Extracellular biosynthesis of silver nanoparticles by some bacteria

S. Minaeian*
Division of Microbiology, Science and Research Campus, Islamic Azad University, Tehran, Iran.
A. R. Shahverdi
Department of Pharmaceutical Biotechnology, Faculty of pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
A. S. Nohi
Division of Microbiology, of Science and Research campus,Islamic Azad University Tehran, Iran.
H. R. Shahverdi
Department of Metallurgy, Tarbiat Modares University, Tehran, Iran.

Abstract:

The development of reliable processes for the synthesis of silver nanomaterials is an important aspect of nanotechnology today. There are few reports in literature on the biosynthesis of silver nanoparticles using microorganisms such as fungi. Here in this study, the production of silver nanoparticles by some microorganisms was investigated. The test strains were cultivated in their special conventional conditions for 24 hours. Silver nitrate at concentration of 10^{-3} M was separately added to the each reaction vessels that were contained the supernatants of Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus and Candida albicans. The silver nanoparticles were characterized by scanning electron microscopy and UV-visible spectroscopy. The silver nanoparticles were effectively produced, by Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae. We did not observe any extracellular biosynthesis activity from other microorganisms such as the Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus and Candida albicans in conditions tested during this investigation.,This is the first report on the production of silver nanoparticles in enterobacteriaceae.

Keyword: Nanoparticle, Extracellular, Reductase

1. Introduction:

An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries [1], as optical receptors [2], catalysts in chemical reactions, biolabelling [3], etc. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration [4-5].It has been known for a long time that in nature a

* Corresponding author

variety of nanomaterials are synthesized by biological processes. For example, the magnetotactic bacteria synthesize intracellular magnetite or greigite nanocrystallites [6], the other examples are diatomas, which synthesize siliceous materials [7-8], and S-layer bacteria that produce gypsum and calcium carbonate layers [9-10].These results showed that micro-organisms could indeed be used for the intracellular synthesis of nanoparticles. We have shown in this investigation some microbial that can synthesize silver nanoparticles.

However, the biosynthesis of silver nanoparticles by free cell system and culture filtrate has not been investigated yet. In this paper, we report on the synthesis of silver nanoparticles by the reduction of aqueous Ag^+ ion by simultaneous reduction of aqueous Ag^+ with the culture broth of some tested bacteria and fungi. Through our screening process involving a number of bacteria we observed that enterobacteria group were potential candidate for rapid synthesis of silver nanoparticles.

2. Materials and methods:

Bacteria:

The test strains were: Bacillus subtilis (PTCC 1023), Lactobacillus acidophilus (PTCC 1608), Klebsiella pneumoniae (PTCC 1053), Escherichia coli (PTCC 1399), Enterobacter cloacae (PTCC 1238) Staphylococcus aureus (PTCC 1112) and Candida albicans (PTCC 5011).

Preparation of cell free microbial extract:

Different culture medium were prepared, sterilized and inoculated with fresh culture of the test strains. The cultured flasks were incubated at 37 oC for 24h and at 30 oC for 24h for C. albicans. After incubation time the cultures were centrifuged at 12000 rpm and their supernatants were used for further experiments.

Biosynthesis of silver nanoparticles:

Silver nitrate at concentration of 10-3 M was separately added to the reaction vessels containing different supernatants (1% v/v). The reaction between different supernatants and Ag+ ions was carried out in the dark or bright condition. Periodically, aliquots of the reaction solution were removed and the absorptions were measured using a UV-Vis spectrophotometer (CECIL 9200). Furthermore, the silver nanoparticles were characterized by scanning electron microscopy (LEO 440i). A drop of each Supernatant is located on Aluminum pieces and let it dry. It can give more information about the shape and surface structure of the particle.

