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Green Synthesis of Silver Nanoparticles Using Lactic Acid Bacteria: Assessment of Antimicrobial Activity

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ABSTRACT

The biosynthesis of silver nanoparticles (Ag-NPs) is a new methodology in nanotechnology with a hopeful implementation in medicine, food control, and pharmacy. The objective of the present research was to conduct a green synthesis of Ag-NPs using the cell-free supernatant of *Lactobacillus plantarum* and *Lactobacillus brevis* and evaluate their antibacterial and antifungal activities. The production of Ag-NPs was confirmed by the color alteration from yellow to brown. Using the UV-visible spectrophotometer, the biosynthesized Ag-NPs indicated an absorption peak at 410 nm. The transmission electron microscope was used for the determination of the size and morphology of the nanoparticles. Nanoparticles appeared in spherical or polyhedral form, poly-dispersed and their diameter ranged from 5 to 40 nm. The X-ray diffraction analysis exhibited the crystalline nature of the particles with a face-centered cubic (FCC) structure. The biosynthesized Ag-NPs were evaluated for their antimicrobial efficiency using the agar well diffusion method. The antibacterial activity of Ag-NPs was more potent against Gram-negative bacteria than Gram-positive bacteria. Ag-NPs synthesized from *Lactobacillus plantarum* recorded the maximum activity against *Escherichia coli* (ATCC® 10536™) and *Pseudomonas* (ATCC® 27853™) bacteria, while those synthesized from *Lactobacillus brevis* recorded the maximum activity against *Escherichia coli* (ATCC® 35218™). Ag-NPs synthesized from *Lactobacillus plantarum* and *Lactobacillus brevis* showed antifungal activity against *Candida albicans* (ATCC® 10231™). The effect of these nanoparticles on *Escherichia coli* (ATCC® 10536™) was examined and imaged by a transmission electron microscope that indicated damage to the plasma membrane and cell wall. In conclusion, the biosynthesized Ag-NPs have applications as antimicrobial agents in the medicine and food industry.

Keywords: Antimicrobial activity, *Lactobacillus brevis*, *Lactobacillus plantarum*, Silver nanoparticles, Transmission electron microscope

INTRODUCTION

At the present, bacterial infections are one of the most common dangers fronting medical treatment due to the emergence of drug-resistant bacteria resulted from the spread of resistance mechanisms (Holmes et al., 2016). The overuse of antibiotics for the treatment of infectious diseases creates complicated mediation problems. Moreover, these drugs cause various side effects and problems, including allergy, hypersensitivity, and immune-suppression. Therefore, there is a need to develop new antimicrobial drugs for the treatment of pathogenic diseases (Jain et al., 2009).

Nanotechnology is a novel field of preparation and use of nanomaterials less than 100 nm in size. Nanoparticles exhibit distinctive properties and a high surface-to-volume ratio (Khan et al., 2014). Nanotechnology enhances the production of different metal nanoparticles, especially silver nanoparticles (Ag-NPs), which have high bactericidal potential. Ag-NPs can be used as an alternative for antibiotic drugs due to their strong effect on multidrug-resistant bacteria (Song and Kim, 2009). It has been demonstrated that Ag-NPs have antibacterial potential against some antibiotic-resistant microorganisms (Birla et al., 2009; Inoue et al., 2010). The antimicrobial properties of Ag-NPs depend on silver ions (Ag^+) which intensely hinder microbial growth through the inhibition of electron transport components, respiratory enzymes, and interference with DNA functions (Li et al., 2006). Silver at a nanoscale (smaller than 100 nm) has a potent toxic effect on a broad group of microorganisms (Elechiguerra et al., 2005). Morones et al. (2005) determined the antimicrobial effect of Ag-NPs on four species of Gram-negative bacteria (*Pseudomonas aeruginosa*, *Salmonella* Typhi, *Escherichia coli (E. coli),* and *Vibrio cholera)* and suggested that Ag-NPs stick to the cell membrane surface and interrupt its function by permeation the bacterial cell wall and freeing of Ag^+ ions.

