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PREPARATION OF SILVER NANOPARTICLES STABILIZED WITH THE PRODUCTS OF MECHANOENZYMATIC HYDROLYSIS OF YEAST CELL WALLS

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Abstract

Data on the synthesis of silver nanoparticles and their stabilization by the products of mechanoenzymatic hydrolysis of yeast biomass are presented. It is shown that the reduction of silver salts by the products of mechanoenzymatic hydrolysis of yeast cell walls appears to be a promising method of obtaining stabilized preparations of nanoscale particles. Formation of silver particles starts on altered cell walls of the yeast. The particles growth occurs due to the reduction of silver salts on seeds by dissolved carbohydrates which are products of cell walls hydrolysis.

Key words: *silver nanoparticles, yeast cell wall, mechanoenzymatic hydrolysis.*

1. INTRODUCTION

Silver in the ion form and as nanoparticles exhibits a broad range of antimicrobial action. Increased attention to the use of silver as biocide is due to its high toxicity against many microorganisms, and the absence of the resistance towards this element in the majority of microorganisms.

The most efficient forms of silver are preparations containing nanoscale particles of this metal, which possesses more pronounced biocide effect than ionic silver (Korenevsky et al. 1993; Revina et al. 2005; Baranova et al. 2005). A substantial part of ionic silver forms insoluble salts in gastrointestinal tract and thus loses the biocide activity. Nanoscale silver in this case possesses higher stability, does not form insoluble salts and can preserve activity for a long time.

The systems containing silver nanoparticles are efficient against various bacteria (colibacillus, salmonella, staphylococcus, enterococcus) (Titova et al. 2011; Koptev et al. 2012). Gel with silver nanoparticles stabilized with organic high-molecular substances is used to treat inflammation caused by *Propionibacterium acne*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*; and show higher activity than ionic silver.

By present, many methods have been proposed to obtain the systems containing colloid silver (Yamamoto et al. 2000; Egorova et al. 2000; Revina et al. 2003; Shin et al. 2008). The majority of studies deal with the compositions in which surfactants are added to prevent agglutination of silver nanoparticles. Stable colloid silver solutions can be obtained by reducing its salts with hydrazine and borohydrides in solutions containing surfactants, for example sodium dodecyl sulphate, polyoxyethylene sorbitane monooleate ("Tween-20"), cetyltrimethylammonium bromide, polyvinyl alcohol (Kotelnikova et al. 2005; Grishchenko et al. 2006).

Especially interesting methods are those in which the compounds of natural origin are used to reduce silver and to stabilize the formed nanoparticles. The usage of polysaccharide matrices as the stabilizing component looks promising.

A simple method to obtain nanocomposites containing silver nanoparticles is based on the processes that occur in aqueous alkaline solutions of the ammonia complexes of silver in the presence of natural polysaccharide arabinogalactan (Mouxing et al. 2006; Haoran et al. 2005). The formation of metal silver "nuclei" (or "seeds") occurs due to the reduction of complex ions by the terminal aldehyde groups of polysaccharide. The addition of alkali results in partial hydrolysis of the polysaccharide and accumulation of reducing agent (oligosaccharides) in solution, which provides the optimal growth of silver seeds.

Methods in which microorganisms are used to reduce silver in aqueous solutions have been developed. It is accepted that ionized carboxylic groups of amino acids, as well as amide groups, are responsible for the initial stage of nanoparticle formation (sorption of the ionic forms of silver). Reducing groups incorporated into aldehydes and ketones are involved in the interaction with silver ions and ensure the growth of seeds (Strizhko et al. 2003). It was demonstrated that the addition of a definite amount of alkali into the solutions of ionic silver containing the dead culture of bacteria *Aeromonas* sp. SH10 can accelerate the formation of metal nanoparticles on cell surface and near it (Wang and Chen 2006). The characteristics of nanoparticles depend on the concentration of the ammonia complexes of silver and on pH of solution. It should be noted that the growing seeds are fixed in the matrix of cell wall polymers and cannot aggregate.

Many microorganisms are able to sorb the silver. The maximal sorption capacity is 15-25 mg of silver per one gram of the dry biomass (Kierans et al. 1991). The ability of the cell walls of microorganisms to sorb the ions of heavy metals with subsequent formation of metal granules is used in chemical technology, in particular to recover metals from industrial effluents (Shpak and Ulberg 2005).

