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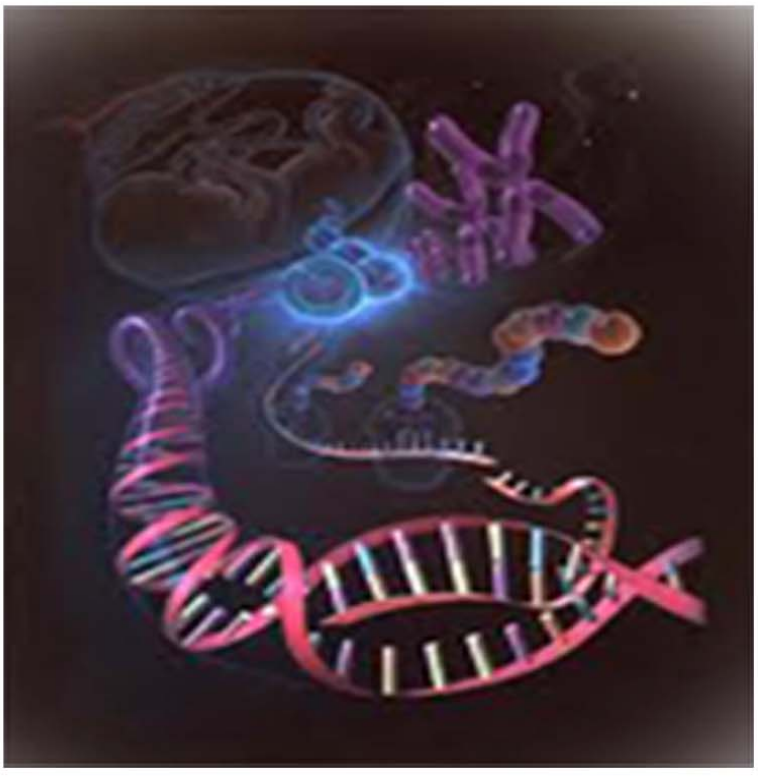

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Research Paper

EVALUATION OF THE ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES SYNTHESIZED WITH *FUSARIUM OXYSPORUM* AND *ESCHERICHIA COLI*

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Silver naoparticles have antibacterial properties and are used in textile, hygiene, refinement, staining, agriculture and livestock industries. The aim of this study was production of silver nanoparticles using *Fusarium oxysporum* and *Escherichia coli*. We used agar diffusion disk for inspection of antibacterial properties of nanosilver colloids. The shape and morphology of nanosilver colloids were evaluated by transmission electron microscopy. The presence of nanosilver colloids was confirmed by UV/Vis spectrophotometry. The mean size of nanoparticles produced by *F. oxysporum* is smaller than those of them in *E. coli*. Results of the present study showed that the quality of nanosilver colloids from *F. oxysporum* is better than those of them in *E. coli*.

Keywords: Silver nanoparticles, Antibacterial activity, Escherichia coli, Fusarium oxysporum

INTRODUCTION

Transition of microparticles to naoparticles is accompany with some changes in physical properties that two most important once are increases in surface to volume ratio (surface: volume) and achieving the size of influences of quantum. Greater surface to volume ratio that occurs with size reduction naturally is resulting in overcoming behavior of atoms on surface to behavior of interior atoms. This phenomenon affects on distinct specificity of material and its interaction with others. Once, particle becomes small enough, it starting to show quantum mechanical behavior. Properties of quantum dots are examples of this kind. This spots sometimes called artifact atoms, because its free electrons behave the same as electron confine whit in atoms and occupy discontinue and virtual energy.

In general with regard to unique specificity of nanoparticles, using appropriate production techniques to yield nanoparticles with pleasant properties, less cost and environmental protection is important challenges in field of nanotechnology. In recent years by using microorganism in production of nanoparticles is so favorite topic. It could be produced extracellular or intracellular

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(Simkiss and Wilbur, 1989; Mann, 1996). Fugues that are known for producing nanosilver were noticed in Table 1.

Despite of recognition antibacterial activity of fugues, there are reported bacterial resistant to silver such as *Escherichia coli* (Shahverdi *et al*., 2007). Even it is possible that silver accumulates in their cell wall and results in a weight increased by 2.9% biomass. With this regard use of them in industrial reprocessing from minerals was reported.

In this study *Fusarium oxysporum* and *E. coli* was used to produce nanosilver that in bout microorganisms silver reduction occurs intercellular.