Chemical and physical methods used for the production of Ag-NPs are costly and include toxic substances that can harm public health and the surrounding environment. The latest studies indicated great attention for the preparation of Ag-NPs from eco-friendly and cost-efficient biological sources using various plant, microbial (bacterial and fungal), and algae extracts (Ratan et al., 2020).

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The purpose of the current research was the biosynthesis of Ag-NPs by extracellular reduction of silver nitrate ($AgNO_3$) using lactic acid bacteria isolated from fermented milk product samples. In addition, the antibacterial and antifungal effects of the Ag-NPs were examined against Gram-positive, Gram-negative bacterial, and fungal species.

MATERIALS AND METHODS

Isolation and identification of *Lactobacillus plantarum* and *Lactobacillus brevis*

Samples of fermented dairy products, including Karish cheese, fermented goat's milk, and Laban Rayeb (concentrated sour milk) were collected from Cairo and Giza governorates in Egypt. All samples were collected in sterile bags, transferred to the laboratory under aseptic cooled conditions, and stored at 3 ± 1 ºC for up to a maximum of 24 hours before the analysis. For the preparation of samples, 10 g of each sample were homogenized in a stomacher lab-blender with 90 ml of sterile sodium citrate solution (2% w/v) for cheese samples and 90 ml of sterile physiological saline (0.85% NaCl w/v) for fermented milk samples for 30 seconds. A loopful from each sample was cultured on de Man, Rogosa and Sharpe (MRS, Oxoid, England) Petri-dishes. All plates were incubated at 37 °C for 48-72 hours under anaerobic conditions. Single colonies were picked from MRS agar plates, purified, and identified using morphological and biochemical characterization (Mathara et al., 2004).

Preparation of cell-free supernatant of *Lactobacillus* strains

Single colonies of *Lactobacillus* isolates were inoculated in nutrient broth (Oxoid, England) and incubated at 37°C for 24 hours, then the culture was centrifuged at 4500 rpm for 10 minutes to prepare the cell-free supernatant. After centrifugation, the precipitated cells at the bottom of the tube were discarded and the cell-free supernatant was collected and used for the synthesis of Ag-NPs (Chaudhari et al., 2012).

Biosynthesis of silver nanoparticles using cell-free supernatant of *Lactobacillus* strains

$AgNO_3$ was a precursor for the biosynthesis of Ag-NPs by *Lactobacillus*. AgNO3 (Sigma-Aldrich) with a concentration of 2 mM was added to *Lactobacillus* cell-free supernatant in a ratio of 1:1 and was mixed well. This step was prepared in a dark condition to avoid oxidation of AgNO3. The pH of the mixture was adjusted to 8.3. The resultant solution was incubated in a shaking incubator at 150 rpm at 37 °C for 24 hours (Dakhil, 2017). After incubation, the color change was observed and the reaction mixture was centrifuged at 10000 rpm for 10 minutes. The supernatant was discarded and replaced with deionized distilled water and re-centrifuged three times at the same speed and time to remove the remaining supernatant. The pellet deposited at the bottom of the tube represented Ag-NPs and then dried in an oven at 40°C for 18-24 hours. The dried powder was collected carefully and stored in sample vials for further analysis (Chaudhari et al., 2012; Sarvamangala et al., 2013).

Characterization of silver nanoparticles

Ultraviolet-visible spectrophotometry

The ultraviolet-visible (UV-Vis) spectrum of the Ag-NPs was measured using a UV-Vis spectrophotometer (Shimadzu, 1600) in the central laboratory of the National Research Center, Egypt. The absorbance was measured in the range of 400-800 nm. Deionized water was used as the blank.

Transmission electron microscopy

The size and shape of the Ag-NPs were determined by transmission electron microscopy (TEM). The sample was prepared by placing a drop of nanoparticle solution on a carbon-coated copper grid and water was allowed to evaporate. The TEM measurements were performed on a JEM – JEOL model 2100 instrument, which was operated at an accelerating voltage of 120 kV (Dong et al., 2019).

