UV-Vis spectroscopy analysis of silver nanoparticles synthesized using thermophilic bacterium strain

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Received: October 21, 2022; Reviewed: November 04, 2022; Accepted: November 11, 2022

Here we report a dependence of the biological synthesis of silver nanoparticles with the participation of a strain of thermophilic bacterium KY_2, isolated from the thermal spring of the Kalbajar region "Yukhari Istisu", on time and concentration. The synthesis of silver nanoparticles was studied in reaction media prepared at various concentrations. Synthesis was observed in a mixture of 50 ml of cell-free supernatant topped with 50 ml of 1 mM silver nitrate ($AgNO_3$) solution, which was detected primarily by a change in the color of the reaction medium from yellow to black. The obtained UV-visible spectrum of the aqueous medium containing silver ions showed a peak at 420 nm which indicates a correspondence of the silver nanoparticles. Synthesis was studied within 5-30 days. Based on the UV-visible spectra, it was found that over time, the number of synthesized silver nanoparticles increases.

Keywords: *UV-Vis spectroscopy, thermophilic bacteria, biological synthesis, silver nanoparticles*

INTRODUCTION

Silver nanoparticles, like other nanoparticles, are characterized by unique properties associated with a high ratio of their surface to volume, which determines their high efficiency (Chernousova & Epple, 2013; Jain et al., 2011; Mohamed et al., 2022). Silver nanoparticles are known to have antibacterial properties against many pathogenic microorganisms. In this regard, smaller silver nanoparticles are most effective, as they have an extremely large specific surface area, which increases the contact area of silver with bacteria or viruses (Ankush et al., 2022; Irfan et al., 2022; Jiale et al., 2011; Li et al., 2010; Salem et al., 2015). In many studies, it was found that silver nanoparticles in combination with antibiotics demonstrate the inhibition of penicillin, ampicillin and novobiocin-resistant bacterial strains - *B. subtilis, Bacillus* sp., *S. nematodiphila,* and *Streptococcus* sp. The research results showed that the effectiveness of antibiotics against test strains increased in the presence of silver nanoparticles. The increase in antimicrobial activity of antibiotics in combination with silver nanoparticles suggested that silver nanoparticles could be used as an adjuvant for the treatment of various diseases caused by bacteria (Gurunathan et al., 2014, 2015; Saravanan & Nanda 2010; Seenivasan et al., 2012; Sharma et al., 2009; Silva et al., 2017).

The mechanism of antimicrobial activity of silver nanoparticles is the adhesion and accumulation of nanoparticles on the bacterial surface. Structural damage to cell membranes leads to an increase in the permeability of bacteria which in turn also depends on the size of the nanoparticles. Studies using E. coli have confirmed that the accumulation of silver nanoparticles on the cell membrane creates breaks in the integrity of the bilayer, which predisposes it to increased permeability and death of bacterial cells (Gurunathan et al., 2014; Li et al., 2010).

The advantage of nanosilver over all existing antimicrobial agents is due to a wide spectrum of antimicrobial activity. Also, microorganisms are not able to develop resistance to silver nanoparticles, therefore they do not transfer resistance to offspring during mutations (Saravanan & Nanda 2010; Sharma et al., 2009).

Silver nanoparticles are widely used in medical devices and consumer products such as surgical instruments, sterilizers, medical catheters, creams, lotions, sprays, household appliances, detergents, toothpastes, soaps, food storage and preservation containers, and antiseptic paints. The production of textile and polymer products for medical and household use, modified with silver nanoparticles, is one of the promising areas since such materials can be used as preventive antimicrobial protective equipment in places where the risk of infection spread increases (Bethu et al.,

2016; Faryad et al., 2022; Gattu et al., 2022; Gurunathan et al., 2013; Mahboob, 2022; Monika et al., 2022; Nehil et al., 2021; Nidhi et al., 2022).

The unique antiseptic properties of nanosilver led to the search for the most advanced methods for the synthesis of these nanoparticles. Nanoparticles can be synthesized by various methods including chemical synthesis, physical methods, biological methods. In the chemical method, the main disadvantage is the use of extremely harmful organic solvents. These solvents have lower biocompatibility which limits their natural use. Physical methods do not use toxic chemicals and usually require fast processing. However, physical methods have some drawbacks such as high energy consumption and longer thermal stability time requirements. In the biological synthesis of silver nanoparticles, toxic reducing agents and stabilizers are replaced by non-toxic molecules produced by living organisms, including bacteria, fungi, and plants. In general, the synthesis of silver nanoparticles using a biological method is the most common environmentally friendly production method (Anand et al., 2015; Balaji et al., 2009; Bhainsa & D'Souza 2006; Ganaie et al., 2015; Gunashova et al., 2021; Swarnendra et al., 2022; Nikhat et al., 2022; Saifuddin et al., 2009).

