Synthesisof silver nanoparticles by some locally*Lactobacillus* spp.and detection of their antibacterial activity

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Abstract:

The study included the rapid synthesis and antibacterial activity of silver nanoparticles synthesized by locally *Lactobacillus* spp.(*Lactobacillus acidophilus, L. gasseri, L. rhamnosus, L. fermentum*). The silver nanoparticles were characterized with Atomic Force Microscopy (AFM) and found theaverage size ranges from 33.71 to39.16 nm. Finally antibacterial activities of the silver nanoparticles were checked against pathogenic bacteria (*Escherichia coli, Acinetobacter baumannii, Staphylococcus aureus and Listeria monocytogenes*).

The zone of inhibiton seems extremely good showing a relatively large zone of inhibition in both gram positive and gram negative bacterial isolates. To the best of our study this is the first report on synthesis of silver nanoparticles by locally *Lactobacillus* spp.

الخلاصة:

تضمنت الدراسة انتاج دقائق الفضة النانوية من عزلات محلية تعود لبكتريا *Lactobacillus* spp. والتي شملت الانواع *L.rhamnosus* *Lactobacillus acidophilus* و *L.gasseri* و *L.fermentum*، ودراسة الفعالية ضد البكتيرية للمادة النانوية المنتجة . درست دقائق الفضة النانوية باستعمال المجهر Atomic Force Microscopy (AFM).

بينت النتائج ان الفضة النانوية المنتجة من العزلات المحلية كانت بحجم تراوحت معدلاته بين 33.71الى 39.16 نانومتر، وعند اختبار الفعالية ضد البكتيرية للفضة النانوية المنتجة تجاه البكتريا المرضية (*Escherichia coli,Acinetobacterbaumannii, Staphylococcus aureus, Listeria monocytogenes*) .

أعطت مناطق تثبيط جيده ضد البكتريا السالبة والموجبة لصبغة كرام . وتعد هذه الدراسة من الدراسات الاولى التي تناولت انتاج الفضة النانوية باستعمال عزلات محلية تعود لبكتريا*Lactobacillus* spp.

Introduction:

Silver nanoparticles are among the most commercialized inorganic nano-particles due to their antimicrobial potential. They have also been used for a number of applications such as non linear optics, spectrally selective coating for solar energy absorption, biolabelling and antibacterial activities [1]. Production of silver nanoparticles can be achieved through different method; Chemical approaches are the most popular methods for the production. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. There is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals [2]. Bio-synthesized silver nanoparticles displayed good antimicrobial efficacy towards Gram-negative and Gram-positive bacteria as well as anti-biofilm potency against biofilm pathogen[3,4].

This is now well known that many organisms, can produce inorganic materials either intra- or extracellularly , Bacteria, being prokaryotes have survived the test of time in enriching ions , synthesizing magnetite nanoparticles, reducing Ag into metal particles, forming nanoparticles , and in generation of cermets[5].The formation of extracellular and intracellular silver nanoparticles by bacteria (*Pseudomonas stulzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella typhus*, and *Staphylococcus currens*) has been investigated[6]. Nanocrystals of gold, silver and their alloys have been synthesizedby the assistance of lactic acid bacterial cells [5].

In this paper, we report on the synthesis of silver nanoparticlesby somelocally *Lactobacillus* spp.

Materials and Methods:

Lactobacillus spp. **Isolates:**

Lactobacillus isolates used in the study included (*Lactobacillus acidophilu, L. gasseri, L. rhamnosus* and *L. fermentum*) isolated from Vaginal and Stool samples obtained from Department of biology / College of Science / Al-Mustansiriya University.

Pathogenic bacteria:

Isolates of *Escherichia coli, Acinetobacte rbaumannii, Staphylococcus aureus and Listeria monocytogenes*were collected from different infections sources from Central Medicine City hospital in Baghdad. Isolates were identified according to [7].

Synthesisof silver nanoparticles by *Lactobacillus spp*:

In a typical procedure of nanoparticles synthesis, every isolate were individually inoculated into sterilized 250 ml of whole milk in 500 ml flask and incubated for curdling at 37°C for 24 hours. The whey was collected by coarse filtration (Whatman 40). The filtrate was pale yellow in appearance, and the pH was typically 4.4. To 5 mL of each sample solution taken in a test tube, 1 mg of $AgNO_3$ was added and kept in the laboratory under ambient conditions. The solution became brown in about 12-16 h [1]. A brown mass gets at the bottom of the test tube after 24, 48, 72 h depending on isolates. Control was whey without $AgNO_3$.

