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Synthesis and Characterization of Silver Nanoparticles as Strong Antimicrobial Agents Using Bacteriocin of A Novel Probiotic Strain of *Lactobacillus Pentosus* S6 (KU92122)

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Research Article

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Abstract

The goal of this study was to use bacteriocin from a probiotic strain of *Lactobacillus pentosus* S6 (KU92122) tosynthesize innovative strong silver nanoparticles (AgNPs). UV-Vis spectroscopy was used to analyze the synthesized nanoparticles, and the maximum absorbance was found to be approximately 450 nm. AgNPs produced were spherical in shape, with an average size of 50 nm, as validated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Fourier transform infrared spectroscopy (FTIR) was used to confirm the presence of distinct functional groups on the surface of nanoparticles. The positive antibacterial activity of produced silver nanoparticles was tested against multidrug resistant bacteria such as *Staphylococcus aureus* and *Bacillus cereus*, as well as challenging plant pathogens such as *Aspergillus niger*, *Pythium aphanidermatum*, *Fusarium oxysporum,* and *Phytopthora parasitica* with an apparent aim to use these AgNPs with bacteriocins as effective antimicrobial agents that can be employed at broader scale in clinical and biocontrol applications especially in medicine, agriculture and the food processing sectors.

Keywords: Silver Nanoparticles; Scanning electron microscopy; Transmission electron microscopy; Fourier transform infrared spectroscopy; Antimicrobial potential

Abbreviations: AgNPs: Silver Nanoparticles SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; FTIR: Fourier Transform Infrared Spectroscopy

Introduction

The increasing tendency of microbial infections, rapid emergence of drug-resistance to recent antibiotics and quick evolution through mutation necessitate development or modification of antimicrobial compounds and alternative treatments [1]. Advanced research in nanotechnology recently comes with the development of Nanoscale objects with prominent antimicrobial actions against multidrug resistant pathogens suggesting a platform to fight against bacterial mutation arch [2]. Among the most advanced Nano technological applications, metal nanoparticles provide the most effective results with their unique mode of actions [3]. In clinical applications for human beings, these are emerging as potential candidates especially against multidrug susceptible as well as multidrug resistant bacteria. Being strong antimicrobial agents, silver nanoparticles also offer potential uses in agriculture for

plant protection. The ambition of nanomaterials in agriculture is to reduce the amount of spread chemicals, minimize nutrient losses in fertilization and increased yield through pest and nutrient management. Nanotechnology has the prospective to improve the agriculture and food industry with novel Nano tools for the controlling of rapid disease diagnostic, enhancing the capacity of plants to absorb nutrients among others. Nanotechnology provides an opportunity for controlled delivery of agrochemicals to break disease resistance and improve plant growth enhancement and nutrient utilization. Nanoscale silver particles with high surface-area-to-volume ratio (size below 100 nm) are of prime interest due to high antimicrobial actions against both gram-positive and gram negative bacteria, viruses and other eukaryotic microorganisms as compared to other metals in their Nano form [4]. Silver nanoparticles because of their unique properties have proven to be effective nanomaterial based fungicides inthe control of some plant fungal diseases responsible for major crop loss. The applications of silver nanoparticles in crop protection, helps in the development of efficient and practical approaches for the management of plant pathogens [5].

Synthesis and production of nanoparticles for their effective use in health, food and agriculture sector primarily is dependent on their physical and chemical properties [6]. The synthesis of silver nanoparticles from microbes is a boon for advance research in nanotechnology. However, maximizing the antimicrobial efficacy of silver nanoparticles could further be achieved by conjugating them to antimicrobial agents of microbes, such as Bacteriocins [7]. Lactic acid bacteria are a clad of gram-positive non-sporing cocci or rods with non-aerobic habit which generally produce bacteriocin as important metabolic end product with strong antagonistic potential and inhibit the growth of pathogenic and deteriorating microorganisms. Bacteriocins are proteinaceous antibacterial compounds, which have been successfully used as: food bio preservative, anti-biofilm agents and additives or alternatives to the currently existing antibiotics and to minimize the risk of emergence of resistant strains. Antimicrobial property of silver nanoparticles can be used to enhance the antimicrobial spectrum of bacteriocins as they themselves have enormous antibacterial potential and hold promise to target food borne pathogenic species [8]. Different approaches can be used to conjugate bacteriocins with nanoparticles to increase the antimicrobial spectrum of the former, which in turn can prove to be an efficient weapon in the fight against different challenging pathogens. The high affinity of silver nanoparticles synthesized by bacteriocin towards sulphur and phosphorus is assumed to play an important role in contributing to its antimicrobial property [9]. The presence of sulfur-containing proteins on bacterial cell membrane leads to the interaction of silver nanoparticles with sulfur-containing amino acids within or outer surface of the cell, which in turn affects bacterial cell permeability. Bacteriocin synthesized silver nanoparticles exert their antibacterial effect by generating holes in the cell wall, leading to the discharge of cell contents and cell death.

