Innovative Romanian Food Biotechnology Vol. 22, 2022
2022 by Galaţi University Press Received March /Accepted July 2022

RESEARCH ARTICLE

SILVER NANOPARTICLES FROM *LACTOBACILLUS DELBRUECKII*: MICROBIAL SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY

GBEMISOLA OGUNLEYE[1], KUBRAT OYINLOLA[1], AUGUSTINA AGUOKOLI[1], *TEMITOPE OYEWO[1]*

[1]*Microbiology laboratory, Department of Biological Sciences, KolaDaisi University, Ibadan, Nigeria.*

*Corresponding author: geegbemz@gmail.com (Gbemisola Elizabeth Ogunleye)

Abstract: Microbial synthesis of nanoparticles is an eco-friendly and non-toxic method of synthesizing silver nanoparticles. Biological synthesis of silver nanoparticles (SNPs) has become a focus of current interest due to their unique properties, thus serving as alternative to chemical and physical methods. This study aimed to investigate the potential of *Lactobacillus delbrueckii* for the synthesis of SNPs and their antibacterial activities against pathogens. SNPs were synthesized by *L. delbrueckii* culture free supernatant and characterized using visual detection, UV-visible spectroscopy, fourier transformed infra-red spectroscopy (FTIR) and scanning electron microscopy (SEM). Antibacterial activity of the biosynthesized SNPs was estimated using agar well method. Biosynthesized SNPs were characterized by a strong plasmon resonance peak at 550 nm and had a broad band between 450 – 650 nm. There were colour changes from yellow to golden brown. FTIR confirmed the presence of hydroxyl, aldehyde, carboxylic acid, amino acid, and esters which are responsible for the stability of silver nanoparticles. Morphologically, biosynthesized nanoparticles were spherical with size range of 1.7 - 10 nm. The biosynthesized SNPs exhibited the highest antibacterial activity with significant inhibitory effects against both Gram positive and Gram negative bacteria, when compared to ciprofloxacin and the culture free supernatant of *L. delbrueckii*.

Keywords: *Lactobacillus delbrueckii,* silver nanoparticles, biosynthesis, inhibitory activity, characterization

RESEARCH ARTICLE

Introduction

Lactobacillus delbrueckii is a member of the lactic acid bacteria (LAB) that plays an important role in the food fermentation. Lactic acid bacteria are ubiquitous, heterogeneous group of bacteria that are significant in food, agricultural and clinical applications. They are Gram-positive bacteria, non-sporing, catalase negative facultative anaerobes and sometimes classified as aero-tolerant anaerobes. They are a non-pathogenic microorganism that produces lactic acid (Mithun *et al*., 2015). LAB is widely used in numerous industrial applications, ranging from starter cultures in the food industrial fermentation to probiotics in dietary supplements, and as bioconversion agents in agro-industrial by product. Due to limited biosynthetic abilities and their high requirements in terms of carbon and nitrogen sources, the natural habitat of LAB is represented by nutritionally rich environments. They are generally associated with plant and animal raw materials, and the corresponding fermented food products, includes dairy, meat, vegetable, and cereal plant environments (Iyare *et al*., 2020). LABs are very important in the food and dairy industries because lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavour enhancers. They are used as a biological agent in the synthesis of nanoparticles (Adebayo-Tayo and Popoola, 2017).

Nanotechnology, the science involving the synthesis and stabilization of various nanoparticles has emerged as multidisciplinary discipline with physics, chemistry, biology, materials science, and engineering, playing active roles (Hidayat *et al*., 2020). Nanotechnology has the potential to increase the efficiency of energy consumption; it helps clean the environment and solves major health problems. It also helps to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology are smaller, cheaper, lighter, yet more functional but are toxic when used at higher concentrations than 10%; they require less energy and fewer raw materials to manufacture (Kabo *et al*., 2019).