X-ray powder diffraction

Pattern of dry nanoparticle powder was obtained with Cu Kα radiation (1.5406 Å; 45 kV, 30 mA). The X-ray powder diffraction (XRD) pattern was analyzed to determine position, peak intensity, and width. The nanoparticle size was calculated using the Scherrer formula as follows:

$D = 0.94\lambda / \beta \cos \theta$

Where, D is the mean diameter of the nanoparticles, β denotes the angular full width at half maximum (FWHM) of the XRD peak at the diffraction angle θ, and λ refers to the wavelength of the X-ray radiation source (Shehzad et al., 2018).

Antibacterial activity of silver nanoparticles

Antibacterial activity of biogenic Ag-NPs was carried using the agar well diffusion method against different types of pathogenic Gram-positive and Gram-negative bacteria as listed in Table 1. Standardized suspension of each tested bacterium (1.5×10^8 CFU/mL) was prepared to a 0.5 McFarland standard by measuring the absorbance at wavelength

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625 nm, and swabbed separately onto sterile Muller-Hinton Agar (MHA, Oxoid, England) plates using sterile cotton swabs. Agar was punched with a sterilized cork borer (6 mm) and 50 µl of biogenic Ag-NPs was added into each well. One petri-dish was cultured for each pathogenic bacterium and used as control. All plates were incubated at 37 °C for 24 hours and the inhibition zones were measured (Charannya et al., 2018).

Antifungal activity of silver nanoparticles

Antifungal activity of biogenic Ag-NPs was assayed against pathogenic fungus, *Candida albicans* (Table 1) using the agar well diffusion method. The concentration of fungal suspension was adjusted to 0.5 McFarland standard in normal saline (at wavelength 530 nm) to achieve concentration of 1-5 × 10^6 CFU/mL. This suspension was streaked on Sabouraud dextrose agar (Oxoid, England) plate by a cotton swab. Wells were made in the agar plates with a sterilized cork borer (6 mm) and 50 µl of biogenic Ag-NPs were added into wells. One Petri-dish was cultured as control. After incubation for 3 days at 28 °C, the inhibition zones were measured (Buszewski et al., 2018).

Table 1. Bacterial and fungal strains used in this study for antimicrobial activity test

Bacterial strains
Gram-positive bacteria
Staphylococcus aureus (ATCC® 25923™)
Enterococcus faecalis (ATCC® 29212™)
Staphylococcus epidermidis (ATCC® 12228™)
Staphylococcus aureus (ATCC® 29213™)
Clostridium perfringens (ATCC® 13124™)
Gram-negative bacteria
Escherichia coli (ATCC® 10536™)
Klebsiella pneumonia (ATCC® 700603™)
Pseudomonas aeruginosa (ATCC® 27853™)
Escherichia coli (ATCC® 35218™)
Neisseria gonorrhoeae (ATCC® 19424™)
Fungal strains
Candida albicans (ATCC® 10231™)

Assessment of effect of silver nanoparticle on *Escherichia coli* by transmission electron microscopy

In order to observe the impact of Ag-NPs on the morphology and structure of *E. coli* (ATCC® 10536™), isolates of *E. coli* were adopted, and each was proliferated to 1×10^8 CFU/ml. After being exposed to 11.25 µg/mL Ag-NPs for 12 hours, the culture was precipitated by centrifugation and washed once with phosphate buffer saline (PBS) and then centrifuged; the precipitates were fixed in 2.5% glutaraldehyde overnight, and rinsed three times with 0.1 M phosphoric acid; the bacteria were dehydrated, paraffin-embedded, and sliced, then double-stained with 3% uranium acetate and lead nitrate before being observed by TEM. The bacteria without Ag-NPs treatment were considered as control (Liao et al., 2019).

RESULTS

Biosynthesis of silver nanoparticles

Culture filtrates of *Lactobacillus plantarum* (*L. plantarum*) and *Lactobacillus brevis* (*L. brevis*) were added to the $AgNO_3$ solution, after 24 hours, the color of the mixture changed to yellowish-brown or brown by optical observation.

Characterization of silver nanoparticles

UV-visible spectroscopy

UV-Vis spectroscopy analysis showed that the formed Ag-NPs had an absorption peak at 410 nm (Figure 1).