The goal of the present work was to obtain stabilized silver nanoparticles using the products of partial hydrolysis of the yeast cell walls as the reducing and stabilizing agent.

2. MATERIALS AND METHODS

The reagents and materials used in the work were chemically pure grade: ammonia, silver nitrate, D-(+)-glucose (Acros organics), yeast *Saccharomyces cerevisiae* (State Standard GOST # 171-81, Novosibirsk Yeast Plant), enzymatic complex "Cellolux 2000" 2000 units/g (Sibbiofarm plant, Berdsk, Novosibirsk Region).

Mechanoenzymatic hydrolysis was carried out according to the procedure described previously (Bychkov et al. 2010).

Reduction of the ammonia complexes of silver. To 200.0 mg of initial yeast or yeast after mechanical and enzymatic treatment, we added 1.00 ml of the working solution containing water - 10.00 ml, 1.00 mM solution of $AgNO_3$ - 1.00 ml, concentrated ammonia solution - 300 µl. The preparations were kept at room temperature for 24 h, and then the samples for electron microscopy were prepared.

Electron microscopy of yeast cells in suspension. A drop (20 µl) of the sample was placed on the film (Parafilm), and a copper mesh covered by formvar film was placed on the top. After 60 seconds, the mesh was taken off, excess liquid was removed using the filter paper, and the sample was dried and placed into a Petri dish. The dried samples were examined with "H-600" transmission electron microscope (Hitachi, Japan).

Electron microscopy of yeast cells in ultrathin sections. Yeast samples were fixed using a 4 % paraformaldehyde on Hank's solution, then dehydration was carried out in the solutions of ethanol with sequentially increasing concentration and acetone. After dehydration, the samples were placed for 12 h in a mixture of acetone and epon-araldite (1:1), and then polymerization was carried out for 48

hours. Ultrathin sections were prepared with a Reichert-Young ultratome (Austria) and examined in "H-600" transmission electron microscope (Hitachi, Japan).

3. RESULTS AND DISCUSSION

A set of experiments with gradual complexification of the system "silver nanoparticles – stabilizer" was carried out in order to correctly achieve the formulated goal. A classic reaction of the reduction of ammonia complex of silver nitrate was chosen as a basis of the method to obtain nanoparticles. Then, using increasingly more complicated reducing/stabilizing agents from monomeric glucose to partially hydrolyzed cell walls, we detected the formation of silver nanoparticles with the help of UV spectroscopy and transmission electron microscopy, and studied their size, shape and stability.

3.1. Formation of nanoparticles stabilized with soluble biopolymers

As the simplest case, we studied the formation of silver nanoparticles through reduction with the help of glucose without adding any stabilizers. Electron microscopy (Fig. 1-a) shows that the resulting particles are spherical; their size distribution is narrow. However, the resulting colloid is unstable and precipitates within 3-5 hours due to the aggregation of particles in the absence of any stabilizing agent.

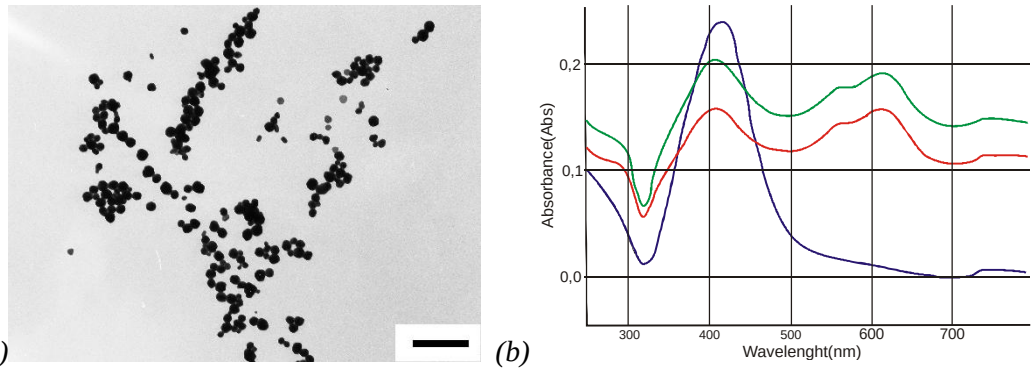


Fig. 1. Silver nanoparticles obtained through reduction with glucose in absence of stabilizing agent: *a* – electron microphotograph of the particles (scale bar – 0.3 μm), *b* – UV spectrum of the initial colloid solution, solution after 3 hours and after 5 hours.

