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## Extracellular Formation of Silver Nanoparticles by the Cell Filtrate of *Actinomyces spp. NSX-333*

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**Abstract:** *In this paper, we have used a biological method of the biosynthesis of silver nanoparticles, using supernatant (culture liquid) of bacterium Actinomyces spp. NSX-333, isolated from Azerbaijani soil. The culture liquid has been obtained by culturing the bacterium at 28 ° C for 5-6 days. It was showed that during the incubation of the culture liquid with AgNO<sub>3</sub> solution, a restoration of silver ions takes place and stable silver nanoparticles are formed. Initially, the nanoparticle formation process was visually observed by a color change of the incubation solution. The nanoparticles have characterized using a UV spectrophotometer, an energy dispersive X-ray spectroscopy (EDXS) and a scanning electron microscope (SEM). UV spectrophotometric analysis of nanoparticles showed the presence of absorption in the range of 410 nm, which is characteristic of silver nanoparticles. EDXS spectrum showed a peak corresponding to the silver nanoparticles. Obtained silver nanoparticles had a spherical shape and size in the range of 20 to 130 nm. Clusters of nanoparticles were also observed.*

**Keywords:** *Actinomyces spp. NSX-333, culture fluid, silver nanoparticles, UV spectroscopy, EDXA spectroscopy, SEM.*

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### 1. INTRODUCTION

One of the fastest growing areas of modern nanotechnology at the present time is the creation and use of nanoparticles of various materials. Special attention has recently drawn to the silver nanoparticles, which are characterized by unique properties. These properties are associated with a high ratio of surface to volume, which determines greater efficiency of their action. Much attention is paid to the functional activity of silver nanoparticles from the standpoint of imparting both bactericide and bacteriostatic properties of various materials and products. They have an extremely large specific surface area. This increases the area of the silver contact with bacteria or viruses, greatly improving its destructive action for microorganisms [5,7,8, 11].

Thus, the use of silver as a nanoparticle allows hundreds of times to bring down the concentration of silver storing all bactericidal properties. Silver nanoparticles are used as a biocide additive - in the form of a modifier. This modifier is destined for creation and production of new materials, coatings and other products with biocide properties of a wide range of activities. Choice of silver nanocomposites for impregnation is due to their significant and indisputable advantages over all existing antimicrobial remedies. With a wide range of antimicrobial activity, silver compounds are, in many ways, devoid of the disadvantages that are related to the problem of resistance of pathogen microorganisms to them [9, 20,22].

A bunch of physical and chemical methods have been developed for the synthesis of nanoparticles of suitable size and shape. However, in spite of their successful application they often remain expensive and require hazardous chemical compounds. Therefore, there is a need to develop effective methods of synthesis, different nanoparticles using biological objects, particularly, microorganisms safe for the environment and humans [6, 10, 21].

At the present time an active search for effective microorganisms (bacteria, yeast, and moldy fungi) is conducted to produce silver nanoparticles [4, 4, 12-14,18]. A series of works are devoted to the biosynthesis of silver nanoparticles using branched bacteria (*Streptomyces rochei*, *S. species*, *S. albidoflavus*, *S. aurefacies*) [1, 2, 15-17, 19].

At the present work branched bacterium *Actinomyces spp.NSX-33* isolated from Azerbaijani environment has been used for synthesis of silver nanoparticles.

## 2. MATERIALS AND METHODS

Bacterial strain *Actinomyces spp.NSX-333*, isolated from Azerbaijani soil, was used as the object. Pure culture bacteria were maintained for further researches in a mineral medium of the following composition (g / l): soluble starch -20;  $K_2HPO_4$ -0,05;  $KNO_3$ -0,1;  $FeSO_4$ -0,005;  $MgSO_4$ -0,05; distilled water-1litre; at 4-6°C.

To study the ability of bacterial strain to form silver nanoparticles, culture was grown aerobically in 250 ml Erlenmeyer flask, containing 100 ml of mineral medium with the above composition by adding yeast extracts in an amount of 2 g/l.

Liquid medium was inoculated with the culture of *Actinomyces spp.NSX-333* and incubated at 30°C within 5-7 days on a rotary shaker. Then the biomass was separated by filtration through a filter paper and the culture liquid was used for the formation of nanoparticles. Culture fluid in an amount of 50 ml was mixed with 50 ml of 1 mM  $AgNO_3$  and was incubated at 30°C in dark conditions within 72-120 hours. Culture fluid without added  $AgNO_3$  was used as a control.

Initially, nanoparticle formation was observed by the color darkening of the incubation mixture. The optical properties of the nanoparticles were investigated by the spectrophotometer "Specord 250 plus" (Germany). Morphological characteristics and the size of the silver nanoparticles were studied by the scanning electron microscope (SEM) "JEOLJSM-7600F" (Japan). Energy-Dispersive X-rays analysis (EDXA)) of the the obtained nanoparticles was used as well.

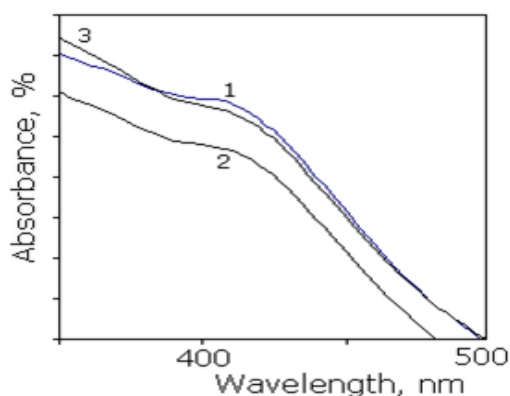
## 3. RESULTS AND DISCUSSIONS

By the incubation of bacterial culture fluid *Actinomyces spp.NSX-333* with 1mM solution of  $AgNO_3$  within 72-129 hours, we observe the darkening of the incubation mixture to a reddish-brown, which is characteristic for silver nanoparticles (fig. 1).