Characterization of silver nanoparticles by Atomic Force Microscopy:

Atomic Force Microscopy image was taken using Park system AFM XE 100. The aqueous silver nanoparticles were deposited onto a freshly cleaved mica substrate. The sample aliquot was left for 1 min and then washed with deionized water and left to dry for15 min. The images were obtained by scanning the mica in air in non contact mode [8].

Antibacterial activity of silver nanoparticles produced by locally *Lactobacillus* isolates:

Silver nanoparticles produced by *Lactobacillus acidophilus, L. gasseri, L. rhamnosus, L. fermentum*were screened for their inhibitory activities against the indicator Gram-negative and Gram-positive bacterial isolates (*E. coli, A. baumannii, S. aureus, L. monocytogenes*), using agar well diffusion-method. Plates were prepared by spreading approximately 10^5cfu/ml culture broth of each indicator bacterial isolates on nutrient agar surface. The agar plates were left for about 15 min before aseptically dispensing the 50µl of each silver synthesized by *Lactobacillus* isolates into the agar wells already bored in the agar plates.

The plates were then incubated at 37°C for 18 - 24 h. Zones of inhibition were measured and recorded in millimeter diameter.

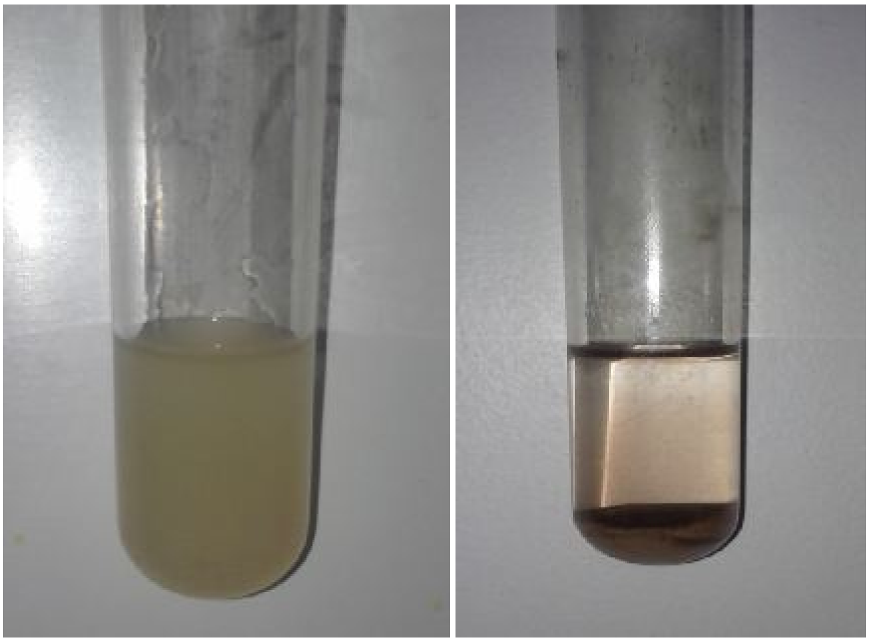
Results and Discussion:

Synthesis and Characterization of silver nanoparticles:

In the present study all *Lactobacillus spp* isolates (*L. acidophilus, L. gasseri, L. fermentum* and *L. rhamnosus*), confirmed as positive for Biosynthesized silver nanoparticles by change of the reaction mixture from pale yellow to brown color (Fig-1) indicating the production of silver nanoparticles (Ag+ to Ag0) this result agree with Minaeian *et. al* [9].

A brown mass gets at the bottom of the test tube after 24h for *L. gasseri* and 48h for *L. fermentum* while the isolates *L. acidophilus* and*L. rhamnosus* produced a brown mass after 72 h.

