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Biological Synthesis of Silver and Gold Nanoparticles by Bacteria in Different Temperatures (37°C and 50°C)

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Major purpose of this study was to investigate the effect of two temperatures of 37°C and 50°C on biosynthesis of extracellular silver and gold nanoparticles by some bacteria. To perform biosynthesis of nanoparticles, the bacterial culture supernatants of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia* and *Yersinia enterocolitica* were treated with aqueous solutions of silver nitrate (25 µL of 1 M) and gold III chloride ions (25 µL of 1 M) and incubated in shaker incubators (at 150 rpm) at 37°C and 50°C. Nanoparticles of silver and gold in the culture were monitored through UV-visible spectrophotometry and confirmed by X-ray diffraction analysis (XRD). The average size and morphology of nanoparticles were determined by transmission electron microscopy (TEM). The results obtained indicated that silver and gold nanoparticles were produced in the bacterial culture supernatants within 24 hr and the rate of production was relatively more at 50°C. XRD analysis confirmed biosynthesis of nanoparticles and UV–visible spectra showed maximum absorption for silver and gold nanoparticles at 410-430 and 510-540 nm respectively. In addition, TEM images illustrated the average size of nanoparticles from 10 to 80 nm. Overall, biosynthesis of nanoparticles is a simple method and does not require more time. In addition, some bacterial stains could produce reductive enzymes for reduction of silver and gold ions and this bioreduction could be affected by temperatures.

Key Words: Biosynthesis, Nanoparticles, Bacteria, Temperature.

The prefix *nano* describes one billionth (10^9 m) in size and metal nanoparticles show different physical, chemical, magnetic, optical and biological properties based on their bulk material (Schmid *et al.*, 1992 and Daniel *et al.*, 2004). Nowadays application of nanoparticles was considered in different area. For instance, silver and gold nanoparticles have been recommended as drugs and gene delivery systems for treatment of cancers (Fayaz *et al.*, 2010, Castro-Longoriaa *et al.*, 2011). Moreover synthesis of biolabeling, delivering, diagnostic and biosensing systems for different applications was investigated using nanoparticles (Binupriya *et al.*, 2010 and Duncan *et al.*, 2010).

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Metal nanoparticles were synthesized by several methods viz., physical, chemical and biological methods. Although, physical and chemical nanoparticles productions need nonliving conditions, animate conditions should be provide for biosynthesis of nanoparticles (Narayanan and Sakthivel 2010). It has been confirmed that some microorganisms have ability to produce nanoparticles by reduction of metal ions and relatively this method has advantage of environmental friendly (Saifuddin *et al*., 2009, Narayanan and Sakthivel 2010). To produce nanoparticles, microbial cells neutralize the metal ions by their reduction into insoluble metal either inside or outside of the cells. The accumulation site of nanoparticles depended on localization of the reductive enzymes. If reductive enzymes secreted by the cells bioreduction occurred out of the cells and if reductive enzymes remain and act inside of the cells, nanoparticles produced within the cells (Narayanan and Sakthivel 2010). Several microorganisms have ability to produce nanoparticles with different sizes and shapes. For example magnetic, silver, gold, palladium and platinum nanoparticles could be produced in the presence of an exogenous electron donor by different microorganisms such as bacteria, fungi, yeasts and viruses (Narayanan and Sakthivel 2010).

In present study we conducted to produce silver and gold nanoparticles by ordinary pathogenic bacteria. In addition, biosynthesis of extracellular nanoparticles was considered by this investigate in order to achieve information concerning to activity of metal ions reductive enzymes produced by bacteria at two different temperatures 37°C as well as 50°C. The temperature of 37°C was selected to evaluate activity of reductive enzymes at optimum growth of the bacteria and 50°C was selected as ambient temperature for activity of these enzymes.

MATERIALS & METHODS

Bacterial Strains

The bacterial strains used in this study were obtained from the Persian Type Culture Collection (PTCC). These strains were *Staphylococcus aureus* (PTCC 1113), *Staphylococcus epidermidis* (PTCC 1453), *Enterococcus faecalis* (PTCC 1394), *Bacillus cereus* (PTCC 1015), *Escherichia coli* (PTCC 1330), *Klebsiella pneumoniae* (PTCC 1053) and *Yersinia enterocolitica* (PTCC 1477). The strains were maintained in nutrient broth medium with 15% glycerol at - 15° C.