Nanoparticles are the building blocks for nanotechnology that possess unique chemical, physical and biological qualities in terms of their size, surface area to volume ratio, optical and magnetic potentials. They can be synthesized using chemical, physical and biological methods. Chemical and physical methods are quite expensive, potentially dangerous to the environment, emitting hazardous chemicals and occupying large space. The development of biological method of synthesizing is an alternative, eco-friendly method and is evolving into an important area of

nanotechnology. The role of microorganisms in nanoparticle synthesis has recently been observed. Among the microorganisms, prokaryotic bacteria have received the most attention in the area of metal nanoparticle biosynthesis. The formation of extracellular and intracellular metal nanoparticles by bacteria like *Escherichia coli*, *Pseudomonas stutzeri*, *P. aeruginosa*, *Plectonema boryanum, Salmonella typhi*, *Staphylococcus aureus, Vibrio cholera*, *Lactobacillus* spp. etc., has been reported (Gupta *et al*., 2012; Popoola and Adebayo-Tayo, 2017). Silver nanoparticles (SNPs) exhibit important physicochemical properties such as pH-dependent partitioning to solid and dissolved particulate matter, as well as biological activities when compared with the regular metal. Silver nitrate is often used as a precursor in the synthesis of the different forms of silver nanoparticles (Rai *et al*., 2009). Silver nanoparticle is an effective bactericidal agent against a broad spectrum of Gram-negative and Gram-positive organisms. Gram-negative bacteria include genera such as *Acinetobacter, Escherichia, Pseudomonas, Salmonella,* and *Vibrio.* Gram-positive bacteria such as *Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus* and *Streptococcus* are also susceptible to silver nanoparticle. It is also noteworthy that, silver nanoparticles enhance the antibacterial activity of various antibiotics. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin against *S. aureus* and *E. coli* increase in the presence of SNPs (Furno *et al*., 2004). This research is aimed at the biosynthesis, characterization and antimicrobial activity of silver nanoparticles using the culture free supernatant (CFS) of *L. delbrueckii.*

Materials and Methods

Culture collection

Lactobacillus delbrueckii and the test pathogens (*Bacillus subtilis*, *Escherichia coli, Klebsiella* spp.*, Staphylococcus aureus, Pseudomonas aeroginosa* and *Salmonella typhi*) were collected from the culture collection of the Microbiology Laboratory, Department of Biological Sciences, KolaDaisi University, Ibadan, Nigeria. *L. delbrueckii* was maintained in a medium consisting of De Mann Rogosa and Sharpe (MRS) broth (LabM, UK) and 12% (v/v) glycerol. The stock culture was stored at 4°C and sub-cultured for subsequent use.

Preparation of culture free supernatant (CFS)

RESEARCH ARTICLE

MRS broth was prepared, sterilized and inoculated with a freshly grown pure culture of *L. delbrueckii.* The culture flask was incubated aerobically for 24 h, at 35°C. After the incubation period, the culture was centrifuged (Himac CR21GII Hitachi, Japan) at 5000 rpm, for 20 min, at 37ºC and the obtained supernatant was used for further analysis.

Synthesis of silver nanoparticles using culture free supernatant of L. delbrueckii

Freshly prepared 50 mL of 1mM $AgNO_3$ (lower concentrations (0.5%) are not toxic) solution in deionized water was added to 10 mL of the CFS and the mixture was incubated at room temperature for 48 h, in the dark.

Characterization of synthesized silver nanoparticles

Visual characterization

The visual detection of the synthesized silver nanoparticles was observed in the reaction mixture for a change in colour in comparison to the control.

UV-Visible spectroscopy analysis

The bio-reduction of silver ions (Ag^+) by the CFS of *L. delbrueckii* in the solution and formation of silver nanoparticles were characterized by UV-Visible spectroscopy with a resolution of 0.5 nm. The absorbance of the sample (2 mL) was measured using UV-visible spectrophotometer (Cecil CE 1011, Cambridge, England) with wavelengths ranging from 250 nm to 750 nm (Natarajan *et al*., 2010).

Fourier Transformed Infra-Red spectroscopy (FTIR) analysis

This analysis was carried out to characterize the synthesized silver nanoparticles from CFS of *L. delbrueckii* using FTIR. The obtained functional groups were used for the characterization. The synthesized SNPs were freeze dried in a freeze-dryer and potassium bromide was added to the freeze dried sample in a ratio of 100:1. The FTIR spectrum was recorded using JASCO FT-IR-6300 Ede in the range of 4000-500 cm^{-1} and a resolution of 4 cm^{-1} (Adebayo-Tayo *et al*., 2017).

