Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Synthesis of silver nanoparticles using *Lactobacillus bulgaricus* and assessment of their antibacterial potential

Síntese de nanopartículas de prata usando *Lactobacillus bulgaricus* e avaliação de seu potencial antibacteriano

Q. A. Naseer[a,b†], X. Xue[c†], X. Wang[c], S. Dang[a,c], S. U. Din[d], Kalsoom[e*], J. Jamil[e]

[a]Jiangsu University, The Affiliated Hospital, Department of General Surgery, Zhenjiang, Jiangsu, China
[b]Jiangsu University, School of Medicine, Department of Immunology and Institute of Laboratory Clinical Diagnostics, Zhenjiang, Jiangsu, China
[c]Pucheng Hospital, Department of General Surgery, Pucheng, Shanxi, China
[d]Quaid I Azam University, Department of Microbiology, Islamabad, Pakistan
[e]University of Swabi, Department of Microbiology, Swabi, KP, Pakistan

Abstract

Many pathogenic strains have acquired multidrug-resistant patterns in recent a year, which poses a major public health concern. The growing need for effective antimicrobial agents as novel therapies against multidrug-resistant pathogens has drawn scientist attention toward nanotechnology. Silver nanoparticles are considered capable of killing multidrug-resistant isolates due to their oligo-dynamic effect on microorganisms. In this research study NPs were synthesized using the gram-positive bacteria *Lactobacillus bulgaricus* and its activity against selected pathogenic strains. *Lactobacillus bulgaricus* pure cultures were isolated from raw milk and grown in "De Man, Rogasa, and Sharp" broth for synthesis of nanoparticles. *Lactobacillus bulgaricus* culture was centrifuged and Cell-free supernatant of it was employed with aqueous silvery ions and evaluated their antibacterial activities against bacterial strains i.e. *Staphylococcus aureus, Staphylococcus epidermidis* and *Salmonella typhi* using agar well diffusion assay. Antibiotic profiling against selected pathogenic strains were also conducted using disc diffusion method. The synthesis and characterization of silver nanoparticles were monitored primarily by the conversion of the pale-yellow color of the mixture into a dark-brown color and via ultraviolet-visible absorption spectroscopy and Scanning electron microscopy respectively. The result showed that that AgNPs with size (30.65-100 nm) obtained from *Lactobacillus bulgaricus* were found to exhibit antibacterial activities against selected bacterial strains. Taken together, these findings suggest that *Lactobacillus bulgaricus* has great potential for the production of AgNPs with antibacterial activities and highly effective in comparison to tested antibiotics.

Keywords: Silver nanoparticles, *Lactobacillus bulgaricus*, biosynthesis, antibacterial.

Resumo

Muitas cepas patogênicas adquiriram padrões multirresistentes nos últimos anos, o que representa um grande problema de saúde pública. A crescente necessidade de agentes antimicrobianos eficazes como novas terapias contra patógenos multirresistentes atraiu a atenção dos cientistas para a nanotecnologia. As nanopartículas de prata são consideradas capazes de matar isolados multirresistentes por causa de seu efeito oligodinâmico em microrganismos. Neste estudo de pesquisa, as NPs foram sintetizadas usando a bactéria Gram-positiva Lactobacillus bulgaricus e sua atividade contra cepas patogênicas selecionadas. Culturas puras de Lactobacillus bulgaricus foram isoladas do leite cru e cultivadas em caldo "De Man, Rogasa e Sharp" para síntese de nanopartículas. A cultura de Lactobacillus bulgaricus foi centrifugada, e o sobrenadante livre de células foi empregado com íons prateados aquosos, avaliando-se suas atividades antibacterianas contra cepas bacterianas, isto é, Staphylococcus aureus, Staphylococcus epidermidis e Salmonella typhi usando ensaio de difusão em poço de ágar. O perfil de antibióticos contra cepas patogênicas selecionadas também foi conduzido usando o método de difusão em disco. A síntese e a caracterização das nanopartículas de prata foram monitoradas principalmente pela conversão da cor amarelo-pálida da mistura em uma cor marrom-escura e por espectroscopia de absorção visível e ultravioleta e por microscopia eletrônica de varredura, respectivamente. O resultado mostrou que AgNPs com tamanho de 30,65-100 nm, obtidas de Lactobacillus bulgaricus, exibiram atividades antibacterianas contra cepas bacterianas selecionadas. Tomados em conjunto, esses achados sugerem que o Lactobacillus bulgaricus tem um grande potencial para a produção de AgNPs com atividades antibacterianas e altamente eficazes em comparação aos antibióticos testados.

Palavras-chave: nanopartículas de prata, *Lactobacillus bulgaricus*, biossíntese, antibacteriano.

