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ORIGINAL ARTICLE

Biosynthesis of Silver Nanoparticles by *Lactobacillus fermentum*

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ABSTRACT
Several methods used to synthesized AgNPs, but each of these methods had disadvantages. Chemical methods have high toxicity and low stability, and physical methods are costly and low-efficient. Recently, researchers have found a new method for synthesized AgNPs, which used plants and microorganisms such as bacteria, fungi, and actinomycetes. This method is called eco-friendly or green synthesis or biosynthesis. The aim of This study was, biosynthesis of AgNPs using Lactobacillus Fermentum, which it is one of the microorganisms in the group of probiotics Consequently, the AgNPs synthesized using this method are very safe and can be used in pharmacology industry and medicine. Lactobacillus Fermentum was cultured in MRS broth and incubated at 37^0c for 24 h and 5% co_2, Then they were centrifuged , and supernatant was added to silver nitrate 0.001 , 0.002, 0.003M, pH 5.6,7,9 and incubated at 28 -35-37^0c. The formation of AgNPs was monitored by change color , UV-vis spectroscopy, TEM, XRD and FTIR. The results showed that Lactobacillus Fermentum had great potential to biosynthesized of AgNPs in size of 13nm.
Keywords: *silver nanoparticles , Biosynthesis, Lactobacillus fermentum,*

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INTRODUCTION

In recent years nanotechnology is the one of the important field of modern sciences [1]. Nanotechnology studied about structures between 1-100 nanometer (nm). This structures in this size, fined the new properties and functions [2].In this fields of research, nanoparticles was very attractived . Among of nanoparticles, the metallic nanoparticles such as Silver, Gold and zinc have shown great functional effect in medicine, pharmacology, industry, electronic, water treatment, anti tumor, anti cancer and textile engineering [3,4].Nanotechnology is usually explained by two different process:" Top-down" and "Bottom-up .In Top-down process, making nanoscale structure from large scale structure by different techniques .In bottom-up process making nanoparticle structures by atom-by atom or molecule by molecule by self assembly or self organization [5-7]. Silver nanoparticles (AgNPs) are very interesting, because they have many unique properties [8]. AgNPs used in biosensors, electronic component, antimicrobial agent, environmental, cosmetic products, drug delivery, optical, catalysis and other areas [4-8].AgNPs was synthesized by bottom-up process in several methods such as chemical reduction of silver ion in aqueous solution [9], Thermal decomposition in organic solutions [10], Chemical reduction, photoreduction [11,12], Radiation, Laser [13,14]. Most of these methods are very expensive and unstable also AgNPs was produced in these methods had been many sideffect and very toxic. Then we can not used of them in medical or pharmacology purposes. Nanobiotechnology is a new branch of nanotechnology then complex of microbiology, physic and chemistry science. Nanobiotechnology was applicated of biological system such as bacteria, fungi, plant for the production of nanoparticles(16).Presently AgNPs are produced by "Green synthesis method. In this method used of biological source including bacteria [1,4,8,17] green algae, *fungi* [16,18-20], *actinomycetes* [20],plant extract [6,7,9,21,22]. Green synthesis is clean, simple, eco friendly and non toxic.On the other hand, some organisms was used, had the pathogenic effect. This subject is a big risk and very dangerous.
In this study we used the *Lactobacillus fermentum(*ATCC: 9338 , PTCC:1638*)*. It is a rod, gram positive and so it's a probiotic bacteria. Probiotic defined as" live microorganisms witch when administered in

adequate amount confer a health benefit on the host"[23]. Then AgNPs produced by *Lactobacillus fermentum* are safe, so fancierly can be used to medical and pharmacology purposed.

MATERIALS AND METHODS

Bacterial used for synthesis of Silver nanoparticles

Lactobacillus fermentum ATCC: 9338 PTCC : 1638 was obtained from microbial collection of Iranian Research Organization for Science and Technology (IROST) .It was inoculated in MRS broth and incubated in 37 c and 5% co_2 for 24-48 h.

