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GREEN BIOSYNTHESIS OF AgNPs BY *Lactobacillus acidophilus* AND THEIR USE

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Silver nanoparticles nowadays are considered one of the most researched materials in science and technology. These nanoparticles are up to a hundred nanometers in size and differ from ordinary metals in their unique physical and chemical properties. Silver nanoparticles also have great potential in biomedicine. They can be used as antimicrobial agents since silver is highly active against bacteria and fungi. Silver nanoparticles can also treat wounds, promoting rapid healing and preventing infections. The paper presents parameters of green biosynthesis of silver nanoparticles using *Lactobacillus acidophilus* UCM B-2691. Using the methods, it was established that silver nanoparticles can be obtained only with the help of the supernatant of the culture liquid of *L. acidophilus* UCM B-2691 by selecting the optimal parameters of green biosynthesis. It was established that nanoparticles with a size of 35 nm can be obtained at a temperature of 50°C. The pH of the medium is also important. To obtain nanoparticles the pH value must be alkaline. The best results were observed when the pH was at 13. The obtained results were checked visually, spectrophotometrically and using the BeNano 90 Zeta nanoparticle size analyse by photon correlation spectroscopy (PCS).

Keywords: nanoparticles, *Lactobacillus acidophilus*, green biosynthesis.

INTRODUCTION

Many physical and chemical methods are used for nanoparticle synthesis. However, because of a growing concern over unnecessary and excessive emissions from production, there is a threat to the environment. Therefore, it is advisable to use “green” methods of metal nanoparticle production. It is also important to optimize various biological methods to achieve a high percentage of product yield and minimal costs for production (Brar *et al*., 2022; Wang *et al*., 2020; Voloshyna *et al*., 2023).

The nanoparticles themselves, their properties, use and production methods play an important role in modern scientific and industrial research. In particular, biological synthesis opens up new horizons for research and use. Undoubtedly, the chemical and physical production of metal nanoparticles is also effective, yet, an energy- and financial-intensive process, without mentioning excessive environmental pollution. Biological methods, such as synthesis by microorganisms (bacteria, fungi) or biological plant extracts are becoming increasingly popular. Biological molecules such as enzymes and metabolites are used to reduce metal ions into nano-sized particles (Brar *et al*., 2022; Voloshyna *et al*., 2023).

The size and shape of the nanoparticles affect their properties which can only be partially explained what makes nanoparticles interesting for further research. Silver nanoparticles (AgNPs) are clusters of silver atoms 1-100 nm in diameter which can be used by scientists as antimicrobial agents (Brar *et al*., 2022; Wang *et al*., 2020; Voloshyna *et al*., 2023).

AgNPs are mostly obtained by chemical, physical and biological methods. Chemical production is in use mode often, even though they are more ecologically

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dangerous (Wang *et al*., 2020; Voloshyna *et al*., 2023). Besides, the conventional methods of nanoparticle production require toxic substances that pollute the environment. One of the safest methods of nanoparticle synthesis remains the biological method by the aid of various biological agents (Voloshyna *et al*., 2023).

Silver nanoparticles are often used in various fields of biomedicine due to their antimicrobial activity since there is a problem of resistance of various microorganisms to antibiotics. AgNPs exert antibacterial properties against a range of gram-negative and gram-positive bacteria. Also, atomic silver is proven to have anti-fungal, anti-bacterial and anti-viral properties (Ndolomingo *et al*., 2020; Voloshyna *et al*., 2024).

The effectiveness of silver nanoparticles is completed by several advantages such as minimized toxicity and stability. However, the use of silver nanoparticles is not limited to their antimicrobial potential but is also used to obtain and develop new biomedical products. For instance, AgNPs can be used in the development process of medicinal substances, biomaterials, optical probes, and orthopedic materials, being a part of various pharmaceutical formulations and medical devices (Prasher *et al*., 2020). It has been found that changing the size, surface and shape of AgNPs affects the cell membrane and cell organelles, so they can be used to reduce pathogenicity and increase drug sensitivity (Prasher *et al*., 2020; Voloshyna *et al*., 2023).

