A comparative account of biogenic synthesis of silver nanoparticles using in-house potential probiotics and their antimicrobial activity against challenging antibiotic resistant pathogens

SHAKSHI SHARMA (✉ shakshi.sharma2193@gmail.com)

Dr Yashwant Singh Parmar University of Horticulture and Forestry <https://orcid.org/0000-0002-1669-0873>

NIVEDITA SHARMA

Dr Yashwant Singh Parmar University of Horticulture and Forestry

NEHA KAUSHAL

Dr Yashwant Singh Parmar University of Horticulture and Forestry

Research Article

Keywords: silver nanoparticles, AgNP, probiotics, Lactobacillus, antibacterial, TEM, FTIR

Posted Date: August 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-806048/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The present work focusses on development of a safe, inexpensive, and more accessible source for biosynthesis of silver nanoparticles. Four different in-house probiotic isolates i.e., *Lactobacillus pentosus* S6, *Lactobacillus plantarum* F22, *Lactobacillus crustorum* F11 and *Lactobacillus paraplantarum* KM1 isolated from different food sources, were used in the current study to check their ability to synthesize silver nanoparticles. All the probiotics synthesized silver nanoparticles shows maximum Surface Plasmon Resonance (SPR) at a peak of 450 nm, which confirms the formation of silver nanoparticles. Scanning Electron Microscopy (SEM) analysis identified the shape and distribution of silver nanoparticles. Transmission Electron Microscopy (TEM) revealed the average size of synthesized nanoparticles in the range of 10-50 nm, with smallest size of 5 nm for silver nanoparticles synthesized by *L. crustorum* F11. Further, Fourier-transform infrared spectroscopy (FTIR) detected the presence of different functional groups responsible for reduction of silver ion to form silver nanoparticles. The antimicrobial activity of these AgNP was also found to be effective against different bacterial and fungal pathogens viz. antibiotic resistant *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Pythium aphanidermatum*, *Fusarium oxysporum* and *Phytophthora parasitica*. However, *L. crustorum* F11 synthesized AgNP showed maximum inhibition against all the bacterial and fungal pathogens, with highest against *S. aureus* (20 0.61mm) and *F. oxysporum* (23). Findings from this study provide a durable and ecofriendly method for the biosynthesis of silver nanoparticles, having strong antimicrobial activity against different multidrug resistant microorganisms.

Introduction

Nanotechnology is a rapidly evolving field of science, that has been flourishing since the beginning of last century. It involves the synthesis of particles at nanoscale level having size less than 100 nm (Arshad, 2017). There is an increase concern worldwide about the rise in antibiotic resistant cases, as harmful pathogenic microorganisms have developed resistance against already used different antibiotics (Khatoon et al. 2019). These multidrug-resistant organisms (MDROs) are multiplying at faster rate, making various health related infections almost untreatable (Lee et al. 2019; Boucher et al. 2009; Peleg and Hooper, 2010). Therefore, nanotechnology is coming forward with a solution to treat these infections as studies revealed that the utilization of nanoparticles provides a potential to manage infection caused by these pathogenic microorganisms (Baptista et al., 2018; Muzammil et al. 2018). Among metallic nanoparticles, silver and gold nanoparticles are most promising nanoparticles because of their large surface area to volume ratio, which has garnered interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Mosallam et al. 2014). Silver nanoparticles (AgNPs) are gaining attention as one of the most promising nanoparticles due to its unique properties (Yusof et al. 2020). AgNPs have strong toxic effect against wide range of microorganisms and can be used as a potential antimicrobial agent (Naseer et al. 2020). There are different physical and chemical methods already being used for the synthesis of nanoparticles, since these are associated with hazardous chemicals exhibiting toxicity (Hemlata et al. 2020), biologically

synthesized metallic nanoparticles are preferred for their ecofriendly nature, cost effectiveness and less hazardous effect.

Probiotics are the live microbes that exert health beneficiary effects to the host cell, when administrated in an adequate amount (Pandey et al. 2010). The utilization of probiotic bacteria to synthesize nanoparticles has been reported in different studies and is a focus of ongoing research nowadays (Akhtar and Pathak, 2017). Probiotics are now being employed in synthesizing or more precisely biosynthesizing various nanoparticles, such as metallic as well as non-metallic nanoparticles. Probiotics synthesized nanoparticles has its application in health industry, food industry, cosmetics and biotechnology. The bacterial genera *Lactobacillus* and *Bifidobacterium* are the most widely used probiotics, that are being used in biosynthesis of silver nanoparticles. These probiotic synthesized silver nanoparticles have been found to possess effective antimicrobial efficacy against a wide range of pathogenic microbes and have enhanced surface area to volume ratio, high catalytic capabilities and tendency to generate reactive oxygen species (Berton et al. 2014). Thus these Probiotics synthesized nanoparticles can be employed as an alternative of antibiotic as they have the ability to inhibit harmful microorganisms present in human body (Hong et al. 2019; Gillor et al. 2008).

