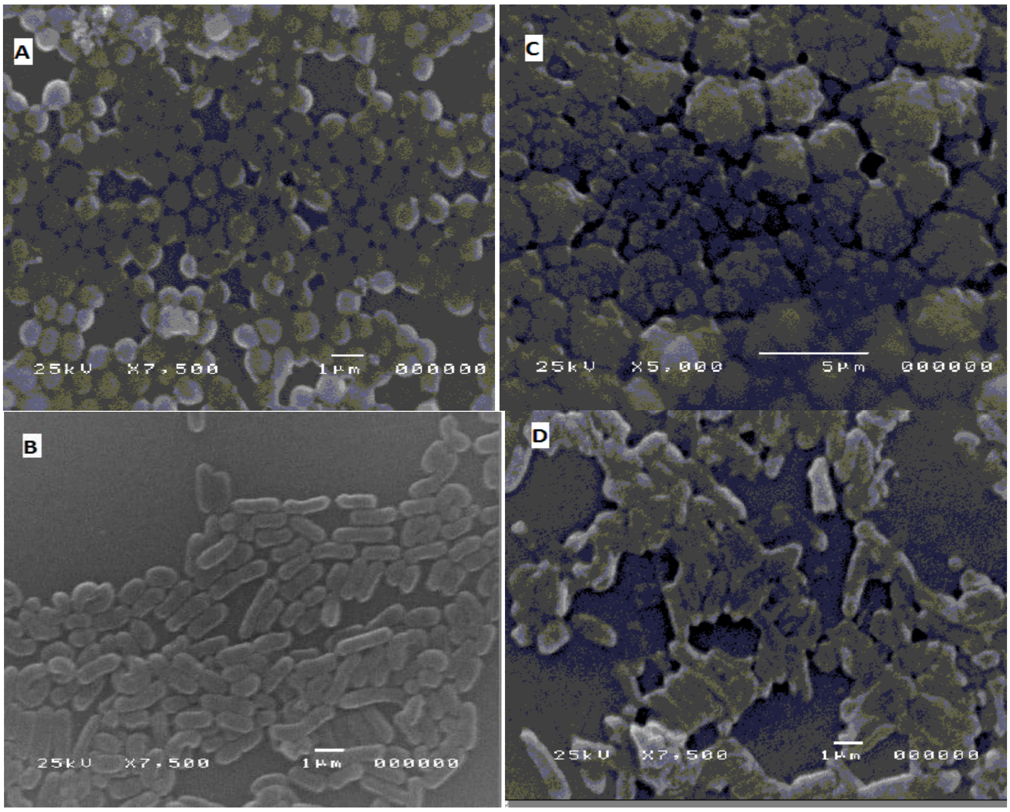

Fig. 2: SEM micrograph of *S. aureus* (A, C) and *E. coli* (B, D). (A, B : control; C, D : treated with Ag-NPs)

DISCUSSION

B. subtilis is selected for the synthesis of AgNPs because of few reports about its synthesis using *bacillus sp.* AgNPs displayed antibacterial activity against the two tested strains. There is no activity noticed for either *B. subtilis* supernatant or $AgNO_3$ and this indicates the antimicrobial activity is due to biosynthesized AgNPs. The use of silver nitrate by Moyer[16] demonstrated that it had potent antibacterial properties against *S.aureus, P. aeruginosa*, and *E. coli* [16]. Due to their small size and high surface area to volume ratio, the bactericidal activity of silver nanoparticles is enhanced[17]. AgNPs reach the cell walls of bacteria causing its degradation leading to the cell death[18]. In terms of its bactericidal impact, the size of nanoparticles is very crucial. The smaller the scale of the AgNPs, the more the activity of antibacterial increases. Small nanoparticles with a wide contact area to volume ratio have adequate bactericidal efficiency even at low concentrations[19, 20].

The characterization of *B. subtilis* AgNO3 was recognized by the colour shift from light yellow to dark brown, this characteristic brown colour provides a convenient signature of their development due to the excitation of Plasmon vibrations in the nanoparticles. Brown colour formation demonstrates the existence of AgNPs[21, 22].

Synthesized AgNPs found to be about 100 nm. The negative value of the Z- potential confirms the repulsion between the particles and thus increases the stability of the formulation. The Z- potential was found to have been -19.1 mV. The electrostatic repulsive forces between the nanoparticles will protect them from forming an association when they are charged negatively. This eliminates the agglomeration of particles in the medium and results in long-term stability[23].

It is reported that the silver has a toxic effect on microorganisms[24], so silver-based combinations have been commonly used for their antibacterial applications[25,26]. It has recently been stated that the antibacterial activity of AgNPs is correlated with free radical formation [27]. Also, the free radicals produced by AgNPs cause damage to the bacterial cell membrane. AgNPs have complicated effects on bacterial cells. In the treated community of AgNPs, however, irregular fragments emerged instead of standard rod-shaped cells. Some scientists have confirmed that the antimicrobial activity of AgNPs have had an effect on Gram-negative bacteria, the concentration of Ag in the nanoparticles is dependent on and was closely connected with the development of "pits" in the cell walls[28,29]. The permeability of cell membrane increased by the negatively charged AgNPs in the bacterial membrane. Interestingly, there is some distinction between Gram positive *S. aureus* and Gram negative *E. coli*. In particular, morphological death of bacterial cells occurs in the case of SEM observations. *S. aureus* was soft and weak in contrast to *E. coli*. This difference was possibly attributable to the difference of the peptidoglycan layer of the bacterial cell between *S. aureus* and *E. coli*. Defense against antibacterial agents like antibiotics, contaminants, chemicals, and degrading enzymes is an essential characteristic of the peptidoglycan layer[30].

CONCLUSION

In conclusion, *Bacillus subtilis'* AgNPs was proved to be a potent antibacterial agent against *S. aureus* and *E. coli*. This biosynthesized AgNPs has many benefits over chemically derived nanoparticles and could be an ideal way to build an environmentally safe protocol.

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