Preparation of supernatants

MRS broth was preparedand sterilized, then 1ml of freshly *L. fermentum* inoculated per MRS flask. They were incubated for 48 h at 37° c and 5% co_2 . After the incubation period, the cultures were centrifuged at 5000 rpm and their supernatan were used for further experiments.

Synthesis of silver nanoparticles

Aqueous silver nitrate solution (10^{-3} M) was prepared and added to supernatants or *Lactobacilluse fermentum* (10% v/v), and incubated in 28°c and darkness . The color of this solution was without color, change of color to brown was showed that silver nanopaticles produced .

UV-Visible Spectroscopy

The reduction of the Ag^+ ions by the supernatant of this bacteria and formation of silver nanoparticles were monitored by UV-visible spectroscopy. After every 10 days change of color was considered and change of conditions was measured by uv-vis spectroscopy. Sampling 1 ml of solution and they were measuring during 350-600 nm absorbance. The UV-Vis spectra of these samples were measured on a Cecil model CE 7250.

Transmission Electron Microscopy (TEM)

Size and shape of the silver nanoparticles formed by the supernatants of the test bacteria was considered by transmission electron microscopy. First Each sample were dispersed by ultrasonicator for10-15 min, to separated distinct particles then immediately one or two drops of the suspension was placed on to carbon coated grids and dried under infrared lamp.

X-Ray Diffraction

The formation of silver nanoparticles was provided by X-ray diffraction (XRD) technique using an X-ray diffractometer (3003 pts , Seifert) with Cu K_α radiation λ = 1.5405 Å over a wide range of Bragg angles as (20–80°C). Glass slides coated with silver nanoparticles produced. For more efficient, the slides were coated with silica gel, then coated with silver nanoparticles solution.

Fourier Transform Infrared (FTIR) spectroscopy

The AgNPs synthesized by *L.fermentum* was studied by FTIR analysis. The sample was dried in freez-drier (lyophilizer) for 24 h, then freeze dried sample was appointed with KBr pellets and analysed using a Thermo Nicolet model: nexus 870 in range of 450-4000 cm^{-1} at a resolution of 4 cm^{-1}.

Identification of best condition for synthesized AgNPs

Identification of best conditions such as pH, temperature incubation, concentration of silver nitrate was helped to established the best condition for biosynthesized of AgNPs by *L.fermentum* .Aqueous silver nitrate solutions in pH 4, 5.6, 8 for pH and different concentration of silver nitrate 0.001, 0.002, 0.003 M were prepared and added to supernatants or *Lactobacilluse fermentum* (10% v/v), and incubated in 28°c and darkness. And three silver nitrate solutions (10^{-3} M) was prepared and added to supernatants or *Lactobacilluse fermentum* (10% v/v), and incubated in 28, 35, 38°c and darkness.

RESULTS AND DISCUSSION

The first symptom of AgNPs formation, was changed color of silver nitrate, it was played a key role to shown that AgNPs was formatted. Wrought of AgNPs by *lactobacillus fermentum* can be easily monitored by color change. Silver nitrate was colorless and after reduction by *L.fermentum* supernatant had brown color. The change of color of reaction is present in figure 1. The color changed to Brown after 7 day of incubation and there was not changed after ward. This observation implied that the reduction of Ag^+ ions take placed in this process. The severity of color due to actuated surface Plasmon vibration in the metal nanoparticles. [24]. These results are consistent with the results reported that articles present in 1 to 22 and 24-32 references.

