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SYNTHESIS OF SILVER NANOPARTICLES USING A THERMOPHILIC BACTERIUM STRAIN ISOLATED FROM THE SPRING YUKHARI ISTISU OF THE KALBAJAR REGION (AZERBAIJAN)

G.Y. Gunashova*

Department of Biophysics and Biochemistry, Baku State University, Baku, Azerbaijan

Abstract. The eco-friendly synthesis of silver nanoparticles using thermophilic bacteria is a safe replacement for the chemical and physical methods. The study is devoted to the synthesis of silver nanoparticles using the thermophilic bacterium strain KY_2 which was isolated from the thermal water spring Yukhari Istisu of Kalbajar region. Morphologically, the cells of this strain are spore-forming rods. On the surface of a solid nutrient medium, it forms cream-colored, round, wavy colonies along the edges. It was found that KY_2 strain actively grows in the temperature range 55-60°C, at pH=7.0-9.0. The synthesis of silver nanoparticles was observed both in the biomass and in the cell-free supernatant of the strain. Characterization of the synthesized silver nanoparticles was done by UV-vis Spectroscopy. The obtained UV-visible spectrum of the aqueous medium containing silver ion, showed a peak at 410-420 nm which corresponds to the Plasmon absorbance of the silver nanoparticles.

Keywords: *Thermophilic bacteria, biosynthesis, nanoparticles, UV-vis Spectroscopy.*

Corresponding Author: *G.Y. Gunashova, Department of Biophysics and Biochemistry, Baku State University, Z. Khalilov 23, AZ1148, Baku, Azerbaijan, e-mail: gunay_a-va1995@mail.ru*

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1. Introduction

Nanoparticles (NPs) of noble metals, especially silver (Ag) with various potential physiological characteristics are widely used in many fields of medicine (Pareek *et al*., 2017). The conducted fundamental studies have shown that silver Nps have unique optical properties due to surface plasmon resonance, highly developed surface and catalytic activity (Lusfyi *et al.*, 2015). One of the valuable properties of nanosilver is a pronounced biological antibacterial activity, due to which silver nanoparticles can be used for environmental and medical purposes, for example for the disinfection of drinking water, in food packaging materials (Deljou & Goudarzi, 2016; Ethiraj *et al*., 2016; Oves *et al*., 2018; Jadoun & Anna Dilfi, 2021). The antibacterial effect of silver NPs is explained by the fact that nanoparticles when bound to the surface of the cell membrane release silver ions that disrupt its permeability and destroy the components of the cell respiratory chain and penetrate into bacteria, inactivate the ability of DNA replication, thereby destroying cells. It has been shown that smaller silver NPs have a larger surface area available for interaction and a greater bactericidal effect than larger silver NPs (Hsueh *et al*., 2015; Dakal *et al*., 2016).

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There are many methods for the synthesis of NPs, which are divided into physicochemical and green (biological) methods (Iravani *et al*., 2014). The chemical synthesis of NPs is widespread, but biosynthesis is more promising for a number of reasons. Green NPs are synthesized in the presence of oxygen, while chemical synthesis often requires anaerobic conditions: reactions are carried out under a vacuum or in an inert gas atmosphere, which increases the cost of NPs production. Biologically obtained particles are stable for several months, while chemically created NPs are prone to aggregation (Fariq *et al.*, 2017; Hamouda *et al*., 2019; Kalpana *et al.*, 2019). Chemical procedures contribute to the formation of some hazardous substances on the surface of NPs, which can cause complications in their use in medicine. During the biosynthesis of NPs, natural reagents are used, which makes the product safe for the environment and humans. Therefore, the development of biological and simple techniques (green chemistry) is necessary for the widespread use of nanomaterials and the prevention of environmental pollution (Kedi *et al*., 2018; Meena & Chouhan 2015; Singh *et al*., 2016). The biological synthesis of metal nanoparticles is carried out using cultures of bacteria, algae, fungi, and plant extracts (Chung *et al*., 2016; Singh *et al*., 2017). Cultures of these organisms have the ability to reduce metal ions with the formation of metal NPs. Bacteria are potential candidates for nanoparticle biosynthesis because they can survive in all sorts of adverse conditions, such as high or low-temperature peaks, varying degrees of alkalinity or acidity, and high salinity concentrations (Akter & Huq 2020; Divya *et al.*, 2016; Fouda *et al.*, 2020; Gunashova *et al.*, 2021).