Scanning Electron Microscopy (SEM) analysis

RESEARCH ARTICLE

SEM of the biosynthesized SNPs from the CFS was used to define the size, shape and morphology of the SNPs. The aqueous solution of synthesized SNPs was freeze dried and subjected to scanning Eelectron microscopy (JEOL, Akishima-shi, JFC-1600, Japan).

Antibacterial activity of SNPs

The antibacterial activity of the biosynthesized SNPs against selected Gram-positive and negative bacteria (*B. subtilis, E. coli, Klebsiella* spp., *Staphylococcus aureus, P. aeruginosa* and *S. typhi*) was determined using agar well diffusion method. The 24-h-old culture of each test isolate was inoculated into 5 mL normal saline, in a test tube, and standardized to 0.5 McFarland. The isolates were seeded on Nutrient agar (LabM, UK) plates allowed to dry, and uniform holes were made on the dried seeded plates using sterile cork borer of 5 mm. Each of them was well filled with 40 µL of the biosynthesized SNPs. The plates were incubated at 37°C, for 24 h and examined for zones of inhibition around the wells (Ghasem *et al*., 2016).

Statistical analysis

Statistical analysis of antimicrobial activities of SNPs was done using SPSS software (version 24, SPSS lnc, Chicago, IL, USA). ANOVA was used to compare the results. $P \geq 0.05$ was considered significant.

Results and discussion

The microbial synthesis of silver nanoparticles using CFS of *L. delbrueckii* was visually characterized in Figure 1. It was observed a change in colour of the mixture from golden yellow to dark brown indicating the production of silver nanoparticles. The colour exhibited by the metallic nanoparticles could be as a result of the coherent excitation of entire free electrons within the conduction band leading to surface plasmon resonance (SPR). The changes in colour were also as a result of coherent and collective oscillations of the surface electrons. This result is in correlation with the study conducted by Kabo *et al.* (2019) who reported the biosynthesis of silver nanoparticles with potent antimicrobial activity using lactic acid bacteria. Also, Adebayo-Tayo *et al.* (2017) have biosynthesized silver nanoparticles using culture free supernatant of lactic acid bacteria isolated from fermented food samples.

RESEARCH ARTICLE

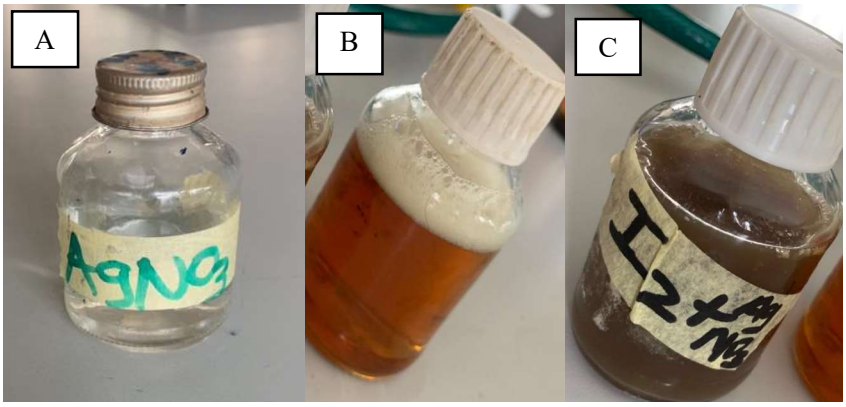

Figure 1. Visual characterization of SNPs synthesized by CFS of *L. delbrueckii*; A- $AgNO_3$; B- CFS of *L. delbrueckii*; C- SNPs synthesized by CFS of *L. delbrueckii*.