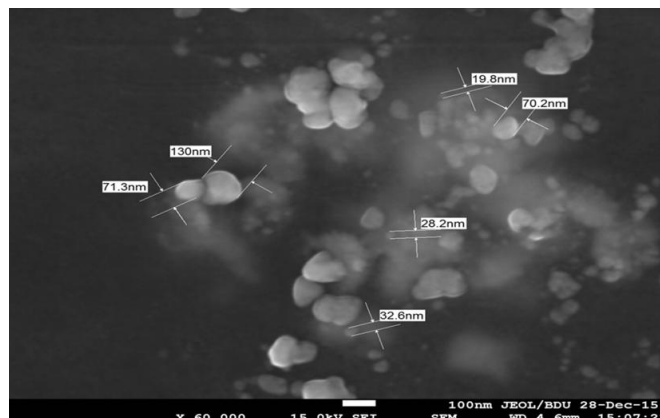
**Figure1.** Color change of reaction mixture during formation of silver nanoparticles by *Actinomyces spp.NSX-333*: a-experiment, b-control

UV spectrophotometric analysis of nanoparticles showed the presence of absorption in the range of 410 nm for 7 hours, which is characteristic of silver nanoparticles (fig.2).



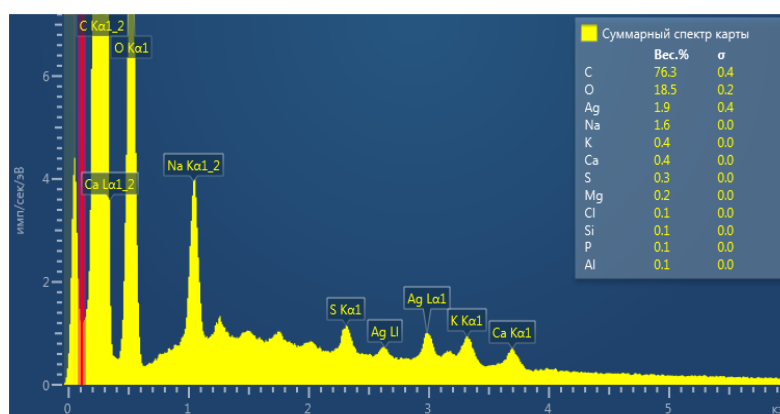
**Figure2.** UV spectr of silver nanoparticles synthesized by *Actinomyces spp.NSX-333*

The morphology and shape of silver nanoparticles were studied by scanning electron microscopy SEM method (fig.3). Have been found that the sizes of nanoparticles distribute in the range of 20-130 nm. In our opinion the wide size distribution is explained with uneven agglomeration of silver clusters in the suspension.



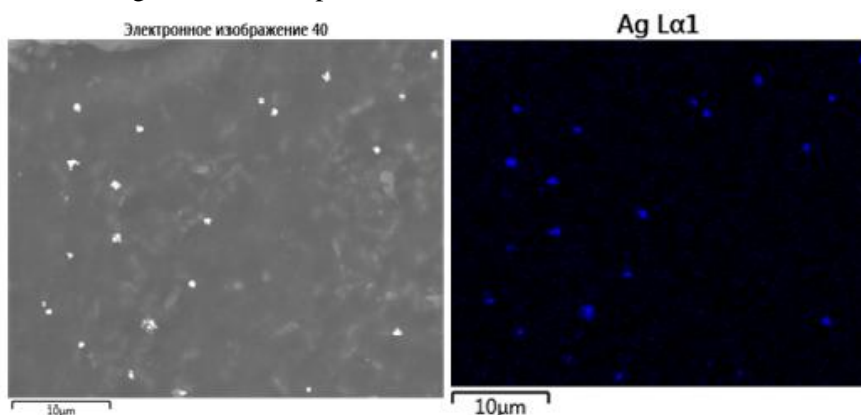
**Figure3.** SEM images of silver nanoparticles synthesized by *Actinomyces spp. NSX-333*

Fig.4 shows the Energy-Dispersive X-ray spectral analysis of obtained nanoparticles' sample and mapping of selected area for the elements distribution. As we can see from the mapping and spectral analysis the presence of peaks AgLa 1 and AgU proved the presence of silver nanoparticles.



**Figure4.** Energy-Dispersive X-rayspectr of silver nanoparticles synthesized by *Actinomyces spp. NSX-333*

The figure 5 shows the mapping the elements of silver nanoparticles in the solution. From this figure it is clear seen the forming of silver nanoparticles.



**Fig5.** Mapping the elements

#### 4. CONCLUSION

The obtained results enable to come to a conclusion, that bacterial culture *Actinomyces spp. NSX-333* is able to form silver nanoparticles extracellularly. Nanoparticles were characterized by using of UV spectrophotometer, EDXA spectrophotometer and scanning electron microscope (SEM). Silver nanoparticles have a spherical form, and their size varies in the range of 20 to 130 nm.

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