# Biosynthesis and characterization of selenium nanoparticles by *Saccharomyces cerevisiae* M437

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## Abstract

**Introduction.** Biosynthesized selenium nanoparticles (SeNPs) hold significant promise for applications in the food industry due to their antimicrobial and antioxidant properties.

**Materials and methods.** The yeast strain *Saccharomyces cerevisiae* M437 was cultivated in yeast peptone dextrose medium. The SeNPs biosynthesis conditions included: *Saccharomyces cerevisiae* M437 biomass, 1 mM sodium selenite ($Na_2SeO_3$), 30°C, 250 rpm, for 7 days. Nanoparticle formation was assessed using light microscopy, UV-visible spectroscopy, and dynamic light scattering (DLS) analysis.

**Results and discussion.** A sodium selenite concentration of 1 mM was selected as optimal, as it ensured high bioreduction efficiency with minimal cytotoxic effects on *Saccharomyces cerevisiae* M437 cells. Throughout the 7-day biosynthesis process, a visible color shift of the biomass was observed from milky white to pink, and finally to deep red, serving as a visual indicator of intracellular SeNPs formation *via* the biological reduction of selenite ions to elemental selenium, one of the allotropes of which exhibits a red color. Light microscopy confirmed the presence of fine particulate inclusions both inside and outside the cells, indicating efficient nanoparticle formation. For more detailed characterization of SeNP morphology and internal structure, scanning transmission electron microscopy (STEM) are planned in future studies.

UV-Vis spectral analysis of the biogenic SeNPs revealed a distinct absorption peak at 252 nm, associated with electronic transitions and surface plasmon resonance. DLS measurements showed an average hydrodynamic diameter of 179.3 nm. The zeta potential value of –16.2 mV suggests moderate colloidal stability, while the polydispersity index of 0.229 indicates a narrow size distribution and low tendency toward agglomeration. The organic capping layer, likely composed of biomolecules derived from yeast cells, contributes to the stability and bioactivity of the nanoparticles. The obtained results are consistent with literature reports on SeNPs synthesized by microorganisms and demonstrate the promise of this biotechnological approach.

**Conclusions.** The intracellular biosynthesis of selenium nanoparticles by *Saccharomyces cerevisiae* M437 was confirmed. The core physicochemical properties of the synthesized nanoparticles were characterized, demonstrating their potential suitability for applications in the food industry.

## Introduction

Selenium nanoparticles (SeNPs) exhibit significant potential for applications in the food industry due to their antimicrobial and antioxidant properties. SeNPs are effective against a broad spectrum of foodborne pathogenic microorganisms, including *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* (Hussein et al., 2022), *Listeria monocytogenes*, *Salmonella enterica* (Abdel-Moneim et al., 2022), *Vibrio parahaemolyticus* (Vinu et al., 2021; Zhang et al., 2021), *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella pneumoniae* (Huang et al., 2020), as well as fungi such as *Fusarium verticillioides*, *Fusarium graminearum,* and *Alternaria alternata* (Hu et al., 2019). These antimicrobial properties enable the use of SeNPs in the production of active food packaging, which can potentially extend the shelf life of food products.

Biopolymers are commonly used as carriers for SeNPs due to their biocompatibility and eco-friendliness (Ao et al., 2023; Ndwandwe et al., 2021). Biopolymer films serve as an alternative to synthetic packaging materials (Jamróz et al., 2019a). For instance, films incorporating SeNPs have been shown to extend the shelf life of hazelnuts, walnuts, potato chips, ham, chicken, and ready-to-eat vegetable mixes (Vera et al., 2018). Similarly, the preservation of freshness of mini-kiwi (Jamróz et al., 2019a) and fish products (Jamróz et al., 2019b) has been demonstrated. SeNPs embedded in polymer matrices damage bacterial cell walls, disrupt DNA structure, and induce apoptosis, exhibiting biocidal activity even against multidrug-resistant strains. Additionally, SeNPs demonstrate the antioxidant activity by reducing oxidative processes in food products, thereby preventing spoilage. Although no specific regulatory framework exists for SeNPs in food packaging, their biocompatibility and efficacy warrant further research in this field (Ndwandwe et al., 2021).

Beyond food packaging, selenium nanoparticles are actively investigated as feed supplements for livestock (Zhang et al., 2023). This approach not only supports animal health and productivity but also enhances selenium intake in human diets through increased selenium content in meat, milk, and eggs (Malyugina et al., 2021). Positive effects of SeNPs on weight gain in broiler chickens (Nabi et al., 2020) and growth in various fish species (Jahanbakhshi et al., 2021), including Nile tilapia (Ghazi et al., 2021; Ibrahim et al., 2021) and grass carp (Zhang et al., 2022), have been reported.

Selenium nanoparticles can be synthesized *via* physical, chemical, or biological methods (Zhang et al., 2023). For biogenic synthesis, microorganisms such as fungi, bacteria, and yeast are utilized (Dutta and Ray, 2024; Gregirchak et al., 2024; Stabnikova et al., 2023).