The synthesized silver nanoparticles using CFS of *L. delbrueckii* was characterized using UV-Visible spectrophotometer, as shown in Figure 2. The SNPs had strong surface plasma resonance (SPR) peak at 550 nm and a broad band at 450 - 650 nm, which indicated the formation of SNPs from the cell free supernatant. SNPs exhibited size and shape, dependent on SPR bands. This result is similar to the study conducted by Kamani and Lim (2013), who reported that SNPs showed a strong SPR peak at 400-550 nm with a broad band and size, indicating the formation of SNPs that varied in shape and size. Adebayo-Tayo *et al.* (2017), also, reported that the UV-Visible spectra of biosynthesized SNPs by *Lactobacillus casei* LPW2 and *Lactobacillus fermentum* LPF6 showed a broad band between 400 - 600 nm at 24 h and 48 h, respectively. In Figure 2 there was also a surface plasmon absorption band between 250-350 nm which is in accordance with the work of Sani *et al.* (2017) who observed a characteristic surface plasmon absorption band at 292 nm, which could be attributed to the presence of proteins.

RESEARCH ARTICLE

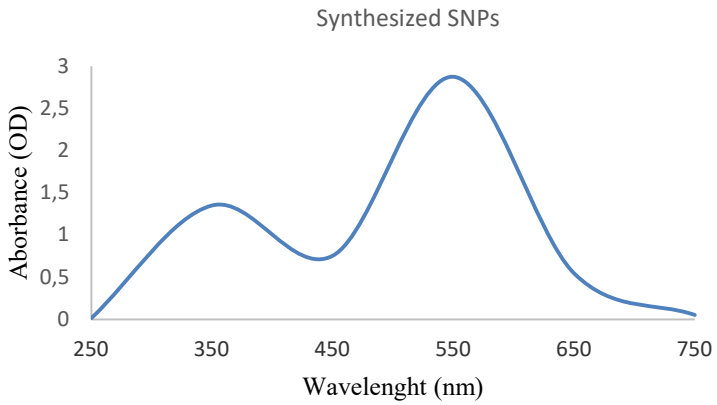

Figure 2. UV-Visible absorption spectra of SNPs synthesized by CFS of *L. delbrueckii*

The whole spectrum was scanned in order to identify the highest and the lowest surface plasmon resonance peaks. Two peaks were observed in Figure 2. The highest absorption peak was at 550 nm while the lowest absorption peak was at 350 nm.

The synthesized SNPs were further characterized by scanning electron microscopy. This is an important tool for the SNPs characterization, as reported by Kamani and Lim (2013); Nanda and Raghavan (2014). The micrograph of SNPs synthesized by CFS of *L. delbrueckii* is shown in Figure 3. The SEM analysis confirmed that SNPs synthesized by CFS of *L. delbrueckii* are spherical with size ranging from 1.7-10.0 nm, which is due to correlation of the absorption spectrum for individual SNPs and the size difference are caused by concentration of reducing agent. This is similar to the result obtained by Ghasem *et al.* (2016), who reported the morphology of SNPs biosynthesized from *Candida albicans* as highly variable, including spherical, rod-like, decahedral, triangular and platelet shapes. However, Vituya *et al.* (2014) reported the SEM of *Bacillus* mediated extracellular synthesis of SNPs to be almost spherical in morphology and some SNPs were aggregated, making the shape to be obscured. Adebayo-Tayo and Popoola, (2017) reported that the SEM for the SNPs biosynthesized by LAB was partially aggregated with particle size ranged from 0.7-10.0 nm.

