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## Research Article

# EXTRACELLULAR BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *LACTOBACILLUS ACIDOPHILUS*

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**ABSTRACT**

Nanobiotechnology is modestly too vast and too rapidly changing to cover exhaustively. Nanoparticles which play important role in the drug delivery systems and for treatment. This is because of its potential of possessing large surface areas and essentially no inner mass, i.e., their surface-to-mass ratio is extremely high. Recently researchers have found a new method for synthesis of silver nanoparticles (AgNPs) called green synthesis, from both plant and microorganisms such as bacteria, fungi and actinomycetes. The aim of the present study was, biosynthesis of silver nanoparticles (AgNPs) using *Lactobacillus* sp. which is one of the group of microorganisms, used as probiotics, which are safe and can be used in pharmacology industries. *Lactobacillus* was cultured in nutrient broth and incubated at 37°C for 24 h. Then they were centrifuged and supernatant was saved. To the supernatant, silver nitrate was added to and incubated for 28h at 35-37 °C. The formation of AgNPs was monitored by colour change and confirmed by the different characterizations of UV-Spec, FTIR, SEM and EDS. This research article confirmed the beneficial uses of extracellular biosynthesis and characterization of silver nanoparticles from *Lactobacillus acidophilus* for the treatment of gastro intestinal treatment.



## INTRODUCTION

Nanotechnology emerges from the physical, chemical, biological and engineering sciences where novel techniques are being developed. Nanoparticles and its biosynthesis is one of the useful application in the field of Nanobiotechnology. The unique properties such as large surface to volume ratio, absorption in the visible range, controlled drug delivery make Nanoparticles to be useful for mankind (Chand Banerjee *et al*.,2014). Currently, metal nanoparticles are beginning to be used like silver (Ag), gold (Au) and copper (Cu) with biological, optical, and magnetic interesting properties, within which silver nanoparticles (AgNPs), is the most studied because of their showed antimicrobial potential to bacteria, virus and fungus (Julio Cesar *et al*., 2018). Nanoparticles are smaller than hundred nanometers in size. Silver (Ag) and gold nanoparticles are promising tool for drug delivery applications (Zarina and Anima, 2014). Biologically synthesised silvernanoparticles have a important role in material science and nanotechnology. The biosynthesis of silver nanoparticles from bacteria offer numerous application in pharmaceutical and other biomedical field (Kanipandian *et al*., 2014). Several studies have demonstrated that physical and chemical properties that AgNPs have helped to increase the efficiency of silver, principally in control disease area (Prabu and Poulose., 2012). The extracellular biosynthesis of silver nanoparticles from *Lactobacillus acidophilus* is a eco-friendly and low cost effective method (Chaudhari *et al*., 2012). These method used to develop new effective antimicrobial agents against multiple antibiotic resistsnce of microorganisms (Franci *et al*., 2015). Therefore this present study has been designed to biosynthesis of Silver nanoparticles (AgNPs) using *L.acidophilus* in order to treat gasterointestinal diseases.

## MATERIALS AND METHODS

### *Sources of Lactobacillus*

*Lactobacillus* is a harmless ecofriendly probiotic which is predominant in curd and other fermented milk products was used for the present study. *Lactobacillus acidophilus* spores manufactured by the company Intra Labs India Pvt Ltd (Inventure) with a brand name- All G were obtained through a pharmacy in Tiruchirapalli, Tamil Nadu.

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***Preparation of cell free bacterial extract***

Lactobacillus spores were prepared, inoculated in sterile nutrient broth (HiMedia, Mumbai) and incubated at 37° C for 24 hours. The bacterial cell free extract was prepared according to (Chaudhari *et al.,* 2012).

***Biosynthesis of silver nanoparticles (AgNPs)***

Silver nitrate was used as a precursor in the biosynthesis of AgNPs. 200 ml of bacterial filtrate was added with 1mM $AgNO_3$ and kept at room temperature for 48 hours in dark for the bioreduction of $AgNO_3$. The primary detection of synthesis of silver nanoparticles was carried out by observing the color change of the medium from pale yellow to reddish brown by the method of **(**Chaudhari *et al.*, 2012).

***Extraction of silver nanoparticles***

The synthesized AgNPs was separated by means of centrifugation (Eppendorf) at 13,000 rpm for 1 hour. The pellet was resuspended in distilled water and again centrifuged for 15 mins at 13,000 rpm this step was repeated thrice. Thus, the supernatant solution obtained was stored at -4°C. The stored supernatant was then poured in a petridish then hot air dried to get AgNPs in powder form for further structural studies.