It is reported that reduction of Ag+ to Ag0 occurs through nitrate reductase enzyme , these enzymes released in the solution can reduce the silver nitrate to silver nanoparticles through capping agents such as proteins[1]. Lactic acid bacteria including *Lactobacillus* spp., *Pediococcuspentosaceus, Enterococcus faecium*, and *Lactococcusgarvieae*, were able to reduce silver, and *Lactobacillus* spp. can be used for a rapid and efficient production of silver nanoparticles [10]. Biosorption and bioreduction of Ag° on cell surface was also reported in *Lactobacillus* spp. at 30° C, pH 4.5 in 24 h by Lin *et. al* [11].



a= Control b= test

Fig -1: Lactobacillus spp in whey (a) without AgNO3 taken as control (b) with AgNO3 (1mg/5 ml)

The knowledge about the reduction of silver ions and formation of silver nanoparticles were still not clear, but believe that protein molecules and enzyme, includes nitrate reductase enzyme act as good regulating agent in silver nanoparticles synthesis. The primary conformation of synthesis of nanoparticles in the medium was characterized by the changes in color from yellowish white to brown[12].

Silver nanoparticles of well defined morphology and size are formed within the periplasmic space of the bacteria. The biogenic method for nanoparticle production is simple, eco-friendly and allows for getting controlled nanoparticles which can be used as catalysts with specific composition, which cannot be synthesized by classical methods. Applications in sensors and medicine are envisaged and the nanoparticles synthesized in the bacteria can be used against the human pathogens[13].

This study covered particle size was analyzed by Atomic Force Microscopy. AFM was used to view then anoparticles both in surface and three Dimensional view, and found the average size of particles (37.12, 34.62, 39.16, 33.71) nm synthesized by *L. acidophilus*, *L. fermentum*, *L .gasseri*, *L. rhamnosus*) respectively Figure (2, 3, 4, 5).

Hasan*etal* [14] showed that the biosynthesized silver nanoparticles were almost spherical, single (25–50 nm) or in aggregates (100 nm), attached to the surface of biomass or were inside and outside of the cells.

Antibacterial activity of silver nanoparticles against pathogenic bacteria:

Antibacterial activity of the silver nanoparticles synthesized by *Lactobacillus spp* isolates were tested against pathogenic bacteria (*E. coli, A. baumannii, S. aureusand L. monocytogenes*) using well diffusion technique. The diameter of inhibition zones around each well with silver nanoparticles is represented in table-1. The highest antibacterial activity was observed against *S. aureus* followed *by L. monocytogenes*, *E. coli* and *A. baumannii*. Yamanaka *et. al* showed that the silver nanoparticles have an antimicrobial effect on *S. aureus and E. coli* [15].Also Mohsen*eta l*[3] showed that the biosynthesized silver nanoparticles have effect against *E. coli* and *S. aureus*.

The silver atoms bind to thiol groups (-SH) in enzymes and subsequently cause the deactivation of enzymes.

Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport [16].

It is also believed that silver can take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R). Silver does this by catalyzing the reaction between oxygen molecules in the cell and hydrogen atoms of thiol groups: water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond [17].

Another one of the suggested mechanisms of the antimicrobial activity of silver was proposed that Ag^+ enters the cell and intercalates between the purine and pyrimidine base pairs disrupting the hydrogen bonding between the two anti-parallel strands and denaturing the DNA molecule [16].

