Black Sea Journal of Health Science

doi: 10.19127/bshealthscience.1551540

Open Access Journal
e-ISSN: 2619 – 9041

Review
Volume 7 - Issue 6: 283-287 / November 2024

EFFECT OF ENVIRONMENTAL FACTORS ON THE PRODUCTION OF SILVER NANOPARTICLES BY YEAST STRAINS

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Abstract: In the presented work, the literature data on the influence of various environmental factors were analyzed on the formation of silver nanoparticles by yeast strains. According to literature information and our obtained results, it was determined that the optimal conditions for the synthesis of silver nanoparticles by the yeast strain *Saccharomyces ellipsoideus* BSU-XR1 were on the 21st day of incubation, on 4-6 days of incubation in different strains of *Saccharomyces cerevisiae*, and between 2-10 days in *Candida* strains. The optimal amount of wet biomass was between 8 and 10 g for *Candida* strains and 10 g for *Saccharomyces* strains. The temperature limit for *Saccharomyces* was observed at 25-35 °C, and for *Candida* at 25-37 °C. For strains, synthesis of silver nanoparticles was optimal in the pH range of 4-10, and pH range of 7 for *Candida* strains. Depending on the concentration of $AgNO_3$ (silver nitrate), salt, the optimal synthesis of silver nanoparticles occurred at concentrations of 0.5 and 1 mM for *Saccharomyces*, and 1 mM for *Candida*. The optimal incubation conditions for both types of strains were under dark environment.

Keywords: Yeast strains, Silver nanoparticles, Incubation time, Amount of biomass, Medium acidity (pH)

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Received: September 17, 2024
Accepted: October 25, 2024
Published: November 15, 2024

Cite as: Jafarov MM, Kariptaş E, Alkishiyeva KS. 2024. Effect of environmental factors on the production of silver nanoparticles by yeast strains. BSJ Health Sci, 7(6): 283-287.

1. Introduction

One of the recent advancements in bionanotechnology is the biological production of silver nanoparticles, which have potential applications in medicine, biotechnology, and the food industry. Therefore, the interaction between microorganisms and silver ions has gained significant attention. As a result, extensive research works have been conducted on obtaining silver nanoparticles from molds (Bhainsa and D'Souza, 2006; Prakasham et al., 2010; Abd El-Aziz., 2012; Guangquan et al., 2012; Honary et al., 2013; Ganbarov et al., 2014), bacteria (Gurunathan et al., 2009; Punjabi et al., 2012; Mousavi et al., 2020), and Actinomycetes (Ganbarov et al., 2016; Hasanova et al., 2017). It has been determined that, depending on the type of microorganism, silver nanoparticles can be produced directly by living cells, as well as by the cell-free culture fluid of the microbial culture (Bozkurt et al., 2017). It has been known that the microbiological synthesis of silver nanoparticles varies depending on the physicochemical parameters (for example, the age of the cell and its mass, temperature, environmental acidity (pH), incubation time, concentration of $AgNO_3$ salt) (Azadaliyeva et al., 2018; Ganbarov et al.,2018; Jafarov et al., 2020; Azadaliyeva et al., 2021). The microbiological synthesis of silver nanoparticles varies depending on several physicochemical parameters, such as cell age and biomass, temperature, environmental pH, incubation time and the concentration of $AgNO_3$.

There is enough information in the literature regarding the acquisition of silver nanoparticles by *Saccharomyces* (Kaler et al., 2013; Roy et., al 2014) and Candida yeasts (Revina et al., 2005; Hassan et al., 2013; Chauhan et al., 2014; Varquez et al., 2014; Saminathan, 2015).

The main goal of the presented article was to collect data on the influence of environmental factors on the formation of silver nanoparticles by yeast strains and to compare it with the strain of *Saccharomyces ellipsoideus* BSU-XR1 isolated from sour milk.

From the above literature data and from our experimental results, it is clear that the formation time of silver nanoparticles varies significantly depending on the yeast strain and species. Of course, this process primarily depends on the incubation conditions and the metabolic characteristic features of the fungal organism.