As the use of probiotics in synthesizing nanoparticles is a novel and current trending research due to multiple benefits of probiotics, in the present paper an attempt has been made to synthesize silver nanoparticles from four in-house potential probiotic cultures i.e. *Lactobacillus pentosus* S6, *Lactobacillus plantarum* F22, *Lactobacillus crustorum* F11 and *Lactobacillus paraplantarum* KM1 that have already been isolated from different food sources and their characterization by using UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). Further their antagonistic spectrum was examined against different challenging pathogens (Bacteria and Fungi).

Materials And Methods

Materials

The four in-house potential probiotic cultures isolated from different food sources were collected from Microbiology laboratory, Department of Basic Sciences, UHF Nauni, Solan, H.P, India, for the biosynthesis of silver nanoparticles and analysis of their antimicrobial potential. Table 1 shows the different in-house cultures collected for the study. All the probiotic isolates were cultivated in de Man, Rogosa and Sharpe (MRS) broth separately for 24h (37°C). The bacterial strains used in the study were obtained from MTCC (IMTECH, Chandigarh), CRI (Kasauli) and IGMCI (Shimla). All the chemicals used in this study were of analytical grade.

Table 1
list of in-house potential probiotic cultures collected for synthesis of silver nanoparticles

Name	Accession number	Food Source
<i>Lactobacillus pentosus</i> S6	KU92122	Siddu
<i>Lactobacillus plantarum</i> F22,	KT865223	Chhang
<i>Lactobacillus crustorum</i> F11	KT865221	Human milk
<i>Lactobacillus paraplantarum</i> KM1	KX671558	Milk
Source: Microbiology research laboratory, Deptt. Of Basic Sciences, UHF Nauni, Solan, H.P, India		

Biosynthesis of silver nanoparticles using probiotic isolates

The collected probiotic strains were inoculated in 100 ml of MRS media and was incubated overnight at 37°C for 24 h. After incubation, the culture was centrifuged at 10,000 rpm for 15 min. 10 ml of culture supernatant from each isolate was separately mixed with 90 silver nitrate (AgNO_3) solution (0.1 mM) and another reaction mixture without silver nitrate was used as control. The prepared solutions were incubated at 30°C (24 h) and kept in dark to avoid any photochemical reactions during the investigation. The change in color in synthesized silver nanoparticles was observed after 24 h. The biosynthesized silver nanoparticles were then characterized by using UV-vis, SEM, TEM and FTIR spectroscopy.

Characterization of silver nanoparticles

UV-Visible Spectroscopy Analysis

Silver nanoparticles were analyzed by UV-Vis spectrophotometer (Shimadzu 1600, Japan). The bio reduction of silver ion in aqueous solution was monitored by periodic diluted sampling of aliquots and subsequently measuring the spectra of the solution on UV-Vis spectrophotometer (Sarvamangla et al. 2013). The absorbance of all the four synthesized silver nanoparticle was read at different wavelengths i.e., 350, 450, 550 and 650 nm.

Scanning electron microscopy (SEM)

The thin film of the different probiotic synthesized nanoparticles was prepared separately on aluminum plate by just dropping a very small amount of the sample on the plate, extra solution was removed using blotting paper and then the film on the plate was allowed to dry overnight. The SEM analysis was performed on a JEOL; model JSM – 6610LV instrument operated at an accelerating voltage of 20 KeV (Sarvamangla et al. 2013).

Transmission electron microscopy (TEM)

Transmission Electron Microscopy was performed by using model FP 5022/22-Tecnai G2 20 S-TWIN. TEM shows the size, shape, appearance and crystal structure of the particles. The grid for TEM analysis

was prepared by placing a drop of the synthesized nanoparticles suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer for 1 h.