It is known that a linear dependence between particle size and the position of absorption peak in the UV spectrum is observed for silver nanoparticles within the size range from 20 to 100 nm. For example, an increase in particle size from 25 nm (390 nm) to 50 nm causes a shift of the peak to the wavelength of 420 nm (Mouxing et al. 2006; Khlebtsov et al. 2007; Chandran et al. 2006; Panacek et al. 2006; Huang et al. 2007). Our experiments confirmed these data (Fig. 1-b). At first, the formation of aggregates manifests itself in the UV spectra as broadening of the initial peak to the longer wavelengths, and then the appearance of additional broad peaks in the region 500-600 nm is observed.

a) $R-COOH + Ag^+ \rightleftharpoons R-COOAg + H^+$

$R-SH + Ag^+ \rightleftharpoons R-SAg + H^+$

b) $R-COOAg + e^- \rightarrow R-COO^- + Ag$

$R-SHAg + e^- \rightarrow R-S^- + Ag$

Fig. 2. Mechanism of the formation of elementary silver in cell wall: *a* – the first stage, the formation of silver salts; *b* – the second stage, silver reduction (Strizhko et al. 2003).

It is known that biopolymer molecules affect the growth of nanoparticles and promote the formation of complicated dendritic structures possessing good stability. According to the mechanism of silver seeds formation in similar systems (Fig. 2), it is necessary that biopolymers had not only reducing groups but also the groups responsible for the primary formation of salts. The most suitable polymers for this purpose are the polymers present in yeast hydrolyzates. Protein molecules present in these hydrolyzates are involved in the formation of salts and in stabilization of the formed dendrites, while low-molecular carbohydrates play the part of reducing reagent.

A microphotograph and UV spectrum of nano-structured dendritic structures synthesized in the presence of soluble biopolymers isolated from yeast cell walls by means of classical enzymatic hydrolysis are shown on Fig. 3. A peak in the region of 420 nm, attributed to the particles about 50 nm in size, provides evidence that these macrostructures are nano-structured in spite of their complicated shape and visible extension. The absence of broadening and additional peaks in the longer wavelength region of the spectrum provides evidence of the absence of aggregates and therefore the stability of resulting particles.

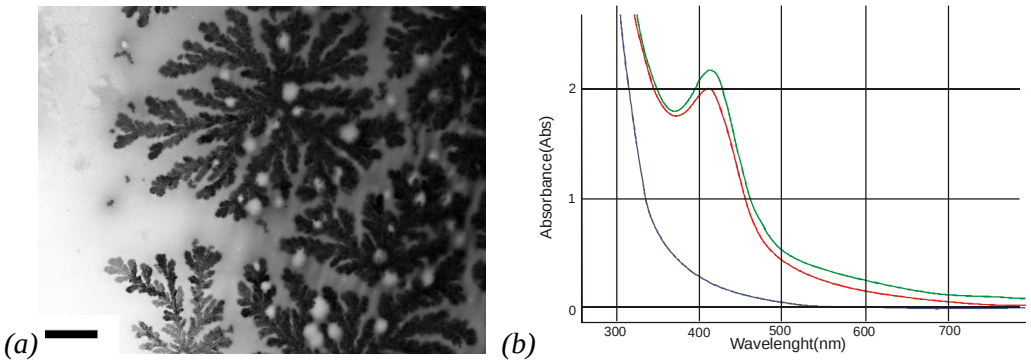


Fig. 3. Nano-structured dendritic formations of silver obtained in the solutions of biopolymers: *a* – electron microphotograph of the formations (scale bar – 2 μm), *b* – UV spectrum of the initial solution of nanostructured formations, solution after 3 hours and after 5 hours.