Biological synthesis of nanoparticles by bacteria

Biosynthesis of silver nanoparticles

A loop full of each organism was inoculated into the flask (100ml) containing 50 ml sterile nutrient broth (Merck) and incubated in the shaker incubator at 150 rpm at 35°C for 24 hours. Then the cells were harvested by centrifugation (at 6000 rpm for 10 minutes) and the supernatant (25 ml) was challenged with 25 µL of 1 M silver nitrate solution (Sigma Aldrich, USA). The solution was dispersed into two flasks: First flask was incubated in shaker incubator (at 200 rpm) in dark conditions at 37°C and the second was incubated in the same conditions at 50°C. An extra flask containing sterile nutrient broth with 1mM of silver nitrate solution was prepared and considered as control (Saifuddin *et al*., 2009).

Biosynthesis of gold nanoparticles

A loop full of each organism was inoculated into the flask (100ml) containing 50 ml sterile nutrient broth (Merck) and incubated in the shaker incubator at 150 rpm at 35°C for 24 hours. The cells were harvested by centrifugation (at 6000 rpm for 10 minutes) and the supernatant (20 ml) was challenged with 25 µL of 1 M gold III chloride solution (Sigma Aldrich, USA). The solution was dispersed into two flasks: First flask was incubated in shaker incubator (at 200 rpm) in dark conditions at 37°C and the second was incubated in the same conditions at 50°C. Control flask containing sterile nutrient broth with 2.5 mM of gold III chloride solution was prepared to assess biosynthesis of gold nanoparticles (Gericke *et al*., 2006).

Characterization of nanoparticles

UV–visible spectral analysis

A change of color was special character of the silver nitrate and gold III chloride solutions when incubated with the bacterial supernatants. The bioreduction of the silver and gold ions in the solution was monitored by UV-visible spectrophotometer (Shimadzu UV-1700) adjusted to the span from 350 to 600 nm (Gericke *et al*., 2006 and Saifuddin *et al*., 2009).

X-ray diffraction analysis (XRD)

In order to prove biosynthesis of the silver

and gold nanoparticles by bacteria, 50 ml of each supernatant was freeze-dried and used for XRD analysis. For this purpose, the samples were calcined under 300°C for 3 h and the diffracted intensities were recorded for silver and gold nanoparticles from 30° to 80° 2θ angles (Sadhasivam *et al*., 2010).

Transmission electron microscope (TEM)

The average size and morphology of nanoparticles was determined by TEM. To observe the nanoparticles, a drop of each supernatant containing the silver or gold nanoparticles was placed on the carbon coated copper grids. Then excess solution was removed by a blotting paper and the grids were dried under Infrared lamp. The dried grids were assessed for observation of the nanoparticles using transmission electron microscope (Ziess, Leo 910) (Kathiresan *et al*., 2009).

RESULTS

Characterization of nanoparticles

In the present study seven bacterial strains were assessed for production of silver and gold nanoparticles. Silver and gold nanoparticles were produced after challenging silver nitrate and gold III chloride ions in the culture supernatants of the bacterial broth cultures. These nanoparticles were characterized and the results were recorded as follows.

UV–visible spectral analysis

As seen in table 1 all the bacteria produce nanoparticles relatively more at 50°C. Although gold nanoparticles produced in the culture supernatants of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia* and *Yersinia enterocolitica* at both temperatures (37°C and 50°C), gold nanoparticles were synthesized in *Staphylococcus epidermidis* supernatant culture only at 50°C. Of all bacteria tested gold nanoparticles didn't produce in *Bacillus cereus* supernatant culture either at 37°C or 50°C. The results obtained from biosynthesis of silver nanoparticles indicated that silver nanoparticles were produced in the supernatant cultures of all the bacterial tested at 50°C. However, biosynthesis of silver nanoparticles in the culture supernatants of *Bacillus cereus*,

Table 1. UV-vis spectra of gold and silver nanoparticles produce by bacteria at two temperatures (37 and 50°C)