2. Effects on Silver Nanoparticles

2.1. Effect of Incubation Period on the Production of Silver Nanoparticles

The dependency of the incubation period was determined on the synthesis of silver nanoparticles by the yeast strain (Azadaliyeva et al, 2018).

It was found that on the 21st and 30th days of incubation $AgNO_3$ salt with the wet biomass of *Saccharomyces*

ellipsoideus strain BSU-XR1, the dark brown colored reaction mixture gave 420 nm wavelength absorption in the UV-VIS spectrophotometer. Scanning electron microscope analysis revealed that, the silver nanoparticles formed were spherical, with sizes ranging from 17.2 to 22.4 nm. During different incubation periods, each fungal strain exhibits a color change, which serves as an early indicator for the formation of silver nanoparticles, depending on the species and strain. For example, the reaction mixture of the commercial strain of *Saccharomyces cerevisiae* yeast started to darken from light yellow to brown after 4 days of incubation, and in the UV spectrophotometer, silver nanoparticles had an absorption at 450 nm wavelength. The reaction mixture of another strain of *Saccharomyces cerevisiae* yeast began to darken on the 6th day of incubation. At this time formed silver nanoparticles showed absorption at 430 nm wavelength (Baranova et al., 2005).

The formation of nanoparticles with the wet biomass of another strain of *Saccharomyces cerevisiae* (a yeast used in baking) has been studied. In this case, the formation of nanoparticles started after 24 h of incubation. The processed nanoparticles were in cuboidal shape, and had a very small size of 67.2 Å (angstroms**).** As a result of the study of the formation of silver nanoparticles with the culture liquid of another strain of the yeast *Saccharomyces cerevisiae,* it was determined that after 48 hours, nanoparticles with a spherical shape and a size of 25 nm were formed. In the UV spectrum, the absorption of nanoparticles was at a wavelength of 413 nm (Roy et al., 2014).

The production of silver nanoparticles by Candida yeasts was slightly different from that of Saccharomyces. The silver nanoparticles produced by the wet biomass of *Candida albicans NCIM-3100* strain during 48 h of incubation showed absorption at 420 nm wavelength in the UV spectrum. The size of silver nanoparticles varied between 20-60 nm and formed aggregates. The nanoparticles were spherical in shape. Another strain produced silver nanoparticles after 96 h of incubation, which showed absorbtion in the UV spectrum at 370 nm and had a size of 80nm. They were spherical in appearance. Silver nanoparticles produced by *Candida albicans* strain ATCCSC5314 within 72 h were 3-60 nm in size. In the UV spectrophotometer, these particles showed absorption at a wavelength of 415 nm. Another strain of yeast fungus *Candida albicans* darkened the color of the reaction mixture during 24 h of incubation and the formed silver nanoparticles had absorption at 400 nm wavelength in UV spectrophotometer. The size of nanoparticles was in the range of 20-100 nm and formed a conglomerate (Varquez et al., 2014; Mare et al., 2015).

The wet biomass of *Candida utilis NCIM3469* yeast strain started to form silver nanoparticles after 24 h of incubation. Silver nanoparticles had an absorbance at a wavelength of 401 nm in a UV spectrophotometer. Nanoparticles were spherical in shape and 20-80 nm in size. In the experiments conducted with the wet biomass of another strain of *Candida utilis*, silver nanoparticles were formed after 48 h. The size of 55% of processed silver nanoparticles was 2-3 nm, and the size of 45% was 8 nm. Nanoparticles showed absorption at a wavelength of 380 nm in a UV spectrophotometer. Cluster formation of silver nanoparticles was determined in the electron microscope (Baranova et al., 2005; Revina et al., 2005).