Figure1) Biosynthesis of silver nanoparticles observation by change color to brown.(a) silver nanoparticles formation as a test (b) silver nitrate as a control

The ultra visible spectrophotometric method was the second reason for AgNPs formation. uv-vis spectrum show in figure 2. It showed that single and strong absorption peak at 420 nm. This band was called the surface Plasmon resonance (SRP). The surface Plasmon resonance band of AgNPs to proved by uv-vis spectrum peak, at 400-450 nm, this symptom, showed that the AgNPs was synthesized (2,3,7). According to Mié s theory, single surface Plasmon band, demonstrated small and spherical nanoparticles but anisotropic particles showed two or three bands[18,25-29]. In this study SRP band showed one strong band that indicatived the spherical shape and small size of AgNPs, which was proved by TEM. These results are consistent with the results reported that articles, present in 1 to 22 and 24-32 references.

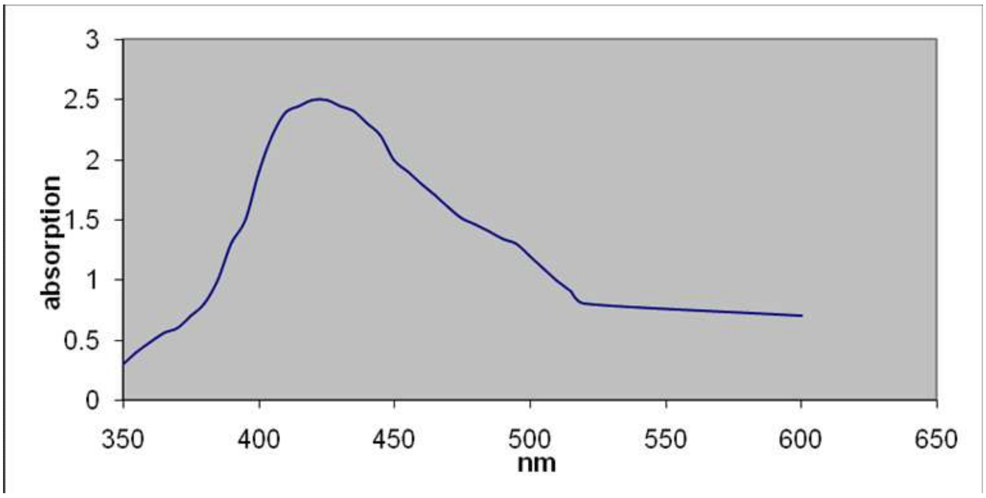

Figure 2) Uv-Vis spectra. The maximum absorbance was at 420 nm

The effected of variable pH, incubated temperature and concentration of silver nitrate to synthesized AgNPs show in figures 3-5.

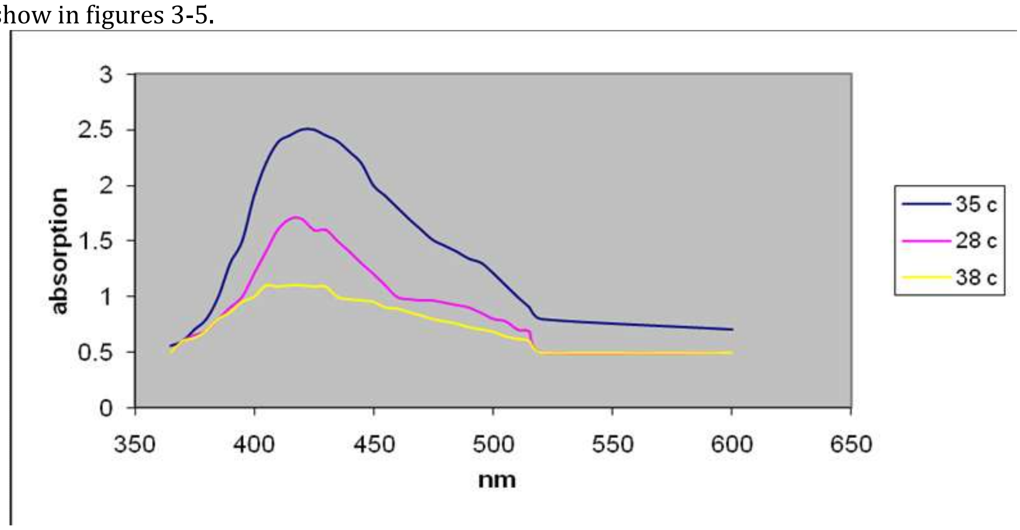

Figure 3) Uv-Vis spectra recorded in different incubation temperature.