*e-mail: kalsoom_wazir@yahoo.com
Received: December 20, 2019 – Accepted: July 13, 2020
† Equal contribution of author in article.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

The synthesis of silver nanoparticles (AgNPs) is an active area of nanotechnology and biotechnology. Applications of nanotechnology in the field of biomedicine are varied and distinctive due to their high structural integrities and diversified properties (Sarvamangala et al., 2013). A variety of physical-chemical methods have been used for the synthesis of AgNPs. Unfortunately, the chemicals used in many of these synthesis protocols are toxic, flammable, and are not easily disposable, which pose as hazards to individuals and the environment (Dakhil, 2017). Hence, the development and use of non-toxic, clean, eco-friendly, and cost-effective methods have drawn considerable interest for the biological synthesis of AgNPs (Aondona, 2018). Few biomimetic approaches have been established that are ecological and sustainable for the synthesis of nanoparticles. One of the fundamental processes in biomimetic is the bio-reductive process via microorganisms, enzymes, fungi, plants, and/or plant extracts (Patra et al., 2014; Klueh et al., 2000). For centuries, silver has been used for the treatment of burns, chronic wounds, venereal disease, fistulae from slivery glands, bone and perianal disease, and numerous bacterial infections. With the discovery of the first antibiotic, the use of silver for the treatment of infections were reduced. However, silver has once again been revived as a potential antimicrobial agent due to limitations in the use of antibiotics and the bacterial resistance against antibiotics (Rai et al., 2009). An antimicrobial use of silver has received widespread acceptance because it controls the growth of many different types of microorganisms, and since silver and its compounds are non-toxic, inorganic, and efficient antimicrobial agents (Jeong et al., 2005). Similarly, AgNPs have also drawn noteworthy attention as potential antimicrobial (i.e., antifungal and antibacterial) agents. This attention has been due to the rapid development in research on metal nanoparticles, and the efficacy of nano sized antimicrobial activity rivaling that of bulk silver metals. Also, AgNPs have a high specific surface area and a substantial fraction of surface atoms (Kim et al., 2008; Lee et al., 2010). AgNPs have strong toxic effects against a wide range of microorganisms, as they exhibit strong bactericidal effects in *Escherichia coli*—which is a model organism of gram-negative bacteria—and in as many as 16 other species of bacteria (Sondi and Salopek-Sondi, 2004). Silver or silver-based compounds are considered as an excellent source against microorganisms and proven its activity against bacterial strains i.e. sixteen species from both gram-positive and gram-negative (Zhao and Stevens Junior, 1998; Spadaro et al., 1974; Tankhiwale and Bajpai, 2009). However, from last few years, the focus of research diverted to produce AgNPs by microorganisms which have antibacterial activity, for this number of experiments based on antibacterial activity using filter paper method was conducted but the dose of sample was not clearly optimized and the antibacterial activity was greatly inhibited by aggregates formation of AgNPs (Martins et al., 2012; Sumi Maria et al., 2015). For commercializing the product and maximizing the antibacterial activity of AgNPs, the solution to these problems need to be sorted out. The present study aimed to synthesize AgNPs extracellular from extracts of *Lactobacillus bulgaricus* as a rapid method of production and to evaluate the antibacterial activities of these AgNPs against selected bacterial strains.

2. Methodology

2.1. Lactobacillus bulgaricus: isolation and identification

The *Lactobacillus bulgaricus* was isolated from raw milk, sample was 10 fold diluted ranging from 10^{-1} to 10^{-10}. At the end of dilution, 100 µL of sample was shifted aseptically with the help of micropipette to the Rogasa, and Sharpe (MRS) agar plates by using sterile glass spreader. Afterward, plates were stored at static incubator for 24 hrs at 37 °C. After incubation, the types and number of colonies were counted and examined. The isolated colonies were sub-cultured in triplicate on freshly prepared MRS plates for purpose to obtain pure culture. The pure strain keep at 4 °C for further analysis. *Lactobacillus bulgaricus* was identified by mean of biochemical and morphological characteristics.

2.2. Synthesis of AgNPs using Lactobacillus bulgaricus

A loop full pure culture of *Lactobacillus bulgaricus* was inoculated in MRS broth and incubated overnight at 37 °C. After incubation the biomass was centrifuged at 5000 rpm for 10 minutes and transfers to sterile bottle and preserved at 4 °C for further use in silver nanoparticle synthesis. The synthesis of AgNPs needs precursor, for which silver nitrate ($AgNO_3$) was used. 10 mL of 1 mM$AgNO_3$ solution was prepared in 1 mL of cell free supernatant. To obtained reaction the solution was incubated overnight at room temperature. Control solutions were also prepared for comparison, i.e., 10 mL of $AgNO_3$ and 10 mL of distal water separately. The synthesis of AgNPs was determined by color changes of solution.

2.3. AgNPs characterization and formation determination

Silver nanoparticles characterization and formation were carried out using various analytical techniques i.e. Visible Spectrophotometry, Scanning electron microscopy(SEM) SEM and Energy-dispersive X-ray spectroscopy(EDS). Vis. Spectrophotometer is widely used technique for silver nanoparticles detection and determination. After visible observation of color changes of solution, the solution was analyzed through spectrophotometer for reduction study. 2 mL of the reaction mixture after incubation time was measured for the absorbance spectra (190-650 nm) using UV-1600 UV Spectrophotometer by Shimadzu Corporation, Japan. Size and morphological features of AgNPs was determined using Scanning electron microscope using standard protocol (Dakhil, 2017). Elemental analysis for presence of AgNPs was carried out following standard protocol of using EDS (Braker, Netherland) connected with Scanning electron microscope with voltage 10 kV and spot size 5.

2.4. Silver nanoparticles antibacterial activity

For antibacterial activity, cell-free supernatant containing AgNPs was carried out using agar well diffusion

assay against selected pathogenic strains on Mueller-Hinton (MH) agar plates. The selected pathogenic bacterial strains *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Salmonella typhi* were obtained from the Microbiology Laboratory of Jiangsu University affiliated hospital. These strains were revived from preservation vials onto MH agar plates and incubated overnight, then MH broth was prepared in three separate test tubes and each test tube was inoculated with individual strain and incubated overnight. MH agar plates were prepared for assay, and wells of 6 mm diameter were formed with borer and inoculated with 50 µL of AgNPs (4 mm) solution and 50 µL of bacterial cell suspension. The plates were then incubated at 30 °C for overnight, and the zone of inhibition was measured.