Vol. 22, 2022
Received March /Accepted July 2022

RESEARCH ARTICLE

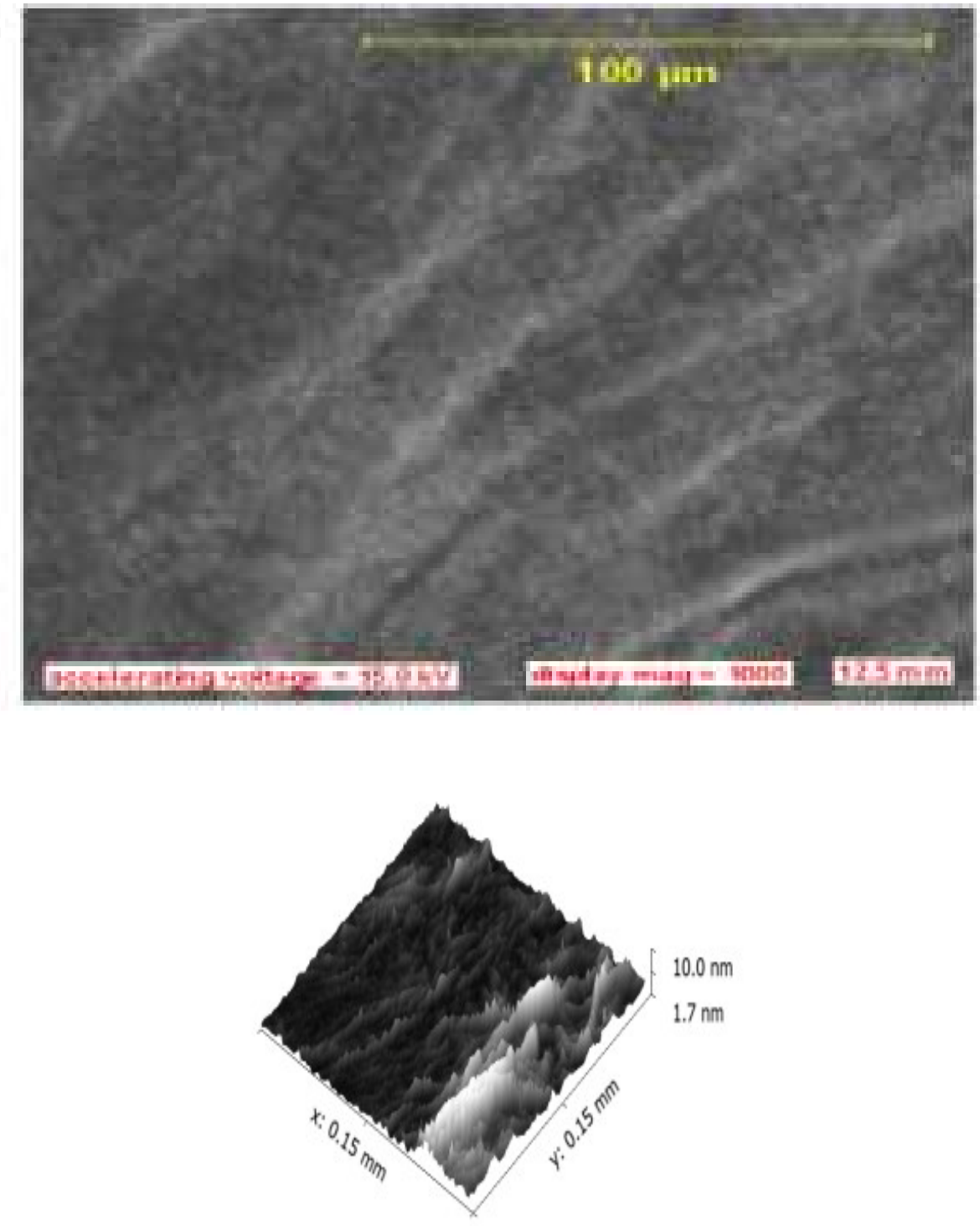

Figure 3. Scanning electron micrograph of SNPs synthesized by CFS of *L. delbrueckii*