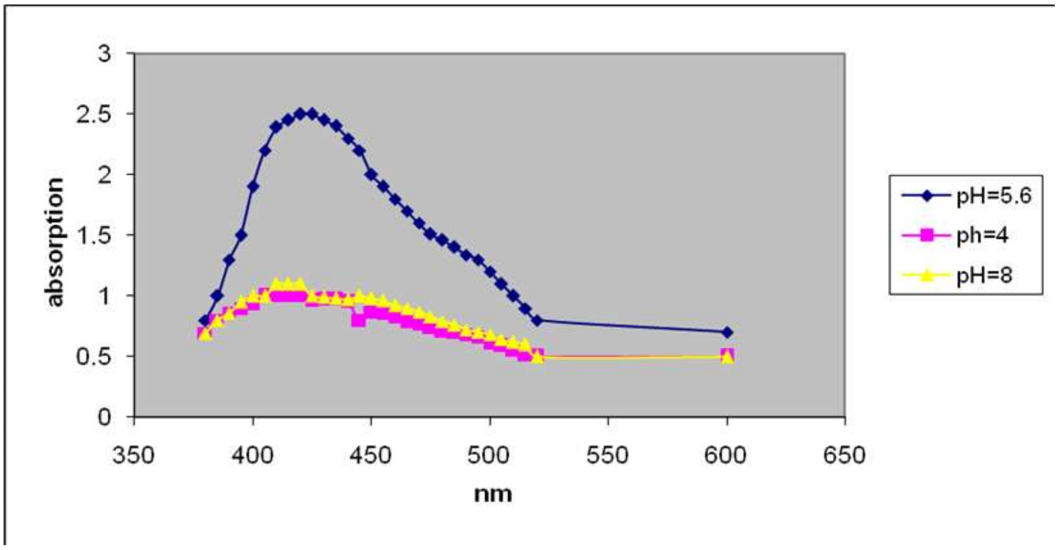

Figure 4) Uv-Vis spectra recorded in different pH.

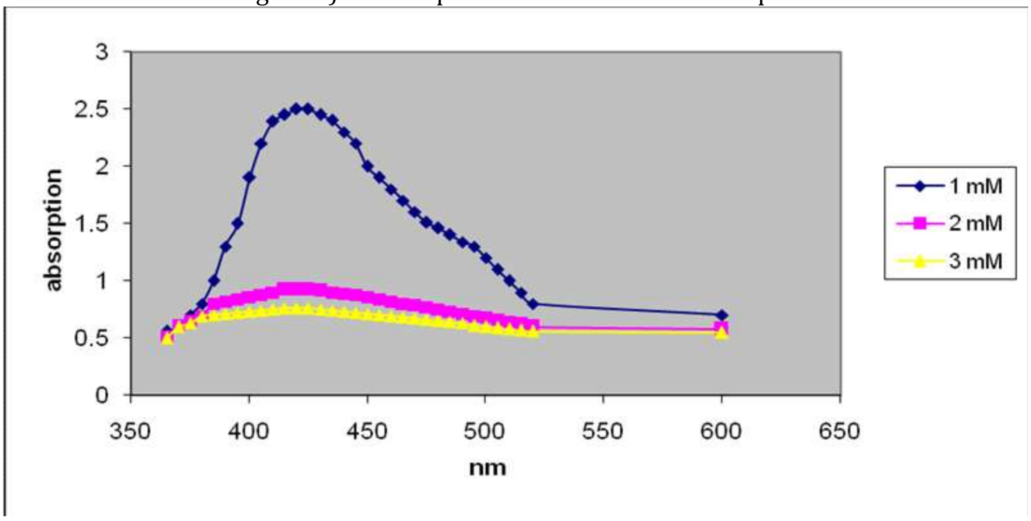

Figure 5) Uv-Vis spectra recorded in different concentration of silver nitrate.