***Characterization of silver nanoparticles***

***UV - visible spectroscopy analysis***

The presence of silver nanoparticles in solution was analysed using UV-Vis spectroscopy in a range from 300 to 900nm at resolution of 1nm. All the UV‐vis spectroscopy measurements were performed on a Perkin Elmer (Model lambda 35), spectrophotometer operated at ambient temperature (Caroling *et al.,* 2013).

***Fourier Transform Infrared analysis (FT-IR)***

The AgNPs synthesized by *L. acidophilus* was studied by FTIR analysis. The interaction between protein and silver salts was recorded by FT-IR (Perkin Elmer) in a range of 400 $cm^{-1}$ to 4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

***Energy Dispersive Spectroscopy (EDS) and Scanning Electrom Microscope (SEM) analysis***

The samples were prepared on a carbon film by drop coating of silver nanoparticles for Energy Dispersive Spectroscopy. SEM used as Elemental analysis on single particle and instrument equipped with a thermo EDS attachment. The SEM analysis was performed on a JEOL; model JSM - 6610LV instrument operated at an accelerating voltage of 20 keV.

## RESULTS AND DISCUSSION

In this study aimed to biosynthesize of AgNPs using the aqueous extract of *Lactobacillus* was screened for ability to synthesize AgNPs. Production of AgNPs was observed after incubating the cell free culture extract with 1mM silver nitrate for 48hrs in dark, since silver ions naturally undergo photo reduction the entire reaction was carried out in dark. Production of AgNPs was indicated by visual change in colour from pale yellow to dark brown. The controls (1mM $AgNO_3$ solution and culture filtrate devoid of $AgNO_3$) showed no colour change which in turn proved the formation of AgNPs by the biomolecules in culture extracts. (Rahi *et al.,* 2014). After incubated 72 hrs the colour of the bacterial extract remained unchanged because of the synthesized AgNPs were well dispersed and the nanoparticles did not aggregate with each other. (Majeed *et al.*2018) (Fig 1).

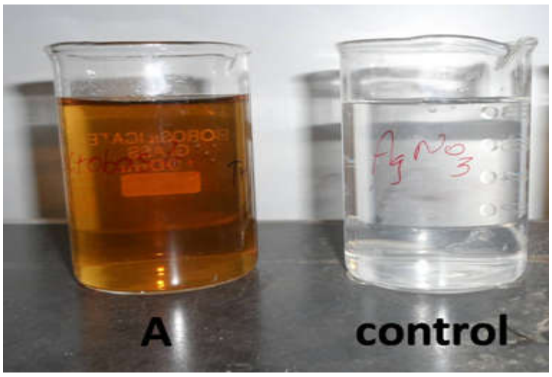


**Fig 1** Biosynthesis of silver nanoparticles A (sample + $AgNO_3$), control ($AgNO_3$)

***Characterization of silver nanoparticles***

***UV - Visible Spectroscopy Analysis***

The process of reaction between the metal ions and biosynthesized AgNP's was monitored using UV spectra through the aqueous solution. After 24hours of incubation color change was observed. In the UV-Vis spectrum absorption peak at 418 nm proves the surface plasmon excitation a typical characteristic of AgNPs (Kathiresan *et al.,*2009). The broadened peak at 418 also indicates the presence AgNP's in large size. The prescence of AgNPs was confirmed at the range was given in the Fig 2.

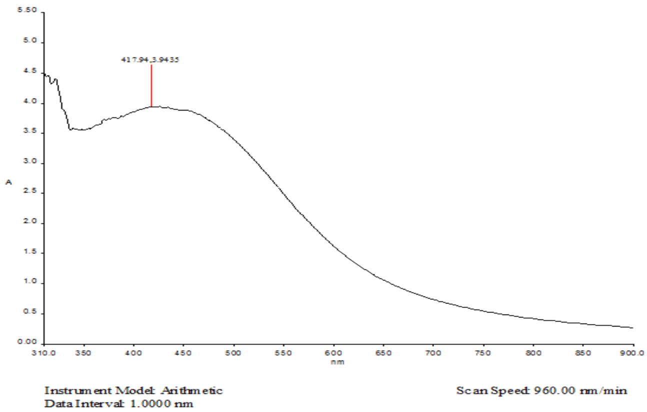


**Fig 2** The absorption of AgNPs are exhibited at strong broad peak at 418nm

***FT-IR analysis***

The interaction between Ag salts and protein molecules are identified by FT-IR analysis. The functional groups are idendified and named using absorbance ratio 400-4000 cm-1 at a resolution of 4 cm-1. The FTIR analysis of AgNPs shown in (Fig.3). The band observed at 3436.60 $cm^{-1}$ showed the presence of alcohol and phenol functional groups with O-H stretching and H-bonded and the band observed at 2079.43 $cm^{1}$ respresented X=C=Y allenes, ketenes functional group. The band observed at 1637.25 $cm^{-1}$ represented -C=C-stretching vibration of alkenes functional group. The functional group of aromatic compounds appeared 1489.80 $cm^{-1}$. The band at 1299.95 could be assigned to C-H wag (-CH2X) alkyl halides functional group.The bands observed at 1384.24 $cm^{-1}$ and 675.34 $cm^{-1}$ represented C-H bend of alkanes and alkynes functional group respectively.

