**Journal of Nanoscience and Nanoengineering**
Vol. 3, No. 1, 2017, pp. 1-5
http://www.aiscience.org/journal/jnn
ISSN: 2471-8378 (Print); ISSN: 2471-8394 (Online)

# Microorganisms-Producers of Nanoparticles of Silver

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## Abstract

Screening among collection microorganisms was conducted to identify producers of nanoparticles. Nanoparticles of silver were received at presence of active strains and were stabilized by chitosan of *Bombyx mori*. Structure and morphology of nanoparticles were studied by UV-spectroscopy and electron microscopy methods. It was established that synthesized nanoparticles of silver have spherical form and size ranging from 1.5 to 5 nm. Received data may be useful for further development of microbiological methods of nanoparticles production.

## Keywords

Microorganisms, Nanoparticles of Silver, Chitosan of *Bombyx mori*

Received: May 30, 2017 / Accepted: July 4, 2017 / Published online: August 1, 2017



## 1. Introduction

The main approaches for synthesis of inorganic nanoparticles (NP) include physical, physical and chemical, chemical, and biological methods. Biological methods of NP formation are the most diverse. From the point of view of formation of particles with predefined parameters, the microbiological methods of NP formation are the most promising ones. As a rule, at the biological formation of NP microbial enzymes serve as a reducer. For example, fungus *Fusarium oxysporum* reduces silver ions with help of $NADH^-$ dependent reductase [1]. However, different cellular components, microbial metabolites or compounds comprising the nutrient medium may contribute to the process of reduction of ions of metals. Biochemical processes taking place during microorganisms' cultivation cause considerable changes in composition of microbial cells and cultural broth, which, in their turn, impact properties of synthesized NP.

NP biosynthesis may take place in different microorganisms [2, 5, 10, 12, 15]. Researchers discovered that synthesis of colloidal particles of silver may be conducted by bacteria *Pseudomonas stutzeri* AG259 [7, 8]. To conduct synthesis, silver ions (usually in the form of silver nitrate) are added to the nutrient medium, while synthesis itself takes place in the periplasmatic space of bacterial cell. Yeast cells reported as applicable for synthesis of NP as well [4, 14]. The growth of NP in this case takes place on surface of cell. It was also established that filamentous fungi of species *Fusarium oxysporum* are capable to extracellular synthesis of NP with aid of enzyme nitrate reductase [9]. In general, the microbiological synthesis of NP is considered as very promising approach, since there is no need for special reducers and stabilizers, and only nutrient media are used for cultivation of microorganisms. Another important advantage of such synthesis is possibility of receipt of the combined antibiotic preparations comprising simultaneously traditional antimicrobial (bactericidal or fungicidal) agent for the certain pathogen and nanoparticles possessing bactericidal and fungicidal features as well.

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The main disadvantages of such synthesis are complexity to control sizes of received NP, and difficulty with extraction of formed NP from the cultural broth and their separation from biomass. At the same time, at the microbiological synthesis of NP, usually, the layer of biopolymer stabilizes formed particles and this fact may be considered as a positive moment. It not only increases stability of NP towards aggregation, but decreases their toxicity to the human body as well [11].

However, not all microorganisms are capable to independent intensive formation of NP. Mainly, because reduction of ions to NP is stipulated by specific enzymes, which are formed by a limited number of microorganisms. Nevertheless, in certain conditions, e.g. at presence of enzymes of other microorganisms or other reducers, almost all microbes may participate in the processes of NP formation, performing duties of substrate, catalyst or donor of electrons [11]. In these regards, search and isolation of microorganisms capable to NP synthesis and study of these processes represent obvious interest.

## 2. Research Significance

At this work presents results of study of biosynthesis process silver of NP by different microorganisms preserved at the culture collection of the Institute of microbiology. Selection of objects was determined by their resistance to different pollutants, including heavy metals, and by their ability to biological sorption of silver, since ability to form NP of metals is considered as a protective function of microorganisms [6].

## 3. Experimental Procedure

Experiments were conducted by addition of solution of silver nitrate (25 mg Ag+/1000 ml) into the cultural broth of 2-3 days old cultures. Standard elective nutrient media characteristic for each type of microorganisms (beef extract peptone broth, Czapek-Dox broth) and poor synthetic medium ($NH_4NO_3$ – 1.0; $K_2HPO_4$ – 0.5; $MgSO_4$ – 0.1; NaCl – 0.5; peptone – 0.5, g/l) were used in this study. Mixtures of cells and silver ions were incubated on shakers (28°C, 150 rpm) for 14 days. Cells' biomass was obtained by centrifugation at 5000 rpm for 15 min. Formation of silver particles was observed visually: by staining of solutions into yellow and brown colors characteristic for NP of silver, and by formation of precipitates of large silver particles.

UV-spectroscopic investigations were conducted on spectrophotometer SPECORD 210 within range 190–1000 nm. Accuracy of UV-photometry with potassium dichromate in accordance with Ph. Eur. ± 0.01.