3. Result

3.1. Identification and characterization of Lactobacillus bulgaricus

Bacterial strain *Lactobacillus bulgaricus* was isolated from raw milk for extracellular synthesis of AgNPs. The morphological characteristics showed that the colony was creamy grey, round in shape, and had a raised elevation and entire margin (Figure 1). Biochemical tests showed that *Lactobacillus bulgaricus* was positive for mannitol fermentation and negative for catalase, citrate, indole, and urease (Table 1).

3.2. Synthesis and characterization of AgNPs

The biosynthesis of AgNPs was successfully achieved by inoculating 1 mL of inoculum in 10 mL of 1 mM silver nitrate solution (1:10). The positive result for the biosynthesis of AgNPs was observed by color changes from yellow to dark brown, while intensity increasing during the time of incubation. Whereas, both controls were observed unchanged (Figure 2), for accuracy in result, different suspension solution of $AgNO_3$ with varying concentration (1-5%) was added to Lactobacillus suspension. The characteristics of synthesized nanoparticles are greatly affected by adding different concentrations of $AgNO_3$to the supernatant. Maximum synthesis of nanoparticle was observed at 4 mM $AgNO_3$ (Figure 3).

3.2.1. Determination of AgNPs using spectrophotometer

The production of AgNPs was confirmed by UV-VIS absorption. AgNPs from *Lactobacillus bulgaricus* was observed by UV-VIS at visible range 410 to 430 nm (Figure 4) which is presumed peak for AgNPs reported in previously by Sikder et al., (2018).

Table 1. Biochemical identification of *Lactobacillus bulgaricus*.

Serial No	Parameter	*Lactobacillus bulgaricus*
1	Growth at different temperatures	
A	15 °C only	+
B	35 °C only	+
C	45 °C only	+
2	NH_3 from arginine	+
3	Sugar fermentation	+
A	Arabinose	+
B	Cellobiose	+
C	Mannitol	+
D	Mannose	+
E	Raffinose	+
F	Rhamnose	_
G	Xylose	+

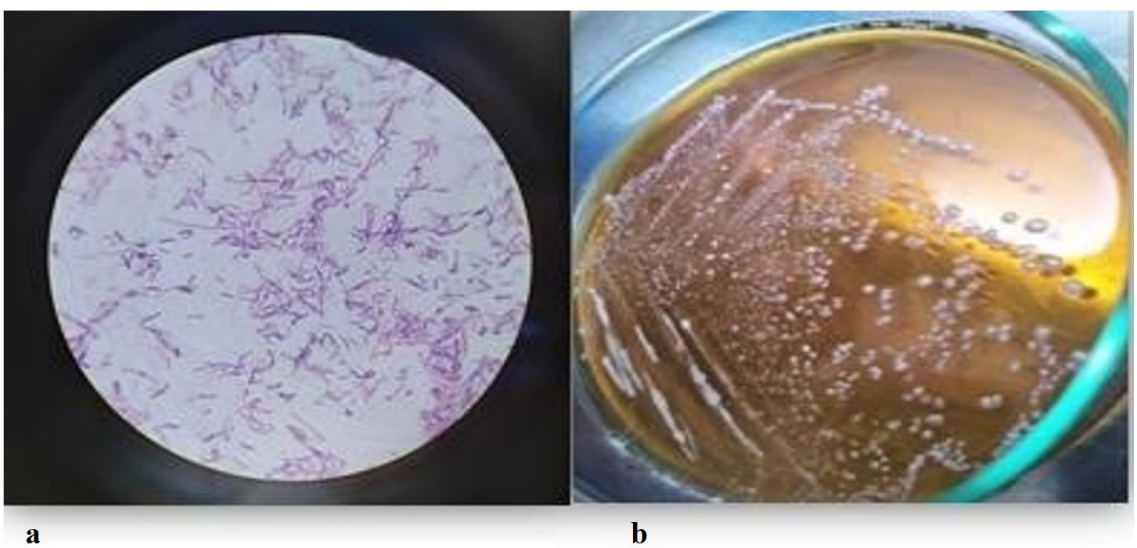

Figure 1. Morphological and microscopic properties of *Lactobacillus bulgaricus*. (a) *Lactobacillus* stained with gram stain under light microscope (X100); (b) *Lactobacillus* on MRS agar medium, 37 °C, 48 h.