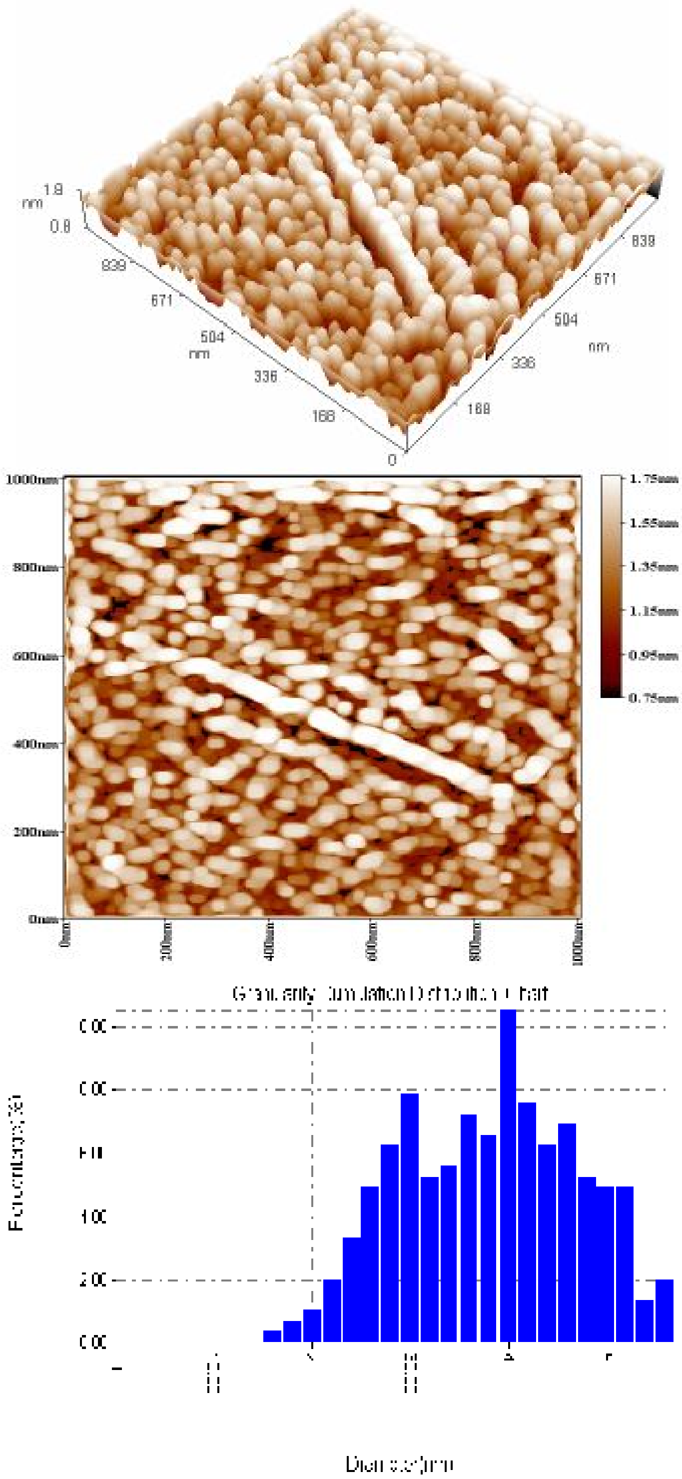


Fig-1: Atomic Force Microscopy image of silver nanoparticles synthesized by *L. acidophilus*.