Fourier transform infrared spectroscopy (FTIR)

The synthesized silver nanoparticles were further characterized by their FTIR analysis. The small amount of synthesized silver nanoparticles was dried in freeze-drier separately (Lyophilizer) for 24 h, then freeze dried sample was appointed with KBr pellets and analyzed using a Thermo Nicolet model: nexus 870 in range of 450-4000 cm^{-1} at a resolution of 4 cm^{-1} (Omidi et al. 2014)

Antibacterial efficacy of silver nanoparticles

Antibacterial assay of synthesized silver nanoparticles was performed against three standard test indicators i.e., antibiotic resistant *Staphylococcus aureus* IGMC, *Listeria monocytogenes* MTCC839 and *Bacillus cereus* CRI using well plate assay (Kimura et al. The inoculum of each pathogen was grown in nutrient broth overnight (37⁰ C). Each pathogen was streaked on a separate nutrient agar plate using sterilized swabs. Wells were punched on nutrient agar plate with the help of a sterile borer. 100 μl of biosynthesized silver nanoparticles (concentration: 200 $\mu\text{g}/\text{ml}$) from all the four different probiotic isolates was added to each well cut on the agar plate separately. The experiment was performed in triplicates.

Antifungal activity of silver nanoparticles

Antifungal activity was checked against three fungal pathogens viz. *Pythium aphanidermatum*, *Fusarium oxysporum* and *Phytophthora parasitica* by well plate assay using dual culture technique (Mew and Rosales, 1984). On the one side of pre-poured sterilized potato dextrose agar (PDA) plates, 7 days old culture bit of indicator fungi was placed with the help of sterile well borer and inoculating loop. On the other side of PDA plate, well was cut with the help of sterile borer followed by addition of 100 μl of 0.1 mM probiotics synthesized AgNP were added to the well. Plates were incubated at 28 $\pm$ 2 $^{\circ}\text{C}$ for 7 days and observed for inhibition of mycelial growth produced around the well. The experiment was performed in triplicates.

Results And Discussion

The utilization of microorganisms for the biosynthesis of nanoparticles, has gained considerable attention in the recent times as compared to the previously used standard methods. This work reports the biosynthesis of AgNPs using four different in-house potential probiotic strain i.e., *Lactobacillus pentosus* S6 and *Lactobacillus plantarum* F22, *Lactobacillus crustorum* F11 and *Lactobacillus paraplantarum* KM1, to check their ability to synthesize them and determination of their antimicrobial activity.

Biosynthesis of silver nanoparticles

Silver nitrate was used as a precursor for the biosynthesis of silver nanoparticles. The first distinctive feature of the formation of silver nanoparticles is the color change in the solution after 24 hours of incubation. Silver nitrate was colorless and after reduction by probiotic isolates, the supernatant changed into brown color. All the four probiotic isolates were capable of synthesizing silver nanoparticles as all of them showed color change after incubation as compared to control (AgNO_3) (Fig. 1 a). The severity of color is due to actuated surface Plasmon vibration in the metal nanoparticles (Prasad et al. 2011). Different microorganisms have different mechanisms of synthesizing nanoparticles. However, nanoparticles are usually formed when microbial cells trapped metal ions on the surface or inside, and then trapped metal ions are reduced to nanoparticles in the presence of enzymes (Xiangqian et al. 2011). Several studies have reported the synthesis of silver nanoparticles using different microorganisms, but much work is needed to be done to improve their efficacy, maintaining their particle size and future safety concerns. Thus, in the present study an attempt has been made to check the ability of different in-house probiotic microorganisms that are isolated from different food sources to synthesize silver nanoparticles. Over the last few decades, there has been growing interest in the use of probiotics as potential alternatives for synthetic antibiotics and anti-inflammatory drugs, not only due to the side effects of synthetic drugs but also to the improper use of antibiotics promotes the development of antibiotic-resistant bacteria. Mikiciuk et al. (2016) depicted the influence of silver nanoparticles on probiotic bacteria. Probiotic bacteria like *Bifidobacterium animalis* subspecies lactis BB-12, *Lactobacillus acidophilus* LA-5, and *Streptococcus thermophilus* ST-Y31 have been taken from fermented milk products to synthesize silver nanoparticles. Ysouf et al. (2020) reported the utilization of biomass of *Lactobacillus plantarum* TA4 for the biosynthesis of silver nanoparticles.