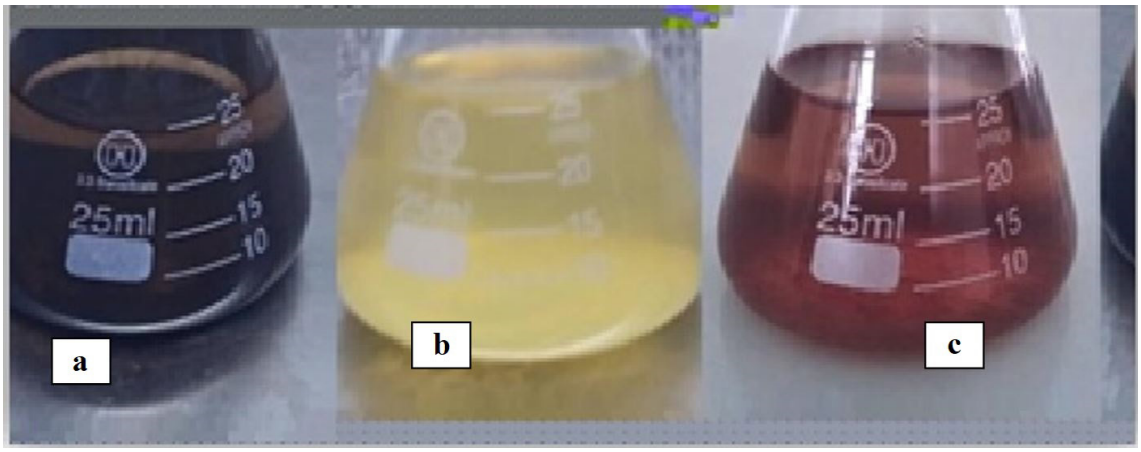

Figure 2. Lactobacillus *bulgaricus* synthesis of Ag-NPs: (a) AgNO3 (control); (b) Reaction mixture before synthesis; (c) Reaction mixture after synthesis.

Suspension at different AgNO3 concentrations					
$AgNO^3$	1%	2%	3%	5%	10%
					

Figure 3. Results of suspension at different $AgNO_3$ concentrations of *Lactobacillus bulgaricus.*

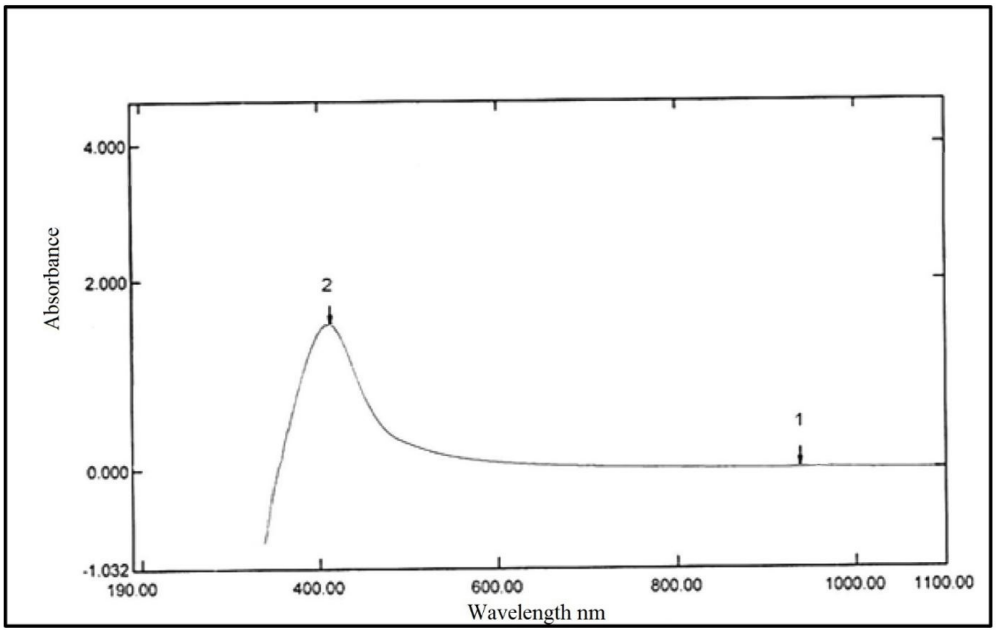

Figure 4. Absorption spectrum of AgNPs synthesized by *Lactobacillus bulgaricus* having clear peak at 410 nm.

3.2.2. SEM confirmation of AgNPs

SEM analysis of AgNPs was performed to determine the size and shapes of AgNPs and dispersed AgNPs with a diameter ranges 30-100 nm and different shapes were observed (Figure 5). The characteristics of synthesized nanoparticles are greatly affected by adding varying concentration of $AgNO_3$ to the supernatant. It was observed in this study that the best concentration for synthesis of nanoparticles was 4 mM $AgNO_3$.

3.2.3. Energy dispersive-X-ray spectroscopy

Energy Dispersive-X-ray Spectroscopy (EDS) was carried out to find out overall compositions of elements in reaction mixture of *Lactobacillus bulgaricus* and silver nitrate after completion of reactions. The EDS analysis showed the significant amount of AgNPs presence, furthermore along with AgNPs other elements were also detected which might be present in media composition (Figure 6).

3.3. Comparative antibacterial activity of antibiotics and AgNPs

The antibacterial activity of AgNPs from *Lactobacillus bulgaricus* were evaluated against three selected bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhi.* An $AgNO_3$ solution (4 mM) and some antibiotics were found to be effective against selected strains. It was found that the antimicrobial activities of biologically synthesized AgNPs were not uniform across bacterial strains. The mean value of zones of inhibition noted for selected bacterial strains were 15-mm for *Staphylococcus aureus,* 17-mm for *S. epidermidis*, and *Salmonella typhi* respectively. The effects of antibiotics against selected bacterial strains were also evaluated according to Birmingham Children's Hospital (2014) guidelines.

We found that the antibiotic activities against selected bacterial strains were not uniform. Furthermore, bacterial strains were found highly sensitive to AgNPs in comparison to tested antibiotics (Table 2 and Figure 7).

Table 2. Comparative analysis of AgNPs and antibiotics against bacterial strains.

Bacterial strains	Antibacterial activity						
	CRO	PEN	AMX	TEC	FOF	IPM	AgNPs
Staph. aureus	S	R	R	R	S	S	S
Staph. epidermis	R	R	R	R	R	S	S
Salmonella	R	R	R	R	R	S	S

AgNPs: silver nanoparticles; AMX: amoxycillin; CRO: ceftriaxone; FOF: fosfomycin; IPM: imipenem; PEN: penicillin; R: resistant; S: sensitive; TEC: teicoplanin.