Considering all of the above, our work was devoted to the synthesis of silver nanoparticles with the participation of thermophilic bacteria.

2. Materials and methods

The KY_2 strain was isolated from the thermal water "Yukhari Istisu" (pH=9.0, t=71°C, mineralization is 4.3 g/l) of the Kalbajar region. For this, a water sample was inoculated on Petri dishes with a nutrient medium of the following composition: peptone-5 g/l, beef extract-1 g/l, yeast extract-2 g/l, sodium chloride-5 g/l, agar-15 g/l and incubated in a thermostat at a temperature of 60°C for 4-5 days. The grown colonies of thermophilic bacteria were isolated in a pure culture according to the Koch method, which is based on the preparation of successive tenfold dilutions of the test sample from the last 2 of which are sown on agar dishes (Gunashova *et al*., 2021).

Despite the fact that several strains (KY_1, KY_2, KY_3) of thermophilic bacteria were isolated, work continued with only one strain of KY_2. First of all, the optimal values of temperature and pH of the medium for the growth of the strain were determined. To do this, the strain was seeded on agar plates and incubated at different temperatures (45°C, 50°C, 55°C, 60°C and 65°C). Optimum temperatures were determined by the diameter of the grown colonies. To settle the optimal pH value of the medium, the strain was inoculated on Petri dishes with nutrient agar with different pH values (6.0, 7.0, 7.5, 8.0, 8.5, 9.0) and incubated at 60°C for one day. The diameter of the grown colonies was used to determine the optimal pH value for strain growth. The morphological and cultural characteristics of this strain were studied. The cultural characteristics of a colony of a strain (shape, size, edge, surface, color, etc.) grown on an agar plate for 24 hours at a temperature of 60°C were described. A magnifying glass was used to accurately describe the colony. To study the cell morphology, we used the method of microscopy (XSP-30 series microscope), the preparation of wet mount and

fixed specimens. After determining the strain's morpho-cultural and some physiological properties, the ability of this strain to synthesize silver NPs was studied. For this, the strain was inoculated on a liquid nutrient medium of the following composition: beef extract-1.0 g/l, yeast extract-2.0 g/l, peptone-5.0 g/l and sodium chloride-5.0 g/l. The nutrient medium was added in 100 ml increments to 250 ml Erlen Meyer flasks and autoclaved at 1 atm. Within 20 minutes. Under sterile conditions the strain was inoculated on appropriate nutrient media in flasks and incubated at 60°C for 2 days. After that, the culture liquid was separated from the biomass by centrifugation at 3000 rpm for 15 minutes. The resulting biomass was centrifuged three times with distilled water to completely remove the remnants of the nutrient medium. 50 ml of silver nitrate solution (1 mM AgNO3) and 50 ml of distilled water were added per 10 g of wet biomass. Also, 50 ml (1 mM) of silver nitrate solution was added to 50 ml of cell-free supernatant. The resulting reaction mixtures were incubated in a thermostat at 60°C under dark conditions. The color change of the reaction medium was observed over time. The formation of silver nanoparticles was determined using a spectrophotometer of the following brand (UV-Vis specord 250 plus).

3. Results and discussion

Only 3 strains of thermophilic bacteria were isolated from the thermal water "Yukhari Istisu" of the Kalbajar region: KY_1, KY_2, KY_3. The KY_2 strain turned out to be the most active in terms of its ability to produce biomass, so further research was continued using this strain.

Fig.1. Agar slant tube culture of KY_2 strain (cultivated at 60°C within 24 hours).

The morphological and cultural characteristics of the KY_2 strain are shown in Fig.2 and Fig.3, respectively, which are described along with some physiological properties (optimal growth temperature and pH of the medium) in Table 1. Cultivation was carried out at 60 ° C for a day on a nutrient agar.