On the other hand, exploitation of bacteriocins in the health care and pharmaceutical industries is moving forward due to their vast potential but also posing a number of limitations and challenges that have yet to be solved. One of the way to overcome these various limitations is the utilization of these nanotechnological approaches to enhance the applicability of bacteriocins, increase their stability, and extend their antimicrobial spectrum against different fungal and bacterial pathogens. Therefore, the present study was carried out with a prime objective to synthesize silver nanoparticles using bacteriocin of in-house novel probiotic strains of *Lactobacillus pentosus* S6 (KU92122) to enhance the antimicrobial spectrum of the synthesized silver nanoparticles against antibiotic resistant bacteria and different fungal pathogens.

Materials and Methods

Isolation and identification of bacteriocin producing ***Lactobacillus*** strain

Samples of fermented local Himalayan food called Siddu (fermented wheat dough) were collected in order to isolate potentially bacteriocinogenic lactic acid bacteria (LAB) from them. After pooling the samples,a stock was produced by combining 0.1 ml of sample with 9.9 ml of sterilized distilled water. It was serially diluted in the dilution range of 10^{-2} to 10^{-12} using serial dilution. The spread plate method was used to mount the samples (0.1ml) from each dilution on sterilized Petri plates containing solidified selected media viz. de Man, Rogosa, Sharpe (MRS) agar for lactic acid bacteria. The plates were incubated in anaerobic jar at 37°C for 48 h. After incubation, individual colonies were selected and purified using streak plate technique. Pure colonies of LAB - isolate S6 growing in predominance were selected for further study. Pure culture so obtained was further preserved in slants and 40% glycerol in deep

freezer (-20°C). The isolate was primarily examined according to their Colony morphology, Catalase reaction (10) and Gram reaction (11). Biochemical tests such as Carbohydrate fermentation, Citrate utilization, H_2S production, Methyl-Red and Voged- Proskauer (MRVP) test and Casein hydrolysis (10) were performed with the selected isolate. Molecular identification of this isolate through 16S r RNA technique revealed it as *Lactobacillus pentosus.* Its genetic sequence was deposited in NCBI, US wide accession number KU92122.

Production and purification of bacteriocin

The MRS broth (1000 ml) was seeded with *Lactobacillus pentosus* S6 KU92122@10 % (*A*=1.0 at 540 nm) and incubated at37 °C for 36 h. The sample was centrifuged at 8000 *g* for 30 min and the supernatant was collected. Partial purification of the sample was done by adding ammonium sulphate at 50 % of saturation level, followed by dialysis for 12 h. The pellet was collected after centrifugation at 8000 *g,* 4°C for 30 min. The pellet was dissolved in phosphate buffer (0.1 M, pH 7.0) and further purified by column chromatography technique on sephadex G-75 column. The bacteriocin positive eluted fraction were pooled together and stored at 4°C for further in silver nanoparticles synthesis.

Antagonistic action of bacteriocin

Antagonistic potential of crude, partially purified and purified bacteriocin was performed against different standard test indicators i.e. *Bacillus cereus* CRI, *Staphylococcus aureus* IGMC using well diffusion method [12].

Well diffusion method (12)

1ml inoculum of each indicator bacteria (1.0 OD) was swabbed properly on prepoured sterilized nutrient agar plates with the help of sterilized cotton buds. The swabbing was done in such a way that indicator culture covers the whole surface of nutrient agar plate. In the well diffusion method, well of 7 mm diameter and 5 mm depth were cut on the lawn laid in the nutrient agar plates with the help of sharp borer. The crude, partially purified and purified bacteriocin from *Lactobacillus pentosus* S6 was poured into the wells then well diffusion method. The plates were then incubated at 37^{O}C for 24 h and the zones of inhibition formed around the wells were measured.

Synthesis of silver nanoparticles using bacteriocin (13)

For the biosynthesis of silver nanoparticles, 10 ml of purified bacteriocin from *Lactobacillus pentosus* S6 (KU92122) was mixed with 90 ml silver nitrate ($AgNO_3$) solution (0.1 mM) and another reaction mixture with only silver nitrate was used as control. The prepared solutions were incubated at 30°C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. The primary detection of synthesized silver nanoparticles was carried out in reaction mixture by observing the color change of the medium from pale yellow to reddish brown.