3. Results and Discussion:

The Erlenmeyer flasks with the Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae supernatants were a Pale yellow color before the addition of Ag+ ions and this changed to a brownish color on completion of the reaction with Ag+ , but the Erlenmeyer flasks with the supernatans of Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus and Candida albicans in conditions test were not changed. The appearance of a light brown color in solution suggested the formation of silver nanoparticles[11]. Thus, it was evident that the metabolites excreted by the culture exposed to silver could reduce silver ions, clearly indicating that the reduction of the ions occur extracellularly through reducing agents released in to the solution by Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae. These reactions only occurred in the light and the nanoparticles were not produced in darkness. The silver nanoparticles were characterized by UV-visible spectroscopy. This technique has proved to be a very useful technique for the analysis of nanoparticles. This event clearly indicating that the reduction of the ions occurs extracellularly through reducing agents released in to the solution by enterobacteriace as it shows the UV_Vis spectra for the

Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae. A strong, but broad, surface plasmon peak located at 430, 419 and 420 nm was observed for the silver nanoparticles prepared using Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae. Figure 1 shows the absorbance spectrum of silver nanoparticles synthesized by Klebsiella pneumoniae. A long tailing on the large-wavelength side may be due to small amount of particle aggregation. Observation of the strong but broad surface plasmon peak has been well known in the case of various metal nanopartcles over a wide size range of 2-100 nm [12].When we challenged the supernatants of Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae with Ag+ ions, it was observed that silver was reduced extracellulary to metallic silver. The solution was extremely stable with no evidence of flocculation of the particles even several weeks after reaction .It is known that silver cations are highly reactive and tend to bind strongly to electron donor groups containing sulfur, oxygen or nitrogen [12]. Figure 2 shows the SEM microgarph recorded from the silver nanoparticle. In this micrograph, silver nanoparticles in the size range 50-100 nm were observed. 4.Conclution:

This is the first report on the extracellular synthesis of nanoparticles by a Klebsiella pneumoniae, Escherichia coli and Enterobacter cloacae. Even though gold / silver nanoparticles have been synthesized using prokaryotes such as bacteria and eukaryotes such as fungi [13, 14], the nanoparticles grow intracellularly , except in the case of recent report in which some bacteria from Enterobacteriaceae were used. In that case, the nanoparticles grew extracellularly, however, depending on the reductase / electron shuttle relationships under these conditions. Biologically synthesized silver nanoparticles could have many applications, in areas such as non-liner optics, spectrally selective coating for electrical batteries, as optical receptors, catalysis in chemical reactions, biolabelling [12] and as antibacterials capacity [15].

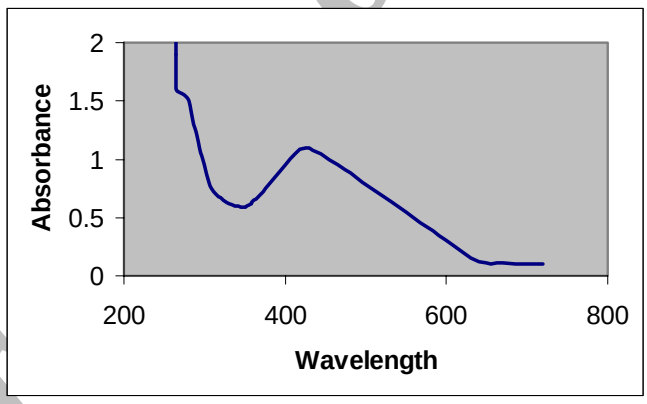

Figure (1): The absorbance spectrum of silver nanoparticles synthesized by *Klebsiella pneumoniae*

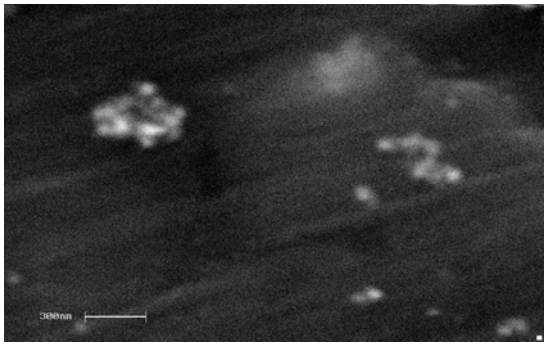

Figure (2): The SEM micrograph recorded from the silver nanoparticle.

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