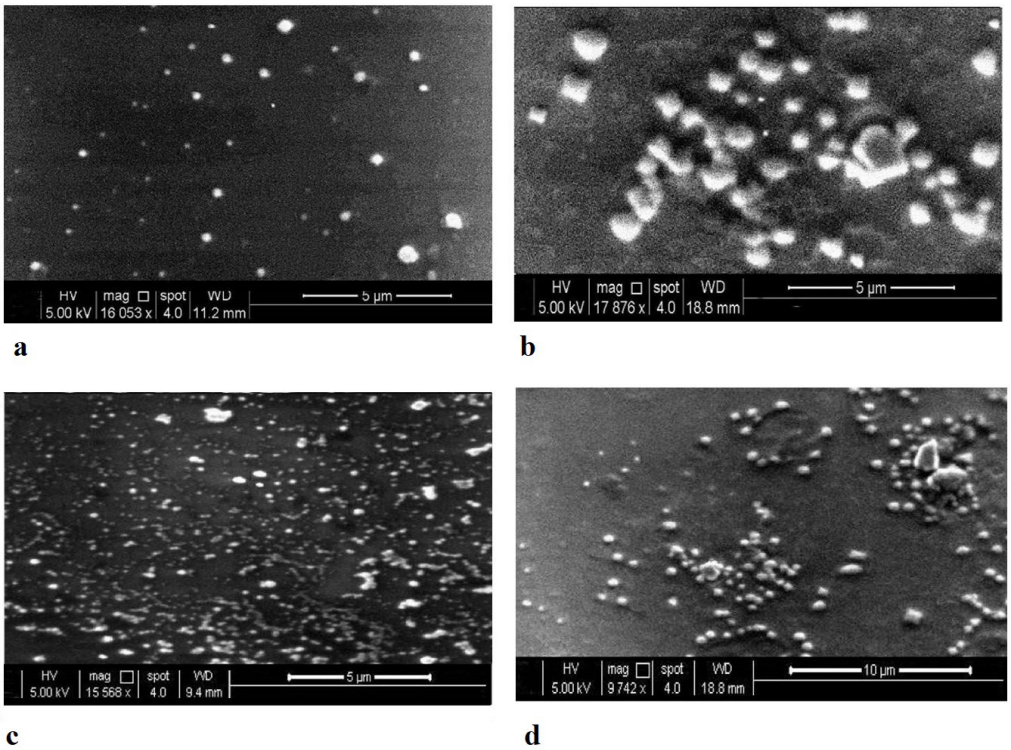

Figure 5. SEM of silver nanoparticles synthesized by *Lactobacillus bulgaricus*. The shape of AgNPs was spherical and size between (30-100 nm), magnification (16053×, 17876×, 15568×, and 9742 ×), the voltage (5 kV) and spot size 4 for (a), (b), (c) and (d) respectively.

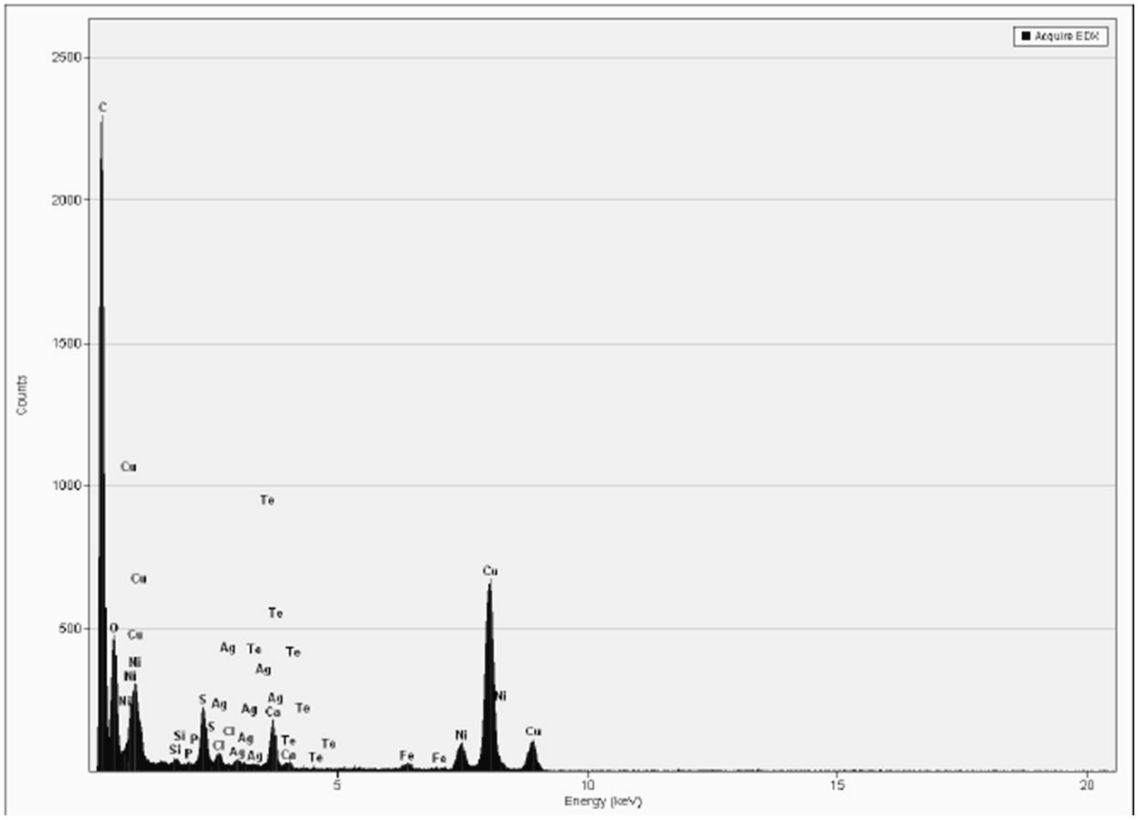

Figure 6. EDS Analysis of AgNPs synthesized by *Lactobacillus bulgaricus*.