Characterization of Silver Nanoparticles UV-Visible Spectroscopy Analysis (14)

The colour change in the reaction mixture was recorded through visible observation. The bio reduction of silver ion in aqueous solution was monitored by periodic diluted sampling of aliquots and subsequently measuring UV-Vis spectra of the solution on UV-Vis spectrophotometer UV-2450. The absorbance of the sample was read using UV visible spectrophotometer at different wavelengths i.e. 350, 450,550 and 650 nm.

Scanning electron microscopy (SEM) (14)

The thin film of the sample was prepared on a small aluminium plate by just dropping a very small amount of the sample on the plate, extra solution were 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.

Transmission electron microscopy (TEM) (13)

Transmission Electron Microscopy was performed by JEOL JSM 1oocx instrument. TEM shows the shape and crystal structure as well as size of the particles. The grid for TEM analysis was prepared by placing a drop of the nanoparticles suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was scanned by a Transmission Electron Microscope.

Fourier transform infrared spectroscopy (FTIR) (15)

The AgNPs synthesized by *Lactobacillus pentosus* S6 was studied by FTIR analysis. The sample was dried in freeze-drier (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}.

Antibacterial efficacy (16)

Antimicrobial assay will be performed against two multidrug resistant test indicators i.e. *Bacillus cereus* CRI, *Staphylococcus aureus* IGMC using well plate assay. One ml inoculum of each indicator bacteria (1.0 OD) was swabbed properly on prepoured sterilized nutrient agar plates with the help of sterilized cotton buds. The swabbing was done in such a way that indicator culture covers the whole surface of nutrient agar plate. In the well diffusion method, well of 7 mm diameter and 5 mm depth were cut on the lawn laid in the nutrient agar plates with the help of sharp borer. 100µl of synthesized silver nanoparticles from *L. pentosus* S6 was added to each well cut on the agar plate. Plates were incubated at 37 ^{0}C for 24-48 h and observed the clear zone production around the well. Antibacterial activity expressed in terms of percent increase:

$$\% \text{ increase} = \frac{Treatement - Control}{Treatement} \times 100$$

Antifungal activity (17)

Antifungal activity of bacteriocin synthesized AgNPs by *Lactobacillus pentosus* S6 was checked against challenging plant pathogens i.e. *Aspergillus niger, Pythium aphanidermatum, Fusarium oxysporum and Phytopthora parasitica* by well plate assay method using dual culture technique. On the one side of prepoured 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 cork borer. 100µl of synthesized AgNPs was added to the well. Plates were incubated at 28±2ºC for 7 days and observed for inhibition of mycelial growth produced around the well. For control, culture bit of indicator fungus was kept in the center of PDA plate and incubated at 28±2ºC for 7 days. Antifungal activity expressed in terms of percent inhibition growth of fungal mycelia as calculated from the equation:

$$\%I = \frac{C-Z}{C} \times 100$$

Where,

Z = Growth of mycelia in treatment

C = Growth of mycelia in control

Results and Discussion

Isolation and identification of bacteriocin producing ***Lactobacillus*** strain

Lactobacillus pentosus S6 was isolated from local Himalayan fermented food - Siddu and its colonies were found round in shape, with elevated elevation, whole edges, and a white colored appearance (Figure 1). The isolated strain was found to be Gram positive rod, with negative catalase, AG - carbohydrate consumption, positive casein hydrolysis, and negative citrate utilization in biochemical assays. On the basis of biochemical testing, the indole tests were determined to be negative, +VP$^+$, absence of H2S production, negative urease test, and facultative anaerobic lactic acid bacteria were discovered. Molecular identification of this isolate through 16S r RNA technique revealed it as *Lactobacillus pentosus.* Its genetic sequence was deposited in NCBI, US wide accession number KU92122.

Similarly, Yelnetty and his colleagues obtained 26 isolates that were classified as LAB based on cell morphology, Gram staining, catalase testing, and proteolytic activity (18). From 20 fruit samples, Naeem and colleagues isolated 15 lactic acid bacteria. The colony morphology and biochemical tests were used to identify each of the 15 samples. The fact that the majority of colonies plated on MRS agar plates were small, whitish to off-white in color, gram positive, catalase negative, and able to thrive in anaerobic conditions showed that these species were linked to lactic acid bacteria (19) .