MATERIALS AND METHODS

Bacterial Cell Culture

For producing biomass required for nanosilver synthesis, fungus *Fusarium oxysporum*, IRAN 31c, were cultured in sabouraud dextrose agar for 5 days then an slice from agar containing fungal mycelia were inoculated into MGYP broth medium with malt extract 3 g/L, yeast extract 3 g/L, peptone 5 g/L, glucose 10 g/L, pH adjusted to 3/5 (Ahmad *et al*., 2003a). It was placed in shaker incubator at 26°C for 4 days. After 4 days bio-mass was passed from filter. After 3 times washing with distilled water, suspend in 100 ml distilled water and incubate at 26°C, 200 rpm. After 3 days culture medium were separated and

Table 1: Fungus Used to Produce Silver Nanoparticles

References	Size (nm)	Microorganisem	No.
(Ahmad *et al*., 2003b)	5?50	*Fusarium oxysporum*	1
(Bhainsa and D'Souza 2006)	5?25	*Aspergillus fumigatus*	2
(Gade *et al*., 2008)	20	*Aspergillus niger*	3
(Vigneshwaran *et al*., 2006)	100	*Phanerochaete chrysosporium*	4
(Vigneshwaran *et al*., 2007)	1.61?8.92	*Aspergillus flavus*	5
(Balaji *et al*., 2009)	10?100	*Cladosporium cladosporioides*	6
(Basavaraja *et al*., 2008)	10?60	*Fusarium semitectum*	7
(Mukherjee *et al*., 2008)	13?18	*Trichoderma asperellum*	8
(Balaji *et al*., 2009)	10?100	*Cladosporium cladosporioides*	9
(Fayaz *et al*., 2010)	5?40	*Trichoderma viride*	10
(Kathiresan *et al*., 2009)	1?100	*Penicillium fellutanum*	11
(Shaligram *et al*., 2009)	23?105	*Penicillium brevicompactum* WA 2315	12
(Mukherjee *et al*., 2001)	12-25	*Verticillium sp.*	13
(Ingle *et al*., 2009)	5?35	*Fusarium solani*	14
(Ingle *et al*., 2008)	5?40	*Fusarium acuminatum*	15
(Verma *et al*., 2010)	10?25	*Aspergillus clavatus*	16

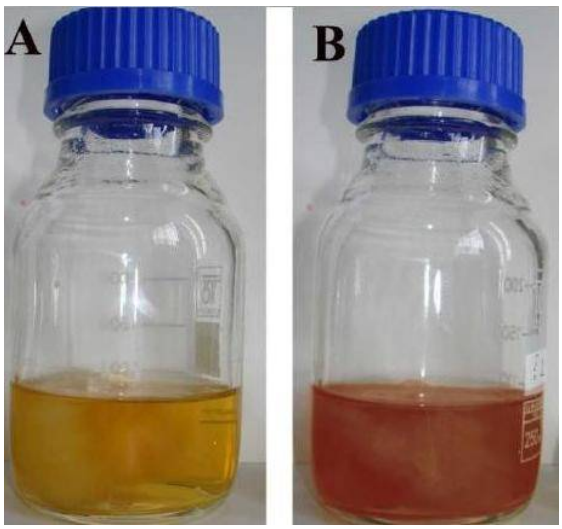
silver nitrate (1 mM) was added to it. After 12 h silver ions were reduced completely and presence of nanosilver was diagnosed by UV-Vis Spectrophotometry.

Biosynthesis of nanosilver by *E. coli*, bacteria were cultured in Luria-Bertani Broth with peptone 10 g/L, malt extract 3 g/L and NaCl 10 g/L for 18-24 h. produced colloid centrifuged at 12000 rpm for 10 min and the supernatant was separated. Silver nitrate 1 mM was added to it after 2 h at RT, UV-Vis Spectrophotometry was performed.

The size and morphology of nanoparticles and its colloids after preparation and placed on copper network were analyzed by FEI/Philips EM 208S transmission electron microscope, in both fungal and bacterial product.

Two blank disk surfaces were plated with nanosilver obtained from these microorganisms. For comparing Antibacterial activity of these surfaces, at first the plate containing LB agar with peptone 10 g/L, malt extract 5 g/L, NaCl 10 g/L, and agar 15 g/L was prepared. E coli growth in LB broth for 18 to 24 h and 100 µl of it were growth on LB agar. After 10 min, blank disks coated with nanosilver were put on the plates and after 24 h the clear zones around these surfaces were related to removal of bacteria in there that were measured and compared together.

Figure 1: The Solution that Underlying by Filtration of Bacterial Cells before (A) and after (B) Adding Silver Nitrate ($Agno_3$)



RESULTS AND DISCUSSION

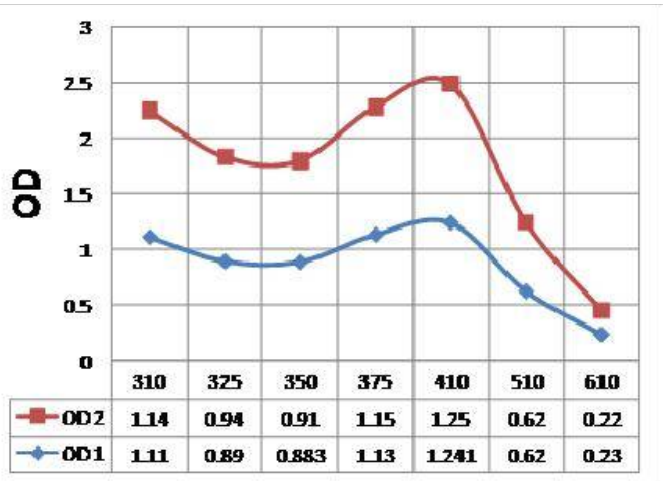
After reduction and nanosilver biosynthesis the yellow solution from cell filtrations by centrifuge of bacterial solution, was changed to strong red (Figure 1) and the cells from centrifuge was changed to strong yellow (Figure 2).