Figure 7. Antibacterial effect of Ag-NPs and antibiotics against (a) *Staphylococcus epidermis* (b) *Salmonella* (c) *Staphylococcus aureus*.

4. Discussion

In recent times the attention of researchers is drawn towards using and exploring microorganisms as a potential source for the production of nanoparticles (Karakas-Sen and Karakas, 2018). Raw milk serves as an excellent source for isolation of *Lactobacillus;* in the current study, raw milk samples were diluted and spread onto MRS agar plates, and isolated bacterial was gram-positive, capable of growing at wide temperature ranges and metabolize different types of sugar (Table 1). The result was compared to characteristics (Bergey's Manual of Determinative Bacteriology) and the genus of isolated bacteria was identified as *Lactobacillus*. *Lactobacillus* was found in raw milk and other dairy products as well (Ranganath et al., 2012). These results are supported by findings from previous studies (Kim et al., 2008; Lee et al., 2010).

Lactobacillus species have previously been used for the synthesis of AgNPs, but approximately 12-14 days were required for this synthesis (Chaudhari et al., 2012a). In the present study, *Lactobacillus bulgaricus* strain was confirmed as positive for biosynthesized silver nanoparticles as media color changes from yellow to brown (Figure 2). The reduction of silver from ionic form Ag^{+} to Ag^{0} occurs by *Lactobacillus bulgaricus,* which serves as a reducing agent. A similar study was conducted in which brown mass settled down at the base of flask after overnight incubation of *Lactobacillus bulgaricus*. Furthermore, it is reported that

the reduction of silver from oxidized to highly reduced form occurred via an enzyme called nitrate reductase. Generally, this enzyme released in the solution and with the help of capping agents (e.g. protein) reduced silver nitrate to silver nanoparticles (David, 2009). Different studies have been conducted before in which biosynthesis of AgNPs occurs using reducing agents i.e. plant extracts and sunlight. The glycolipid bio-surfactant extracted from *Pseudomonas* sp. was also used as reducing agent (Das et al., 2016; Mukherjee et al., 2002).

In our study, the synthesis of AgNPs was confirmed by UV-VIS absorption spectra of the reaction mixture of *Lactobacillus bulgaricus* and observed peak in the visible region ranges at 410-430 for the AgNPs, which is presumed peak for AgNPs (Figure 4). The results showed similarity with another study in which AgNPs were produced by *Lactobacillus* species with absorbance at 410 nm and observed through UV-Visible spectrophotometry (Sani et al., 2018). The peak formation at 410 nm is reported before as confirmation of the formation of AgNPs (Jaffat et al., 2017). Other researchers also have been reported that the absorption spectra peaks forAgNPs ranges 391-440 nm, whereas the peak at 292 nm could be considered for the presence of proteins (Lateef et al., 2015).

Scanning electron microscopy was used to determine the morphology of AgNPs produced by *Lactobacillus bulgaricus* isolated from raw milk. The size and shapes of AgNPs and dispersed AgNPs with a diameter range 30-100 nm and different shapes were observed using SEM analysis (Figure 5). The result of SEM analysis is quite similar with a study done before in which the images of the silver nanoparticles synthesized by *Lactobacillus* showed some variability in shape and size, which range between 2-20 nm (Dhoondia and Chakraborty, 2012). SEM has been used in several studies to ascertain the size and shape of silver nanoparticles (Omidi et al., 2014).

Energy Dispersive-X-ray Spectroscopy analysis is used for both quantitative and qualitative studies of total element composition in a study sample. In our study, EDS was carried out to find out the overall compositions of elements in reaction mixture of *Lactobacillus bulgaricus* and silver nitrate after completion of reactions. The EDS analysis showed a significant amount of AgNPs presence, furthermore, along with AgNPs, other elements were also detected which might be present in media composition (Figure 6). In many previous studies, EDS has been used for the determination of AgNPs synthesis and reduction study (Sarvamangala et al., 2013; Senthil Prabhu et al., 2014).

In this study, the antibacterial activities of AgNPs from *Lactobacillus bulgaricus* were evaluated against three selected bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermis*, *and Salmonella.* An $AgNO_3$ solution (4 mM) and some antibiotics such as cephalexin, dicloxacillin, ampicillin and amoxicillin were found to be effective against selected strains. It was found that the antimicrobial activities of biologically synthesized AgNPs were not uniform across bacterial strains. The mean value of zones of inhibition noted for selected bacterial strains were 15-mm for *Staphylococcus aureus* 17-mm for *Staphylococcus epidermis*, *and Salmonella typhi* respectively. The effects of antibiotics against selected bacterial strains were also evaluated according to CLSI guidelines. We found that the antibiotic activities against selected bacterial strains were not uniform. Furthermore, bacterial strains were found highly sensitive to AgNPs in comparison to tested antibiotics (Table 2 and Figure 7). It was showed that the silver nanoparticles have an antimicrobial effect on *S. aureus and E. coli* (Chaudhari et al., 2012b).