MATERIALS AND METHODS

Characterization of *Lactobacillus acidophilus* UCM B-2691

In this work, a lyophilized strain of *Lactobacillus acidophilus* was used, which is a homofermentative, microaerophilic, short-chain gram-positive microorganism with a rod-like morphology approximately 2-10 μm in size with class II A bacteriocins. They show important thermostability and preservation of activity in a wide pH range and a strong inhibition against food spoilage and pathogenic bacteria, which makes them an important class of biopreservatives. *Lactobacillus* bacteria exist in a variety of environments from dairy products to the human gastrointestinal tract. They are, as a rule, gram-positive, immobile, do not form spores, have a round or rod-shaped nucleus and produce lactic acid as an end product of enzymatic metabolism. *L. acidophilus* is a Gram-positive bacillus that grows optimally between 37 and 42°C and can grow at temperatures up to 45°C. It reaches its highest growth at a pH between 5.5 and 6.0, and its growth ceases at a pH of 4.0. *L. acidophilus* is an obligate homofermentative organism that ferments carbohydrates to form lactic acid and is one of the least oxygen-tolerant LABs.

Cultivation of *Lactobacillus acidophilus* UCM B-2691

The lyophilized culture of *L. acidophilus* UKM B-2691 was obtained for scientific research at the Institute of Microbiology and Virology named after D. K. Zabolotny National Academy of Sciences of Ukraine. The museum plant was transplanted into a liquid nutrient medium MRS for research, and the first and second-generation seed material was obtained (24 h, 37ºC, 160 rpm). In parallel, they were sown on a dense medium of MRS, controlling the purity of the culture. After reviving the culture, it was used to accumulate biomass and metabolites.

Obtaining Working Material for Green Synthesis

The 2nd generation culture was sown on MRS liquid medium and cultivated for 48 hours at 37ºC, 160 rpm. Then centrifugation was performed at 3500 rpm for 30 min. After

centrifugation, the supernatant and cells were collected separately. Sterile distilled water was added to the cells in the amount of the selected supernatant and left for 24h for the final destruction of the cells.

Conducting Biogenic Synthesis of Silver Nanoparticles

Biosynthesis of nanoparticles using *Lactobacillus acidophilus* UCM B-2691 was carried out in two ways – using cell lysate and their supernatant.

After these manipulations, both experimental solutions were poured into 30 ml sterile measuring glasses and 100 mM $AgNO_3$ was added. After adding salt, the pH level of the cell lysate solution and the supernatant solution was measured and adjusted to the required pH levels. 3 pH levels were chosen for the experiment: 3, 7, and 13. Proofing was done using HNO_3 and NaOH, respectively. A temperature gradient of 5°C, 25°C and 50°C was established. So, 3 different pH levels and a control solution were supplied for each selected temperature. For 24 hours, the vials with the experimental solutions of both variations of the methods were kept in thermostats according to the selected temperatures.

Research of Silver Nanoparticles

Determination of the Size of Silver Nanoparticles by Photon Correlation Spectroscopy

To determine the size of nanoparticles, the method of photon correlation spectroscopy (FCS) was used using the BeNano 90 Zeta nanoparticle size analyzer. One of the advantages of using the device when determining the size of metal nanoparticles is the non-invasiveness of the method for samples, that is, the structure of molecules is not destroyed during the study. Also, 1-2 ml of the sample is enough to prepare the experimental solution. This method allows you to obtain results with high repeatability, speed and accuracy. The measurement process is almost completely automatic, which helps to reduce errors in the work process. The chosen method makes it possible to investigate the quality of experimental samples and therefore to maximize the efficiency of further use of nanoparticles. In the protocols of the conducted experiments, you can find data on the average size of the formed nanoparticles (Z-average), the value of the distribution of nanoparticles in the solution (Area %), the standard error of measurement and the value of polydispersity (PdI). Using the polydispersity index (PdI), you can find out the degree of distribution of nanoparticles in the samples - a decrease in the value of polydispersity indicates a more homogeneous solution in terms of the measured nanoparticles size and shape.

