Advances in Biology & Earth Sciences
Vol.2, No.2, 2017, pp.168-174

# THE STUDY OF MORPHOLOGICAL AND CULTURAL PROPERTIES OF ACTINOMYCETES FORMING SILVER NANOPARTICLES

**S.A. Hasanova[1], S.M. Guliyeva[1], S.A. Kelbiyeva[1],G.C. Suleymanova[1] R.F.Babayeva[1], Kh.G. Ganbarov[1*]**

[1]Baku State University, Baku, Azerbaijan

**Abstract.** In the presented work, 8 strains of pigment forming actinomycetes, capable of forming silver nanoparticles, were investigated.The study of their morphological and cultural properties showed that all these strains belong to the genus *Streptomyces*.It was revealed that the formation of silver nanoparticles is observed in only 4 strains.

***Keywords:*** *actinomycetes , morphological and cultural properties ,genus, Streptomyces*.

***Corresponding Author:*** *Prof. Khudaverdi Ganbarov, Department of Microbiology, Baku State University, Baku, Azerbaijan, e-mail: khuda1949@mail.ru*

***Manuscript received***: *2May 2017*

## 1. Introduction

Microorganisms are widely used in biotechnology, including nanobiotechnology, which studies the production and application of metal nanoparticles [11, 2].

In recent years, noble metal nanoparticles have been the subject of targeted research due to their unique optical, electronic, mechanical, magnetic and chemical properties that significantly distinguish them from ordinary metals.These special properties of nanoparticles are associated with their small size and with a large surface area[1].Due to its unique properties, nanoparticles have become widely used in the diagnosis and treatment of cancer, in gene therapy, as a DNA marker in DNA sequencing, in magnetic resonance imaging (MRI), as a cell marker and antibacterial agent [5, 8].

At present, materials with silver nanoparticles are widely used. This interest is due to the fact that the inclusion of Ag nanoparticles in the material gives it fungicidal and antimicrobial properties.It is known that colloidal silver solutions are effective against more than 650 species of microorganisms, while antibiotics are usually active against 5-10 species. It is also necessary to note that addiction to silver nanoparticles is not produced, as is the case with conventional antibiotics.This is due to the fact that unicellular organisms cannot mutate into forms resistant to silver nanoparticles [9, 7].

For the synthesis of nanoparticles of unimpaired form and size, many physical and chemical methods have been processed, but despite their successful application, they often remain expensive and require the use of hazardous chemical compounds. Therefore, there is a need for the development of environmentally and efficiently safe methods for obtaining various nanoparticles using biological objects [6, 10].

Synthesis of nanoparticles with the help of microorganisms is carried out by bacteria, fungi, yeast, actinomycetes [4, 6, 3]. In microorganisms, the synthesis of nanoparticles can proceed intracellularly and extracellularly, as a result of which nanoparticles such as metal nanoparticles (Au, Ag, Ft, Pt, Cu, Cd, Ni, etc.) are synthesized, nanoparticles of metal alloys (Au-Ar, Au- Pt, Ag-Pt, etc.), nanoparticles of metal sulfides (ZnS, PbS, CdS, etc.), nanoparticles of magnetic and nonmagnetic oxides (Fe2O3, Fe3O4, TiO2, ZnO, CuO2, etc.) [7].Despite the stability, the biologically produced nanoparticles are heterogeneous, and their synthesis is rather slow. To overcome these problems, it is necessary to conduct research to identify new promising producers and to identify their morphological and cultural properties.

In the previous one we found that only in 4 strains there was formation of silver nanoparticles. In strains Streptomyces (BDU-C19, BDU-12, BDU-37), the formation of silver nanoparticles was observed only in biomass, and in the strain Streptomyces sp. BDU-27, the formation of silver nanoparticles was observed both in biomass and in culture liquid (1).

The aim of the study was to study the morphological and cultural properties of these four strains of actinomycetes forming the silver nanoparticles.

## 2. Materials and methods

8 strains of pigment-forming actinomycetes isolated from various soils of Azerbaijan were used in the studies (BDU-C19;BDU-8;BDU-D3;BDU-12;BDU-27;BDU-24;BDU-37;BDU-41).Pure cultures of actinomycetes were maintained on the Gauz mineral medium in a refrigerator at 4-6 °C.