UV-Visible spectroscopy

The UV-Vis spectroscopy analysis was the second characterization method used for the confirmation of the synthesis of silver nanoparticles. The process of reaction between the metal ions and biosynthesized silver nanoparticles was monitored using UV spectra through the aqueous solution. The presence of nanoparticles synthesized by *Lactobacillus pentosus* S6, *Lactobacillus plantarum* F22, *Lactobacillus crustorum* F11 and *Lactobacillus paraplantarum* KM1 was confirmed by obtaining a spectrum in different wavelength range i.e., 350, 450, 550 and 650nm respectively (Fig 1 b). The highest absorbance peak for all of the four isolates was found at the wavelength of 450 nm, which was specific for silver nanoparticles synthesis. Dhoondia and Chakraborty (2012) observed the maximum absorption peak at 430 nm for silver nanoparticles synthesized using *Lactobacillus mindensis*. Malathi et al. (2015) monitored the synthesis of silver nanoparticles from *Lactobacillus* by UV-spectroscopic analysis. The peak was observed between 400-450 nm indicating the presence of silver nanoparticles.

Scanning Electron Microscopy (SEM)

The synthesized silver nanoparticles were further characterized by Scanning Electron Microscopy (SEM). SEM reveals the external morphology and texture of silver nanoparticles. Fig 2 (a, b, c and d) depicts the SEM analysis of silver nanoparticles synthesized from different probiotics. From the SEM analysis, it can

be observed that silver nanoparticles from all the four probiotic isolates were spherical in shape and were found as an individual particle and also present in aggregates. Tayo and Papoola (2017) reported that SEM is an important tool for AgNP characterization. Production of silver nanoparticles from *Lactobacillus* sp. with varying shape i.e., rectangular to spherical was observed (Prabhu et al. 2014). Arshad A (2017) examined SEM analysis of silver nanoparticles synthesized from *Bacillus* sp, are spherical and pseudo-spherical in shape.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is a valuable tool used to visualize size of synthesized silver nanoparticles. Fig 3 (a, b, c and d) and Table 2 depicts the TEM images of silver nanoparticles synthesized from *L. pentosus* S6, *L. plantarum* F22, *L. crustorum* F11 and *L. paraplantarum* KM1 respectively. Silver nanoparticles of different size were synthesized by all the four isolates. *L. pentosus* S6 synthesized silver nanoparticle has a mean size of 50nm, whereas, for silver nanoparticle synthesized from *L. plantarum* F22 it is 20 nm. AgNP from *L. plantarum* F22 has a size of 10 nm and for *L. paraplantarum* KM1 it is 50 nm. The silver nanoparticles synthesized in this work were in the same approximate range as reported by other authors for silver nanoparticles synthesized from lactic acid bacteria and other microorganisms (Sintubin et al. 2009; Kalimathu et al. 2008). However, out of all the four probiotics synthesized silver nanoparticles, one synthesized using *L. crustorum* F11 was found to be smaller (10 nm) of all. Size plays an essential role in nanoparticles applications; smaller particles have a relatively large surface area as compared to larger ones; this increases their interaction with harmful pathogens and consequently trigger more toxic and adverse effect. Many researchers also observed the almost same range of silver nanoparticles synthesized using probiotics just like our findings. Garmasheva et al. (2016) reported the TEM analysis of silver nanoparticles synthesized from lactic acid bacteria and found that the size of nanoparticles was between 10 and 40 nm. Prabhu et al. (2014) also observed the size of silver nanoparticles synthesized by *Lactobacillus* sp. and found the average size of about 20-40 nm. Ranganath et al. (2012) observed the TEM picture of the silver nanoparticles formed by *Lactobacillus* sp. VRS-2. The TEM Technique used to visualize size and shapes of biosynthesized silver nanoparticles have predominantly shown spherical shape structures with size ranging between 2nm - 20 nm and have shown the presence of individual as well as number of aggregates of silver nanoparticles.

Table 2: Transmission Electron Microscopy (TEM) analysis of synthesized nanoparticles by different probiotics

Probiotic culture	Size of synthesized nanoparticles (nm)
<i>Lactobacillus pentosus</i> S6	50
<i>Lactobacillus plantarum</i> F22	20
<i>Lactobacillus crustorum</i> F11	10
<i>Lactobacillus paraplantarum</i> KM1	50

Fourier transform infrared spectroscopy (FTIR)