The wet biomass of *Candida guilliermondii BSU-217* and *Candida macedoniensis BSU-GA48* and *Candida macedoniensis BSU-MI44* yeast strains and the darkening of the reaction mixture color (from light yellow to dark brown) of $AgNO_3$ salt changed between 3-10 days of incubation. The synthesis of silver nanoparticles by the wet biomass of *Candida macedoniensis BSU-MI44* strain occurred on the 3rd-7th days of incubation, after the 7th day, the formation of nanoparticles weakened and after the 10th day of incubation, the formation of nanoparticles decreased sharply. Intensive formation of silver nanoparticles by *Candida guilliermondii BSU-217* yeast biomass occurred on 3-10 days of incubation, and then the process weakened, while *Candida macedoniensis BSU-MI44* yeast biomass was observed on 3-7 days of incubation. After day 7, the rate of nanoparticle formation slowed, and after day 10 of incubation, nanoparticle formation was dramatically reduced (Dzhafarov et al., 2016; Bozkurt et al., 2017; Ganbarov et al., 2019).

2.2. Effect of Biomass Amount on the Biosynthesis of Silver Nanoparticles

It has been determined that the amount of wet biomass plays a crucial role in the synthesis of silver nanoparticles by yeast fungi (Dzhafarov et al., 2016; Azadaliyeva et al., 2021). Thus, *Saccharomyces ellipsoideus BSU-XR1* strain incubated with different biomasses during analysis in a UV spectrophotometer in the reaction mixture of 5 g of biomass and silver nitrate salt at a wavelength of 400 nm suitable for silver nanoparticles, at a wavelength of 420 nm at 10 g, and at a wavelength of 15 g It had absorption at a wavelength of 415 nm. The scanning electron microscope showed that the silver nanoparticles have a spherical shape and different sizes. The size of nanoparticles formed varied with the amount of biomass: 8.6-16.9 nm with 5g of biomass, 22.4nm with 10g, and 17.2nm with 15g. Therefore, depending on the amount of wet biomass, the size of the silver nanoparticles formed was also different. The optimal biomass of *Saccharomyces ellipsoideus BSU-XR1* strain for the synthesis of silver nanoparticles was 10 g.

It is clear from the literature that the optimal biosynthesis of silver nanoparticles in *Candida albicans NCIM-3100* strain was carried out in 10 grams of wet biomass, in another strain of *Candida albicans* and in yeast fungus *Candida glabrata*, the formation of silver nanoparticles was also carried out with 10 g of wet biomass. Some authors have used 8-10 g of wet biomass in the production of silver nanoparticles by the yeast *Candida albicans* (Varquez et al., 2014; Mare et al., 2015).

The formation of silver nanoparticles was manifested in 8 g of wet biomass of yeast strain *Candida utilis NCIM3469*, and in 8-9 g of another strain of *Candida utilis* species (Revina et al., 2005).

2.3. Effect of Temperature on the Biosynthesis of Silver Nanoparticles

The temperature factor has a great role in microbial cultures synthesizing silver nanoparticles (Dzhafarov et al., 2017; Ganbarov et al., 2018).

The production of silver nanoparticles by *Saccharomyces ellipsoideus BSU-XR1* yeast was studied depending on the temperature, and it was determined that the formation of silver nanoparticles was observed in mixtures incubated at 25 °C and 30 °C due to color change. Spectrophotometric analysis of the taken samples showed that absorption (peak) at 410-413 nm wavelength was observed in the samples incubated at 25 °C and 30 °C. Scanning electron microscopy revealed the presence of spherical silver nanoparticles, with sizes 22.4 nm and 17.2 nm. It was found that the optimal temperature limit for this strain to synthesize silver nanoparticles is in the range of 25-30 °C.