5. Conclusions

The findings of the present study suggest that non-pathogenic bacteria are useful in the extracellular synthesis of AgNPs. There is an increasing necessity to develop environmentally friendly methods for nanoparticle synthesis that do not use toxic chemicals. *Lactobacillus bulgaricus*is considered to be a probiotic that has been labeled as friendly bacteria and is found in the enteric system as well as in the oral cavity. Bio-$Ag^{0\text{-}6}$ exhibited excellent antibacterial activity against Gram-negative bacteria and a reduced level of activity against Gram-positive bacteria. The MIC and MBC tests revealed that the antibacterial activity was more pronounced against Gram-negative organisms than Gram-positive organisms possibly due to the differences in cell wall and membrane structure. In the present study AgNPs from lactobacillus exhibited significant antibacterial activity against various gram positive bacteria which is a simple, stable, non-toxic, and ecological method for synthesizing bioactive AgNPs as compared to previous reported study. These biologically synthesized AgNPs were found to be antibacterial. Hence, this novel method for synthesizing AgNPs may represent a promising alternative to traditional chemical and physical methods since our method is free from toxic chemicals and is cost-effective.

Acknowledgements

We thank Let Pub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

References

AONDONA, I.P., 2018. Biosynthesis of eco-friendly silver nanoparticles using dry fluted pumpkin telfairia occidentalis leave extracts reducing agent. *International Journal of Trend in Scientific Research and Development.*, vol. 2, no. 3, pp. 1041-1047. http://dx.doi.org/10.31142/ijtsrd11354.

BIRMINGHAM CHILDREN'S HOSPITAL, 2014. *Guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis in the UK (Version 2) an evidence based guideline*. Birmingham: Birmingham Children's Hospital.

CHAUDHARI, P.R., MASURKAR, S.A., SHIDORE, V.B. and KAMBLE, S.P., 2012a. Effect of biosynthesized silver nanoparticles on *Staphylococcus aureus* biofilm quenching and prevention of biofilm formation. *Nano-Micro Letters*, vol. 4, no. 1, pp. 34-39. http://dx.doi.org/10.1007/BF03353689.

CHAUDHARI, P.R., MASURKAR, S.A., SHIDORE, V.B. and KAMBLE, S.P., 2012b. Antimicrobial activity of extracellularly synthesized silver nanoparticles using *Lactobacillus* specie obtained from

VIZYLAC capsule. *Journal of Applied Pharmaceutical Science*, vol. 2, no. 3, pp. 25-29.

DAKHIL, A.S., 2017. Biosynthesis of silver nanoparticle (AgNPs) using Lactobacillus and their effects on oxidative stress biomarkers in rats. *Journal of King Saud University Science.*, vol. 29, no. 4, pp. 462-467. http://dx.doi.org/10.1016/j.jksus.2017.05.013.

DAS, A.J., KUMAR, R., GOUTAM, S.P. and SAGAR, S.S., 2016. Sunlight irradiation induced synthesis of silver nanoparticles using glycolipid bio-surfactant and exploring the antibacterial activity. *Journal of Bioengineering & Biomedical Science*, vol. 6, no. 5. http://dx.doi.org/10.4172/2155-9538.1000208.

DAVID, A., 2009. *Bioinspired synthesis of magnetic nanoparticles*. Ames: Iowa State University. Master of Science. http://dx.doi.org/10.2172/967072.

DHOONDIA, Z.H. and CHAKRABORTY, H., 2012. *Lactobacillus* mediated synthesis of silver oxide nanoparticles. *Nanomaterials and Nanotechnology*, vol. 2, pp. 15. http://dx.doi.org/10.5772/55741.

JAFFAT, H.S., ALDUJAILI, N.H. and ABDUL HASSAN, A.J., 2017. Antimicrobial activity of silver nano particles biosynthesized by *Lactobacillus* mixtures. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 8, no. 1, pp. 1911-1924.

JEONG, S.H., YEO, S.Y. and YI, S.C., 2005. The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. *Journal of Materials Science*, vol. 40, no. 20, pp. 5407-5411. http://dx.doi.org/10.1007/s10853-005-4339-8.

KARAKAS-SEN, A. and KARAKAS, E., 2018. Isolation, identification and technological properties of lactic acid bacteria from raw cow milk. *Bioscience Journal*, vol. 34, no. 2, pp. 985-999. http://dx.doi.org/10.14393/BJ-v34n2a2018-34517.

KIM, K.-J., SUNG, W.S., SUH, B.K., MOON, S.K., CHOI, J.S., KIM, J.G. and LEE, D.G., 2008. Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. *Biometals*, vol. 22, no. 2, pp. 235-242. http://dx.doi.org/10.1007/s10534-008-9159-2. PMid:18769871.

KLUEH, U., WAGNER, V., KELLY, S., JOHNSON, A. and BRYERS, J.D., 2000. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *Journal of Biomedical Materials Research*, vol. 53, no. 6, pp. 621-631. http://dx.doi.org/10.1002/1097-4636(2000)53:6<621::AID-JBM2>3.0.CO;2-Q. PMid:11074419.

LATEEF, A., ADELERE, I.A., GUEGUIM-KANA, E.B., ASAFA, T.B. and BEUKES, L.S., 2015. Green synthesis of silver nanoparticles using keratinase obtained from a Strain *Bacillus safensis*LAU 13. *International Nano Letters*, vol. 5, no. 1, pp. 29-35. http://dx.doi.org/10.1007/s40089-014-0133-4.