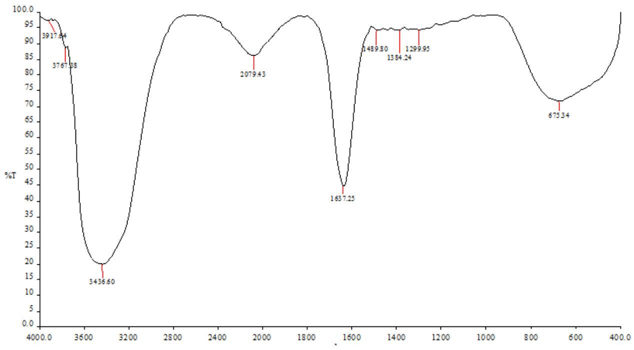


**Fig 3** FTIR spectral analysis of AgNps synthesized by *Lactobacillus*

### *EDS Analysis*

Silver is the most ingredient element. Biosynthesized nanoparticles from *lactobacillus* extract, which suggests the presence of silver. Generally metallic silver nanoparticles show a typical strong signal peak at 3 keV, due to surface plasmon resonance (Magudapatty *et al*. 2001; Kaviya *et al*. 2011; Das *et al*. 2013). Figure 4 shows presence of elements such as SI, MG, CU, CL, FE, S, C are shown. In the present investigation, the synthesized silver nanoparticles show strong absorption in the range 3keV.

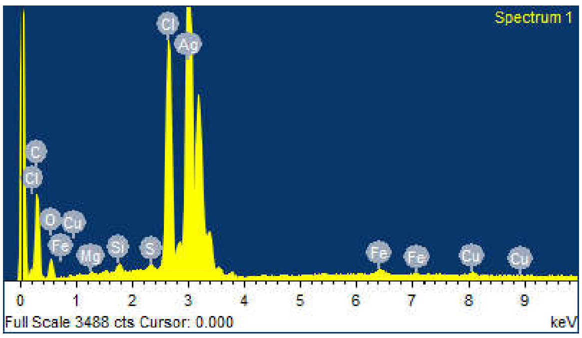


**Fig 4** EDS spectrum of synthesised AgNPs using *L. acidophilus* extract

### *SEM analysis*

SEM was used to study the topology of nanoparticles and the process of synthesis from EPS. The EPS-AgNPs synthesis from the biofilm was observed. (Fig.5). SEM images revealed the presence of spherical shaped EPS-AgNPs at 35,000X, and the dispersion of particles was visualized at 10,000X.

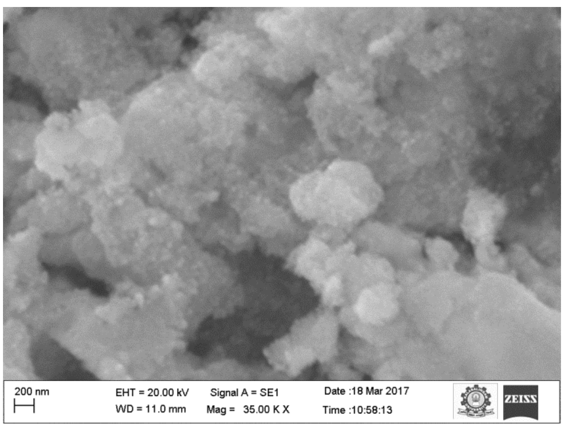


**Figure 5** SEM image of AgNP's biosynthesized from *L. acidophilus*

## CONCLUSION

In this study, the biosynthesis of AgNPs by a common probiotic *lactobacillus* used to treat gasterointestinal diseases was investigated. The AgNPs were synthesized extracellularly and the results were measured visually and UV Spectrophotometrically. The synthesized nanoparticles were characterized through UV-visible spectrophotometer shows the surface Plasmon Resonance and high peak at 418nm. FTIR results indicates that the involvement of protein in bioreduction of $AgNO_3$ into silver nanoparticle. Then the Nanoparticles powder was prepared and involved for microscopically studies are Scanning Electron Microscope (SEM) which shows the shape of spherical nanoparticles. The EDS analysis also proved the presence of elemental silver (at 3KeV).

Therefore, it has been concluded that *L. acidophilus* have beneficial role can be potentially applied for treat gasterointestinal diseases. This technique can be useful for the development of new therapeutic agents.

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