*The formation of silver nanoparticles* .Cultures of actinomycetes were grown aerobically (250 ml Erlenmeyer flasks containing 100 ml of medium) on the Gausen liquid mineral medium with the following composition (g / l of distilled water): soluble starch-20;K2HPO4-0,5;KNO3-1;FeSO4-0,01;MgSO4-0,5;NaCl-0,5; distilled water -1l ; pH- 7,2-7,4.The liquid medium was inoculated with the actinomycete culture and incubated at 28-30 ° C for 10-14 days.After incubation, the biomass was separated by filtration through a filter paper.Biomass and culture liquid(cell free) filtrate were separately checked for the possibility of participation in the formation of silver nanoparticles.

The biomass was washed three times with sterile distilled water to remove the remaining components of the nutrient medium.After washing, the biomass was resuspended in 100 ml of sterile distilled water and held at 28-30 ° C for 24 hours.Then the suspension was filtered and biomass was obtained which was used to prepare the nanoparticles.For this, the biomass was mixed with 100 ml of an aqueous solution of 1 mM silver nitrate(AgNO3) and incubated in a thermostat at 28-30 °C under dark conditions.

To study the participation of the filtrate of the actinomycete culture liquid in the formation of silver nanoparticles 50 ml of the culture fluid filtrate was mixed with 50 ml of a solution of 1 mM silver nitrate (AgNO3) and incubated in a thermostat at 28-30° C under dark conditions.As a control in the reaction medium, a filtrate of an actinomycete culture liquid without silver nitrate was used.

*Research of silver nanoparticles.* Optical properties of silver nanoparticles were investigated on a spectrophotometer “SPECORD 250 plus” (Germany). To study the morphological properties of actinomycete strains, a light microscope ”Kruss”(Germany).

## 3. Results and discussions

The initial characterization of the formation of silver nanoparticles in the reaction mixture was carried out by visual observation of the color change of the solution in a dark brown color. After addition of 1 mM ArNO3 to the biomass and filtrate of the culture fluid of actinomycetes, a change in the color of the solution was observed. It should be noted that when using culture liquid in the experiments, the color change was observed already on the 7th day, and in the case of biomass, the color of the solution changed after 10-14 days. The color of the suspension, which did not contain silver ions, was pale yellow (pic.1,a), and in the presence of silver ions the solution changes color to a yellowish brown color (Fig.1,b).

**Fig.1.** Change in the color of the reaction during the formation of silver nanoparticles

It was shown that of the eight strains of actinomycetes used as objects of investigation for the formation of silver nanoparticles, only in four strains silver nanoparticles were observed. In the strains of actinomycetes (BDU-C19, BDU-12, BDU-37) silver nanoparticles were detected in a biomass study, in the actinomycete strain of BDU-27, silver nanoparticles were found in biomass and culture fluid. And the formation of silver nanoparticles was not observed in strains of actinomycetes (BDU-24, BDU-41, BDU-D3 and BDU-8).Screening of these pigment-forming strains of actinomycetes by the ability to form silver nanoparticles are shown in the Table 1.

Further, the morphological and cultural properties of the strains of actinomycetes forming nanoparticles were studied.

Morphological features of actinomycetes (colony size, shape, color, edges of the colony, structure, etc.) are studied by inoculation to the surface of the solid nutrient medium of Gaus. From the results obtained, we came to the conclusion that the strain of actinomycete BDU-27 belongs to the genus Streptomyces sp. This strain forms small colonies immersed in the substrate with a diameter of 2-10 mm.

**Table 1**. Screening of strains of actinomycetes by the ability to form silver nanoparticles

| Strains | Signs of silver nanoparticle formation by color change | |
|---|---|---|
| | in biomass | In the culture liquid |
| 1.BDU- 27 | + | + |
| 2.BDU-C19 | + | _ |
| 3.BDU -12 | + | _ |
| 4.BDU-37 | + | _ |
| 5.BDU-24 | _ | _ |
| 6.BDU-41 | _ | _ |
| 7.BDU-D3 | _ | _ |
| 8.BDU- 8 | _ | _ |

The surface part of the colony is covered with an aerial mycelium, which has a thicker coating than the substrate mycelium. In addition, when studying the morphological features of this strain, it was found that it forms a pigment of a blue-violet color. This pigment is synthesized by the substrate myciliia of this strain as a secondary metabolite. During the study of the morphological features of the strain *Streptomyces sp*. BDU-27 also found out that their colonies are whitish-gray or whitish-blue, have a dry consistency, soft with a smooth surface and have a round or oval shape with uneven edges (Fig.2).