Spectrophotometric Analysis for the Presence of Silver Nanoparticles

A ULAB 102 UV spectrophotometer was used to analyse the obtained samples for the presence of silver nanoparticles. The optical density of the samples was measured to a wavelength range of 320 nm to 500 nm in steps of 5-10 nm. Distilled water was used as a control. The wave range was chosen according to a literature review of studies of the peak of the plasmon resonance spectrum of metal nanoparticles. The obtained results were used to plot graphs of the dependence of the optical density of the samples on the wavelength.

Statistical Analysis

The results of calculating the average size of nanoparticles were calculated immediately on the BeNano 90 Zeta device. Statistical processing of the results was carried out using Microsoft Office Excel 2016 software.

RESULTS AND DISCUSSION

An experiment was conducted using the lysate of *L. acidophilus* UCM B-2691 cells and the supernatant for the synthesis of silver nanoparticles. It is known that this type of bacteria can reduce silver ions Ag^+ to elemental argentum Ag^0 thanks to enzymes, metabolites, proteins and cofactors.

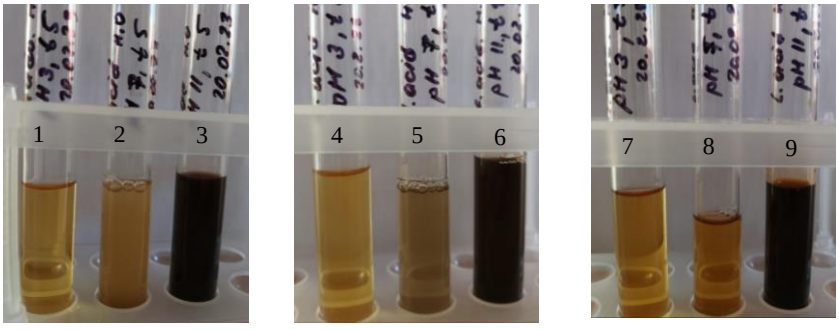
First of all, it should be noted that experiments using the supernatant of *L. acidophilus* UCM B-2691 cells as a substrate for the synthesis of silver nanoparticles are not widespread, but also give excellent results, compared to experiments with cell lysate of this culture as the main component of the synthesis of silver nanoparticles. Thus, taking into account the negative results in the course of the experiment with the solution of *L. acidophilus* UCM B-2691 cell lysate, namely the formation of a silver mirror and a transparent final solution, and the positive results with the use of the supernatant solution of this culture, it can be assumed that this technique is effective for using the supernatant of *L. acidophilus* UCM B-2691 cells as a substrate for the green synthesis of silver nanoparticles.

The general scheme of the experiment is shown, where it is possible to determine the main mechanisms of experimenting, presented in the table. 1. The pH range was changed from 3 to 13 and the temperature from 5 to 50°C. The first control of the possible formation of silver nanoparticles was recorded by a change in color (Fig. 1).

Table 1. Results of qualitative analysis of the synthesis of silver nanoparticles

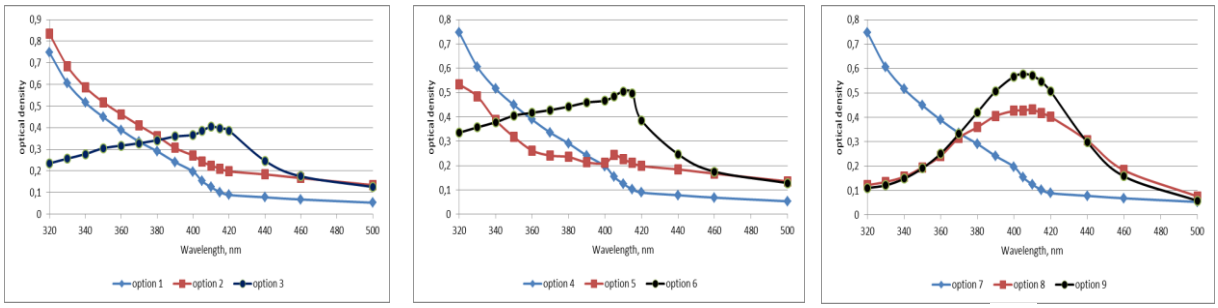
option	t, °C	pH	Color change
1		3	Light yellow
2	5	7	Light yellow
3		13	Dark brown
4		3	Deep yellow
5	25	7	Yellow-gray
6		13	Dark brown-red
7		3	Deep yellow
8	50	7	Deep yellow
9		13	Dark brown-red