RESEARCH ARTICLE

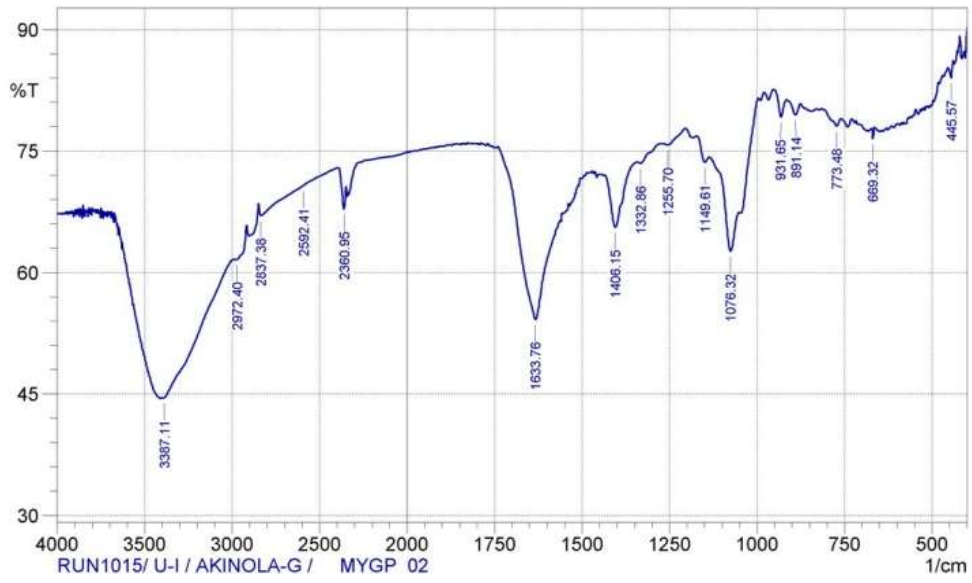

Figure 4: FTIR spectrum of SNPs synthesized by CFS of *L. delbrueckii*

FTIR spectra were further used to characterize the synthesized SNPs. It can be identified the functional groups responsible for the reduction of silver ions. The FTIR spectrum of the biosynthesized SNPs is shown in Figure 4. The observed characteristic peaks ranged from 3387.1 cm^{-1} to 445.57 cm^{-1}. The peak at 3387.1 cm^{-1} showed the presence of O-H stretch of alcohol. The absorption peak at 2972.4 cm^{-1} and 2837.38 cm^{-1} could be attributed to C-H stretch of aldehyde groups. The peaks at 2592.4 cm^{-1}1 indicate the presence of NH_2^+ stretch of primary amide (bonded) and OH stretching vibration. The absorption peak at 2360.95 cm^{-1} could be attributed to the presence of COOH vibrating stretch of overtone. The intense absorption peak at 1633.76 cm^{-1} showed the presence of C=C stretch of terminal olefin plus N-H bend of secondary amide. The peaks at 1406.15 cm^{-1}, 1332.86 cm^{-1} and 1255.7cm^{-1} corresponded to C-H in plane bend, N=N bend of secondary amine and C-O of acetic ester groups. The absorption peak at 1149.61 cm^{-1} was identified as C-O out of plane bending and 1076.32cm^{-1} indicated C-N stretch of aliphatic amines. The peaks at 931.65 cm^{-1} and 891.14 cm^{-1} showed the presence of C-O, CCH and vibrating ring of pyranose. The presence of C-H stretching vibration of monosubstituted benzene was shown by the peak at 773.48 cm^{-1}. The absorption peaks at 669.32 cm^{-1} up to 445.57 cm^{-1} indicated the presence of acetylenic -C≡C-H bend of alkynes. From all indications with the observed functional groups, aldehydes, esters, alcohol, amino acids and proteins may be responsible for the stability and bio-reduction of silver nitrate to SNPs. These functional groups are present as a result of the

RESEARCH ARTICLE

presence of biomolecules from the cell membrane of *L. delbrueckii* involved in the biosynthesis process. Carbonyl groups from the amino acid residues and peptides of protein has strong ability to bind silver, while protein forms a coat covering the metal nanoparticles and aids its stabilisation in the medium. Similarly, Hidayat *et al.* (2020) reported the presence of proteins and amino acids as stabilizing agent for SNPs. Shankar and Pradhan (2017) reported that amino acids, hydroxyl, carbonyl and aldehyde functional groups were present in biosynthesized SNPs and they were responsible for the SNPs bio-reduction.