Bacteria	maximum absorbance ($AuCl_3$ at 37°C)	maximum absorbance ($AuCl_3$ at 50°C)
Staphylococcus aureus	555.5	581.5
Staphylococcus epidermidis	-	583
Enterococcus faecalis	554.5	568.5
Bacillus cereus	-	-
Yersinia enterocolitica	562	577
Escherichia coli	550.5	565.5
Klebsiella pneumoniae	558.5	571
Bacteria	maximum absorbance ($AgNo_3$ at 37°C)	maximum absorbance ($AgNo_3$ at 50°C)
Staphylococcus aureus	-	413
Staphylococcus epidermidis	-	387
Enterococcus faecalis	-	435.5
Bacillus cereus	414.5	445
Yersinia enterocolitica	394.5	485.5
Escherichia coli	412	487
Klebsiella pneumoniae	-	413

Escherichia coli and *Yersinia enterocolitica* was nil at 37°C. In addition our finding from UV–visible spectra showed that maximum absorption for silver was at 410-430 nm while for gold was at 520–580nm.

Figure 1 showed UV-vis spectrum of culture supernatant of *Yersinia enterocolitica* after treatment with silver nitrate and gold III chloride ions.

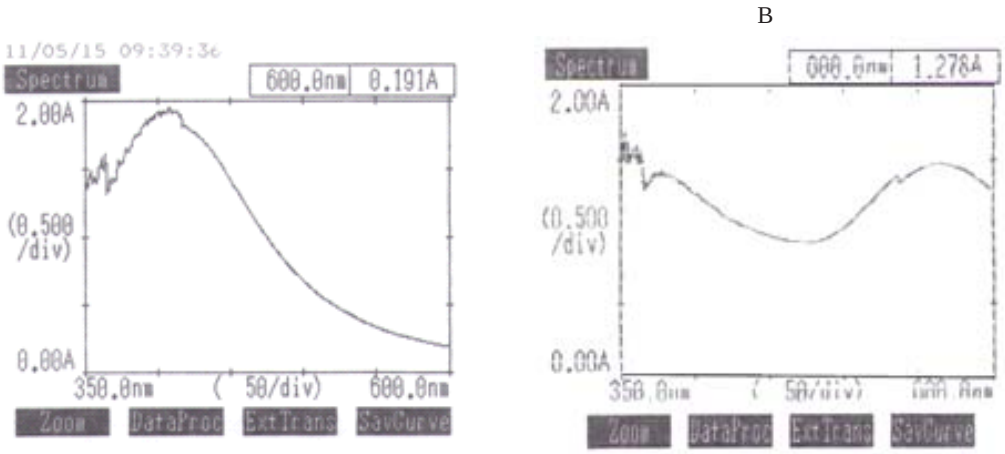

Fig. 1. UV-vis spectrum and color changing of *Yersinia enterocolitica* broth culture supernatant after treatment with silver nitrate (a) and gold III chloride ions (b).