Some morphological features of the strain of actinomycete *Streptomyces sp.* BDU-27 such as the shape of sporophores, the thickness of the mycelium, etc. are studied using a microscope. It was found that this mycelium is divided into primary (substrate) and secondary (air) mycelium. Diameter of mycelium from 0.5-2 mm and have a straight or wavy form, and form spore-bearing hyphae, on the surface of which spore strands are located, which consist of 3- 50 or more spores (Fig.2).

A strain of actinomycete *Streptomyces sp.* BDU-C19. During the study of the morphological features of *Streptomyces sp*. BDU-C19 strain, it was found that this strain has a well developed aerial mycelium as well as a substrate mycelium.In the strain *Streptomyces sp*. BDU-C19 the colony size in diameter reaches 10-15 mm, the colony color is whitish-gray or whitish-pink, and has a rounded shape with uneven edges. Edges of the colony with cutouts or with protrusions, the surface of the colony is smooth or has a cotton-like coating. On the surface of the hyphae, they found 3- 4 sporogenoussporangia. Spore strands are wavy and occur in short or long form (Fig.3)

**Fig.2.** Type of colony strain of actinomycetes *Streptomyces sp*. BDU – 27

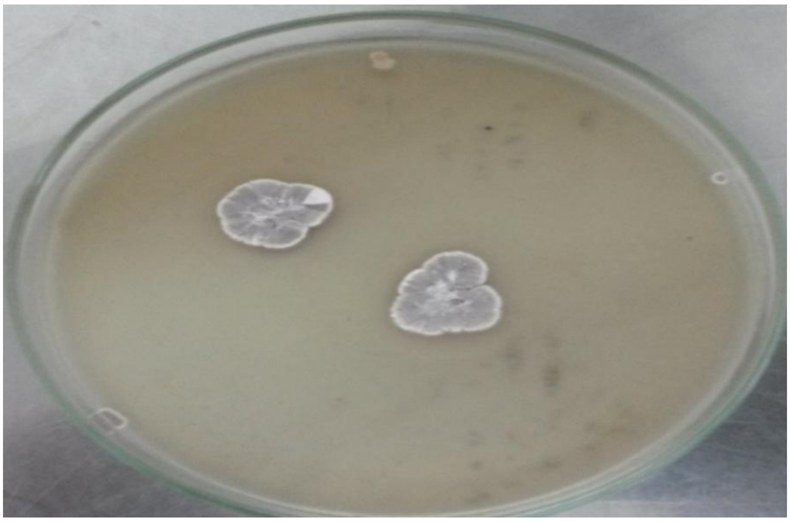

**Fig.3.** Type of colony strain of actinomycetes *Streptomyces sp*. BDU – 19

Another pigment-forming strain of actinomycete, studied during the study for the formation of silver nanoparticles is the strain BDU-12. During the study of the morphological features of this strain, it was found that this strain also belongs to the genus *Streptomyces sp.* In the strains of this section, the aerial mycelium is pink, light pink or pinkish-violet in color.And the substrate mycelium in the mineral agar is colorless.

Colonies of the strain BDU-12 grown on the surface of Gausa solid nutrient medium have a whitish pink color, dry consistency, solid, rounded with uneven edges, the surface of the colony has fringed or cottonishroughness. The colony is small in size and in diameter reaches 5-10 mm. The aerial mycelium is well developed than the substrate mycelium (Fig.4).

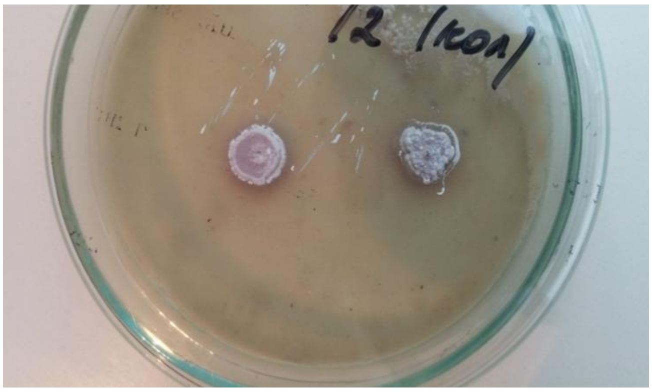


**Fig.4.** Type of colony strain of actinomycetes *Streptomyces sp*. BDU – 12

For the research work, to study the synthesis of silver nanoparticles with pigment-forming actinomycetes, a strain of actinomycete *Streptomyces sp.* BDU-37 was also studied. In this strain, the aerial mycelium is well developed than the substrate mycelium. When studying the morphological features of the strain *Streptomyces sp*. BDU-37 it was found out that this strain has a small colony in diameter of 3-8 mm, round or oval in shape with uneven edges, grayish-violet in color, the surface of the colony is smooth or cotton-like. Spores are located singly at the tips of slightly branched sporophores.Spore-bearing filaments are straight or wavy, long. On the surface of sporiferous filaments can settle from 3 to 50 spores (Fig.5).