Table 1. Antibacterial activity of $AgNO_3$, *L. delbrueckii* CFS (LDCFS), synthesized SNPs by CFS of *L. delbrueckii* (LDSNPs) and Ciprofloxacin (control) against test bacteria

	Zones of Inhibition (mm)			
Test bacteria	**$AgNO_3$**	**LDCFS**	**LDSNPs**	**Ciprofloxacin**
E. coli	$8^{d}\pm 0.27$	$10^{c}\pm 0.38$	$22^{a}\pm 0.53$	$16^{b}\pm 0.42$
S. typhi	-	$8^{c}\pm 0.73$	$17^{b}\pm 0.28$	$22^{a}\pm 0.63$
S. aureus	$8^{d}\pm 0.43$	$10^{c}\pm 0.61$	$21^{a}\pm 0.54$	$16^{b}\pm 0.58$
B. subtilis	$10^{d}\pm 0.56$	$12^{c}\pm 0.72$	$20^{a}\pm 0.51$	$19^{b}\pm 0.40$
Klebsiella sp.	$11^{c}\pm 0.49$	$9^{d}\pm 0.54$	$29^{a}\pm 0.42$	$17^{b}\pm 0.59$
P. aeruginosa	-	$8^{c}\pm 0.33$	$18^{a}\pm 0.57$	$15^{b}\pm 0.45$

The mean values reported with different superscript in each row indicated significant difference ($P \geq 0.05$)

The antibacterial activity of SNPs synthesized by CFS of *L. delbrueckii* against some test bacteria was statistically evaluated (Table 1). The SNPs synthesized by the CFS of *L. delbrueckii* (LDSNPs) exhibited the highest antimicrobial activity against *E. coli*, *S. aureus*, *B. subtilis* and *Klebsiella* spp., with a zone of inhibition of 22 mm, 21 mm, 20 mm and 29 mm, respectively. *S. typhi* and *P. aeruginosa* are however resistant to the synthesized SNPs. Ciprofloxacin which was the positive control, also had antibacterial activity against the tested bacteria but it was not as high as that of the synthesized SNPs, except against *S. typhi.* It was observed that the antibacterial activity exhibited by LDCFS and $AgNO_3$ had the lowest inhibition range for all isolates tested.

RESEARCH ARTICLE

The synthesized SNPs had a bactericidal effect on both Gram-positive and Gram-negative bacteria (Priyadaishini *et al.* 2013; Adebayo-Tayo and Popoola, 2017) which was as a result of interaction with the DNA of the bacteria, thus making them to lose the ability to replicate, thus, leading to the cell death. This observation is in agreement with the study conducted by Priyadaishini *et al.* (2013), who reported that Gram-negative bacteria, *E. coli*, showed a greater antibacterial activity compared to that of the Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus pyogenes*, being probably attributed to their thick cell walls. Adebayo-Tayo and Popoola (2017) reported that *S. aureus*, a Gram-positive organism was the most susceptible to the biosynthesized SNPs. This was also in correlation with Hidayat *et al.* (2020) findings, who reported that the inhibitory effects of SNPs were more evident on Gram-negative bacteria, due to their cell composition and thickness of the cell wall (Rajathi *et al*., 2012). Sadhasivam *et al*. (2010) observed the highest antimicrobial activity of SNPs against *B. subtilis* and *Candida albicans*.

Conclusions

The findings of this study demonstrated an eco-friendly method of synthesizing silver nanoparticles using culture free supernatant of *L. delbrueckii*. *L. delbrueckii* was able to reduce $AgNO_3$ for the production of SNPs. Changes in colour indicated that SNPs were synthesized. The UV-Visible absorbance spectra confirmed the strong plasmon resonance peak at 550 nm with spherical shape and varying sizes (1.7-10.0 nm). The functional groups present in the synthesized SNPs were hydroxyl, aldehyde, esters and amino acids groups, respectively. The synthesized SNPs showed a potential antibacterial activity against Gram-positive and Gram-negative bacteria compared to ciprofloxacin and $AgNO_3$.

Acknowledgments. The authors would like to thank KolaDaisi University, Ibadan, Nigeria for its contributions in the provision of some equipment to carry out this research.

References

1. Adebayo-Tayo B.C., and Popoola A.O. (2017) Biogenic synthesis and antimicrobial activity of silver nanoparticles using exopolysaccharide from lactic acid bacteria. International Journal of Nano Dimension, 8, 61-69.
2. Adebayo-Tayo B.C., Popoola A.O., and Ajunwa O.M. (2017) Bacterial synthesis of silver nanoparticles by culture free supernatant of lactic acid bacteria isolated from fermented food samples. Biotechnological Journal International, 19, 1-13.