Biosynthesis of nanosilver in sample was analysis by UV-Vis Spectrophotometry. In the spectra, absorbance pike in 400-420 wavelengths is related to surface plasmon resonance of nanosilver. Figure 3 show the spectra from colloidal nanoparticles biosynthesized by *Fusarium oxysporum* and *E. coli*. It shows that the spectrum from the fungi is stranger, asymmetric and softer in compare to the Bactria. Increasing in ratio of absorption in specific wavelengths with the same concentration of nanosilver, is related to increasing of nanosilver that is biosynthesized in colloid.

In addition asymmetry and flatter produced spectra showed an even particle size dispersion

Figure 2: The Solution that Underlying by Filtration of Fungal Cells Before (A) and After (B) Adding Silver Nitrate ($AgNo_3$)

Figure 3: UV-Vis Spectra of Nanosilver Colloids Produced of Fungus *Fusarium oxysporum* (Red) and Bacteria *E. coli* (Blue)

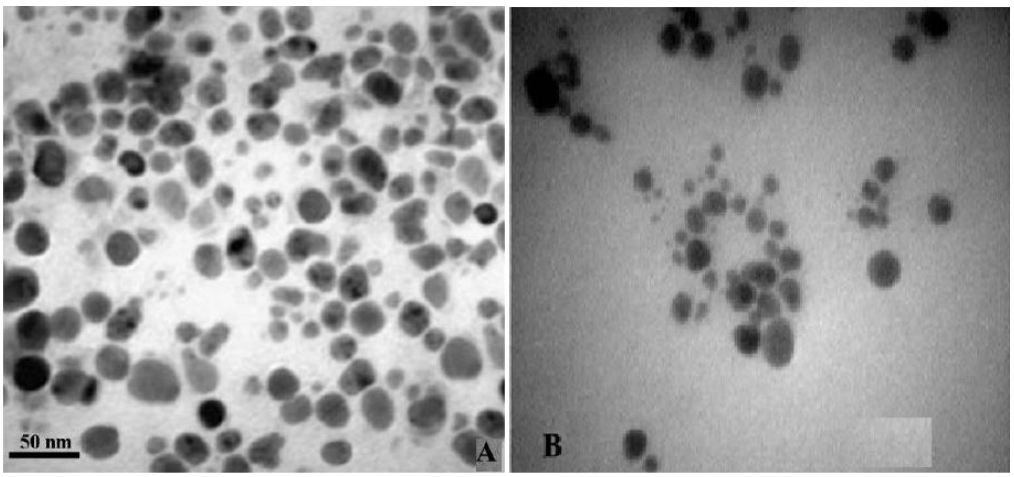


that is according to Figure 4. TEM in this figure shows dispersion of size of nanosilver produced by *Fusarium oxysporum* were 5-50 nm and for *E. coli* is 10-60 nm.

The plate that is related to antibacterial activity of surfaces coated with nanoparticles synthesized by bacteria and fungi was shown in Figure 5. As it is seen in this figure, bacteria did not grow around the blank disk. The hallow zone around the samples is due to antibacterial activity. In the case of *Fusarium oxysporum* fungus the hallow zone is 1 mm bigger than bacteria that it indicates more antibacterial activity of fungal colloidal product.

Principally it is shown that antibacterial activity of silver nanoparticles is related to its size and morphology. Nanoparticles size indicated its surface that is in contact with bacteria. It means that with nanoparticles size reduction increases surface to volume ratio and it result in increases

Figure 4: Transmission Electron Microscopy (TEM) Observations of Nnanosilver Produced of Fungus *Fusarium oxysporum* (a) and Bacteria, *E. coli* (b)



nanoparticles contact surface with bacterial cell, therefore nanoparticles antibacterial activity improves. For example nanoparticles size reduction from 10 µm to 10 nm increase contact surface area (Pal *et al*., 2007). Hence it is appears that nanoparticles antibacterial activity of *Fusarium oxysporum* that is smaller in size than bacteria according to TEM image, is more appropriate that was shown in Figure 5.

Figure 5: The Plate Containing Disk Coated with Nanosilver Colloids, Production of Fungus *Fusarium oxysporum* (a) and Bacteria, *E. coli* (b)

CONCLUSION

The results of this study were showed that *Fusarium oxysporum* can generally produce silver nanoparticles with maximal size of 50 nm. Silver nanoparticles were produced extracellularly by these fungi and they were secreted with proteins and enzymes. Thereby, the extraction procedure is not necessary and thus the production cost was diminished and extraction problems were avoided.

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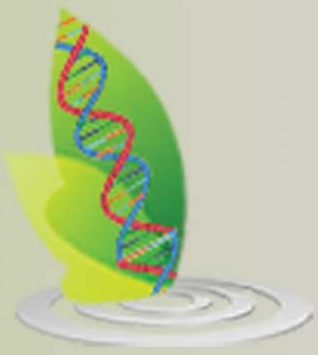

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