Selenium nanoparticle biosynthesis has also been explored using different yeast species (Table 1). For example, *Kluyveromyces lactis* GG799 (Song et al., 2021), *Candida utilis* ATCC 9950 (Kieliszek et al., 2020), marine yeast *Yarrowia lipolytica* NCIM 3589 (Hamza et al., 2017), and genetically modified yeast *Pichia pastoris* (Elahian et al., 2017) have been utilized. Lashani et al. investigated SeNPs biosynthesis using *Yarrowia lipolytica* ATCC 18942, demonstrating antimicrobial activity against *Serratia marcescens*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, as well as anti-biofilm activity against *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The biogenic SeNPs also exhibited antioxidant activity (Lashani et al., 2024). SeNPs produced using the supernatant of *Candida albicans* TIMML-1306 showed significant antifungal effects against *Candida albicans* and *Candida glabrata* (Bafghi et al., 2022). In the case of selenium nanoparticles biosynthesis using cell-free aqueous extracts of *Magnusiomyces ingens* LH-F1, inhibition of *Arthrobacter* sp. W1 growth was observed (Lian et al., 2019).

**Table 1**

**Biosynthesis of selenium nanoparticles using yeast species**

| Yeast | SeNPs biosynthesis parameters | SeNPs characteristics | Source |
|---|---|---|---|
| *Pichia pastoris* | YPD broth, 4 mM $SeO_2$, 30°C, 96 h | Intracellular, spherical, size 70–180 nm | Elahian et al., 2017 |
| *Yarrowia lipolytica* NCIM 3589 | MGYP broth, 4 mM $Na_2SeO_3$, 30°C, 120 rpm, 48 h | Intracellular, spherical, size 30–60 nm | Hamza et al., 2017 |
| *Yarrowia lipolytica* ATCC 18942 | GYP broth medium, 2.5 mM $Na_2SeO_3$, 30°C, 160 rpm, 48 h | Intracellular, spherical, size 37–180 nm | Lashani et al., 2024 |
| *Candida albicans* TIMML-1306 | Supernatant after yeast cultivation was added to 5 mM $Na_2SeO_4$, 28°C, 24 h | Spherical, average diameter size 38 nm | Bafghi et al., 2022 |
| *Candida utilis* ATCC 9950 | YPD medium, 30 mg $Na_2SeO_3$, 200 rpm, 24 h | Intracellular, spherical, size 20–40 nm | Kieliszek et al., 2020 |
| *Magnusiomyces ingens* LH-F1 | Cell-free extract from cell lysate, 2 mM $SeO_2$, pH 7, 30°C | Spherical/quasi–spherical, size 70–90 nm | Lian et al., 2019 |
| *Nematospora coryli* | Yeast biomass, 1 mM $Na_2SeO_3$, 28°C, 130 rpm, 48 h | Intracellular, spherical, size 50–250 nm | Rasouli, 2019 |
| *Kluyveromyces lactis* GG799 | YPD broth with yeast biomass, 1.2 mM $Na_2SeO_3$, 30°C, 200 rpm, 24 h | Intracellular, spherical, size 80–150 nm | Song et al., 2021 |

Note: YPD, Yeast extract, peptone, dextrose; MGYP, Malt extract, glucose, yeast extract, peptone; GYP, Glucose, yeast, peptone.

However, each microbial system has its advantages and limitations. One of the key advantages of using yeast, particularly *Saccharomyces species*, is their ease of handling and inherent safety, as they do not require specialized biosafety measures unlike many bacterial and fungal strains (Ao et al., 2023; Grasso et al., 2020).

*Saccharomyces cerevisiae* yeast is one of the most widely used organisms for SeNPs biosynthesis (Table 2) due to its availability and metabolic versatility. The intracellular biosynthesis of SeNPs has been studied using baker's yeast *Saccharomyces cerevisiae* and their antioxidant activity has been confirmed (Faramarzi et al., 2020; Fath-Alla et al., 2024). Cultivation of *Saccharomyces cerevisiae* GVT263 on agricultural waste resulted in the biosynthesis of crystalline rod-shaped SeNPs (Goud et al., 2016). SeNPs with antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,

*Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus subtilis* were produced using *Saccharomyces cerevisiae* MTCC 36. The antibacterial activity of SeNPs is attributed to their ability to disrupt bacterial cell walls and generate reactive oxygen species, inducing oxidative stress in microorganisms (Hariharan et al., 2012). Controlled intracellular SeNPs biosynthesis, followed by their release from cells, was studied using *Saccharomyces cerevisiae* BY4741. SeNPs transport out of cells occurs via exocytosis or vesicular secretion (Pereira et al., 2018). Additionally, the biosynthesis of SeNPs under oxygen-limited conditions using *Saccharomyces cerevisiae* yielded nanoparticles with a protein coating containing carbonyl and hydroxyl groups, thereby enhancing their hydrophilicity and stability (Zhang et al., 2012).