RESEARCH ARTICLE

3. Furno F., Morley K.S., Wong B., Sharp B.L., Arnold P.L., Howdle S.M., Bayston R., Brown P.D., Winship P.D., and Reid H. J. (2004) Silver nanoparticles and polymeric medical devices: A new approach to prevention of infection. Journal of Antimicrobial Chemotherapy, 54, 1019-1024.
4. Ghasem R., Fahimeh A. and Aureza K. (2016) Mycosynthesis of Silver nanoparticles from *candida albicans* and its Antibacterial Activity against *Escherichia coli* and *Staphylococcus aureus*. Tropical Journal of Pharmaceutical Research, 15, 371-375.
5. Gupta S., Sharma K. and Sharma R. (2012) Myconanotechnology and Application of Nanoparticles in Biology. Recent Research in Science and Technology, 48, 36-38.
6. Hidayat M.Y., Nor Aini A.R., Rosfarizan M., and Uswatun H.Z. (2020) Microbial Mediated Synthesis of Silver Nanoparticles by *Lactobacillus plantarum* TA4 and its Antibacterial and Antioxidant Activity. Journal of Applied Sciences, 10, 69-73.
7. Iyare H.E, Ogunbiyi O.J, and Ariredіegha O.V. (2020) Isolation and Characterization of Microorganisms obtained from pap of different market sources in Benin city. Journal of Basic and Applied Research International, 26, 97-103.
8. Kabo R.W., Wathuto O., Daniel L., Phatsimo M., Baemedi L., Mbaki M., Krishna B. K., and Ntebaleng M. (2019) Biosynthesis of silver nanoparticles with potential antimicrobial activity using lactic acid bacteria. European Journal of Biomedical and pharmaceutical sciences, 6, 135-141.
9. Mithun S., Dipak V., and Sheela S. (2015) Isolation and identification of *Lactobacilli* from raw milk samples obtained from Aarey milk colony. International Journal of Scientific and Resources Publication, 5, 1-5.
10. Nanda A. and Raghavan C.M. (2014) Antimicrobial efficacy of synthesized AgNPs from Exopolysacharides (EPS) produced by *Bacillus subtilis* of Medico-clinical sinks. International Journal of Chemical Technology Research, 6, 2914-2919.
11. Natarajan K., Selvara S., and Murty V.R. (2010) Microbial production of silver nanoparticles., Digest Journal of Nanomaterials and Biostructures, 5, 135-140.
12. Popoola A.O. and Adebayo-Tayo B.C. (2017) Green synthesis of silver nanoparticles using supernatant from *Lactobacillus casei* LPW2 cultured in modified exopolysaccharides selection medium. Journal of Applied Sciences International, 13, 1-8.

RESEARCH ARTICLE

13. Priyadaishini S., Gopinath V., Priyadharsshini N.M., Muibarak A. and Velusamy P. (2013) Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus cereus* and its biomedical applicant. Colloids Surface B, 102, 232-237.
14. Rai, M. Yadav A. and Gade A. (2009) Silver Nanoparticles: As a New Generation of Antimicrobials. *Biotechnology Advance*, 27, 76-83.
15. Rajathi K., Vijaya D.R., Anarkali J. and Sridhar S. (2012) Green synthesis, characterization and In-vitro Antibacterial activity of Silver Nanoparticles by using *Tinospora cordifolia* leaf extract. International Journal of Green Chemistry and Bioprocess, 2, 15-19.
16. Sadhasivam S., Shanmugan P. and Yun K. (2010) Biosynthesis of Silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic micro-organisms. Colloids and Surfaces Biointerface, 81, 358-362.
17. Sani N.J., Aminu B.M., and Mukhtar M.D. (2017) Eco-friendly Synthesis of Silver nanoparticles using *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from Kindrimo (locally fermented milk) in Kano State, Nigeria. Journal of Pure and Applied Sciences, 10, 481-488.
18. Shankar K.G., Pradhan N. Masilamani K. and Fleming A.T. (2017) Silver nanoparticles from *Trianthema portulacastrum:* Green synthesis, characterisation, antibacterial and anticancer properties. Asian Journal of Pharmaceutical and Clinical Research, 10, 308-313.