LEE, J., KIM, K.-J., SANG, W., GUK, J. and GUN, D., 2010. The silver nanoparticle (Nano-Ag): a new model for antifungal agents. In: D.J. POZO PEREZ, ed. *Silver nanoparticles*. London: IntechOpen. http://dx.doi.org/10.5772/8510.

MARTINS, N.C.T., FREIRE, C.S.R., PINTO, R.J.B., FERNANDES, S.C.M., PASCOAL NETO, C., SILVESTRE, A.J.D., CAUSIO, J., BALDI, G., SADOCCO, P. and TRINDADE, T., 2012. Electrostatic assembly of Ag nanoparticles onto nanofibrillated cellulose for antibacterial paper products. *Cellulose*, vol. 19, no. 4, pp. 1425-1436. http://dx.doi.org/10.1007/s10570-012-9713-5.

MUKHERJEE, P., SENAPATI, S., MANDAL, D., AHMAD, A., KHAN, M.I., KUMAR, R. and SASTRY, M., 2002. Extracellular synthesis of gold nanoparticles by the fungus fusariumoxysporum. *ChemBioChem*, vol. 3, no. 5, pp. 461-463. http://dx.doi.org/10.1002/1439-7633(20020503)3:5<461::AID-CBIC461>3.0.CO;2-X. PMid:12007181.

OMIDI, B., HASHEMI, S.J., BAYAT, M. and LARIJANI, K., 2014. Biosynthesis of silver nanoparticles by *Lactobacillus fermentum*. *Bulletin of Environmental. Pharmacology and Life Sciences*, vol. 3, no. 12, pp. 186-192.

PATRA, J.K., GOUDA, S., DHAL, N.K. and THATOI, H., 2014. Comparative evaluation of antibacterial properties and phytochemical profile of selected mangrove plants from Bhitarkanika, India. *Journal of Biologically Active Products from Nature.*, vol. 4, no. 2, pp. 136-148. http://dx.doi.org/10.1080/22311866.2013.868706.

RAI, M., YADAV, A. and GADE, A., 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, vol. 27, no. 1, pp. 76-83. http://dx.doi.org/10.1016/j.biotechadv.2008.09.002. PMid:18854209.

RANGANATH, E., RATHOD, V. and BANU, A., 2012. Screening of *Lactobacillus* spp. for mediating the biosynthesis of silver nanoparticles from silver nitrate. *IOSR Journal of Pharmacy*, vol. 2, no. 2, pp. 237-241. http://dx.doi.org/10.9790/3013-0220237241.

SANI, N.J., AMINU, B.M. and MUKHTAR, M.D., 2018. Eco-friendly synthesis of silver nanoparticles using *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from kindrimo (locally fermented milk) in Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, vol. 10, no. 1, pp. 481-488. http://dx.doi.org/10.4314/bajopas.v10i1.92S.

SARVAMANGALA, D., KONDALA, K., SIVAKUMAR, N., BABU, M.S. and MANGA, S., 2013. Synthesis characterization and antimicrobial studies of AgNP's using probiotics. *International Research Journal of Pharmacy*, vol. 4, no. 3, pp. 240-243. http://dx.doi.org/10.7897/2230-8407.04352.

SENTHIL PRABHU, S., RESHMA, K.M., SANHITA, P. and RESHMA, R., 2014. Production of bacteriocin and biosynthesis of silver nanoparticles by lactic acid bacteria isolated from yoghurt and its antibacterial activity. *Scrutiny International Research Journal of Microbiology and Biotechnology.*, vol. 1, no. 3, pp. 7.

SIKDER, M., LEAD, J.R., CHANDLER, G.T. and BAALOUSHA, M., 2018. A rapid approach for measuring silver nanoparticle concentration and dissolution in seawater by UV-Vis. *The Science of the Total Environment*, vol. 618, pp. 597-607. http://dx.doi.org/10.1016/j.scitotenv.2017.04.055. PMid:28411867.

SONDI, I. and SALOPEK-SONDI, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, vol. 275, no. 1, pp. 177-182. http://dx.doi.org/10.1016/j.jcis.2004.02.012. PMid:15158396.

SPADARO, J.A., BERGER, T.J., BARRANCO, S.D., CHAPIN, S.E. and BECKER, R.O., 1974. Antibacterial effects of silver electrodes with weak direct current. *Antimicrobial Agents and Chemotherapy*, vol. 6, no. 5, pp. 637-642. http://dx.doi.org/10.1128/AAC.6.5.637. PMid:15825319.

SUMI MARIA, B., DEVADIGA, A., SHETTY KODIALBAIL, V. and SAIDUTTA, M.B., 2015. Synthesis of silver nanoparticles using medicinal *Zizyphus xylopyrus* bark extract. *Applied Nanoscience*, vol. 5, no. 6, pp. 755-762. http://dx.doi.org/10.1007/s13204-014-0372-8.

TANKHIWALE, R. and BAJPAI, S.K., 2009. Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. *Colloids and Surfaces. B, Biointerfaces*, vol. 69, no. 2, pp. 164-168. http://dx.doi.org/10.1016/j.colsurfb.2008.11.004. PMid:19131217.

ZHAO, G. and STEVENS JUNIOR, S.E., 1998. Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion. *Biometals*, vol. 11, no. 1, pp. 27-32. http://dx.doi.org/10.1023/A:1009253223055. PMid:9450315.