The ability of this strain to synthesize silver nanoparticles was studied and it was found that the strain has this ability. This was primarily observed by the color change of the reaction medium within 20 days, from yellow to dark brown, almost black in the cell-free supernatant and from white to brown in the biomass (Fig. 3 and Fig. 4).

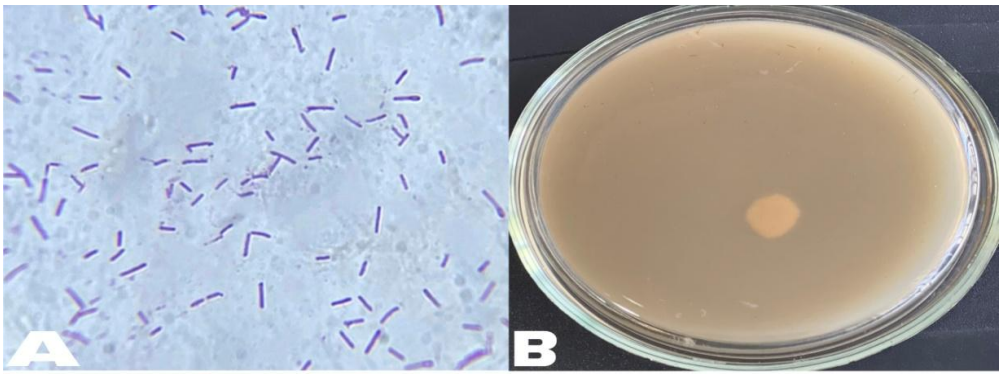

Fig. 2. A: Morphology of cells of the KY_2 strain under a microscope (magnification x1000); **B:** Colony of the KY_2 strain

Table 1. Characteristics of the KY_2 strain

Morphological characteristics				**Cultural characteristics**	**Some physiological characteristics (optimal temperature and pH)**
Shape	**Size**	**Gram strain**	**Spore**	Creamy white color, round in shape, wavy in margin, elevation is convex.	Actively grow in the temperature range 55-60°C, at a pH value 7.0-9.0.
Rods	0.3-1.2 x1.5-4 µm	Positive	Spore forming		

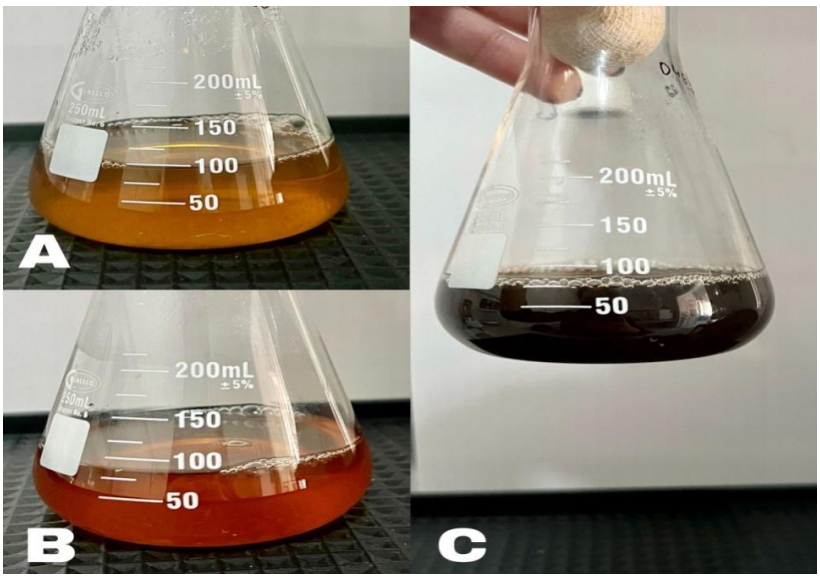

Fig. 3. Change in color of the reaction medium (cell-free supernatant) at 60°C
A-initial color; **B**-within 5 days; **C**-within 15 days