It is known from the literature that the formation of silver nanoparticles in *Candida albicans NCIM-3100* strain occurs at a temperature of 25 °C, in another strain of the yeast *Candida albicans* and in the yeast *Candida glabrata* - at a temperature of 37 °C, in another strain of the yeast *Candida albicans* - at a temperature of 30 °C, in *Candida albicans ATCC SC5314* strain – 37 °C, formation of nanoparticles with *Candida albicans* fungus culture liquid occurred at 30°C temperature, in *Candida glabrata ATCC90030, Candida krusei ATCC6258, Candida albicans ATCC90028* strains - at 35 °C temperature. Formation of nanoparticles in *Candida utilis NCIM 3469* strain at 30 °C, in another strain of this species – at 28 °C, in *Candida diversa JA1* strain – at 28 °C, in *Candida guillermondi* fungus at 30°C, in yeast fungi *Candida utilis 22* and *Kluyveromyces marxianus* – recorded at 30 °C. The optimal temperature for the formation of silver nanoparticles in *Candida guillermondi BDU-217 strain* was 25 °C, and in *Candida macedoniensis BDU-MI44* strain it was 30 °C (Mare et al., 2015; Bozkurt et al., 2017; Ganbarov et al., 2019).

Depending on the species and strain, the formation of nanoparticles in *Saccharomyces* yeast fungi took place under different temperature conditions. For example, the yeast strain *Saccharomyces cerevisiae* used in baking used silver nanoparticles at a temperature of 30°C, but another fungus *S. cerevisiae* used in baking carried out this process optimally at a temperature of 35°C, some strains of the species *Saccharomyces cerevisiae* used silver nanoparticles at 25 °C and 27 °C formed at temperatures. The optimum temperature for the formation of silver nanoparticles for the extrophilic yeast *Saccharomyces cerevisiae* was 22 °C. Optimal synthesis of silver nanoparticles by the yeast *Saccharomyces bourlardii* took place at a temperature of 35 °C. Thus, it is clear from the above literature data that depending on the type and strain of the fungus, the optimal temperature for the formation of silver nanoparticles can change (Baranova et al., 2005; Roy et al., 2014).

2.4. Effect of Initial Environmental Acidity on the Biosynthesis of Silver Nanoparticles

As an environmental factor, initial environmental acidity also has an effect on the production of silver nanoparticles by yeast fungi (Jafarov et al., 2020). The formation of silver nanoparticles by *Saccharomyces ellipsoideus BSU-XR1* strain was determined depending on the acidity of the environment, and the color change in the reaction mixtures was noticeable starting from the 7th day of incubation. During the spectrophotometric analysis, it was determined that the absorption peak between the wavelength of 408-412 nm was observed in the reaction mixtures with pH of 6.0, 7.0, 8.0. The silver nanoparticles produced by this strain at pH 7.0 were characterized by their spherical appearance in the scanning electron microscope. The size of nanoparticles was equal to 22.4 nm.

It is known from the literature that silver nanoparticles can be actively synthesized during the incubation of *Saccharomyces cerevisiae* yeast cells with silver nitrate salt when the pH of the solution is above 8. Metabolism products collected by the yeast played the role of regulator for the formed silver nanoparticles. It is believed that silver nanoparticles are formed as a result of the reduction of silver ions by secretion, and this is one of the possible causes of formation. The research showed that, microbial cultures can synthesize a higher quantity of silver nanoparticles in alkaline environments compared to acidic ones. When the acidity of the environment exceeds pH 10, it causes the death of cells. Microorganisms synthesize more nanoparticles under neutral alkaline conditions than under acidic conditions. *Saccharomyces cerevisiae*, a yeast used in baking, was able to synthesize silver nanoparticles in a wide pH range (pH 4-10). Optimum biosynthesis of silver nanoparticles for extrophilic yeast was pH 2.5. Although the yeast *Saccharomyces boulardii* can synthesize silver nanoparticles in a wide pH range, the optimal pH was 7 (Baranova et al., 2005, 26, Varquez et al., 2014). *Candida guillermondi BSU-217* and *Candida macedoniensis BSU-MI44* yeast fungi were also able to synthesize silver nanoparticles in the pH range of 7 (Mare et al., 2015; Bozkurt et al., 2017).

In general, it can be noted that the optimal biosynthesis of silver nanoparticles in *Saccharomyces* and *Candida* yeasts occurs mainly in a neutral environment (pH 7). The results obtained using the strain *Saccharomyces ellipsoideus BSU-XR1* confirm this again.