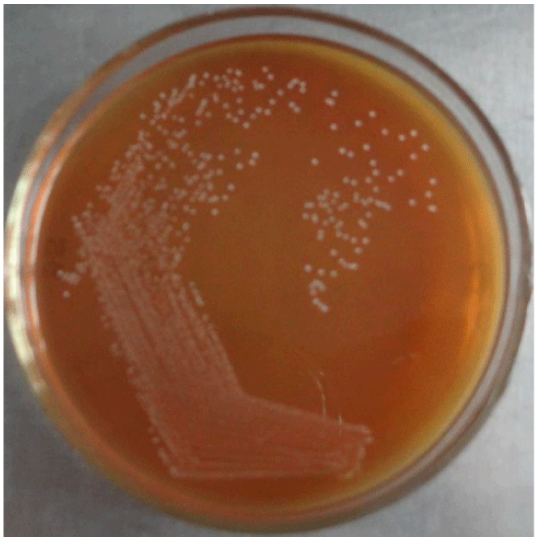

Figure 1: Colony morphology of Lactobacillus pentosus S6 (KU92122)

Production and purification of bacteriocin

Partial purification of bacteriocin from *Lactobacillus pentosus* S6 (Figure 1) was achieved by adding (NH4)2SO4 to the culture supernatant at 50 % level of saturation. It expressed higher activity of 6×10^3 AU/ml as compared to 2×10^3 AU/ml of crude preparation. Complete purification of bacteriocin from *Lactobacillus pentosus* S6 was achieved after gel exclusion column chromatography. Fractions 12–30 were pooled together (Figure 2) and the activity of purified bacteriocin finally increased to 8×10^3 AU/ml. The antagonistic spectrum of partially purified and purified bacteriocin was tested against *Staphylococcus aureus* and *Bacillus cereus.* The purified bacteriocin showed maximum zone of inhibition i.e. 20 mm and 18 mm against *Staphylococcus aureus* and *Bacillus cereus* (Figure 3), followed by partially purified bacteriocin with 18 mm and 16 mm zones, whereas it was only 10 and 12 mm for culture supernatant for *Staphylococcus aureus* and *Bacillus cereus* respectively. Bacteriocins are basically small peptides/ protein in nature, secreted extracellularly by bacteria and are gaining much attention due to their GRAS (generally recognized as safe) status for various applications. Due to their GRAS status, they are considered as a safe alternative to chemical preservatives in food. Bacteriocins are believed to be safe for human consumption since they can be degraded by the action of gastrointestinal proteases. Moreover, because they are ribosomally synthesized, their characteristics can be modified to enhance their activity spectrum [20]. Bacteriocins are known to exhibit antimicrobial activity against various other bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* [21].Currently, their efficacy against antibiotic resistant bacteria is catching attention of their possible role in clinical applications. Todorov and Dicks [22] reported the Bacteriocin production by *Lactobacillus pentosus* ST712BZ isolated from Boza with activity unit of purified bacteriocin to be 6400AU/ml. Prabhu *et al.* [23] reported the Production of bacteriocin by lactic acid bacteria isolated from yoghurt and their use in biosynthesis of silver nanoparticles. Sreedevi *et al.* [24] observed the synthesis, characterization and antibacterial studies of silver nanoparticles using purified bacteriocin from *Lactobacillus plantarum.*

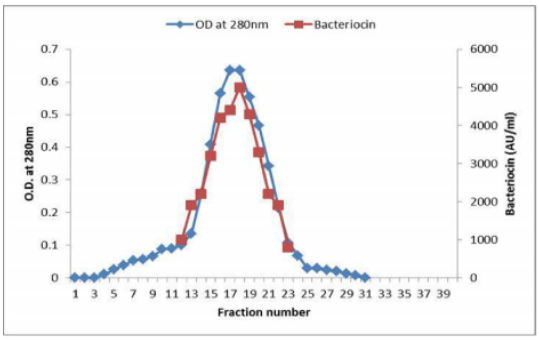

Figure 2: Elution profile of purified bacteriocin from Lactobacillus pentosus S6 on Sephadex G-75 column

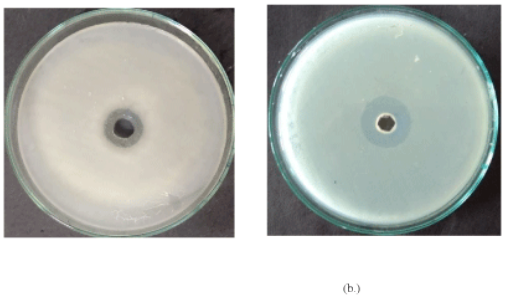

Figure 3: Antagonistic spectrum of purified bacteriocin from Lactobacillus pentosus S6 against (a) Bacilluscereus (b) Staphylococcus aureus