**Table 2**

**Biosynthesis of selenium nanoparticles using yeast *Saccharomyces cerevisiae***

| Yeast | SeNPs biosynthesis parameters | SeNPs characteristics | Reference |
|---|---|---|---|
| *S. cerevisiae* | SDB medium, 25 µg $Na_2SeO_3$, 32°C, 120 rpm, 96 h | Intracellular, spherical, size 709 nm | Faramarzi et al., 2020 |
| *S. cerevisiae* | SDB medium, 0.025 g $Na_2SeO_3$, 30°C, 24 h | Intracellular, spherical, size 34-125 nm | Fath-Alla et al., 2024 |
| *S. cerevisiae* GVT263 | Agro-waste based yeast fermented broth, 2 mM $Na_2SeO_3$, 18-20°C, 24 h | Intracellular, rod-shaped, size 170-240 nm | Goud et al., 2016 |
| *S. cerevisiae* BY4741 | YNB medium, 1 mM $Na_2SeO_3$, 30°C, 150 rpm, 48 h | Intracellular, spherical, size 20-30 nm | Pereira et al., 2018 |
| *S. cerevisiae* | Malt juice, 100 mg $Na_2SeO_3$, pH 4, 30°C, 36 h | Intracellular, spherical, size 71.14 ± 18.17 nm | Wu et al., 2021 |
| *S. cerevisiae* | YPD medium, 0.5 mM $Na_2SeO_3$, 30°C, 120 rpm, 36 h | Extracellular, spherical, size 100 nm | Zhang et al., 2012 |
| *S. cerevisiae* MTCC 36 | MY medium, 2 mM $Na_2SeO_3$, 30°C, 200 rpm, 24 h | Crystalline, size 30-100 nm | Hariharan et al., 2012 |

Note: SDB, Sabouraud dextrose broth; YNB, Yeast nitrogen base; MY, Malt yeast.

The aim of the present study was to investigate the characteristics of selenium nanoparticles produced using *Saccharomyces cerevisiae* M437 for potential applications in the food industry.

## Materials and methods

### Culture media for storage and cultivation of *Saccharomyces cerevisiae* M437

**Medium 1** for storage in slant agar tubes and subculturing of *Saccharomyces cerevisiae* M437: Sabouraud medium, consisting of 9 g/L enzymatic peptone, 1 g/L yeast extract, 40 g/L glucose, and 15 g/L microbiological agar, pH 5.6.

**Medium 2** for cultivating *Saccharomyces cerevisiae* M437 for intracellular SeNPs biosynthesis: YPD (Yeast Peptone Dextrose) medium, consisting of 20 g/L peptone, 10 g/L yeast extract, and 20 g/L glucose, pH 6.3.

### Cultivation of yeast *Saccharomyces cerevisiae* M437

The *Saccharomyces cerevisiae* M437 strain was selected from a live culture collection for use in the experiments. This strain was stored in a laboratory refrigerator at 4°C in test tubes containing slanted agar (Medium 1). For inoculum preparation, yeast cells were washed from the agar surface to obtain a suspension with a concentration of $10^5$-$10^6$ cells/mL. The resulting inoculum, constituting 5% of the medium volume, was added to 100 mL of pre-sterilized liquid YPD medium (Medium 2). The liquid medium was prepared by mixing and dissolving the appropriate components in distilled water, followed by sterilization in an autoclave at 121°C for 15 minutes. Cultivation of the inoculum was carried out in 250 mL Erlenmeyer flasks containing 100 mL of liquid YPD medium at 30°C and 250 rpm for 48 hours until an optical density at 600 nm ($OD_{600}$) of 2.5 was reached.

### Intracellular biosynthesis of selenium nanoparticles

**Obtaining yeast biomass.** After 48 hours of cultivation, *Saccharomyces cerevisiae* M437 biomass was harvested by centrifugation (4000 rpm, 20 min). To remove soluble medium components and extracellular metabolites, the cells were washed three times with sterile distilled water. The supernatant was discarded, and sterile distilled water was added to the cell pellet. The cells were resuspended and centrifuged again (4000 rpm, 20 min). This process was repeated three times.

**Preparation of sodium selenite as a precursor for selenium nanoparticles.** A 1 M sodium selenite ($Na_2SeO_3$) solution was prepared by dissolving 17.2948 g of $Na_2SeO_3$ salt in 100 mL of distilled water. After complete dissolution, the solution was sterilized via cold filtration using a sterile syringe filter with a 0.22 μm pore size. The 1 M sodium selenite solution was stored in a sterile glass tube.

**Biosynthesis procedure.** The washed yeast cells were resuspended in 100 mL of sterile distilled water in a 250 mL flask, and the sodium selenite solution was added to achieve a final concentration of 1 mM. Intracellular SeNP biosynthesis was carried out under the following conditions: 30°C, 250 rpm using an ES-20/60 Biosan SIA orbital shaker-incubator (Latvia). For the first control, a flask with washed yeast in sterile distilled water without sodium selenite was used. For the second control, a flask with a 1 mM sodium selenite solution was used. Observations were conducted over seven days, during which the cell color changed from milky white to pink and then red, indicating nanoparticle formation.

### Characteristics of biosynthesized selenium nanoparticles

**Light microscopy.** For microscopy of *Saccharomyces cerevisiae* M437, a "crushed drop" preparation was made. Samples were collected after seven days of intracellular SeNPs biosynthesis. Under aseptic conditions, 2 mL of cell suspension was collected, and a few drops were placed on a microscope slide and stained with methylene blue. A coverslip was placed on top, and excess moisture was removed with filter paper. A drop of glycerin was applied to the coverslip, and microscopy was performed using an XS-3330 LED MICROmed light microscope (China) with an immersion objective (total magnification: ×1000).

**UV-Visible spectroscopy.** On day seven, the SeNPs biosynthesis process was terminated. Yeast cells were separated by centrifugation (5000 rpm, 40 min). The supernatant was analyzed using a UV-Vis spectrophotometer (Thermo Spectronic UV300, Spectronic Unicam, England). Four milliliters of the supernatant were transferred in a quartz cuvette, and the absorption spectrum was measured in the wavelength range of 200-800 nm with a resolution of 2 nm (Hashem et al., 2023).