MATERIALS AND METHODS

The object of the study was a strain of thermophilic bacterium KY_2 isolated from the thermal spring of the Kalbajar region, "Yukhari Istisu" (pH=9.0, t=71° C, mineralization 4.3 g/l). In our previous work, we synthesized silver nanoparticles using this strain (Gunashova, 2022). Synthesis was observed both in the biomass and in the cell-free supernatant of the strain. In this work, we studied the dependence of bacteriological synthesis on time and concentration. For this, the strain was cultivated on a liquid nutrient medium with the following composition: beef extract -1.0 g/l, yeast extract - 2.0 g/l, peptone - 5.0 g/l, sodium chloride - 5.0 g/l. Incubation was carried out for 48 hours at 60°C. After the incubation period, the biomass of the strain was separated from the culture fluid by centrifugation for 15 minutes at 3000 rpm. From the obtained cell-free supernatant, mixtures were prepared with a solution of silver nitrate (1 mM AgNO3) in various ratios: 50 ml of c.f.s.+ 50 ml of silver nitrate solution, 99 ml of c.f.s + 1 ml of silver nitrate solution, 90 ml of c.f.s. + 10 ml of silver nitrate.

As a control, the sterile liquid nutrient medium (l.n.m.) noted above was mixed with a solution of silver nitrate (1 mM $AgNO_3$) in an amount of 50 ml (Fig.1). The resulting mixtures were incubated in a dark thermostat at a temperature of 60°C for 5-30 days. The color change of the reaction mediums was periodically observed, and the samples were also analyzed in a UV-Vis spectrophotometer (UV-Vis specord 250 plus German).

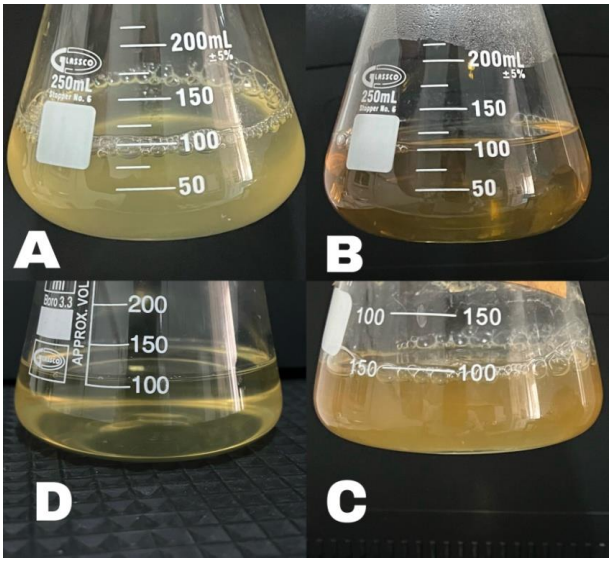

Fig. 1. A – 50 ml c.f.s.+50 ml (1 mM $AgNO_3$); **B** - 99 ml c.f.s.+1 ml (1 mM $AgNO_3$); **C** - 90 ml c.f.s.+10 ml (1 mM $AgNO_3$); **D** - 50 ml l.n.m. +50 ml (1 mM $AgNO_3$).

RESULTS AND DISCUSSION

The reaction mixtures obtained in various ratios (50 ml c.f.s.+50 ml (1 mM $AgNO_3$); 99 ml c.f.s.+1 ml (1 mM $AgNO_3$); 90 ml c.f.s.+10 ml (1 mM $AgNO_3$)) and control mixture (50 ml l.n.m. + 50 ml (1 mM $AgNO_3$)) were incubated in a dark thermostat at 60°C for 5-30 days. In mixtures prepared in the ratio of 99+1, 90+10, and in the control mixture no color change was observed, while in the mixture prepared in the ratio of 50 ml c.f.s.+50 ml (1 mM $AgNO_3$), the color changed from yellow to dark brown, almost black (Fig. 2).