After the synthesis of silver nanoparticles, their concentration was measured using UV-Vis spectrometry and it was determined that at a temperature of 50°C and a pH of 13, the formation of nanoparticles occurred faster and the resulting nanoparticles had an absorption peak at a wavelength of 405 nm.



a b c

Figure 1. Visual image of synthesized silver nanoparticles at the temperature 5°C (a), 25°C (b), 50°C (c) and different values of pH 3 (1, 4, 7), pH 7 (2, 5, 8), pH 11 (3, 6, 9)



a b c

Figure 2. Spectrometric image of silver nanoparticles at temperature 5°C (a), 25°C (b), 50°C (c) and different values of pH 3 (1, 4, 7), pH 7 (2, 5, 8), pH 11 (3, 6, 9)

Thus, when comparing the sizes of nanoparticles obtained during cultivation at a temperature of 5°C (Figs. 1 a and 2 a) and a pH level of 3 (option 1), it can be seen that the nanoparticles were synthesized in the form of large clusters with an average size of 1375 nm, which were determined using BeNano 90 Zeta. Moreover, the difference in size exists due to the different pH values. Visual control was confirmed spectrophotometrically. From Fig. 2 it can be seen that there is a slight absorption peak only in variant 3, which is at 410 nm, in which we record the average particle size of 137 nm. At a neutral pH level of 7 and a temperature of 25°C (option 2), the results were almost the same when kept at a temperature of 5°C (Fig. 1 b and Fig. 2 b). After analysing the results of the study at a temperature of 25°C with different pH values and its proof, it can be seen that in the reaction mixture, where the pH was acidic (option 4), nanoparticles with an average size of 1450 nm were formed, which indicates the formation of cluster compounds and a large number of free biological molecules. However, in variant 5, with a pH level of 7, particles with a size of 965 nm were formed, which makes it clear that sufficiently large particles are formed under these conditions. After setting the pH to 11 and a constant temperature of 25°C (option 6) in the solution, the synthesis of particles with a size of 7193 nm was observed, but in the solution with the pH level adjusted to the introduction of salt, nanosilver with a size of 162 nm was formed, which gives this method an advantage in the given data synthesis characteristics. Also, under these parameters, the absorption peak of the studied variants 4, 5, and 6 was checked spectrophotometrically. The absorption peak at 415 nm was recorded only in variant 6, which indicates the formation of nanosilver particles.

But when the temperature increased to 50°C (options 8 and 9), we already recorded nanoparticles with a size of 35 nm at pH 13 and 98 at pH 7, with the values recorded spectrophotometrically, since in Fig. 2 s it can be seen that the absorption peak occurs at 410 and 405 nm in variants 8 and 9, respectively. Therefore, analysing the obtained results, we can say that pH and temperature are important in the synthesis of silver nanoparticles in the culture liquid of *L. acidophilus* UCM B-2691. The optimal temperature should be at the level of 50°C, and the pH should be alkaline.

CONCLUSIONS

The optimal conditions for the production of silver nanoparticles using *Lactobacillus acidophilus* UCM B-2691 were selected. Synthesis of nanosilver was observed by adding silver nitrate to the culture supernatant at 50°C and pH 13. A range of colors from light to dark brown was observed. Nanoparticle size analysis showed the formation of silver with z-ave: 34.66 nm, PdI: 0.242, Intercept: 0.87 and absorption peak at 405 nm.

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