Formation of silver nanoparticles was further confirmed, by analyzing the characteristic functional groups present in silver nanoparticles. FTIR spectrum is used to identify different functional groups, responsible for the reduction and stabilization of silver ions to silver nanoparticles. The FTIR spectrum of synthesized silver nanoparticles was presented in Fig 5 (a, b, c and d) respectively. *L. pentosus* S6 synthesized silver nanoparticles (Fig 4a) shows absorbance peak at 1710cm^{-1} which correspond to C=O stretching vibration of ketone. The absorbance peak at 2273cm^{-1} corresponds to N=C=O stretching and presence of isocyanate functional group, whereas absorbance peak at 1367cm^{-1} indicated C-O stretching vibration of an ester (Beekes et al. 2007). *L. crustorum* F11 synthesized silver nanoparticles (Fig 4b) shows absorbance peak at 3222cm^{-1} referred to O-H stretching and presence of carboxylic acid, whereas absorbance peak at 1628cm^{-1} revealed C=C stretch and the presence of alkene. The peak at 1146cm^{-1} represents O-H bond of the aliphatic ether, 1062cm^{-1} corresponds to O-H stretch of primary alcohol, 1925cm^{-1} peak indicated C=C=C stretch of allene. For silver nanoparticles synthesized from *L. plantarum* F22 (Fig 4c) the absorbance peak at 1695cm^{-1} corresponds to C=N stretching of imine group. The absorbance peak at 1324cm^{-1} could be attributed to C-N bond of aromatic amide, and absorbance peak at 1635cm^{-1} represented C=C stretching of alkenes. Silver nanoparticles synthesized by *L. paraplantarum* KM1 (Fig 4d) shows peak at 1695cm^{-1} had C=O stretch of conjugated aldehyde and peak at 1941cm^{-1} was identified as C-H stretch of aromatic compound. The peak at 2161cm^{-1} represented S-C=N stretch of thiocyanate and 1647cm^{-1} peak pointed out the C=N stretch of sulfone (Fig. 5d). All the four probiotic isolates used in the study contain important functional groups (hydroxyl, protein, and carboxyl) in the cell membrane, which plays an important role in the reduction of silver nitrate and thus capable of biosynthesizing silver nanoparticles. Viorica et al., (2017) studied the FTIR spectrum of silver nanoparticles synthesized from *Lactococcus lactis* and observed absorption bands at 1650cm^{-1} revealed the presence of amide, whereas amide II region (N-H bending and C-N stretching) occurred at 1550cm^{-1} . Milanowski et al., (2017) observed peak at $1530\text{-}1630\text{cm}^{-1}$ which could be attributed to amide II vibrations, for silver nanoparticles synthesized by *L. casei*.

Antibacterial efficacy of silver nanoparticles

The antimicrobial efficacy of synthesized AgNPs was assessed against challenging pathogens i.e., *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* (Fig). All the four-probiotic synthesized AgNPs was found to exhibit strong antibacterial activity against the bacterial pathogens. However, out of all *L. crustorum* F11 synthesized AgNPs showed maximum zone of inhibition against all the microbial strains with maximum zone of inhibition of 20 0.61mm against *S. aureus*, 14 1.01 mm for *L. monocytogenes* and 12 7.07 for *B. cereus* respectively (Fig. 5). However, Several Studies reported the antibacterial activity of lactic acid bacteria synthesized AgNP against different pathogens, while our study concluded the effectiveness of major probiotics against harmful challenging pathogens. Moodley et al. (2018) reported the antibacterial activity of silver nanoparticles against *K. pneumoniae* and *S. aureus*. Kumar et al. (2016) observed the antimicrobial efficacy of silver nanoparticles synthesized from *Lactococcus amylophilus* GV6 against *P. aeruginosa* MTCC 424, *B. subtilis* MTCC 121, *S. aureus* MTCC 96, *E. coli* MTCC 43 and *Klebsiella pneumoniae* MTCC 109 by using agar well plate assay and reported the zone of inhibition of 1.5 cm against *S. aureus* MTCC 96. Yusof et al. (2020) observed the antibacterial activity of biosynthesized silver nanoparticles from *Lactobacillus plantarum* TA4 against *S. aureus*, *S. epidermidis*, *E. coli* and *Salmonella* by agar well diffusion method.

Antifungal activity of probiotics synthesized silver nanoparticles

The antifungal activity of synthesized silver nanoparticles was assessed against three fungal pathogens viz. *Pythium aphanidermatum*, *Fusarium oxysporum* and *Phytophthora parasitica*. Here also, *L. crustorum* F11 synthesized AgNP showed maximum inhibition against all the three fungal pathogens i.e., 23 ± 0.37 for *F. oxysporum*, 20 ± 1.01 against *P. parasitica* and 32.6 ± 0.48 against *P. aphanidermatum* F11 (Fig. 6). Matei et al. (2020) investigated the antifungal activity of silver nanoparticles synthesized by lactic acid bacteria against *Aspergillus ochraceus*, *Penicillium expansum* and *Aspergillus flavus* and observed maximum antifungal activity for *Penicillium expansum* (15.87 ± 1.01).