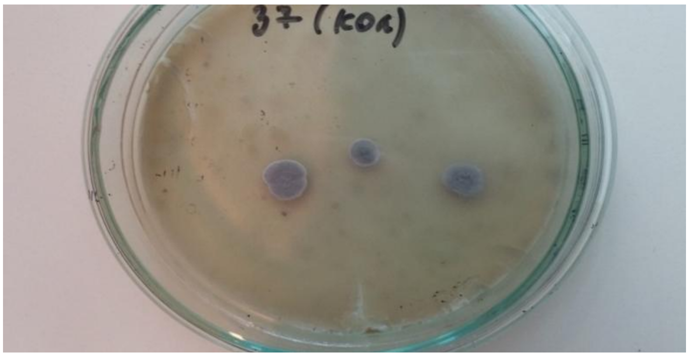


**Fig.5.** Type of colony strain of actinomycetes *Streptomyces sp*. BDU – 37

Thus, as a result of the study, it was found that of the 8 strains of actinomycetes studied, only 4 strains showed the ability to form silver nanoparticles. The study of morpho-cultural properties of 4 strains forming nanoparticles showed that they all belong to the genus Streptomyces and only one of them is Streptomyces sp. BDU-27 forms nanoparticles in both the culture fluid and in biomass.

## References


1. Forough M., Fahadi K., (2011) Biological and green synthesis of silver nanoparticles, *Turkish Journal of Engineering and Environmental Sciences*, 34(4), 281-287.
2. Ganbarov K.G., Ahmadov I.S., Ramazanov M.A., Musayev E.M., Eyvazova Q.I., Aghamaliyev Z.A., (2014) Silver nanoparticles synthesized by the Azerbaijanian environmental isolates Aspergillusniger, *The Journal of Microbiology, Biotechnology and Food Sciences*, 4(2), 137-141.
3. Ganbarov Kh.G., Jafarov M.M., Hajiyeva F.T., Huseynova S.I., Bozkurt H.C., Ramazanov M.A., Aghamaliyev Z.A., Eyvazova Q.I., Ahmedov I.S., (2016), Mycogenic Formation of Silver Nanoparticles by the Azerbaijanese Environmental Isolate Candida macedoniensis BDU-MI44, *International Journal of Research Studies in Biosciences (IJRSB),* 4(5), 1-5.
4. Gasanova S.A., Guliyeva S.M., Eyvazova G.I., Agamaliyev Z.A., Shahgeldiyeva N.A., Ramazanov M.E., Ganbarov K.G., (2015) Investigation of formation of silver nanoparticles of Streptomyces sp. BDU-17, *Microbial Biotechnology: Fundamental and Applied Aspects,* 7, 326-334 (in Russian).
5. Ghorbani H.R., Safekordi A.A., Attar H., Sorkhabadi S.M., (2011) Biological and non-biological methods for silver nanoparticles synthesis, *Chemical and Biochemical Engineering Quarterly*, 25(3), 317-326.
6. Ingale A.G., Chaudhari A.N., (2013) Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach, *J. Nanomed. Nanotechol.*, 4(165), 1-7.
7. Li X., Xu H., Chen Z.S., Chen G., (2011) Biosynthesis of nanoparticles by microorganisms and their applications, *Journal of Nanomaterials*, 10, 1-16.
8. Naqvi S.Z.H., Kiran U., Ali M.I., Jamal A., Hameed A., Ahmed S., Ali N., (2013) Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria, *International Journal of Nanomedicine*, 8, 3187-3195.
9. Nikitina E., (2011) Preparation of silver nanoparticles by the methods of green chemistry and investigation of their antifungal activity and antibacterial properties, Moscow, 15 p. (in Russian)
10. Singh D., Rathod V., Fatima L., Kausar A., Vidyashree, Anjum N., Priyanka B., (2014) Biologically Reduced Silver Nanoparticles from *Streptomyces sp*. VDP-5 and its Antibacterial Efficacy,*International Journal of Pharmacy and Pharmaceutical Science Research,* 4(2), 31-36.
11. Zinchenko A.I., (2014) Nanoparticles as transporters of diagnostic and therapeutic substances, Institute of Microbiology NAS of Belarus, 36-50 (in Russian).