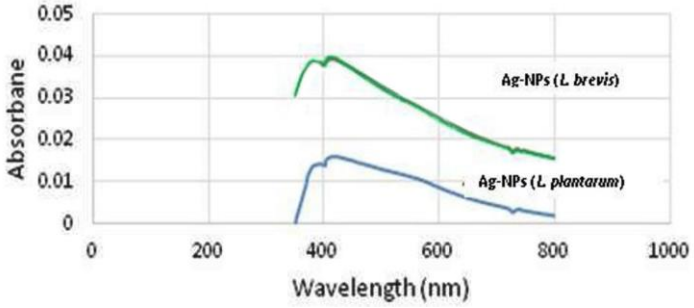

Figure 1. UV-visible absorption spectra of Ag-NPs synthesized by cell-free supernatant of *Lactobacillus brevis* and *Lactobacillus plantarurm*. The absorption spectra of Ag-NPs exhibit a strong broad peak at 410 nm.

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Transmission electron microscopy characterization

TEM results showed that most of the biogenic Ag-NPs were in spherical and polyhedral form, and poly dispersed nanoparticles (Figures 2 and 3). The diameter of_Ag-NPs was within the range of 5-40 nm. Selected area electron diffraction (SAED) patterns of the Ag-NPs presented in concentric circles and the Ag-NPs appeared in the form of crystals as can be seen in Figure 4.

X-ray diffraction pattern

The XRD patterns of the formed Ag-NPs were displayed in Figure 5. Ag-NPs exhibited unique diffraction peaks of metallic face-centered cubic (FCC) silver phase at 42.66° (111), 48.68° (200), 69.32° (220), and 82.12° (311) in 2θ.

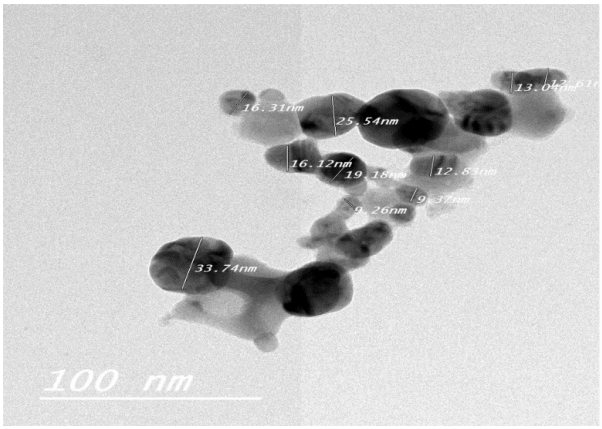

Figure 2. Transmission electron microscope micrograph of silver nanoparticles biosynthesized from *Lactobacillus plantarum*

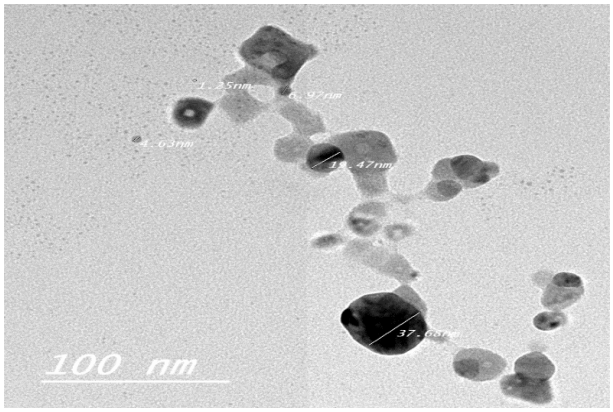

Figure 3. Transmission electron microscope micrograph of silver nanoparticles biosynthesized from *Lactobacillus brevis*

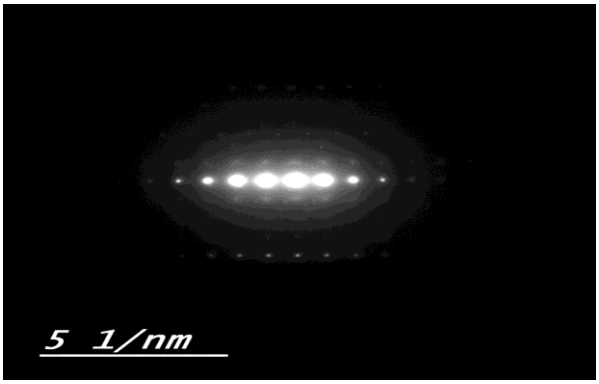

Figure 4. Selected area electron diffraction (SAED) patterns of the silver nanoparticles biosynthesized by *Lactobacillus plantarum*