Although all the probiotic synthesized silver nanoparticles exhibited strong antimicrobial activity, but the one synthesized from *L. crustorum* F11 stands apart, as it showed the maximum antagonistic spectrum against different bacterial and fungal pathogens. This strong inhibitory effect could be attributed to its smaller size (10 nm) as compared to the other probiotic synthesized silver nanoparticles. Similar work is reported by Lu et al. (2013) where they examined size dependent antimicrobial activity of AgNPs against oral pathogenic bacteria. They observed that AgNPs having a size of 5 nm has a stronger antagonistic activity as compared to the one with size of 15 and 55 nm respectively. Morones et al. (2005) suggested that antimicrobial activity of silver nanoparticles was size dependent with higher antagonistic effect from nanoparticles whose size ranges from 1~10 nm. The small particle size helps AgNPs to adhere to the cell wall and penetrate the cell easily, which in turn enhances their antimicrobial activity against harmful pathogenic bacteria.

Conclusions

This study was carried out with the sole purpose to biosynthesize silver nanoparticles using health beneficial probiotic isolates and their antagonistic potential against challenging microbial strains. All of the four different in-house probiotic isolates used in this study were found to be safe and effective for the synthesis of silver nanoparticles. SEM analysis depicted the morphology and TEM analysis evaluated the size of different probiotic synthesized silver nanoparticles (20, 10 and 50nm). FTIR analysis identified different organic compound responsible for reduction and formation of silver nanoparticles. Furthermore, these biosynthesized silver nanoparticles revealed pronounced antimicrobial activity against different challenging bacterial and fungal pathogens. The results in our study recommend using probiotics, as a novel and ecofriendly tool for biosynthesizing silver nanoparticles, having effective antimicrobial activity and thus can be used against different health deteriorating multi drug resistant microorganisms.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest

Acknowledgment

The authors thanks Mr. Arjun, Technician, Indian Institute of Technology, Mandi, Himachal Pradesh, India for technical support and help in obtaining TEM and FTIR results.

Abbreviations

SEM

Scanning Electron Microscope

TEM

Transmission Electron Microscope

FTIR

Fourier Transform Infrared Spectroscopy

AgNP

Silver Nanoparticles

AgNO₃

Silver Nitrate

References

Arshad A (2017) Bacterial synthesis and applications of nanoparticles. *Nano Sci Nano Technol* 11(2):119. [Google Scholar]

Khatoon N, Alam H, Khan A (2019) Ampicillin Silver Nanoformulations against Multidrug resistant bacteria. *Sci Rep* 9:6848. <https://doi.org/10.1038/s41598-019-43309-0>