So, the use of polymers incorporated into the yeast biomass allows one to obtain stabilized nanostructured silver formations. This gives us reason to assume the promising character of the application of partially hydrolyzed yeast cell walls as the stabilizing matrix.

3.2. The formation of nanoparticles stabilized with cell wall polymers

To evaluate the efficiency of mechanoenzymatic treatment as the preparation of cell walls for the reduction and stabilization of nanoparticles, we carried out experiments with native, mechanically activated and mechanoenzymatic hydrolyzed yeast biomass.

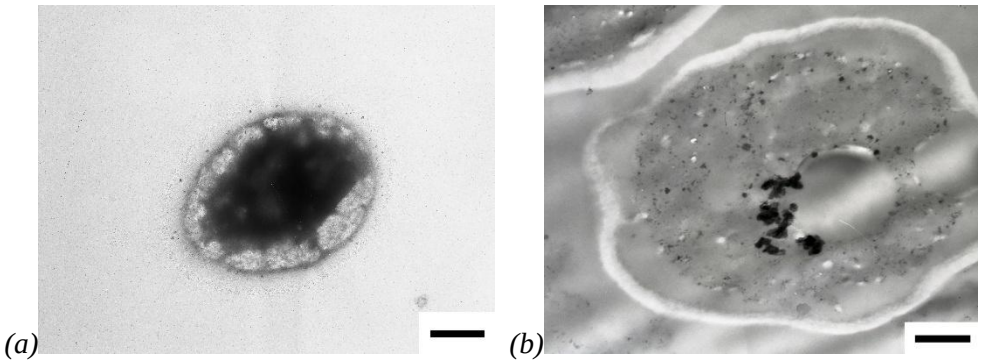


Fig. 4 Microphotographs of initial yeast treated with ammonia solution of silver nitrate: *a* – image of the intact cell on the formvar film (scale bar – 1 μm), *b* – ultrathin section of cells (scale bar – 1 μm).

In the case of the treatment of initial *S. cerevisiae* cells with the ammonia solution of silver nitrate, only insignificant silver reduction is observed (Fig. 4). Two parts of the initial cell differing from each other in composition are clearly seen in the microphotograph (Fig .4-a): a weakly contrasted cell wall and strongly contrasted intracellular part. In the perinuclear space, we also see nanoscale (15-25 nm) silver particles that are likely to be formed through reduction with carbohydrates present in the initial yeast preparation.

The observed poor ability of initial cell walls to reduce silver (Fig. 4-b) may be related with the small number of available groups that are able to serve as nucleation centres. In the native wall, polysaccharides form stable structures in which the concentration of free reducing end groups is not high (Biryuzova 1993; Kalebina and Kulaev 2001). In this case, a part of dissolved silver is only able to penetrate into the cell through the channels of the cell wall and get reduced by intracellular components.

It was demonstrated previously (Bychkov et al. 2012) that non-destroyed cells after mechanical treatment have distorted shape; the integrity of the majority of cells is destroyed, and the supramolecular structure is disordered. The walls of destroyed cells look like ribbons weakly contrasted during the treatment with the ammonia solution of silver nitrate (Fig. 5). Similarly to the case of initial yeast, we may assume that the low reactivity is connected with insufficient content of the necessary reducing functional groups.

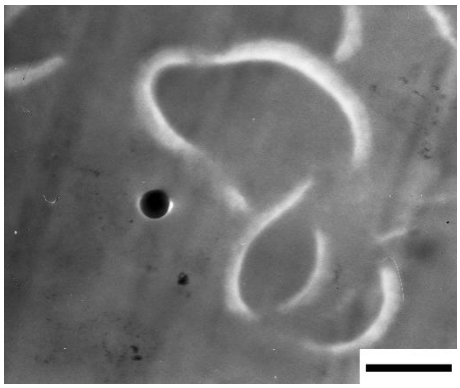


Fig. 5. Yeast cell walls after mechanical activation and treatment with the ammonia solution of silver nitrate (ultrathin section; scale bar – 0.5 μm).

Comparative experiments showed that the reactivity of cell wall components in enzymatic hydrolysis increases substantially during mechanical treatment. Some disturbance is likely to occur in the interactions between the structural elements of cell wall (glucan, glycoproteins, chitin), but the disturbance is insufficient for the formation and growth of silver nuclei. So, the observed changes in cell morphology only weakly affect the supramolecular arrangement of the structural polymers of cell walls but they create conditions for the interaction of structural elements with the solutions of enzymes.