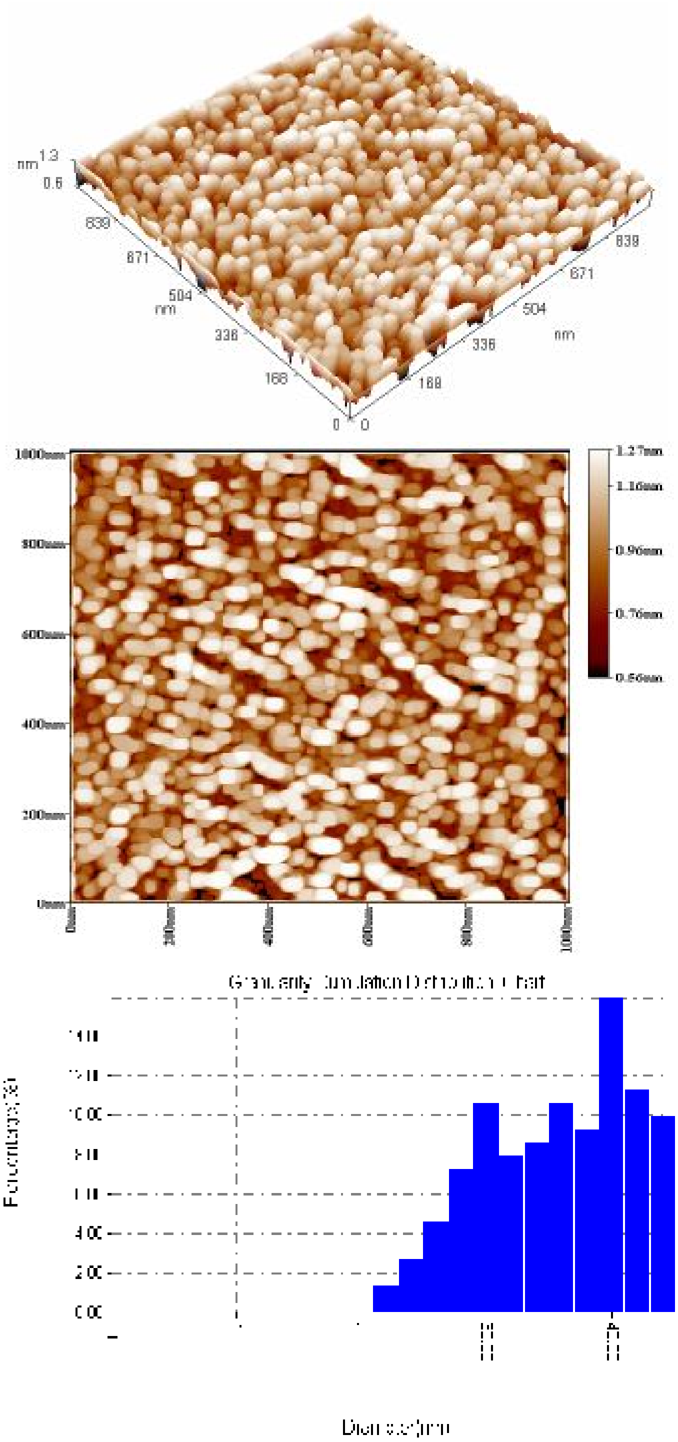


Fig-2: Atomic Force Microscopy image of silver nanoparticles synthesized by *L.fermentum*.

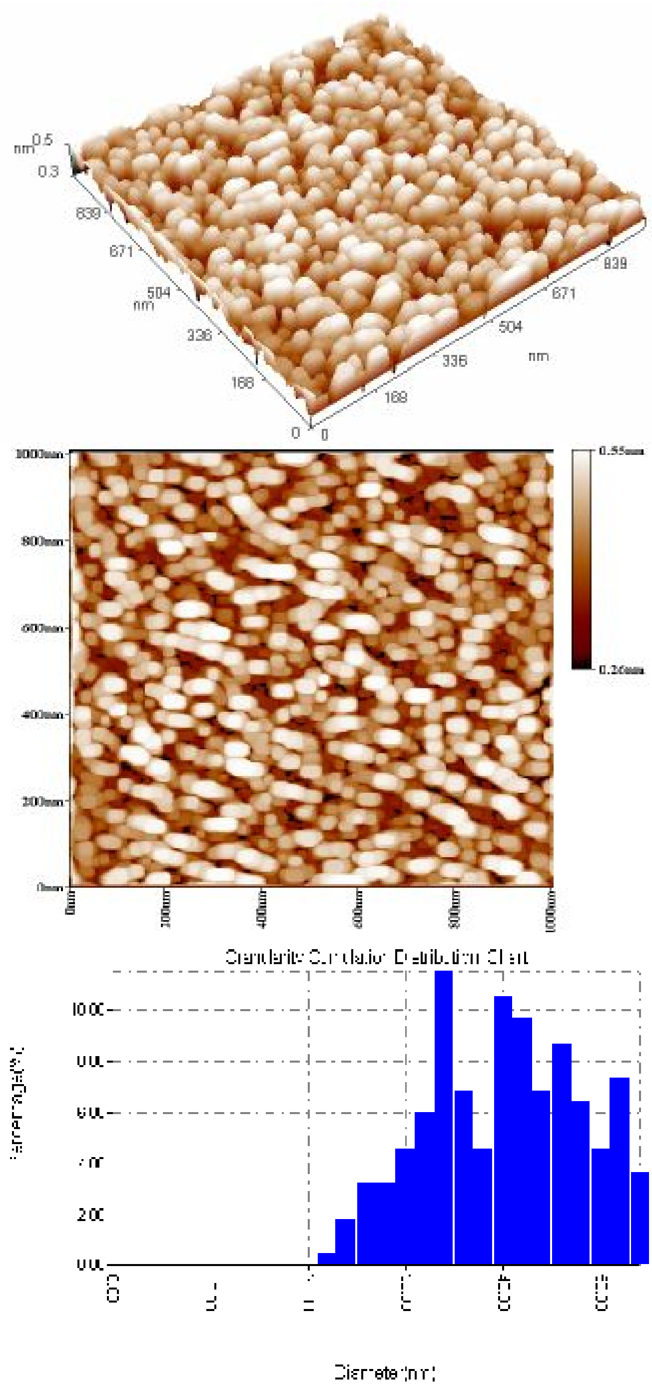


Fig -3: **Atomic force microscopy image of silver nanoparticles synthesized by *L.gasseri*.**