- Yusof HM, Rahman NA, Mohamad R, Zaidan UH (2020) Microbial Mediated Synthesis of Silver Nanoparticles by *Lactobacillus plantarum* TA4 and Its Antibacterial and Antioxidant Activity. Appl Sci 10(19):6973. <https://doi.org/10.3390/app10196973>
- Hong L, Kin WS, Lee SM, Kang SK, Choi YJ, Cho CS (2019) Pullulan Nanoparticles as Prebiotics Enhance the Antibacterial Properties of *Lactobacillus plantarum* Through the Induction of Mild Stress in Probiotics. Front Microbio 10:1–12. <https://doi.org/10.3389/fmicb.2019.00142>
- Gillor O, Etzion A, Riley MA (2008) The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol 81:591–606. <https://doi.org/10.1007/s00253-008-1726-5>
- Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48:1–12. <https://doi.org/10.1086/595011>
- Peleg AY, Hooper DC (2010) Hospital-acquired infections due to gram-negative bacteria. N Engl J Med 362:1804–1813. <https://doi.org/10.1056/nejmra0904124>
- Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-Strategies to Fight Multidrug Resistant Bacteria-"A Battle of the Titans". Front Microbiol 9:1–26. <https://doi.org/10.3389/fmicb.2018.01441>
- Rajawat S, Qureshi MS (2012) Comparative study on bactericidal effect of silver nanoparticles, synthesized using green technology, in combination with antibiotics on *Salmonella typhi*. J Biomater Nanobiotechnol 3:480–485. <https://doi.org/10.4236/JBNB.2012.34049>
- Muzammil S, Hayat S, Fakhar EAM, Aslam B, Siddique MH, Nisar MA (2018) Nanoantibiotics: future nanotechnologies to combat antibiotic resistance. Front Biosci 10:352–374. <http://doi.org/10.2741/e827>
- Hemlata, Meena PR, Singh AP, Tejavath KK (2020) **Biosynthesis of silver nanoparticles using *Cucumis prophetarum* aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines.** ACS Omega 5(10):5520–5528. <http://doi.org/10.1021/acsomega.0c00155>
- Lee NY, Ko WC, Hsueh PR (2019) Nanoparticles in the Treatment of Infections Caused by Multidrug-Resistant Organisms. Front Pharmacol 10(1153):1–9. <http://doi.org/10.3389/fphar.2019.01153>
- Mosallam SF, Amer E, Diab RG (2014) Potentiated anti-microsporidial activity of *Lactobacillus acidophilus* CH1 bacteriocin using gold nanoparticles. Exp Parasitol 144:14–21. <http://doi.org/10.1016/j.exppara.2014.06.002>
- Akhtar N, Pathak K (2017) Probiotics as a Tool to Biosynthesize Metallic Nanoparticles: Research Reports and Patents Survey. Recent Pat on Drug Del Form, 11: 5–18. [10.2174/1872211311666170313124335](https://doi.org/10.2174/1872211311666170313124335)

- Mikiciuk J, Mikiciuk E, Wronska A, Szterk A (2015) Antimicrobial potential of commercial silver nanoparticles and the characterization of their physical properties toward probiotic bacteria isolated from fermented milk products 51(4): 222–229. <https://doi.org/10.1080/03601234.2015.1120614>
- Kalimuthu K, Suresh Babu R, Venkataraman D, Bilal M, Gurunathan S (2008) Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloid Surf B Biointer 65:150–153. <http://doi.org/10.1016/j.colsurfb.2008.02.018>
- Garmasheva I, Kovalenko N, Voychuk S, Ostapchuk A, Livinska O, Oleschenko L (2016) *Lactobacillus* species mediated synthesis of silver nanoparticles and their antibacterial activity against opportunistic pathogens *in vitro*. Bioimpacts 6(4):219–223. <http://doi.org/10.15171/bi.2016.29>
- Matei A, Matei S, Matei GM, Cogalniceanu G, Cornea CP (2020) Biosynthesis of silver nanoparticles mediated by culture filtrate of lactic acid bacteria, characterization and antifungal activity. EuroBiotech J 4:97–103. <http://dx.doi.org/10.2478/ebtj-2020-0011>
- Berton V, Montesi F, Losasso C, Facco DR, Toffan A, Terregino C (2014) Study of the interaction between silver nanoparticles and Salmonella as revealed by transmission electron microscopy. J Prob Health 3:1–5. DOI:10.4172/2329-8901.1000123
- Xiangqian L, Huizhong X, Zhe Sheng C, Guofang C (2011) Biosynthesis of Nanoparticles by Microorganisms and Their Applications. J of nanomat 11:1–16. <https://doi.org/10.1155/2011/270974>
- Viorica RP, Pawel P, Kinga M, Michal Z, Katarzyna R, Boguslaw B (2017) *Lactococcus lactis* as a safe and inexpensive source of bioactive silver composites. Appl microbio biotechn 101(19):7141–7153. <https://doi.org/10.1007/s00253-017-8443-x>
- Haris P, Severcan F (1999) FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. Journal of Molecular Catalysis B: Enzymatic 7:207–221. [http://doi.org/10.1016/S1381-1177\(99\)00030-2](http://doi.org/10.1016/S1381-1177(99)00030-2)
- Prasad KS, Pathak D, Patel A, Dalwadi P, Prasad R, Patel P, Selvaraj K (2011) Biogenic synthesis of silver nanoparticles using *Nicotiana tobaccum* leaf extract and study of their antibacterial effect. Afr J of Biotechn 10(41):122–8130. <https://doi.org/10.5897/AJB11.394>
- Lu Z, Rong K, Li J, Yang H, Chen R (2013) Size dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. J Mater Sci Mater Med. 2013 24(6):1465–1471. <http://doi.org/10.1007/s10856-013-4894-5>
- Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechn 16(10):2346–2353. <http://doi.org/10.1088/0957-4484/16/10/059>