**The hydrodynamic diameter determination.** The hydrodynamic diameter of biosynthesized SeNPs was measured using a Zetasizer Nano ZS (Malvern, UK), based on dynamic light scattering (DLS). Zeta potential and polydispersity index (PDI) were also determined. All measurements were performed at a constant temperature of 25°C in a neutral medium (pH = 7.0). Each analysis was repeated three times to ensure the reliability of the results (Nwoko et al., 2021).

## Results and discussion

### Selection of sodium selenite concentration

One of the key parameters influencing the efficiency of selenium nanoparticle biosynthesis is the concentration of the precursor – sodium selenite ($Na_2SeO_3$). Based on an extensive review of current scientific literature on microbial SeNP biosynthesis, a concentration of 1 mM $Na_2SeO_3$ is widely regarded as optimal for many microorganisms (Table 3).

This concentration balances high selenite reduction efficiency with minimal toxicity to cells. For instance, using *Acinetobacter* sp. SW30 biomass, amorphous SeNPs with antioxidant and antimicrobial properties were synthesized at 1.0 mM sodium selenite. However, at concentrations above 2 mM, crystalline nanorods with antitumor activity were produced but exhibited high cytotoxicity (Wadhwani et al., 2017). Another study describing intracellular SeNP biosynthesis using the culture medium of *Saccharomyces cerevisiae* reported that lower concentrations of selenite salts produced smaller SeNPs with higher antioxidant activity. Nevertheless, the overall process efficiency was reduced due to low yield, limited productivity, and high polydispersity (Faramarzi et al., 2020). Additionally, Shoeibi and Mashreghi (2017) investigated SeNP biosynthesis using *Enterococcus faecalis* at sodium selenite concentrations ranging from 0.19 mM to 2.97 mM. Higher precursor concentrations resulted in the formation of fewer nanoparticles.

**Table 3**

**Sodium selenite concentrations for selenium nanoparticle biosynthesis**

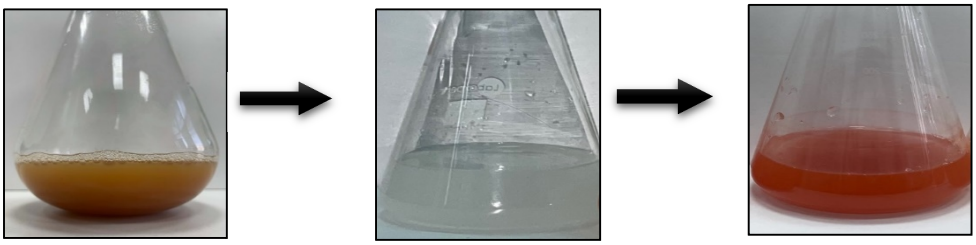
| Microorganism | Culture medium | SeNPs biosynthesis conditions | Reference |
|---|---|---|---|
| *Bacillus subtilis* AL43 | LB broth | pH 7,2, 30°C, 150 rpm, 24 h, 1 mM $Na_2SeO_3$ at the beginning of the cultivation process | Abdel-Moneim et al., 2022 |
| *Streptomyces* sp E3 | NB | 32°C, 180 rpm, 72 h, 1 mM $Na_2SeO_3$, biomass | El-deeb et al., 2023 |
| *Vibrio natriegens* ATCC14048 | LB broth | 30°C, 12 h, 1 mM $Na_2SeO_3$ at the beginning of the cultivation process | Fernández-Llamosas et al., 2017 |
| *Paenibacillus terreus* | TSB broth | 37°C, 150 rpm, 24 h, 1 mM $Na_2SeO_3$ at the beginning of the cultivation process | Nile et al., 2023 |
| *Saccharomyces cerevisiae* BY4741 | YNB, added with amino acids | 30°C, 150 rpm, 48 h, 1 mM $Na_2SeO_3$ at the beginning of the cultivation process | Pereira et al., 2018 |
| *Streptomyces* sp. M10A65 | ISP2 medium | 28°C, 120 rpm, 24-48 h, 1 mM $Na_2SeO_3$, biomass | Ramya et al., 2020 |
| *Nematospora coryli* | YPG broth | 28°C, 130 rpm, 48 h, 1 mM $Na_2SeO_3$, biomass | Rasouli, 2019 |
| *Lysinibacillus macroides* DS15 | LB broth | 35°C, 180 rpm, 36 h, 1 mM $Na_2SeO_3$ at the beginning of the cultivation process | Zhang et al., 2019 |

Note: LB, Luria-Bertani; YPG, Yeast extract, peptone, dextrose; TSB, Tryptone-soy; YNB, Yeast nitrogen base; ISP2, International Streptomyces Project-2 medium, containing yeast extract, malt extract, and dextrose.

Based on this evidence, a sodium selenite concentration of 1 mM was selected for selenium nanoparticle biosynthesis using *Saccharomyces cerevisiae* M437. Although yeast holds significant promise for SeNPs biosynthesis, the number of studies in this area remains limited (Nie et al., 2023).

**Intracellular biosynthesis of selenium nanoparticles**

After adding sodium selenite to *Saccharomyces cerevisiae* M437 cells, a color change from milky white to light pink was observed on the second day. Over the seven-day observation period, the color intensified, reaching a distinct red by the seventh day (Figure 1).