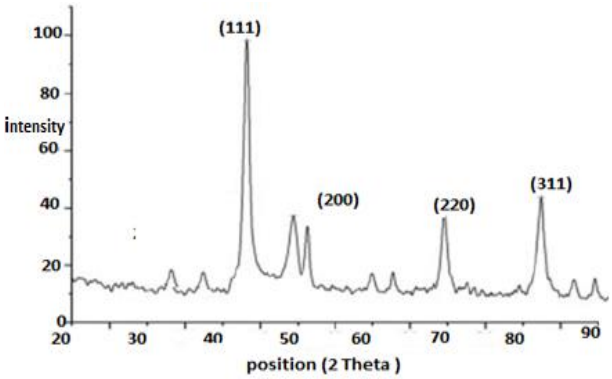

Figure 5. X-ray diffraction (XRD) pattern of silver nanoparticles biosynthesized by cell-free supernatant of *Lactobacillus brevis* and *Lactobacillus plantarum*

Antibacterial and antifungal activity of silver nanoparticles

Biosynthesized Ag-NPs prevented the growth of Gram-positive bacteria as well as Gram-negative bacteria (Table 2). The inhibition zones of Gram-negative bacteria were larger than those of Gram-positive bacteria for Ag-NPs synthesized from the culture filtrate of *L. plantarum*. The zone of inhibition against Gram-negative bacteria ranged 15-22 mm, the largest zone was against *E. coli* (ATCC® 35218™) while the smallest zone was against *Klebsiella pneumoniae* and *Neisseria gonorrhoeae*. The inhibition zone of Ag-NPs synthesized from the culture filtrate of *L. plantarum* against Gram-positive bacteria ranged 16-18 mm, the largest zone was against *Staphylococcus aureus* (ATCC® 25923™) whereas the smallest zone was against *S. epidermidis* and coagulase-negative *S. aureus* (ATCC® 29213™). The inhibition zone of Gram-positive bacteria was larger than that of Gram-negative bacteria for Ag-NPs synthesized from the culture filtrate of *L. brevis*. The diameter of inhibition zones against Gram-positive bacteria was within the range of 14-21 mm, the largest zone was against *Enterococcus faecalis* while the smallest zone was against *S. epidermidis* and coagulase-negative *S. aureus.* On the other hand, the inhibition zone diameter against Gram-negative bacteria ranged 13-22 mm, the greatest and the lowest zones were against *E. coli* and *Pseudomonas aeruginosa*, respectively. Antifungal activity of Ag-NPs was tested against *C. albicans* (Table 2) using the agar well diffusion method. The inhibition zones of Ag-NPs biosynthesized from culture filtrates of *L. plantarum* and *L. brevis* were 17 mm and 18 mm, respectively.

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Table 2. Antifungal and antibacterial activities of silver nanoparticles (Ag-NPs) biosynthesized from *Lactobacillus* species

Strain \ Inhibition zone diameter* (mm)	Ag-NPs biosynthesized by *Lactobacillus plantarum*	Ag-NPs biosynthesized *by* *Lactobacillus brevis*
Gram-positive bacteria		
Staphylococcus aureus (ATCC® 25923™)	18	16
Enterococcus faecalis (ATCC® 29212™)	17	21
Staphylococcus epidermidis (ATCC® 12228™)	16	14
Staphylococcus aureus (ATCC® 29213™)	16	14
Clostridium perfringens (ATCC® 13124™)	17	17
Gram-negative bacteria		
Escherichia coli (ATCC® 10536™)	22	16
Klebsiella pneumoniae (ATCC® 700603™)	15	15
Pseudomonas aeruginosa (ATCC® 27853™)	18	13
Escherichia coli (ATCC® 35218™)	16	22
Neisseria gonorrhoeae (ATCC® 19424™)	15	16
Fungus		
Candida albicans (ATCC® 10231™)	17	18

* Diameter of the zone of inhibition was determined using the agar well diffusion method.