Synthesis of silver nanoparticles using bacteriocin

The formation of silver nanoparticles was monitored with color change. Bacteriocin from *Lactobacillus pentosus* S6 accession number KU92122 showed their ability for extracellular biosynthesis of silver nanoparticles. Changing the color of reaction mixture from yellow to reddish brown after incubation of mixtures for 24 h at 37°C indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag^0 into silver nanoparticles Ag^+ via the active molecules present in the bacteriocin (Figure 4). The color exhibited by silver nanoparticles was a result of the coherent excitation of entire free

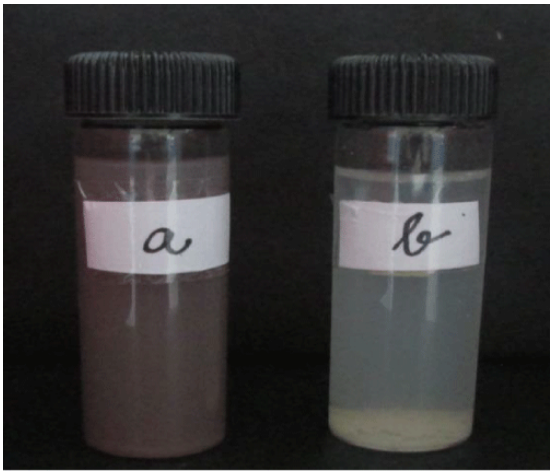

Figure 4: Biosynthesis of silver nanoparticles observation by change color to brown (a) synthesized silvernanoparticles (b) silver nitrate as control

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electrons within the conduction band, leading to surface plasmon resonance (SPR). Nanotechnology in the past two decades has opened doors to unlimited opportunities for solving several problems associated with a wide range of biological products. The collaborative use of these potential bacteriocins along with silver nanoparticles has allowed the development of new antimicrobial weapons of multiple applications [25]. In the food sector, the interaction between nanoparticles and bacteriocins holds high prospectus to be beneficial in increasing the antimicrobial potency/ spectrum of the latter. The interaction may also lead to a reduction in the requirement of high bacteriocin dosage, and an extension in the shelf life of food [26]. The conjugation of these peptides to silver nanoparticles surface is also widely researched for targeted gene therapy and bio-diagnostics. These synthesized AgNPs hold a great potential to be used as drug-delivery carriers, can be used as vehicles to transport drug molecules to target zones and thereby improve therapeutical efficacy; furthermore, express synergism with synthetic antibiotics regarding antibacterial properties. They have boosted the agricultural application by increasing the nutrient uptake by plants and also inhibiting the pathogens infecting the plants. Silver nanoparticles synthesized from bacteriocin (AgNPs) are intensively explored nanostructures for unconventional and enhanced biomedical applications, due to their size-related attractive physicochemical properties and biological functionality, including their high antimicrobial efficiency and non-toxic nature. AgNPs-based Nano systems and nanomaterials are suitable alternatives for drug delivery, wound dressing, tissue scaffold, and protective coating applications. Moodley *et al.* [27] reported synthesis of silver nanoparticles produced by *Pediococcus acidilactici*, High brown coloration was observed after incubation indicating completion of AgNPs synthesis. Pandit *et al.* [28] synthesized silver nanoparticles from the extract of Cymbopogon citrates and conjugated them with nisin to form a nano-bacteriocin conjugate. Similarly, synthesis of silver nanoparticles by bacteriocin producing *Lactobacillus* species isolated from yoghurt was reported by Prabhu *et al.* [23].

Characterization of nanoparticles

UV-Visible spectroscopy

The reaction mixture of synthesized silver nanoparticles was analyzed using UV-Vis spectrophotometer. Silver nanoparticles have free electrons, which give the SPR

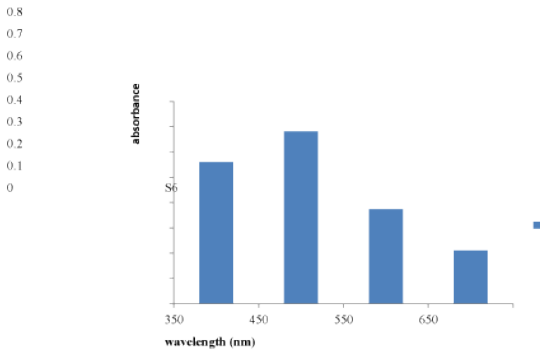


Figure 5: UV-Vis spectra of synthesized AgNPs from bacteriocin of Lactobacillus pentosus S6 at different wavelength