**a** **b** **c**

**Figure 1. Stages of intracellular biosynthesis of selenium nanoparticles by *Saccharomyces cerevisiae* M437: a – native yeast grown on YPD medium; b – washed yeast biomass; c – yeast on day 7 of nanoparticle biosynthesis**

The color change from milky to red indicates intracellular SeNP formation, resulting from the biological reduction of selenite oxyanions ($SeO_3^{2-}$) to elemental selenium ($Se^0$), one of whose allotropic forms exhibits a characteristic red color. Intracellular yeast metabolites facilitate this process (Tan et al., 2018). Similar results have been reported in studies where SeNP biosynthesis was accompanied by characteristic color changes. For instance, the formation of biogenic SeNPs using the supernatant of *Anabaena variabilis* NCCU-441 was confirmed by a color change from sky blue to orange-red (Afzal et al., 2021). SeNPs biosynthesis using *Lactobacillus paracasei* HM1 (El-Saadony et al., 2021) and *Bacillus subtilis* (Chandramohan et al., 2018) resulted in culture medium color changes from yellow to dark red within 48 hours. Likewise, using *Saccharomyces cerevisiae* culture fluid, SeNP formation was indicated by a color change from pale yellow to dark orange within four days (Faramarzi et al., 2020).

On days 5–7, biosynthesized intracellular SeNPs began to release in the solution. It was indicated by the pale red color of the supernatant after centrifugation. Literature analysis confirmed this observation. Pereira et al. demonstrated that SeNPs were synthesized intracellularly by *Saccharomyces cerevisiae* BY4741 within 48 hours and were subsequently released into the extracellular medium by 96 hours without compromising cell integrity (Pereira et al., 2018). Further analysis showed that intracellular SeNPs were concentrated near or protruded from the cell membrane, and extracellular SeNPs possessed well-defined organic shells. The presence of extracellular nanoparticles suggests a vesicular transport mechanism, where yeast cells expel excess selenium via vesicle-like structures (Wu et al., 2021). This intracellular biosynthesis and subsequent extracellular release is advantageous, as it simplifies nanoparticle isolation and purification compared to solely intracellular biosynthesis, which requires cell disruption methods such as ultrasonication, enzymatic cell wall degradation, mechanical disruption with glass or metal beads, or chemical lysis using bases and anionic detergents (Sajnóg et al., 2023). Studies on using *Yarrowia lipolytica* also showed that SeNPs are synthesized intracellularly, are associated with cell membranes, and may be localized in various cellular compartments, indicating the presence of multiple nucleation sites for selenite reduction and intracellular nanoparticle formation in *Yarrowia lipolytica* cells (Lashani et al., 2024).

The light microscopy was performed on day 7 to confirm SeNP formation and localization. Analysis revealed that, compared to native yeast cells with homogeneous cytoplasm, samples with synthesized SeNPs exhibited scattered or clustered inclusions within and outside the cells, potentially indicating SeNPs and their localization (Figure 2). Future studies are planned to employ scanning electron microscopy (SEM) to determine the detailed morphology of the synthesized nanoparticles and their interactions with cellular organelles. SEM enables rapid acquisition of high-resolution 3D images of nanoparticle surfaces with minimal sample preparation. In addition, transmission electron microscopy (TEM) will be used to provide ultra-high-resolution imaging for analyzing the internal structure of the formed nanoparticles (Kumar, 2021).

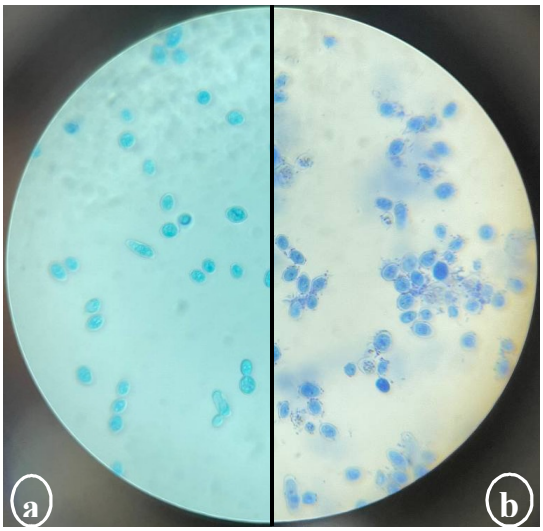



**Figure 2. Cells of *Saccharomyces cerevisiae* M437:**
**a – native yeast cells;**
**b – yeast cells with biosynthesized selenium nanoparticles; magnification ×1000**

**UV-Visible spectroscopy**

UV-vis spectroscopy is a common method to confirm SeNP formation by detecting their characteristic red color absorbance at specific wavelengths, attributed to surface plasmon resonance (SPR) excitation – an effect that arises when conduction electrons in a thin metallic layer are excited by incident light (Fath-Alla et al., 2024). During the SeNPs biosynthesis process, we visually observed a color change not only in the yeast cells but also in the reaction mixture itself, suggesting the release of SeNPs into the extracellular medium. UV-Vis spectroscopy analysis revealed distinct absorption peaks within the 200–800 nm wavelength range, with two maxima at 207 and 252 nm (Figure 3).