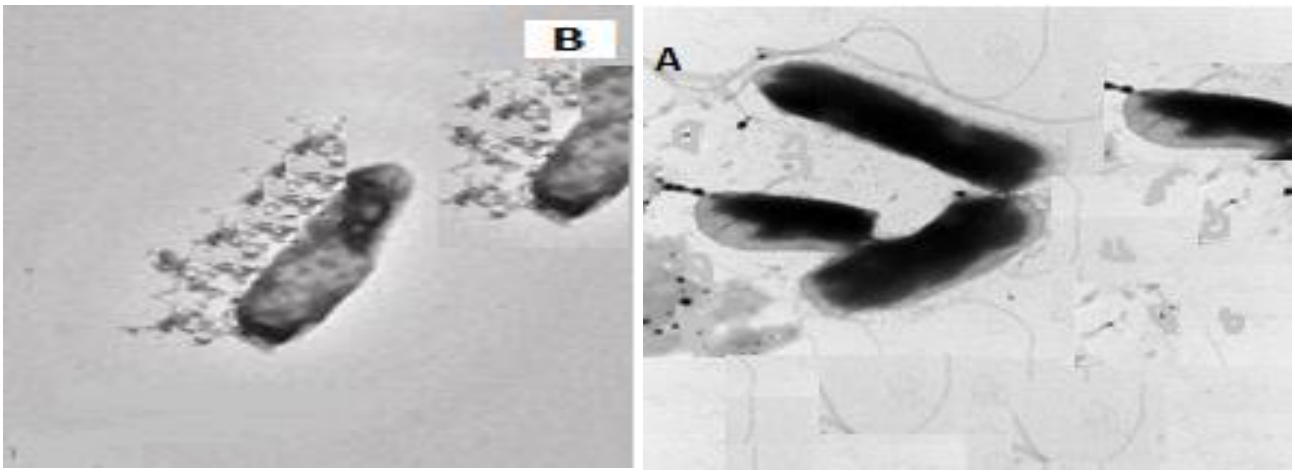

Figure 6. Transmission electron microscopy of *Escherichia coli* (ATCC® 10536™) before **(A)** and after treatment **(B)** with biogenic Ag-NPs.

Morphology and structure of *Escherichia coli* (ATCC® 10536™) after treatment with Ag-NPs by transmission electron microscopy

After treatment with Ag-NPs for 12 hours, the bacteria of *E. coli* (ATCC® 10536™) were collected for structural and morphological examinations by TEM. Ag-NPs-treated bacteria showed that the cell wall became significantly thin or even disappeared, crumpled, and released the cell contents (Figure 6B), in contrast to the untreated bacteria, which indicated the intact morphology of rod-shaped bacteria (Figure 6A).

DISCUSSION

Synthesis of metallic NPs has been established by microorganisms which are considered as potential bio-factories (Mukherjee et al., 2002). Cell-free culture of *Lactobacillus* is the easiest and simplest technique used for the synthesis of size-controlled Ag-NPs (Kalimuthu et al., 2008). Most organisms cannot synthesize Ag-NPs, only the bacteria which contain the "silver resistance machinery" can create Ag-NPs. The mechanism of production of Ag-NPs by culture filtrate of bacteria is not entirely understood. However, it is hypothesized that the enzymes produced by microorganism as reductase and nitrate reductase have a great role in the bio-reduction of silver ions to Ag-NPs (Duran et al., 2005). Nicotinamide adenine dinucleotide (NADH) and NADH-dependent enzymes play an important role in the biogenic synthesis of nanomaterials. The reduction is induced by electron transport from NADH by NADH-dependent reductase as an electron carrier (Thakkar et al., 2010). *Lactobacillus* can produce nitrate reductase at pH above 6 which causes biological reduction of Ag+ to Ag and creation of Ag-NPs (Ranganath et al., 2012). *Lactobacillus* species have the

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ability to secrete the aldehydic group through additional polysaccharides which are responsible for the reduction of Ag+ to Ag (Durán et al., 2011).