2.5. Effect of $AgNO_3$ Salt Concentration on the Biosynthesis of Silver Nanoparticles

According to Azadaliyeva et al., (2021), yeast strains produce silver nanoparticles depending on the concentration of $AgNO_3$ salt. The synthesis of silver nanoparticles by *Saccharomyces ellipsoideus BSU-XR1* yeast cells was observed on the 7th day of incubation in

the reaction mixtures containing 0.5 and 1.0 mM silver nitrate. During the spectrophotometric analysis, absorption peaks at 405 and 408 nm wavelength were observed in the samples of silver nitrate salt incubated at concentrations of 0.5 and 1.0 mM. The scanning electron microscope showed that the silver nanoparticles have a spherical shape and different sizes. The size of nanoparticles was equal to 22.4 nm in 0.5 mM mixture of silver nitrate salt and 35.5 nm in 1.0 mM concentration. These results are similar to those obtained in the yeast *Saccharomyces cerevisiae.* Thus, the size of the silver nanoparticles produced by this fungus at concentrations of 0.5 and 1.0 mM silver nitrate salt was 34.2 nm and 37.5 nm, respectively.

Various strains of the baking fungus *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have produced silver nanoparticles at 1.0 mM concentrations of silver nitrate salt (Baranova et al., 2005; Kaler et al., 2013; Roy et al., 2014). The extromophilic yeast was tolerant to a higher concentration of silver nitrate salt and was able to form silver nanoparticles at a concentration of 0.3 mM of AgNO3 salt (Ganbarov et al., 2014). *Candida yeasts* were able to produce silver nanoparticles at concentrations of 1.0 mM of silver nitrate salt (Hassan et al., 2013; Mare et al., 2015; Bozkurt et al., 2017; Ganbarov et al., 2019).

It is known that silver nitrate salt has a toxic effect on microorganisms. So, yeast fungi studied by various researchers were able to produce silver nanoparticles at a very low concentration (0.1-0.3 mM) of silver nitrate salt.

The effect of dark and light factor on the formation of silver nanoparticles is great (Dzhafarov et al., 2021). Incubation of the reaction mixture of silver nitrate salt with wet biomass of *Saccharomyces ellipsoideus BSU-XR1 strain* was carried out in two environments (dark and light). As a result of the analysis of the reaction mixture of this strain with silver nitrate salt in the UV-spectrophotometer, it was determined that the absorption peak was observed at the wavelength of 414 nm in the sample incubated in the dark environment, and at the wavelength of 409 nm in the sample incubated in the light environment. However, when viewed under a scanning electron microscope, spherical silver nanoparticles with a diameter of 17.2 nm were identified in a sample incubated in a dark environment and silver nanoparticles were not observed in the sample incubated in the light environment.

It should be noted that microbiological synthesis of silver nanoparticles was mainly carried out in dark conditions (in a thermostat) (Khudaverdi et al., 2013; Dzhafarov et al., 2021).

3. Discussion and Conclusion

Thus, the optimal incubation periods were reported as the 21th day for *Saccharomyces ellipsoideus BSU-XR1*, between the 4th and 6th days for various strains of *Saccharomyces cerevisiae,* and between the 2nd and 10th days for *Candida* strains. The optimal amount of wet biomass was 10 g for *Saccharomyces* strains and 8-10 g for *Candida* strains.

The temperature limit for *Saccharomyces* was observed at 25-35 °C, and for *Candida* at 25-37 °C. Synthesis of silver nanoparticles was in the pH range of 4 to 10 for *Saccharomyces* strains, and pH 7 was optimal for *Candida* strains. Depending on the concentration of $AgNO_3$ salt, optimal synthesis of silver nanoparticles occurred at concentrations of 0.5 and 1 mM for *Saccharomyces* and 1 mM for Candida. The most suitable incubation conditions for both yeasts turned out to be a dark environment.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	M.M.J.	E.K.	K.S.A.
C	34	33	33
D	34	33	33
S	34	33	33
DCP	34	33	33
DAI	34	33	33
L	34	33	33
W	34	33	33
CR	34	33	33
SR	34	33	33

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

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