The samples were monitored in UV-Vis spectra ranges (300-800 nm). In mixtures of 99:1, 90:10, and in the control mixture the absorption spectra characteristic of silver nanoparticles were not observed. (Fig. 3). The non-formation of silver nanoparticles in the control mixture obtained by mixing a sterile liquid nutrient medium with a solution of silver nitrate (1 mM $AgNO_3$), in contrast to a mixture of 50 ml of cell-free supernatant with 50 ml of the solution of silver nitrate (1 mM $AgNO_3$), proved that metabolites in the bacterial supernatant, and not the components of the nutrient medium are involved in the synthesis of silver nanoparticles.

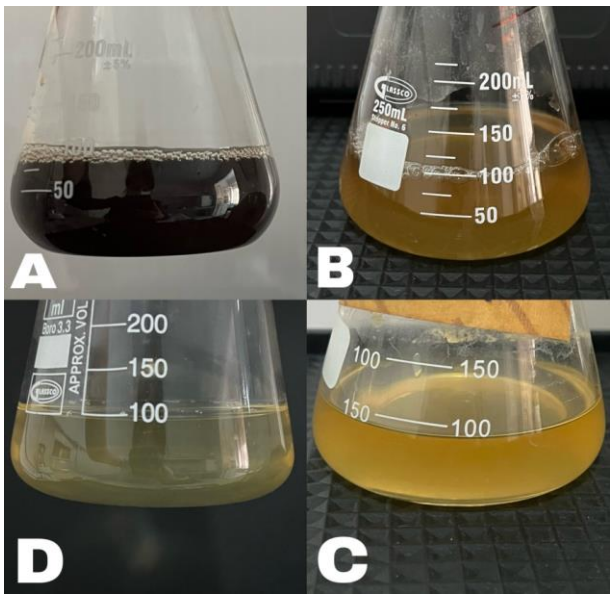

Fig. 2. A – 50 ml c.f.s.+50 ml (1 mM $AgNO_3$); **B** - 99 ml c.f.s.+1 ml (1 mM $AgNO_3$); **C** - 90 ml c.f.s.+10 ml (1 mM $AgNO_3$); **D** - 50 ml l.n.m. + 50 ml (1 mM $AgNO_3$)

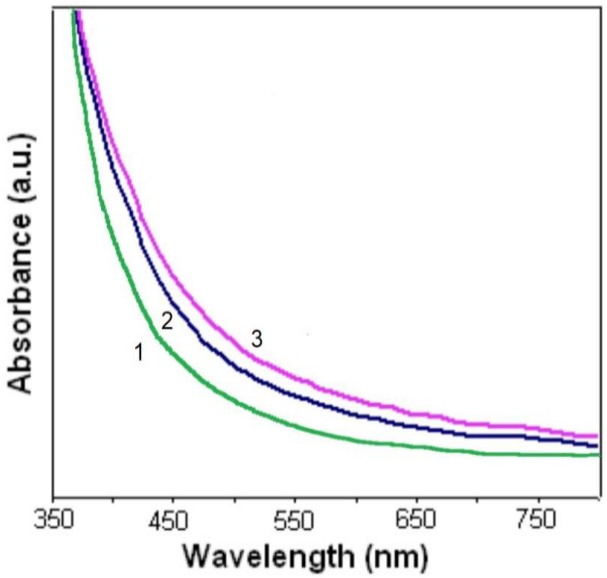

Fig. 3. UV-vis adsorption spectra of mixtures: **1** - control (50 ml l.n.m.+50 ml (1 mM $AgNO_3$)); **2** - 99 ml c.f.s.+1 ml (1 mM $AgNO_3$); **3** - 90 ml c.f.s.+10 ml (1 mM $AgNO_3$).