Addition of $AgNO_3$ to the supernatant of *Lactobacillus* bacteria produced Ag-NPs (at pH 8.3) and a reddish-brown coloration was obtained as a result of stimulation of surface plasmon resonance (SPR) of Ag-NPs (Sarvamangala et al., 2013; Natarajan et al., 2014; Sreedevi et al., 2015). The synthesis of Ag-NPs was confirmed by UV-Vis spectroscopy with collective excitation of conduction electrons in the metal determining the absorption band of the formed Ag-NPs which was at 410 nm (Figure 1). This absorption peak was also reported in Ag-NPs biosynthesized by *Neurospora crassa* (Longoria et al., 2011), *B. licheniformis* (Kalimuthu et al., 2008), *Aspergillus flavus* (Vigneshwaran et al., 2007), *Aeromonas* sp. SH10 (Saifuddin et al., 2009), *Bacillus subtilis* (Mouxing et al., 2006), and marine *Lactobacillus* species (Matei et al., 2015). The results of TEM indicated that the diameter of Ag-NPs biosynthesized from culture filtrates of both *Lactobacillus* species was approximately 5-40 nm that is in concordance with previous studies (Kalimuthu et al., 2008; Sintubin et al., 2009). The examination confirmed the crystalline nature of SAED patterns with clear lattice borders (Figure 4) as reported before (Gannimani et al., 2014).

Rajesh et al. (2014) reported that the average crystal size of Ag-NPs synthesized by *Lactobacillus acidophilus* culture filtrate, determined using the Scherrer equation, was 33 nm. They also found that Ag-NPs had a unique diffraction peak of metallic FCC silver phase at 38.31°, 44.41°, 64.58°, and 77.52° in 2θ.

Ag-NPs biosynthesis using bacteria is influenced by different factors, such as strain type, physical circumstances (including pH), temperature, time, concentration, and the type of metallic salt (Srivastava and Constanti, 2012). Many studies have been implemented to test the antibacterial activity of silver ions and zero-valent Ag-NPs and to apply them in various products, including bio-medical products for the cure of burns and bacterial diseases. Ag-NPs have a greater affinity to react with the cell surface than the silver ions. Moreover, the large surface area of nanoparticles permits their functionalization with different targets, such as antibodies and particular cell types, and prevents the growth of affected cells without upsetting the normal cells. The toxicity of Ag-NPs was studied in numerous studies and found that the production of reactive oxygen species (ROS) were the main causes of toxicity (Agnihotri et al., 2014). Ag-NPs destroy the bacterial cell membrane by endocytosis, penetrate the cells, and interact with glutathione or proteins to generate hydroxyl radicals or ROS. The generated ROS destruct the DNA and prevent the growth of the bacterial cell.

Commonly, the antibacterial effect of Ag-NPs depends on different factors, including their diameter, shape, and surface chemistry (Kora and Sashidhar, 2015; Wang et al., 2015). Bao et al. (2011) investigated the antibacterial activity of Ag-NPs depending on their size ranging from 10 to 100 nm and reported that nanoparticles of 10 nm presented a potent effect. They also mentioned that *E. coli* MTCC 443 and *S. aureus* NCIM 5201 were resistant to Ag-NPs regardless of their size. Moreover, the surface charge of nanoparticles affects efficiently the antibacterial activity of Ag-NPs. Kora and Sashidhar (2015) reported the antibacterial activity of Ag-NPs, synthesized using two plants (gum olibanum and gum ghatti), against different bacteria and found that the antibacterial activity of the Ag-NPs synthesized with gum olibanum was less than that of nanoparticles synthesized with gum ghatti. A bulk of studies have indicated that Ag-NPs are hopeful applicants for the treatment of bacterial diseases although Ag-NPs have an adverse effect on human health when used in high amounts due to high cytotoxicity (Agnihotri et al., 2014).

In the current study, there was a variation in the antibacterial efficiency of Ag-NPs between Gram-positive and Gram-negative bacteria resulting from the variance in the composition of their cell walls (Table 2). Inhibition zones of Ag-NPs biosynthesized from the culture filtrate of *L. plantarum* were 16-18 mm while those of Ag-NPs biosynthesized from the culture filtrate of *L. brevis* were 16-21 mm against Gram-positive bacteria. At the same time, the inhibition zones of Ag-NPs biosynthesized from the culture filtrate of *L. plantarum* were 16-22 mm while those of Ag-NPs biosynthesized from culture filtrate of *L. brevis* were 13-22 mm against Gram-negative bacteria.