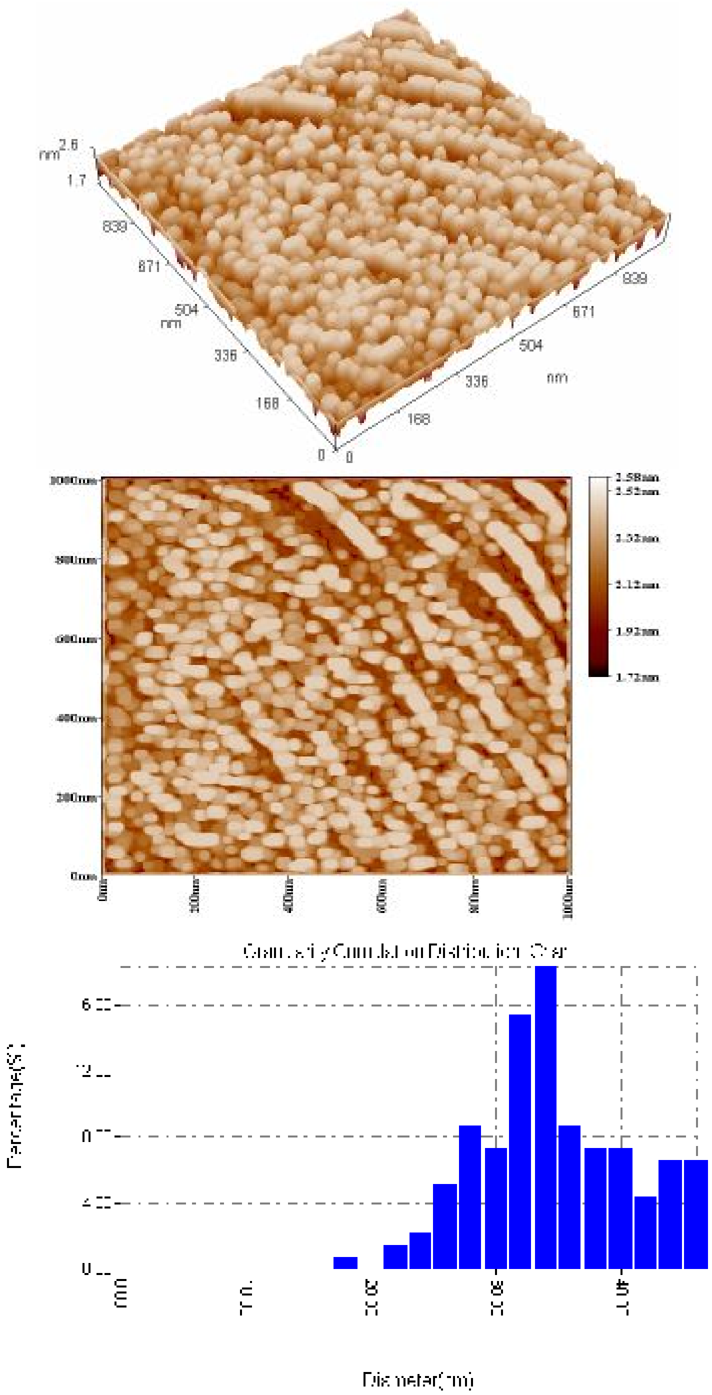


Fig-4: Atomic force microscopy image of silver nanoparticles synthesized by *L.rhamnosus*

Table-1: Antibacterial activity of silver nanoparticle synthesized by locally *Lactobacillusspp* isolates.

	Inhibition zone diameter (mm)			
Silver synthesized by *Lactobacillus spp*	***E.coli***	***A. baumannii***	***S.aureus***	***L.monocytogenes***
L.acidophilus	16	18	21	17
L.gasseri	20	16	24	19
L.rhamnosus	16	16	23	23
L.fermentum	17	18	20	22

Conclusion:

The present study demonstrated the synthesis of silver nanoparticles using locally *Lactobacillus* isolates (*L. acidophilus*, *L. gasseri* , *L. fermentum* and *L. rhamnosus*). The synthesized silver nanoparticles were characterized by using Atomic Force Microscopy. Antibacterial activity was observed against gram positive and gram negative pathogenic bacteria.

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