The aim of this examination was obtained to best condition for synthesized AgNPs by *L. fermentum* . Results showed that pH=5.6 and 30˚c and silver nitrate 1mM, were the best condition for biosynthesized of AgNPs by *L. fermentum*.
The morphology and size of the AgNPs were indicated by the transmission electron microscopy (TEM) images(1-22 and 24-32). The TEM image show in figure 6. The image was confirmed that AgNPs were spherical in shape and average size of them were 13.75 nm .Also all of the particles were single and pure. The small size of AgNPs was very useful because they could easily made a way in to cell membrane and this subject is very important to medical and pharmacologic purposed (30).

Figure 6) TEM image of silver nanoparticles synthesis by *L.fermentum*

The third method to improved of AgNPs formation was X Ray diffractometry (XRD). The chemical composition and material often determined by X-Ray diffraction analysis (30). Figure 7 show that XRD diagram. It was established that AgNPs synthesized by *L.fermentum* and they were crystalline. The average diameter of AgNPs was calculated by scherreŕ s formula (16,29,30). T=0.9λ/β cosθ, (t) is crystalline size,(β) is the width of peak at half maximum intensity of a specific phase in radians , (λ) is the wave length of incident rays, (θ) is the center angel of the peak in radian (1,8,10,12,16). So the AgNPs synthesized by *L. fermentum* were crystallime and size of them were calculated to be 14 nm by used of this formula and consideration of width of the [111] diffraction then called Bragg ́s reflection.

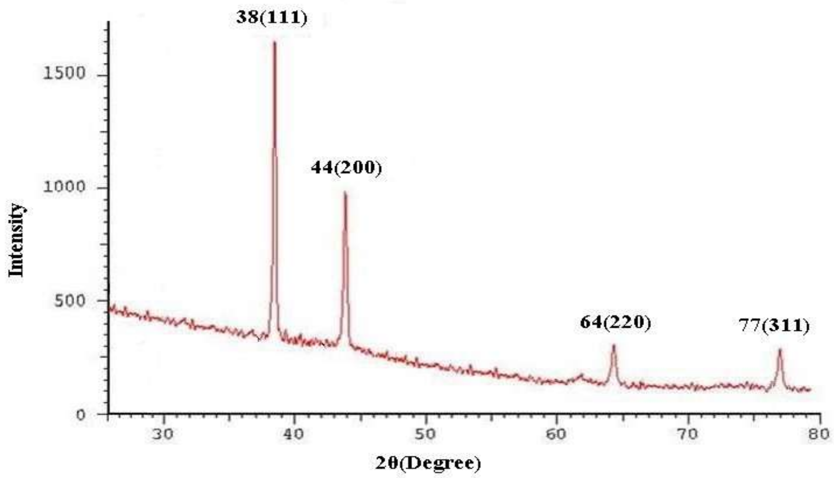

Figure 7) XRD spectrum of silver nanoparticles synthesized by *L.fermentum*

At last, figure 8 showed the FTIR analysis. In FTIR spectrum showed that the band at 3.843, 2.640, 3.337, 1.636, 1.383 cm-1 identified N-H group of amines, O-H group, C=O group and C-N group and peak at 1.058 cm-1 was recognized silver nanoparticles.(30-32). So the peak presented in this area provided that potential of *L. fermentum* supernatant to synthesized AgNPs. This results are consistent with the results reported that articles, present in 1 to 22 and 24-32 references.