Gram-positive bacteria are more resistant to the antibacterial action of Ag-NPs due to the presence of a denser cell wall than Gram-negative bacteria (Yin et al., 2015). Moreover, the lipopolysaccharides of the cell wall of Gram-negative bacteria have a greater negative charge than Gram-positive bacteria, which stimulates the adhesion of Ag-NPs, causing the sensitivity of Gram-negative bacteria to Ag-NPs (Bonnet et al., 2015). Electrostatic interaction between positively charged NPs and negatively charged bacterial cells is significant for the efficiency of nanoparticles as antibacterial materials (Kim et al., 2009).

Ag-NPs biosynthesized using culture filtrates of *Lactobacillus* spp. showed the inhibition zones of 17-18 mm against *C. albicans* due to the ability of Ag-NPs to adhere and saturate the fungal hyphae (Kim et al., 2009). It was also suggested that Ag-NPs cause damage to the cell membrane and subsequently prevent the ordinary budding route (Kim et al., 2009; Chadek et al., 2011; Elgorban et al., 2016). Bocate et al. (2019) studied the antifungal activity of Ag-NPs synthesized from fungi (*Fusarium oxysporum*) against three toxigenic species belonging to the genera *Aspergillus* (*Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus*) and found that these nanoparticles can destruct the cell membrane and cause the cell death. Matei et al. (2015) reported that Ag-NPs biosynthesized from the cell-free supernatant of *L. plantarum* showed antifungal potency against the spoilage strains of fungi belonging to *Aspergillus*, *Fusarium*, and *Penicillium* genera and visual microscope investigation indicated hyphal changes of *Fusarium*.

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The effect of Ag-NPs on *E. coli* (ATCC® 10536™) was studied and morphological changes were investigated using TEM. The untreated bacteria indicated the intact morphology of rod-shaped bacteria. TEM images demonstrated that Ag-NPs were distributed and located at the surface of the plasma membrane of the bacterial cells. This could be due to the destruction of the plasma membrane and cell wall as a result of protein peroxidation and inactivation of membrane lipids, which causes membrane permeability resulting in potassium outflow, which was supported by the findings of a study conducted by Gopinath et al. (2017). The binding force between the bacteria and metal particles is dependent on the surface area of interaction. Sondi and Salopek-Sondi (2004) reported the action mode of the penetration of Ag-NPs nanoparticles into *E. coli* and studied their effects on the bacterial plasma membrane structure could increase the membrane permeability and the entrance of Ag-NPs inside the bacterial cell. That resulted in DNA damages and alteration of the bacterial membrane, which may affect bacterial metabolism, including bacterial respiration, resulting in cell death as a consequence of the consumption of ATP (El-Naggar et al., 2017; Sur et al., 2018; Dakshayani et al., 2019; Hamouda et al., 2019). Li et al. (2008) investigated the antibacterial effect of Ag-NPs on *E. coli* by TEM and found great damage to the bacterial cell membrane and inhibition of the function of some enzymes.

CONCLUSION

The current study established a modest method to prepare Ag-NPs from 1 mM AgNO3 using cell-free supernatant of lactic acid-producing bacteria. The diameter of biosynthesized Ag-NPs ranged from 5 to 40 nm and the form was spherical or polyhedral. The extracellular production of Ag-NPs using cell-free supernatant of *L. plantarum* and *L. brevis* is a safe, non-toxic, and cost-effective substitute to the toxic and expensive chemical and physical methods. These biosynthesized Ag-NPs from biological sources represent potential biomedical applications of promising antibacterial agents.

DECLARATIONS

Authors' contribution

Sohier M. Syame contributed to the planning of ideas, funding, and management of the research, laboratory work, results, and writing of the original draft. Asmaa S. Mansour participated in the laboratory work, writing, reviewing, and editing the final version of the manuscript. Doaa D. Khalaf, E. S. Ibrahim, and Gaber E. S. contributed to the practical part of the research.

Competing interests

The authors have not declared any conflict of interest.

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