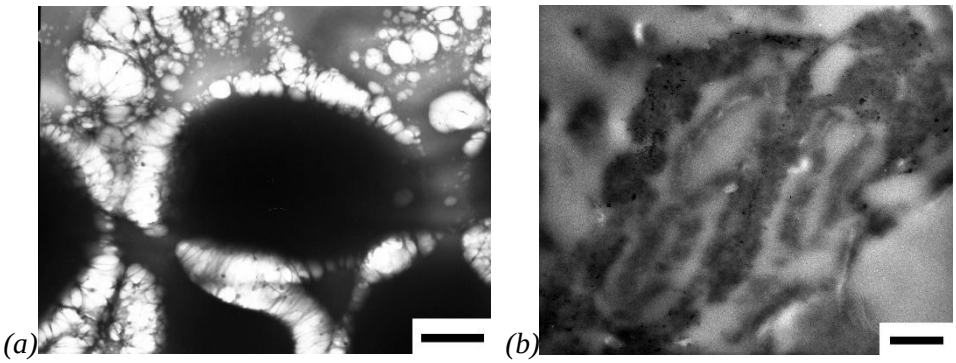


Fig. 6. Yeast subjected to mechanoenzymatic hydrolysis and treated with the silver nitrate: *a* – cells on formvar film (scale bar – 1 μm), *b* – silver nanoparticles, ultrathin section (scale bar – 0.4 μm).

Under similar conditions, we studied the samples after mechanical treatment and subsequent enzymatic hydrolysis. Electron microscopic data (Fig. 6) show that the products of mechanoenzymatic hydrolysis are more active in the process of preparation nanoscale silver particles than the initial yeast cells and the cells after mechanical activation.

The fragments of cell walls in the preparations after mechanical and enzymatic treatment are coated with silver particles, which is the evidence of increased reducing capacity of cell walls, maybe due to an increase in the concentration of the end carbonyl groups of carbohydrates. The size of the formed silver particles does not exceed 65-75 nm; varying treatment conditions we can obtain the particles within the size range of 15-80 nm.

During mechanical and enzymatic treatment, cell wall polysaccharides are hydrolyzed (total polysaccharide content of the dry cell walls is about 70 %), the degree of their polymerization decreases, and the cell wall gets additional carbonyl groups necessary for silver reduction.

Changes in silver reduction in the liquid phase were detected in the presence of the products of cell hydrolysis. A part of carbohydrates become water-soluble as a result of hydrolysis, and these carbohydrates are extracted into solution. As a consequence, reduction processes occur also within the intercellular space. Separate nanoparticles (60-75 nm) obtained by reduction from the aqueous solution by carbohydrates with lower polymerization degree are present. It was shown that mechanical treatment and enzymatic hydrolysis results in the formation of 7.8 % (of cell mass) soluble carbohydrates that are able to participate in the formation of the particles of this size.

In general, the samples of mechanically treated and enzymatic hydrolyzed product possess higher reducing ability than the initial cells do. The number of spatially stabilized nanoparticles is larger than that for the treatment of native *S. cerevisiae* cells.

The results presented here show that the reduction of silver salts by the products of mechanoenzymatic hydrolysis of the yeast cell walls appears to be a promising method of obtaining stabilized preparations of silver nanoparticles. The formation of the “nuclei” of silver particles starts on distorted yeast cell walls, while their growth occurs due to the reduction of silver salts on nuclei through the interaction with dissolved carbohydrate products of cell wall hydrolysis.

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REFERENCES

Baranova EK, Mulyukin AL, Kozlova AN. Interaction of ions and cluster silver in aqueous and aqueous-organic solutions with cells *Candida utilis* and *Saccharomyces cerevisiae*. // High Tech 2005; 5: 33-7 (in Russian).

Biryuzova VI. Ultrastructural organisation of yeast cell. Moscow: Nauka, 1993. 222 p (in Russian).