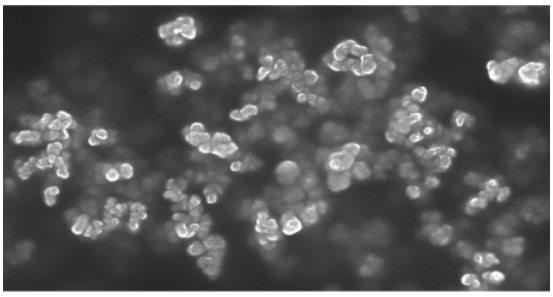


Figure 6: SEM image of silver nanoparticles synthesized by bacteriocin from L. pentosus S6

absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The absorption spectrum of nanoparticles was studied at different wavelengths *viz.* 350, 450, 550, 650 nm. The absorption spectrum was found maximum at a wavelength of 450 nm, which was specific for the synthesis of silver nanoparticles (Figure 5). Dakhil [29] showed the synthesis of silver nanoparticles using *Lactobacillus* sp. and observed the absorption peak at 410 nm. Absorbance associated at wavelengths in this range is typical of spherical AgNPs due to their surface Plasmon. Ranganath *et al.* [30] analyzed the silver nanoparticles synthesized by *Lactobacillus* sp. using UV-Vis spectrophotometer. The absorbance was measured in the range 300-650nm, which included the Plasmon absorbance peak of the silver nano particles centered at 430 nm.

Scanning Electron Microscopy

The synthesized silver nanoparticles were further characterized by scanning electron microscope. A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing

various signals that can be used to obtain information about the surface topography and composition. SEM is a useful tool for studying the size, shape and morphology of synthesized silver nanoparticles from bacteriocin. Figure 6 showed the SEM micrograph of the biosynthesized SNPs by *Lactobacillus pentosus* S6 KU92122. SEM analysis of AgNPs had shown individuals silver nanoparticles as well as their aggregates. The morphology of the silver nanoparticles was highly variable i.e. uniform and spherical. Moodley *et al.* [27] reported the spherical shape of the nanoparticles visualized during SEM analysis. Jemel *et al.* [31] reported that the SEM images show individual silver nanoparticles which are predominantly spherical in shape as well as number of aggregates with no defined morphology.

Transmission Electron Microscopy

TEM was used to determine the morphology (in terms of size and shape) of silver nanoparticles synthesized by bacteriocin from *Lactobacillus pentosus* S6 accession number KU92122. The micrograph (Figure 7) revealed spherical shaped nanoparticles with a mean size of synthesized silver nanoparticles i.e. 100 nm. Ndikau *et al.* [32] showed that the synthesized silver nanoparticles formed were spherical in shape with an average diameter of 17.96 ± 0.16 nm. Das *et al.* [33] reported the rapid synthesis of silver nanoparticles which were in the range of 70 -90 nm with spherical shape. Sani *et al.* [34] analyze TEM of silver nanoparticles synthesized from *Lactobacillus delbrueckii* subsp. *bulgaricus* and their particle size ranged from 1.4 to 8.9nm.

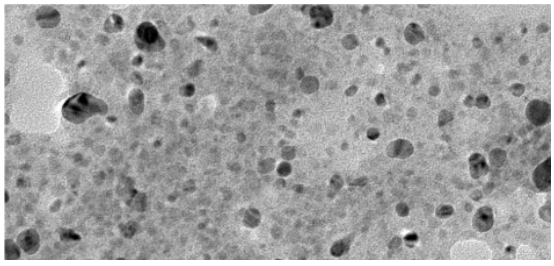


Figure 7: TEM image of silver nanoparticles synthesized by bacteriocin from L. pentosus S6

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to characterize and identify the chemical composition of the silver nanoparticle. FTIR analysis was conducted to determine the biomolecules responsible for capping and subsequent synthesis of AgNPs. FTIR spectroscopy can be used to measure the particle formation. It is widely used to study the nature of surface adsorbents in the nanoparticles. FTIR spectrum of *L. pentosus* S6 accession number KU92122 showed the band at 3674 cm^{-1} peak which represents O-H stretch of alcohol, peak at 1742 cm^{-1} indicates C=O stretch of lactone, 1521 cm^{-1} peak corresponds N-O stretch of nitro compound and the peak at 1079 $cm^{-1}$was identified as C-N stretch of amine respectively. The peak at 536 was identified as C-Br stretch of halo compounds, 1993 peak shows C=C=C stretch of allene, the peak at 2139 represents N=N=N stretch of azide and 1652 corresponds to C=N stretch of imine respectively (Figure 8). The above results of FTIR analysis revealed the presence of alcohol, amine, nitro compound and lactone functional groups which were responsible for the silver nanoparticles formation. These results were similar with the observation of Udayasoorian *et al*. [35] and Nanda and Raghavan [36] who equally characterized silver nanoparticles by FTIR and reported the presence of same functional groups. The FTIR measurement indicated that the structure of the protein was not affected because of its interaction with Ag^{+} ions or nanoparticles. The presence of functional group stretch i.e. O-H, C=O, N=O and C-N were responsible for the reduction of silver nitrate and thus synthesizing silver nanoparticles. Apart from these functional groups, some other groups were also observed i.e. C-Br stretch of halo compounds, N=N=N stretch of azide and C=N stretch of imines respectively. The presence of these functional groups in bacteriocin synthesized silver nanoparticles impart them novelty and uniqueness and proves them as strong antimicrobial agents which have applications in different industries including clinical applications as well as biocontrol agent in agriculture and food sector. Sutha [37] reported the FTIR spectrum of silver nanoparticles synthesized by *Lactobacillus acidophilus*. The band observed at 3436.60 cm^{-1} confirms the presence of alcohol and phenol functional groups with O-H stretching and H-bonded and the band observed at 2079.43 cm^{-1}