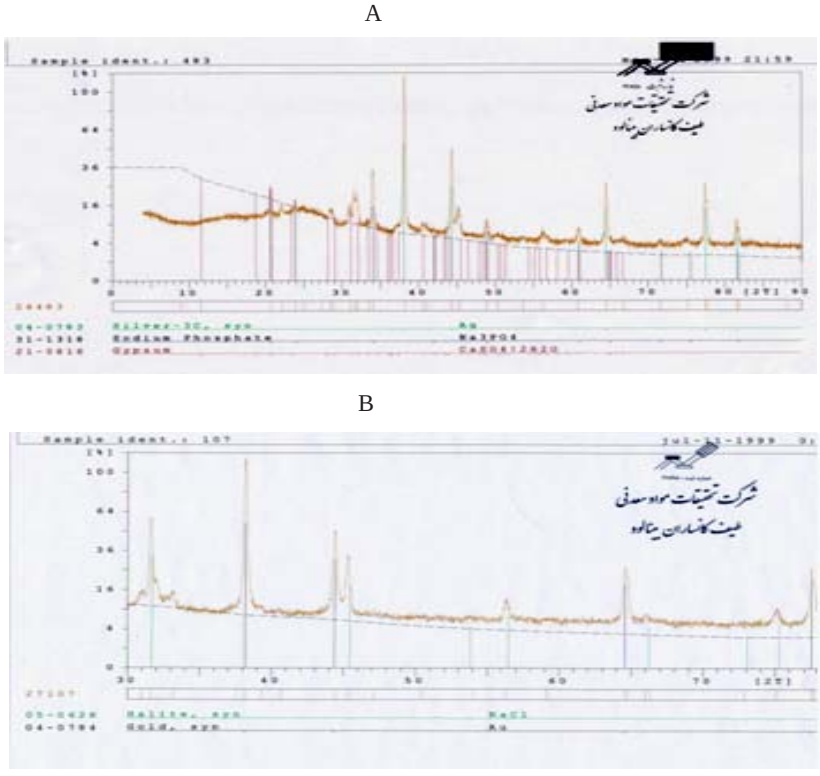

Fig. 2. The XRD spectrum of freeze-dried powder of silver (a) and gold (b) nanoparticles formed in culture supernatant of *Yersinia enterocolitica* after 24 h of incubation.

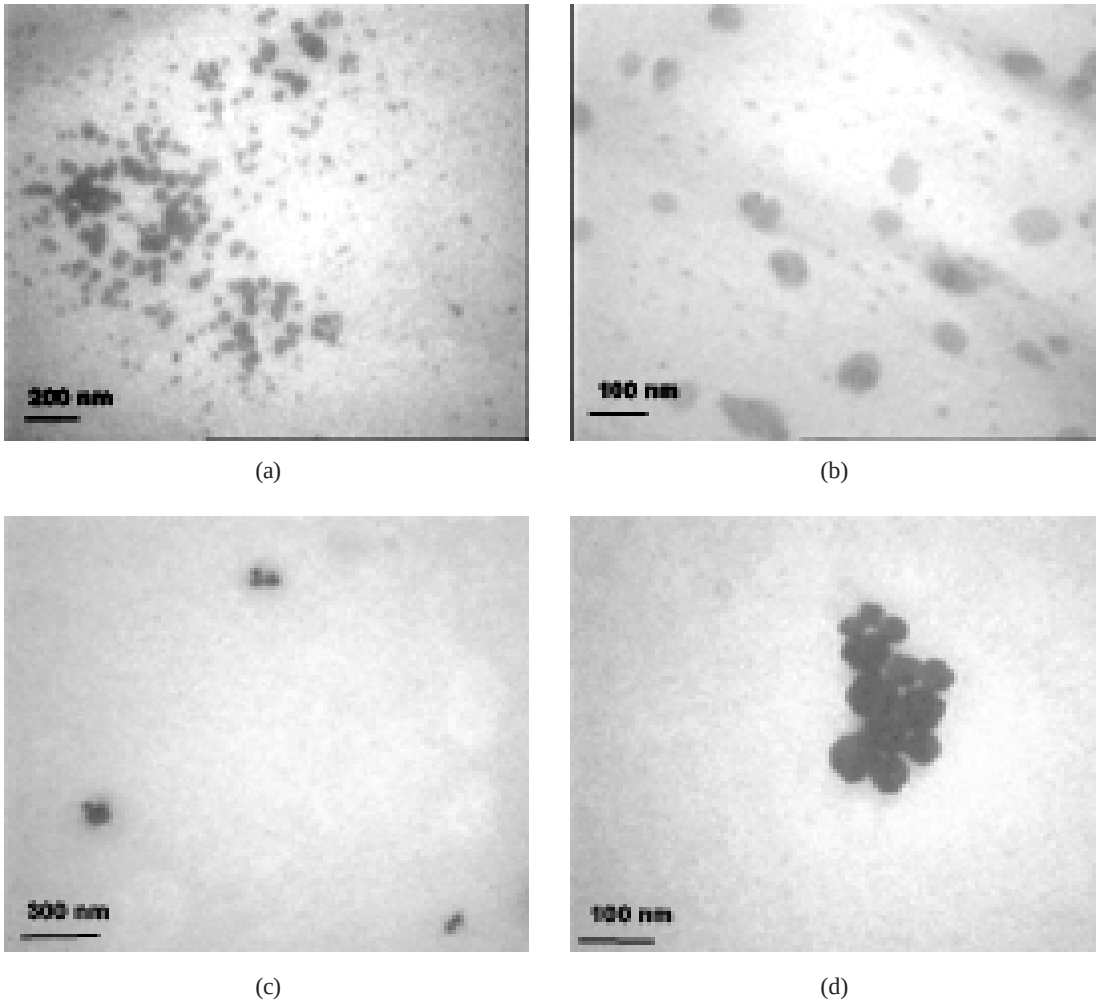

Fig. 3. Transmission electron micrographs recorded from a region of a drop coated film of silver nitrate (a and b) and gold chloride(c and d) solutions treated with the culture supernatant of *Yersinia enterocolitica*