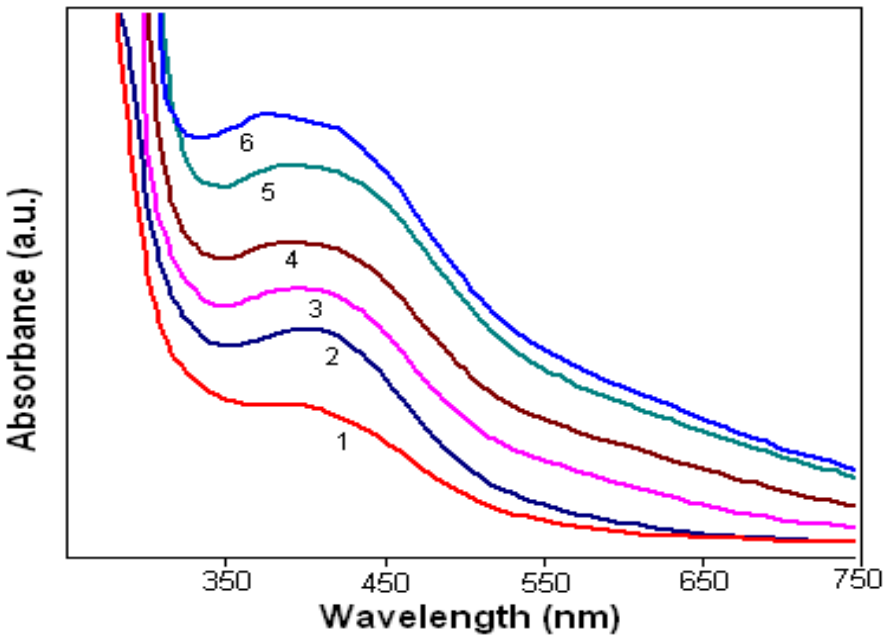

Fig. 4. UV-visible adsorption spectra of silver nanoparticles synthesized in a mixture prepared in a ratio 50ml c.f.s.+50 ml (1 mM $AgNO_3$): 1-in 5 days; 2-in 10 days; 3-in 15 days; 4-in 20 days; 5-in 25 days; 6-in a month.

The synthesis of silver nanoparticles after 5, 10, 15, 20, 25, and 30 days of incubation formed a broad surface plasmon resonance absorption band between 400–420 nm. The intensity of the band was increased by increasing the storage period up to 1 month in a dark thermostat, indicating the synthesis of silver nanoparticles was increased during the storage period. UV-Visible absorption spectra revealed an absorption maximum wavelength of 420 nm for silver nanoparticles (Fig.4).

CONCLUSION

This work is devoted to the synthesis of silver nanoparticles with the participation of a thermophilic bacterium strain. Given the unique properties of silver nanoparticles and the practical significance of an efficient method for the synthesis of these nanoparticles, this study is relevant and has future prospects. We studied the dependence of the bacteriological synthesis of

nanoparticles on time and concentration. The reaction mixtures were prepared in three different ratios of cell-free supernatant with 1 mM silver nitrate solution (50:50, 99:1, 90:10). Synthesis was observed only in a mixture prepared in a ratio of 50:50, which first of all manifested itself in a color change from yellow to dark brown. The studied sample was incubated for 5-30 days and periodically analyzed in a UV-Vis spectrometer. The maximum peak was obtained at a wavelength of 420 nm. It was found that over time up to a month, the number of synthesized nanoparticles increases.

Research in this direction continues.

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Termofil bakteriya ştamının iştirakı ilə sintez edilmiş gümüş nanohissəciklərin UV-Vis spektroskopik təhlili

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Təqdim olunan elmi işdə Kəlbəcər rayonunun "Yuxarı İstisu" termal su mənbəsindən ayrılmış, termofil bakteriya olan KY_2 ştamının iştirakı ilə gümüş nanohissəciklərin bioloji sintezinin zamandan və konsentrasiyadan asılılığından bəhs edilir. Gümüş nanohissəciklərin sintezi müxtəlif konsentrasiyalarda hazırlanmış reaksiya mühitlərində araşdırılmışdır. Üzərinə 50 ml gümüş nitrat (1 mM $AgNO_3$) məhlulu əlavə edilmiş 50 ml kultural maye qarışığında sintez müşahidə edilmişdir ki, bu da ilk növbədə, reaksion mühitin rənginin sarıdan qara rəngə dəyişməsi ilə aşkar edildi. Tərkibində gümüş ionları olan reaksion qarışığın əldə edilən UV-vis spektrları gümüş nanohissəciklərə xas olan 420 nm dalğa uzunluğunda udulmanın baş verməsini göstərmişdir. Sintez 5-30 gün ərzində öyrənildi. UV-vis spektrları əsasında müəyyən edilmişdir ki, zaman keçdikcə sintez edilən gümüş nanohissəciklərin miqdarı artır.

Açar sözlər: *UV-Vis spektroskopiya, termofil bakteriyalar, bioloji sintez, gümüş nanohissəciklər*