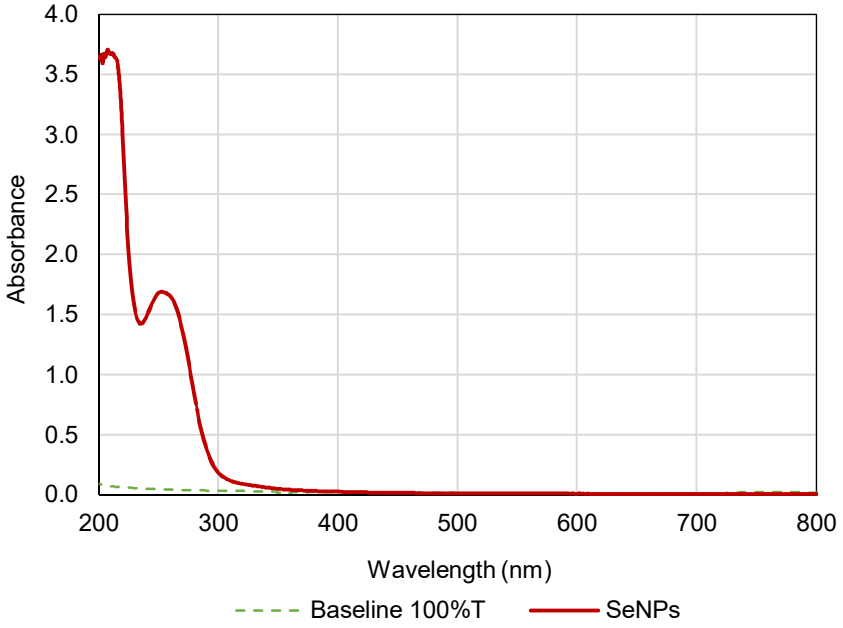



**Figure 3. UV-Vis spectrum of biosynthesized selenium nanoparticles using *Saccharomyces cerevisiae* M437**

The results (Figure 3) are consistent with earlier findings on SeNP biosynthesis using the supernatant of *Aspergillus terreus*, where absorbance measurements in the 200–800 nm range showed two major peaks at 218 and 284 nm. The first absorbance peak may correspond to interband and fundamental electronic transitions, such as transitions from lower energy levels to the conduction or upper bands, while the second peak is likely associated with coherent oscillation of free electrons across the nanoparticle surface known as SPR (Saied et al., 2023). Similarly, biosynthesized SeNPs obtained using a cell-free aqueous extract of *Monascus purpureus* ATCC16436 showed a maximum absorption peak at 593 nm after 30 minutes of biosynthesis, also measured in the 200–800 nm range (El-Sayed et al., 2020). Comparable results were obtained for SeNPs biosynthesis using the probiotic strain *Bacillus subtilis* BSN313, where UV-Vis spectra after 24 hours of biosynthesis showed a transition point at 362 nm and a prominent absorption peak at 650 nm (Ullah et al., 2021). Biosynthesis of SeNPs using *Lactobacillus paracasei* HM1 also followed similar trends, with an absorption maximum of 300 nm within the extended 200-1000 nm range (El-Saadony et al., 2021).

**Characterization of biosynthesized selenium nanoparticles**

The sizes of the selenium nanoparticles were determined using a nanosizer. Our analysis showed that the majority of nanoparticles had a size of 225.6 nm, while only 2% were significantly smaller – 42.18 nm. Consequently, the average hydrodynamic diameter was 179.3 nm (Figure 4).

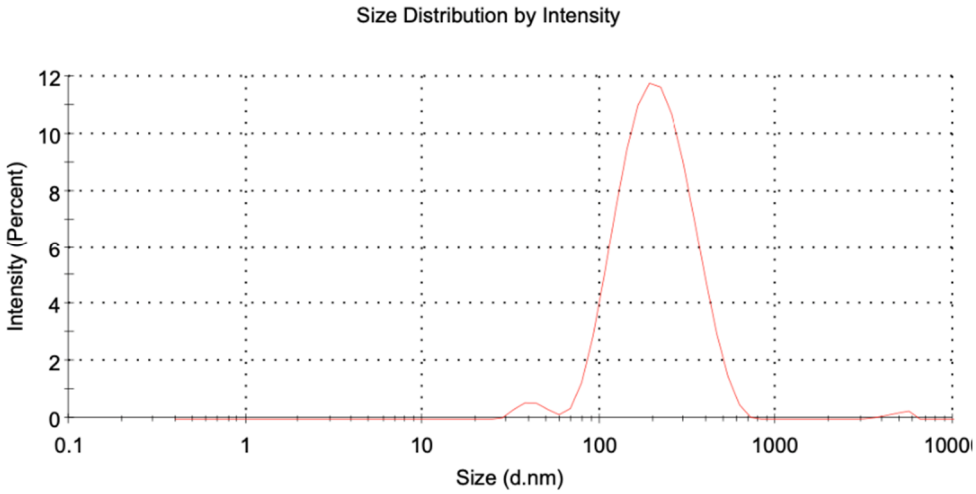



**Figure 4. Sizes of biosynthesized selenium nanoparticles**

This value considerably exceeds the typical dimensions obtained by electron microscopy techniques such as transmission (TEM) or scanning (SEM) electron microscopy, where nanoparticles are often in the tens of nanometers, depending on biosynthesis conditions. This discrepancy arises from the dynamic light scattering (DLS) method employed by the nanosizer, which measures not only the crystalline core of the nanoparticles but also the surrounding organic coating composed of biomolecules (e.g., proteins, lipids, polysaccharides). This organic coating influences the hydrodynamic radius of nanoparticles in solution, increasing their apparent size. Therefore, the larger nanoparticle sizes obtained through DLS, compared to those measured by electron microscopy, should be considered a positive indicator, as the presence of the organic shell is key to colloidal stability, bioavailability, and biological activity of SeNPs (Hashem et al., 2023). This has been corroborated by multiple studies. For instance, Fath-Alla et al. (2024) reported that the sizes of spherical SeNPs synthesized using *Saccharomyces cerevisiae* ranged from 34 to 125 nm as measured by TEM, whereas the average size determined by nanosizer was 173.9 nm (Table 4).