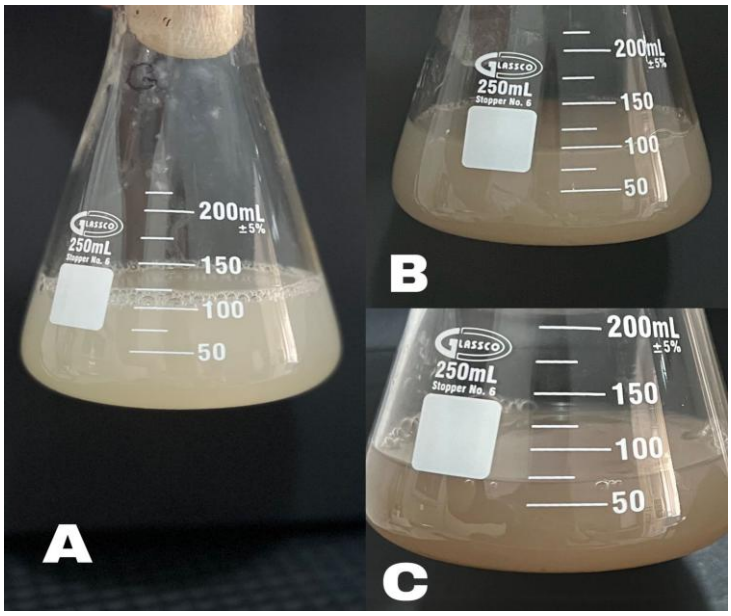

Fig. 4. Change in color of the reaction medium (biomass) at 60°C
A-initial color; **B**-within 5 days; **C**-within 15 days

Reaction mixtures that changed color during the experiment were analyzed and absorption wavelength of 410-420 nm was observed in UV-vis spectrophotometer (Fig.5). Thus, this absorption was fit to the characteristics absorption of silver nanoparticles.

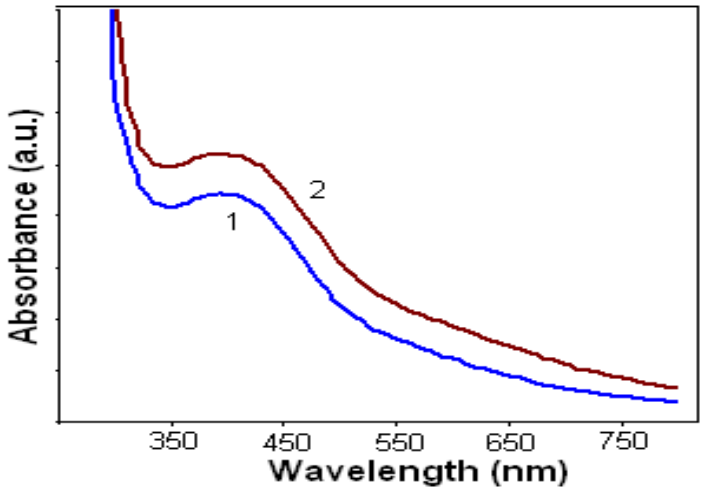

Fig. 5. UV-visible spectra of silver nanoparticles: (1-in cell-free supernatant, 2-in biomass)

3. Conclusion

In this work, the biological synthesis of silver nanoparticles was performed with the participation of a strain of thermophilic bacterium KY_2, which was isolated from the thermal water "Yukhari Istisu" of the Kalbajar region. It was found that the cells of the strain are rod-shaped capable of forming spores. The optimal temperature and pH of the

medium for the growth of the strain turned out to be 55-60°C, pH=7.0-9.0, respectively. The synthesis of silver nanoparticles using KY_2 strain was observed by changing the reaction medium's color and by measuring the samples in a UV-Vis spectrophotometer. The color of reaction medium changed within 20 days from yellow to dark brown, almost black in the cell-free supernatant and from white to brown in the biomass. In a UV-vis spectrophotometer an adsorption wavelength of 410-420 nm was obtained, which corresponded to the characteristic adsorption of silver nanoparticles.

Research in this direction is ongoing.

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