X-ray diffraction (XRD) analysis

X-ray diffraction pattern confirmed existence of the crystalline structures of silver and gold nanoparticles. A broadening of the intense peaks also verified the presence of silver and gold nanoparticles. Figure 2 showed the XRD spectrum of silver and gold nanoparticles formed in culture supernatant of *Yersinia enterocolitica* after 24 h.

Transmission electron microscopy (TEM) analysis

Transmission electron microscopy showed the formation of nanoparticles with an average particle size of 10-80 nm. TEM images confirmed the accumulation of nanoparticles extracellular. As shown in Figure 3 the dark spots in the images represented the silver and gold nanoparticles produced in the supernatant culture of *Yersinia enterocolitica* ..

DISCUSSION

Nowadays, enormous attention has been considered on nanoparticles because of their special properties such as physicochemical characteristics including catalytic activity, optical, electronic, magnetic and antibacterial properties (Morones *et al.*, 2005 and Panacek *et al.*, 2006).

In order to use nanoparticles as medicinal

purpose, biological synthesis method prefer because of same size of nanoparticles and being the environmental friendly . Several microorganisms reduce metal ions by different reducing enzymes and reducing agents such as carbohydrates, quinine derivates and several electron transfer shuttles (Narayanan and Sakthivel 2010). In enzymatic reduction, the site of accumulation of insoluble metals depends on the location of the reductive enzymes. In the case of extracellular silver and gold nanoparticles, the bacteria secrete reducing enzymes in culture during their growth (particularly in the Exponential phase) and synthesis of nanoparticles out of the cells (Narayanan and Sakthivel 2010).

In present study, we provided special conditions for some ordinary pathogenic bacteria to produce extracellular silver and gold nanoparticles. Seven bacterial strains were assessed for production of silver and gold nanoparticles. The results obtained indicated that all bacteria had potential for production of both nanoparticles , however *Bacillus cereus* showed its ability only for production of silver nanoparticles. On the other hand, different responses were observed for nanoparticles production at different temperatures. Our finding illustrated that extracellular silver and gold nanoparticles were produced relatively more at 50°C. In addition, the transmission electron microscope images showed that silver nanoparticles were spherical and single however gold nanoparticles were irregular and clusters.

Parallel with our investigate Klaus and his colleges in 1999 isolated *Pseudomonas stutzeri* AG259 from silver mine with ability for production of silver nanoparticles after challenging with silver ions in the laboratory scale. Fayaz and his colleges in 2010 confirmed the production of extracellular silver nanoparticles by fungus *Trichoderma viride*.

Concerning to the production of nanoparticles by bacteria Shahverdi *et al.*, in 2007 investigated on rapid biosynthesis of silver nanoparticles using culture supernatants of Enterobacteria. They used culture supernatants of *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumonia* and verified production of silver nanoparticles by them.

Escherichia coli and *Klebsiella pneumonia* also were used by us and our results were parallel to their report. In addition our finding indicated that out of all bacteria tested, *Bacillus cereus* could reduce gold ions neither 37°C nor 50ºC.

In addition, biosynthesis of silver and gold nanoparticles in this study was carried out in function of two temperatures (37°C and 50°C). Our finding showed production of nanoparticles comparatively was more at 50°C. It means activity of reductive enzymes produced by the bacteria was relatively more at their ambient temperature.

Regarding to the effect of temperature on nanoparticles production Gericke *et al.*, 2006 opined that the rate of gold nanoparticles formation was related to the temperature and using high temperature at the ambient increased the levels of nanoparticles production. On the other hand, Saifuddin *et al.*, in 2009 reported that the microwave radiation warm up the bacterial culture supernatant and consequently thermal energy increased the enzymatic reactions for production of silver nanoparticles.

Overall, our study verified production of extracellular silver and gold nanoparticles by bacteria and temperature as special factor must be considered for optimization of their biosynthesis.

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