Overall, SeNP sizes vary widely depending on the biosynthesis method and conditions (Table 4). For example, during SeNP biosynthesis using Saccharomyces cerevisiae, nanoparticle sizes ranged from 75 to 709 nm (Faramarzi et al., 2020). SeNPs biosynthesized by Bacillus subtilis exhibited an average diameter of 530 nm (Ullah et al., 2021). Much smaller nanoparticles were obtained using Lactobacillus acidophilus, 34.13 nm (Alam et al., 2020) and Monascus purpureus, 46.58 nm (El-Sayed et al., 2020).

The zeta potential of the biosynthesized SeNPs, which was -16.2 mV (Figure 5), indicating satisfactory stability and dispersion of the nanoparticles in suspension.

**Table 4**

**Sizes of selenium nanoparticles biosynthesized using microorganisms**

| Microorganism | Average Size, nm (Z-average) | Reference |
|---|---|---|
| *Candida pseudojiufengensis* | 14 | Ali et al., 2024 |
| *Saccharomyces cerevisiae* | 173.9 | Fath-Alla et al., 2024 |
| *Bacillus subtilis* BSN313 | 530 | Ullah et al., 2021 |
| *Penicillium corylophilum* | 72 | Salem et al.,2021 |
| *Penicillium verhagenii* | 91.2 | Nassar et al., 2023 |
| *Aspergillus Flavus* | 49.72 | Mohammed et al., 2024 |
| *Eurotium cristatum* | 231.7 | Li et al., 2024 |
| *Trichoderma* sp. | 211.4 | Arunthirumeni et al., 2022 |
| *Fusarium semitectum* | 92.33 ± 48.5 | Abbas and Abou Baker, 2020 |
| *Yarrowia lipolytica* ATCC 18942 | 110 | Lashani et al., 2024 |
| *Saccharomyces cerevisiae* M437 | 179.3 | Own research |

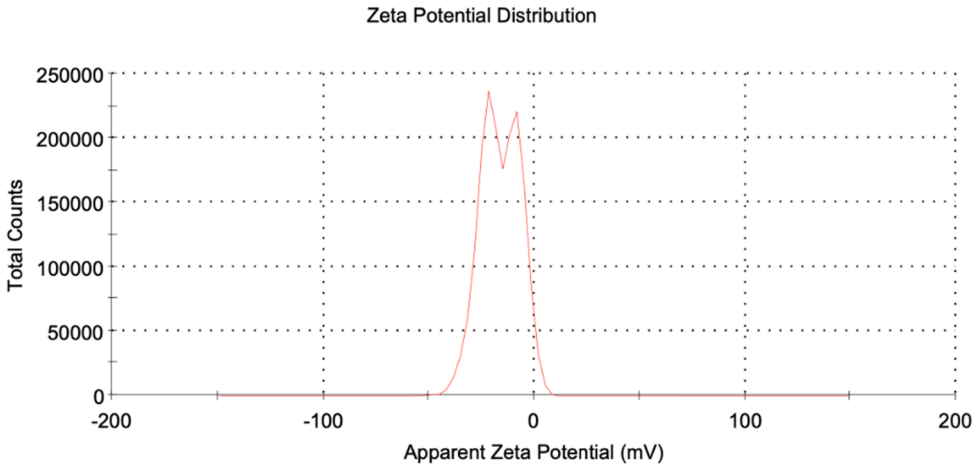



**Figure 5. Zeta potential of biosynthesized selenium nanoparticles**

Zeta potential is a key parameter for evaluating colloidal stability, with values varying across studies. The zeta potential of SeNPs biosynthesized by Saccharomyces cerevisiae ranged from -7.06 to -10.3 mV (Faramarzi et al., 2020), whereas SeNPs produced using Bacillus subtilis exhibited a more negative value of -26.9 mV (Ullah et al., 2021). In contrast, SeNPs biosynthesized by Lactobacillus acidophilus showed a positive zeta potential of +37.86 mV (Alam et al., 2020), while those obtained using *Monascus purpureus* had a zeta potential of –24.01 mV (El-Sayed et al., 2020).

The polydispersity index (PDI) of the SeNPs solution was 0.229, indicating high polydispersity, a low tendency toward agglomeration, and a relatively narrow range of nanoparticle sizes. PDI reflects nanoparticle size homogeneity and distribution variance. For SeNPs biosynthesized using *Saccharomyces cerevisiae*, the PDI values ranged from 0.189 to

0.989 (Faramarzi et al., 2020), for those biosynthesized with *Bacillus subtilis*, the PDI was 0.282 (Ullah et al., 2021). The PDI of SeNPs obtained using *Lactobacillus acidophilus* was 0.285 (Alam et al., 2020), and using *Monascus purpureus*, it was 0.205 (El-Sayed et al., 2020). The higher the polydispersity index (PDI), the less uniform the nanoparticle size distribution in the solution. Conversely, a lower PDI value indicates a more uniform nanoparticle size distribution and a reduced tendency for agglomeration in the sample (Skóra et al., 2021).