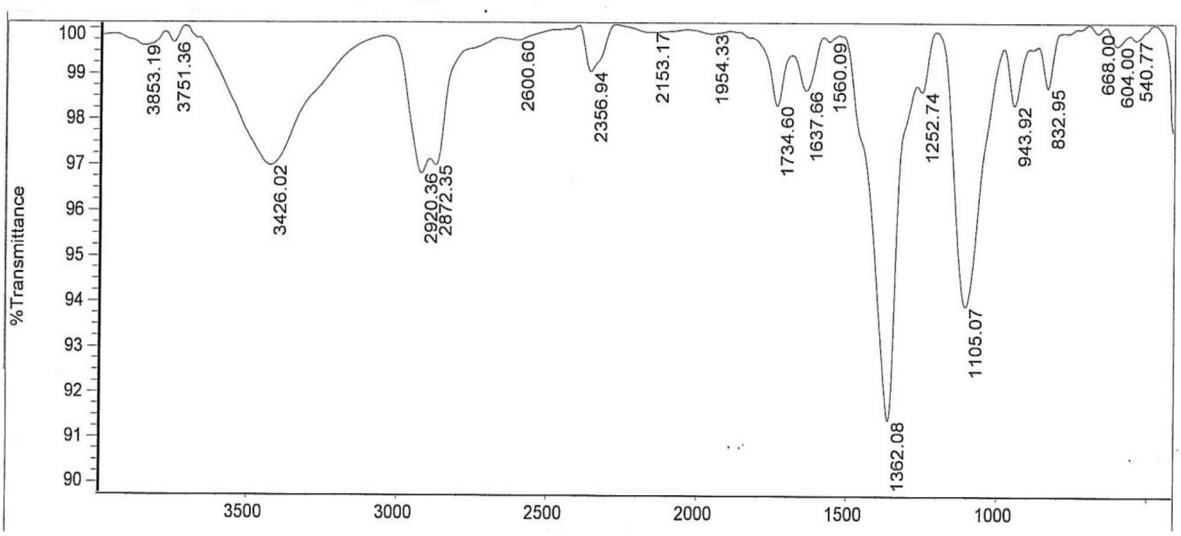

Figure 8)FTIR spectrum of silver nanoparticles synthesized by *L.fermentum*

Stability of AgNPs synthesized by L.fermentum was monitored for 10 month after synthesized, result of them showed in table 1. AgNPs biosynthesized was called test and compared to AgNPs that prepared of Nano Nasb Pars Company as a control. Results were shown that color solution of controls were changed to gray and turbid after six month but the test color was brown and clear after 10 month . In control flask AgNPs were sediment and absorptions of them in 420 nm were decreased but in test flask sediment was not seen and absorptions of them in 420 nm, not changed.

Table 1) Stability of AgNPs synthesized by *L.fermentum*

Time	Color change		Sedimentation		Abs/420	
	Test	control	Test	control	Test	control
1 month	Brown	Brown	Not	Not	2.6	2.7
2 month	Brown	Brown	Not	Not	2.5	2.68
3 month	Brown	Brown	Not	Not	2.55	2.67
4 month	Brown	Brown	Not	Not	2.5	2.5
5 month	Brown	Brown	Not	Not	2.56	2.5
6 month	Brown	Dark Brown to black	Not	Yes	2.5	1
7 month	Brown	Dark Brown to black	Not	Yes	2.54	0.8
8 month	Brown	Dark Brown to black	Not	Yes	2.5	0.7
9 month	Brown	Dark Brown to black	Not	Yes	2.53	0.6
10 month	Brown	Dark Brown to black	Not	Yes	2.5	0.5

Then stability of AgNPs formatted was excellent, and particulars was not altered after 10 month .This stability is a great for applied purposes of AgNPs . These tests were not done in other researches.

CONCLUSION

According to results, we have successfully extra cellular synthesized of AgNPs by *Lactobacillus fermentum* supernatants. This way is simple, safe, cost effective and ecofriendly. AgNPs produced by this method are very small, average particles size is 15 nm and spherical and stable without agglomeration .Also stability of AgNPs biosynthesized, was tested and showed that excellent stability without change color , agglomerate or sedimentation. In other hand *Lactobacilluses* are the major group of probiotics, then this bacteria are safe and without pathogenic effects. So we can confidencely used of them in medical and pharmacology purposed.

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