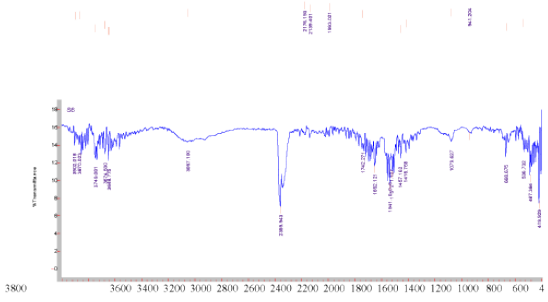
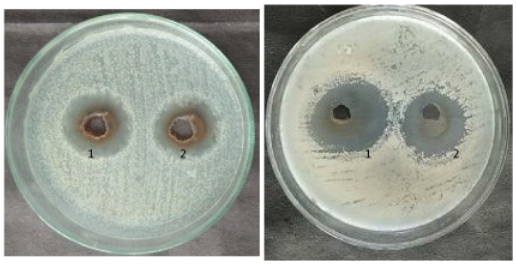


Figure 8: FTIR analysis of silver nanoparticles synthesized by bacteriocin from L. pentosus S6

represented X=C=Y allenes, ketone functional group band observed at 1637.25 cm^{-1} represented C=C stretching with vibration of alkenes functional group.

Antimicrobial potential of silver nanoparticles

The antibacterial effect of synthesized silver nanoparticles was studied against two bacterial pathogens viz. *Bacillus cereus*, *Staphylococcus aureus*. The zone of inhibition of synthesized silver nanoparticles of *L. pentosus* S6 along with control (only bacteriocin) was presented in (Figure 9). Out of *Bacillus cereus* and *Staphylococcus aureus,* the maximum antibacterial activity of synthesized silver nanoparticles was found against *S. aureus* i.e. 20 mm zone of inhibition as compare to control having 15 mm zone of inhibition, with an % increase of 25 % over control, whereas for *B. cereus,* maximum zone of inhibition was found for AgNPs i.e. 19 mm and for control it was 16 mm, having a % increase of 15 % over control respectively. The silver nanoparticles showed efficient antimicrobial activity compared to other salts due to their extremely large surface area, which provide better contact with microorganisms. The bacteriocins themselves are naturally occurring proteins and their use in synthesizing silver nanoparticles has enhanced the antibacterial activity of these proteins against different challenging pathogens. Sreedevi *et al.* [24] reported the antibacterial activity of AgNPs synthesized from bacteriocin of *Lactobacillus plantarum* against *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia* and the maximum zone of inhibition was found against *Bacillus subtilis* i.e. 25 mm.



(a) *Bacillus cereus* (b)*Staphylococcus aureus*

Figure 9: Antibacterial activity of AgNP's against different pathogens (1) synthesized AgNP's (2) only bacteriocin (control)

Antifungal activity of synthesized silver nanoparticles

The antifungal activity of synthesized silver nanoparticles was evaluated against four fungal pathogens *viz. Aspergillus niger, Pythium aphanidermatum Fusarium oxysporum and. Phytopthora parasitica* (Table 1, Figure 10). Bacteriocin synthesized AgNPs from *Lactobacillus pentosus* S6 showed maximum antifungal activity against *Fusarium oxysporum* i.e. 41.67 % followed by 28.57% inhibition against *Phytopthora parasitica* and 27.77 % inhibition against *Aspergillus niger.* Minimum % inhibition of AgNP's was found against *Pythium aphanidermatum*

Table 1: Antifungal activity of bacteriocin synthesized AGNP from Lactobacillus pentosus S6