Bychkov AL, Korolev KG, Lomovsky OI. Obtaining mannanoligosaccharide preparations by means of the mechanoenzymatic hydrolysis of yeast biomass. Applied Biochemistry and Biotechnology 2010; 162: 2008-14.

Bychkov AL, Ryabchikova EI, Lomovsky OI. Mechanoenzymatic hydrolysis of yeast biomass. Journal of International Scientific Publications: Ecology & Safety 2012; 6: 25-36.

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnology Progress 2006; 22: 577-83.

Egorova EM, Revina AA, Kondratieva VS. Method for producing of nanostructured metal particles. 2000 Patent № RU 2147487.

Grishchenko LA, Medvedeva SA, Aleksandrova GP, Feoktistova LP, et al. Redox reactions of arabinogalactan with silver ions and formation of nanocomposites. Russian Journal of General Chemistry 2006; 7: 1111-7.

Haoran Z, Qingbiao L, Yinghua L, Daohua S, et al. Biosorption and bioreduction of diamine silver complex by *Corynebacterium*. Journal of Chemical Technology and Biotechnology 2005; 80: 285-90.

Huang J, Qingbao L, Sun D, Yinghua L. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomus camphora* leaf. Nanotechnology 2007; 18: 1-11.

Kalebina TS, Kulaev IS. The role of proteins in the formation of the molecular structure of the cell wall of yeast. Biochemistry (Moscow). Special issue: Biological chemistry reviews. 2001; 41: 105-130 (in Russian).

Khlebtsov NG, Bogatyrev VA, DykmanLA, Khlebtsov BN. Gold nanostructures with plasmon resonance for biomedical research. Russian Nanotechnologies 2007; 2: 69-86.

Kierans M, Staines AM, Bennett H, Gadd GM. Silver tolerance and accumulation in yeasts. Biology of Metals 1991; 4: 100-6.

Koptev VYu, Titova MN, Kazantseva AS, Bychkov AL. Suture material with a new anti-bacterial coating on the basis of the silver nanoparticles prevents the development of postoperative complications in the form of a purulent infection. Materials of XI Siberian Veterinary Conference «Topical Problems in Veterinary Medicine» (Novosibirsk, March 22-23 2012) : Proceedings. – Novosibirsk: NSAU. 2012. p.101-2.

Korenevsky AA, Sorokin VV, Karavayko GI. The interaction of silver ions with cells *Candida utilis*. Microbiology 1993; 62: 1085-92 (in Russian).

Kotelnikova NE, Lashkevich OV, Panarin EF. Method for producing of silver-based cellulosic materials. 2005 Patent № RU 2256675.

Mouxing F, Qingbiao L, Daohua S, Yinghua L, et al. Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. Chinese Journal of Chemical Engineering 2006; 14: 114-7.

Panacek A, Kvitek L, Prucek R, Kolan M, et al. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry B 2006; 110: 16248-53.

Revina AA. The system modification of objects by nanoparticles. 2003 Patent № RU 2212268.

Revina AA, Baranova EK, Mulyukin AL Sorokin VV. Some characteristics of the impact of silver cluster on *Candida utilis* yeast cells. Researched in Russia 2005; 8: 1403-9 (in Russian).

Shin Y, Bae JT, Arey BW, Exarhos GJ. Facile stabilization of gold-silver alloy nanoparticles on cellulose nanocrystal. Journal of Physical Chemistry 2008; 112: 4844-8.

Shpak AP, Ulberg ZR. Colloid chemical foundations of nanoscinece. Publisher: "Akademperiodika". Kiev, 2005. 466 p (in Russian).

Strizhko LS, Zakharova VI, Korenevskiy AA. Biosorbents for the recovery of precious metals from industrial solutions. Non-ferrous metals 2003; 2: 40-4 (in Russian).

Titova MA, Shkil NA, Koptev VYu, Bychkov AL. Assessment of antibacterial and therapeutic effectiveness of preparation comprising nanoparticles of silver on bovine mastitis. Veterinary Medicine 2011; 3-4: 103-4.

Wang J, Chen C. Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotechnology Advances 2006; 24: 427-51.

Yamamoto T, Wada Y, Sakata T, Mori H, et al. Microwave-assisted preparation of silver nanoparticles. Chemistry Letters 2000; 33: 158-9.