- Moodley JS, Krishna SBN, Pillay K, Sershen, Govender P (2018). Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. Adv Nat Sci Nanosci Nanotechnol 9(1): 1–9 Google Scholar
- Kumar KK, Mahalakshmi S, Harikrishna N, Reddy G (2016) Production, characterization and antimicrobial activity of silver nanoparticles produced by *Lactobacillus amylophilus* GV6. E J OF Pharmaceut Med Res 3(7):236242
- Sintubin L, De Windt W, Dick J, Mast J, Van Der Ha D, Verstraete W, Boon N (2009) Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl microbio biotechn 84(4):741–749. <https://doi.org/10.1007/s00253-009-2032>
- Ranganath E, Rathod v, Banu A (2012) Screening of Lactobacillus spp. for mediating the biosynthesis of silver nanoparticles from silver nitrate. IOSR J of Pharm 2(2):237–241. <http://dx.doi.org/10.9790/3013-0220237241>
- Dhoondia ZH, Chakraborty H (2012) Lactobacillus mediated synthesis of silver oxide nanoparticles 2(15): 1–7 <https://doi.org/10.57722F55741>
- Sarvamangala D, Kondala K, Sivakumar N, Babu MS, Manga S (2013) Synthesis, characterization and antimicrobial studies of AgNPs using probiotics. Int Res J Pharm 4:240–243. <http://doi.org/10.7897/2230-8407.04352>
- Omidi B, Hashemi SJ, Bayat M, Larijani K (2014) Biosynthesis of silver nanoparticles by *Lactobacillus fermentum*. B of Environm Pharmaco and. L Sci 3(12):186–192 Google Scholar
- Mew TW, Rosales AM (1986) Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. Phytopath76: 1260–1264
- Beekes M, Lasch P, Naumann D (2007) Analytical applications of Fourier transform-infrared (FTIR) spectroscopy in microbiology and prion research. Vet Microbiol 123(4):305–319. <https://doi.org/10.1016/j.vetmic.2007.04.010>
- Milanowski M, Pomastowski P, Railean V, Rafinska K, Ligor T, Buszewski B (2017) Biosorption of silver cations onto Lactococcus lactis and Lactobacillus casei isolated from dairy products. PLoS ONE 12(3):e0174521. <https://doi.org/10.1371/journal.pone.0174521>
- Naseer QA, Xue X, Wang X, Dang S, Din SU, kalsoom, Jamil J (2020) Synthesis of silver nanoparticles using *Lactobacillus bulgaricus* and assessment of their antibacterial potential. Braz J of Biol 84 <https://doi.org/10.1590/1519-6984.232434>
- Kimura H, Sashihara T, Matsusaki H, Sonomoto K, Ishizaki A (1998) Novel bacteriocin of Pediococcus sp. ISK-1 isolated from well-aged bed of fermented rice bran. Ann N Y Acad Sci 13(864):345–348.

Figures

Figure 1

(a) Silver nanoparticles synthesized by four in-house probiotic isolates showing color change: (1) control (AgNO₃) (2) *L. pentosus* S6 AgNP (3) *L. crustorum* F11 AgNP (4) *L. plantarum* F22 AgNP (5) *L. paraplantarum* KM1 AgNP; (b) UV-Vis spectra of synthesized AgNPs at different wavelengths

(a)

(b)

(c)

(d)

Figure 2

Scanning electron microscope (SEM) micrograph of silver nanoparticles synthesized by (a) *L. pentosus* S6, (b) *L. crustorum* F11 (c) *L. plantarum* F22 (d) *L. paraplantarum* KM1

(a)

(b)

(c)

(d)

Figure 3

Transmission electron microscopy (TEM) micrograph of silver nanoparticles synthesized by (a) *L. pentosus* S6, (b) *L. crustorum* F11 (c) *L. plantarum* F22 (d) *L. paraplantarum* KM1

Figure 4

FTIR spectrum of silver nanoparticles synthesized by (a) *L. pentosus* S6, (b) *L. crustorum* F11 (c) *L. plantarum* F22 (d) *L. paraplantarum* KM1

Figure 5

Antibacterial activity of *L. crustorum* F11 synthesized silver nanoparticles against different bacterial pathogens

Figure 6

Antifungal activity of *L. crustorum* F11 synthesized silver nanoparticles against different fungal pathogens

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Doc1.docx](#)
- [G](#)