Pathogens	Control (mm dia)	Bacteriocin synthesized AgNP (mm dia)	% Inhibition
Aspergillus niger	90	65	27.77
Pythium aphanidermatum	92	57	27.17
Fusarium oxysporum	72	42	41.67
Phytopthora parasitica	70	50	28.57

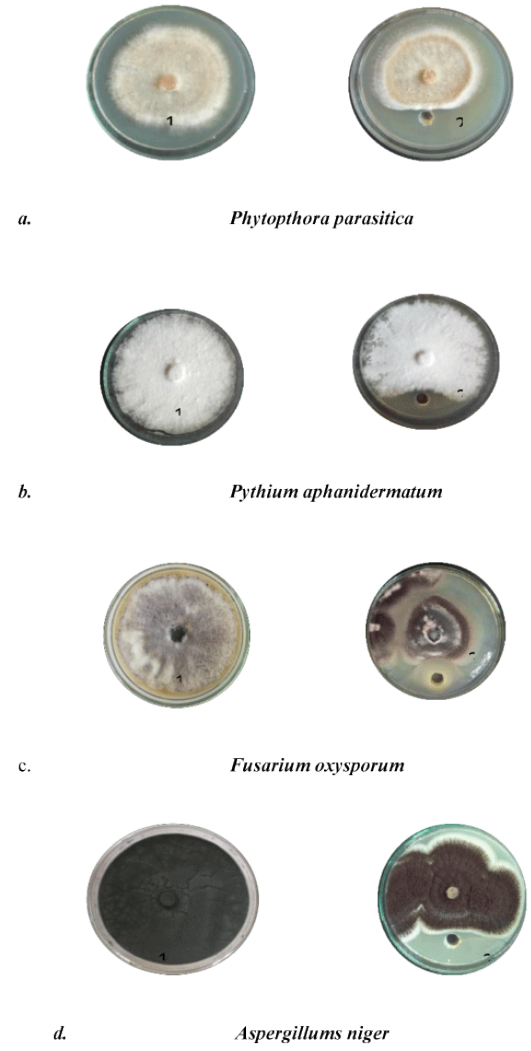


Figure 10: Antifungal activity of bacteriocin synthesized AgNP's against different pathogens: (1) Control, (2) Bacteriocin synthesized AgNP's.

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i.e. 27.17 %. Matei *et al.* [38] investigated the antifungal activity of silver nanoparticles synthesized by lactic acid bacteria against *Aspergillus* and *Fusarium* and found 11 mm zone of inhibition against *Aspergillums* and 18 mm against *Fusarium* respectively. In some cases, according to zeta potential, negative charge is present on the surface of both bacteriocin and fungal strains, so there is no interaction between them because of the similar charge and hence no antifungal activity was observed. But when bacteriocin synthesized silver nanoparticles were formed, they showed antifungal activity against various strains due to the presence of positive charge on its surface, which interacts with the fungal strains and causes its inhibition. The surface charge potential, described as the Zeta potential, plays a significant role in terms of the stability of AgNPs in aqueous solution [39]. This may be due to the large surface area of positively charged nanoparticles which allow their binding to the negatively charged fungal cell membrane. However, silver and gold are more commonly used and investigated nanoparticles for conjugation with bacteriocins as they are expected to have a synergistic effect on the antimicrobial properties [26]. This notion was also supported by the finding that bacteriocin are able to freely adhere to and penetrate bacterial cells, whilst they are unable to enter fungal cells at low concentrations. The Bacteriocin synthesized AgNP's produced in this study show no alteration in bioactivity between bacterial and fungal organisms, suggesting that their mode of action remains unaffected by the difference in the cell wall structures of these organisms [27].

Conclusion

In this study a successful attempt has been made for the synthesis of novel silver nanoparticles using bacteriocin from *Lactobacillus pentosus* S6 (KU92122).The silver nanoparticles had been characterized by UV-Vis, FTIR, SEM and TEM analysis. The UV-Vis spectral studies confirmed the surface plasmon resonance of biosynthesized silver nanoparticles. Biomolecules were responsible for reducing and capping of AgNP's, which had been confirmed by FTIR measurements. SEM studies revealed spherical and uniform shaped silver nanoparticles and the average particle size was confirmed by TEM analysis. The biosynthesized silver nanoparticles were found to have pronounced antimicrobial activity against challenging multidrug resistant bacterial pathogens like *Bacillus cereus* and *Staphylococcus aureus* and fungal plant pathogens such as *Aspergillus niger, Pythium aphanidermatum Fusarium oxysporum* and *Phytopthora parasitica* suggesting their future vital role in food, pharmacy and agriculture.

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