BIOSYNTHESIS OF SILVER NANOPARTICLES AFTER CULTIVATING MUTANT YEAST ON CULTURE MEDIA OF DIFFERENT COMPOSITIONS

Koval R.V., Skrotska O.I.
National University of Food Technologies, kovalrost119@gmail.com

Abstract

Current work is devoted to the determination of the optimal parameters of the biosynthesis of silver nanoparticles using a mutant strain of Saccharomyces cerevisiae yeast. Optimal parameters of nanoparticles biosynthesis were established: cultivation of yeast on YPD 1 medium, the use of cell-free aqueous extract of mutant yeast, 1.5 mM $AgNO_3$, 45°C.

Keywords: *silver nanoparticles, Saccharomyces cerevisiae, culture media, temperature*.

Introduction. Nanoparticles of various metals are attracting more and more attention due to the vide range of advantages they have over their macro analogues. Silver nanoparticles (AgNPs) are of the greatest interest because of their unique physicochemical and biological properties, namely anticancer, antiviral and antimicrobial activity. Traditional physical and chemical methods of silver nanoparticles synthesis require high temperatures and use toxic substances, making them expensive and harmful to the environment. These shortcomings are absent in the biological method of obtaining silver nanoparticles, which is based on the use of various biological objects for the formation of AgNPs.

We have already shown the possibility of obtaining silver nanoparticles using a cell-free aqueous extract of mutant *Saccharomyces cerevisiae* yeast and determined the optimal silver nitrate concentration for biosynthesis [1]. Currently, it is expedient to determine the optimal culture medium composition for yeast cultivation and the optimal temperature for the biosynthesis of nanoparticles.

Materials and methods. The mutant strain of *Saccharomyces cerevisiae* yeast was obtained by exposing a suspension of *S. cerevisiae* M437 cells to the ultraviolet irradiation. Mutant cells were cultivated on three different culture media: Reader's medium (glucose – 20 g/l, $(NH_4)_2SO_4$ – 3 g/l, $MgSO_4 \times 5H_2O$ – 0,7 g/l, NaCl – 0,5 g/l, K_2HPO_4 – 0,1 g/l, KH_2PO_4 – 1 g/l), YPD 1 (glucose – 20 g/l, yeast extract – 10 g/l, peptone – 10 g/l) and YPD 2 (glucose – 20 g/l, yeast extract - 10 g/l, peptone - 20 g/l). Cultivation was carried out under the following parameters: 30°C, 320 rpm, 24 hours. To obtain a cell-free aqueous yeast extract, the biomass was separated by centrifugation and washed three times to get rid of the culture medium remnants with bidistilled water. The resulting biomass was resuspended in sterile bidistilled water for 72 hours. The precipitate was separated by centrifugation, and the resulting cell-free extract was filtered through a sterile syringe filter with a 0.22 μm membrane. The biosynthesis of silver nanoparticles was carried out by adding a solution of silver nitrate ($AgNO_3$) to the final concentration of 0.5 mM or 1.5 mM to the cell-free aqueous extract of mutant yeast, after which the samples were kept under static conditions at 30, 35, 40, 45 and 50 °C. The synthesis of silver nanoparticles was confirmed by measuring the absorption spectra of the samples in the 350-650 nm wavelength range.

Results and discussion. During the experiment, a shift in color from transparent to dark brown was observed in all samples on the 15th day, which indicates the bioreduction of silver ions and formation of AgNPs. While analyzing the absorption

spectra of the studied samples, a pronounced absorption peak was recorded with an average wavelength at which the absorption maximum was recorded being about 420 nm, which indicated the presence of silver nanoparticles in the reaction mixture. The optical density value increased over time, which indicates an increase in the concentration of AgNPs in the solution [2].

It is worth of noting that the composition of the culture medium in which the yeast was cultivated significantly influenced the biosynthesis of nanoparticles. Thus, when using the cell-free extract obtained after the cultivation of mutant *S. cerevisiae* M437 on Reader's synthetic medium, the maximum optical density value at a concentration of 0.5 mM $AgNO_3$ was 1.5 times lower than when using YPD 1 medium at the same silver nitrate concentration. This effect can be explained by the fact that the mechanism of silver nanoparticles synthesis is based on the reduction of Ag^+ to Ag^0, in which the biomolecules of cell-free yeast extract, namely enzymes, take part. In turn, the qualitative and quantitative composition of such biomolecules significantly depends on the nature and concentration of the components in the culture medium for yeast cultivation [3]. As stated previously, YPD medium contains natural components such as peptone and yeast extract, which are absent in Reader's medium. Thus, it can be assumed, that the presence of these compounds in culture media is crucial for the synthesis of biomolecules that participate in the formation of AgNPs.

The light absorption spectra analysis of the samples kept at different temperatures indicates that the higher the biosynthesis temperature is, the higher the absorption maximum is obtained, and therefore the synthesis of nanoparticles proceeds better. For instance, the highest absorption value was recorded in samples with a biosynthesis temperature of 45 and 50 ℃. However, extremely high temperatures might inhibit the biosynthesis of silver nanoparticles [4]. For instance, during biosynthesis at a temperature of 50 ℃, a decrease in the light absorption maximum was observed over time, which may indicate a low stability of the process. Considering this, the most favorable temperature for the biosynthesis of AgNPs using a cell-free aqueous extract of mutant *S. cerevisiae* M437 yeast is 45 ℃.

Conclusions. In summary, we have investigated the process of silver nanoparticles biosynthesis under different temperature parameters using a cell-free aqueous extract of mutant yeast cultivated on culture media of different composition.

References:

1. Skrotska O. I., Koval R. V. Vplyv kontsentratsii nitratu sribla na biosyntez nanochastynok pry vykorystanni mutantnykh drizhdzhiv // Naukovi problemy kharchovykh tekhnolohii ta promyslovoi biotekhnolohii v konteksti reintehratsii: materialy XII Mizhnarodnoi naukovo-tekhnichnoi konferentsii, m. Kyiv, 7 lystopada 2023. – Kyiv: NUFT, 2023. P. 33.

2. Biosynthesis of silver nanoparticles mediated by culture filtrate of lactic acid bacteria, characterization and antifungal activity / A. Matei та ін. The EuroBiotech Journal. 2020. Т. 4, № 2. С. 97–103. URL: https://doi.org/10.2478/ebtj-2020-0011.

3. Rapid Synthesis of Silver Nanoparticles fromFusarium oxysporumby Optimizing Physicocultural Conditions / S. S. Birla та ін. The Scientific World Journal. 2013. Т. 2013. С. 1–12. URL: https://doi.org/10.1155/2013/796018.

4. Bolbanabad E. M., Ashengroph M., Darvishi F. Development and evaluation of different strategies for the clean synthesis of silver nanoparticles using Yarrowia lipolytica and their antibacterial activity. Process Biochemistry. 2020. Т. 94. С. 319–328. URL: https://doi.org/10.1016/j.procbio.2020.03.024.