Zeta potential and PDI values from various studies are summarized in Table 5.

**Table 5**

**Zeta potential and polydispersity index of selenium nanoparticles biosynthesized using microorganisms**

| Microorganism | Zeta potential, mV | PDI | Reference |
|---|---|---|---|
| *Lactobacillus acidophilus* | +37.86 | 0.285 | Alam et al., 2020 |
| *Candida pseudojiufengensis* | +34 | 0.15 | Ali et al., 2024 |
| *Eurotium cristatum* | -15.5 | 0.277 | Li et al., 2025 |
| *Saccharomyces cerevisiae* | -22.4 | 0.503 | Fath-Alla et al., 2024 |
| *Monascus purpureus* | -24.01 | 0.205 | El-Sayed et al., 2020 |
| *Bacillus subtilis* BSN313 | -26.9 | 0.282 | Ullah et al., 2021 |
| *Penicillium verhagenii* | -32 | - | Nassar et al., 2023 |
| *Trichoderma* sp. | - | 0.287 | Arunthirumeni et al., 2022 |
| *Yarrowia lipolytica* ATCC 18942 | -34.51 | 1 | Lashani et al., 2024 |
| *Saccharomyces cerevisiae* M437 | -16.2 | 0.229 | Own research |

**Mechanisms of intracellular selenium nanoparticles biosynthesis**

The intracellular biosynthesis of SeNPs biosynthesis is a complex process based on microbial mechanisms that reduce selenite to elemental selenium, resulting in nanoparticle formation. Key enzymes involved in selenite reduction include sulfite reductase and thioredoxin reductase, which utilize electron donors such as NADPH or NADH to catalyze the reduction reaction (Wang et al., 2018). In *Bacillus mycoides*, thiol compounds like glutathione and N-acetyl-L-cysteine participate in selenite reduction. Other metabolites, such as 4-hydroxybenzoate and indole-3-acetic acid, are produced by *Bacillus mycoides* in response to stress and contribute to SeNPs biosynthesis (Baggio et al., 2021). In *Stenotrophomonas maltophilia*, a homolog of alcohol dehydrogenase is involved in the biosynthesis of SeNPs (Lampis et al., 2017). In *Pichia pastoris*, selenite reduction is mediated by NADH-dependent cytochrome b5 reductase (Elahian et al., 2017).

Thus, various biomolecules are involved in the intracellular biosynthesis of selenium nanoparticles. The localization of SeNPs also varies. In most cases, they are initially formed intracellularly and may later be released into the extracellular medium. For example, the biosynthesis of SeNPs using *Stenotrophomonas maltophilia* involves intracellular formation followed by their release into the extracellular medium (Lampis et al., 2017). When *Rhodococcus aetherivorans* was used for SeNP biosynthesis, the resulting nanoparticles were surrounded by an organic shell that enhanced their stability and prevented aggregation (Presentato et al., 2018). Additionally, in *Shewanella oneidensis*, modulation of the

extracellular electron transfer (EET) pathway has been demonstrated through genetic alteration of the CymA gene, which encodes a key component of the electron transport chain. Such modifications influence the localization, composition, and size of the synthesized nanoparticles (Tian et al., 2017).

In summary, intracellular biosynthesis of selenium nanoparticles is a complex and multifactorial process dependent on the specific enzymatic systems of microorganisms. Understanding these mechanisms is crucial for the further development of biotechnological approaches for the synthesis of SeNPs with tunable properties for medical, environmental, and industrial applications.

## Conclusions

Selenium nanoparticles exhibit considerable potential for applications in the food industry. Their pronounced antimicrobial properties enable effective suppression of a wide range of foodborne pathogens, making them particularly promising for use in active food packaging systems aimed at extending product shelf life. Among the various approaches to SeNP synthesis, biosynthesis using microorganisms such as bacteria, fungi, or yeast is regarded as one of the most efficient methods. Yeast of the genus *Saccharomyces* is of particular interest due to its ease of cultivation, safety profile, and lack of strict biosafety requirements. In this study, *Saccharomyces cerevisiae* M437 was identified as a promising candidate for the intracellular biosynthesis of selenium nanoparticles.

The study confirmed the ability of strain M437 to synthesize SeNPs intracellularly. Light microscopy revealed the presence of inclusions both inside and outside the yeast cells, indicating nanoparticle formation followed by extracellular release. The release of SeNPs into the culture medium was observed between days 5 and 7 of cultivation, facilitating subsequent recovery and purification. UV-Vis spectroscopy analysis revealed a distinct absorption peak at 252 nm, characteristic of SeNPs and likely associated with surface plasmon resonance. DLS analysis indicated a mean hydrodynamic diameter of 179.3 nm, encompassing both the nanoparticle core and the surrounding organic capping layer. This organic shell plays a key role in maintaining colloidal stability, enhancing bioavailability, and contributing to the biological activity of SeNPs. A measured zeta potential of –16.2 mV suggests the stability of the nanoparticles in suspension. A polydispersity index of 0.229 reflects a high degree of uniformity in the nanoparticle suspension.

Despite the promising properties of *Saccharomyces cerevisiae* in SeNP biosynthesis, research in this area remains limited. Nevertheless, the results obtained in this study demonstrate that *Saccharomyces cerevisiae* M437 is capable of producing biogenic SeNPs with characteristics suitable for future applications in food-related technologies.

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