Morphology of nanostructured films systems were studied on atomic-force microscope AFM Agilent 5500 (USA) at room temperature. Silicon cantilevers with rigidity 9.5 N/m with frequency 145 kHz was used in the work. Maximum field of scanning at AFM by X, Y was 15×15 $\mu m^2$, by Z – 1 μm.

Chitosan of *Bombyx mori* was obtained by method [13].

## 4. Discussion of Results

Twenty-two filamentous fungi and seventeen bacteria were studied. Majority of studied microorganisms expressed more or less reasonable ability to NP synthesis (table 1). Nevertheless, for majority of microbial cultures the yield of NP of silver was inconsiderable (less than 10% out of all silver available). It was established that nanoparticles appeared mainly after 24-48 h, depending on certain microorganism and the highest synthesizing activity was observed after 48 h for majority of studied cultures. Longer contact with silver salt causes aggregation of NP and precipitation of sediment; especially it was characteristic for filamentous fungi.

**Table 1.** Results of qualitative screening among microorganisms for NP synthesis.

| № | Microorganism | Color | Color change | | |
|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h |
| Filamentous fungi | | | | | |
| 1. | *Acremonium sp.* | Yellowish-brown | - | ± | + |
| 2. | *Alternaria sp.* | No coloring | - | - | - |
| 3. | *Alternaria pluriseptata* | Light-brown | ± | + | ± |
| 4. | *Asperegillus niger* | No coloring | - | - | - |
| 5. | *Aspergillus terreus 1* | Yellow-brown | + | 3+ | 3+ |
| 6. | *Aspergillus terreus 2* | Light-brown | - | + | + |
| 7. | *Aspergillus terreus 3* | No coloring | - | - | - |
| 8. | *Aspergillus glaucus* | Greyish | - | - | - |
| 9. | *Aspergillus flavus* | No coloring | - | - | - |
| 10. | *Aspergillus versicolor* | Pinky-greyish | - | - | + |
| 11. | *Aspergillus albus* | Pinky | ± | + | + |
| 12. | *Aspergillus oryzae* | Yellow | + | + | + |
| 13. | *Cladosporium cladosporioides* | No coloring | - | - | - |
| 14. | *Cladosporium sp. 1* | Greyish | - | - | - |
| 15. | *Cladosporium sp. 2* | Light-brown | + | - | - |
| 16. | *Nocardia sp.* | Yellow | + | 2+ | - |
| 17. | *Penicillium sp. 1* | Black | 2+ | 3+ | 3+ |
| 18. | *Penicillium sp. 2* | Yellowish | - | + | + |
| 19. | *Penicillium roseo-purpureum* | Greyish | - | + | - |
| 20. | *Trichoderma harzianum* | Greyish | ± | + | ± |
| 21. | *Trichurus terrophilus* | Light-pinky | ± | + | - |
| 22. | *Verticillium dahliae* | Dark brown, black fine sediment | + | + | + |
| Bacteria | | | | | |
| 1. | *Arthrobacter globiformis* | Brown | + | 2+ | 2+ |
| 2. | *Bacillus badius* | Light-yellow | - | + | + |
| 3. | *Bacillus megatherium* | No coloring | - | - | - |
| 4. | *Bacillus sp. 1* | Light-brown | + | + | 2+ |
| 5. | *Bacillus sp. 2* | Light-yellow | + | + | - |
| 6. | *Bacillus sp. 3* | Light-yellow | ± | + | - |
| 7. | *Bacillus subtilis* | Light-yellow | - | + | + |
| 8. | *Pseudomonas stutzeri* | Brown | + | 3+ | 3+ |
| 9. | *Pseudomonas putida 1* | Brown | + | + | - |
| 10. | *Pseudomonas putida 2* | Brown | + | 2+ | 2+ |

| № | Microorganism | Color | Color change | | |
|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h |
| 11. | *Pseudomonas sp.* | Light-yellow | + | + | + |
| 12. | *Rhodococcus erytropolis* | Yellow | + | 2+ | 2+ |
| 13. | *Rhodococcus sp.* | Yellow | + | 2+ | 2+ |
| 14. | *Streptomyces fradia* | Light-grey | - | ± | - |
| 15. | *Streptomyces grizeorubiginosus* | Light-yellowish | - | ± | ± |
| 16. | *Streptomyces iyakirus* | No coloring | - | - | - |
| 17. | *Streptomyces sp.* | Pink | + | + | - |

It was established that strains of filamentous fungi from genera *Acremonium*, *Penicillium* and *Aspergillus*, and bacteria from genera *Pseudomonas*, *Rhodococcus* and *Arthrobacter* were among the most active producers of silver NP. At that, the highest activity was observed after 2 days of cultivation for all studied groups of microorganisms.

In course of NP formation by the different cultures it was established that process of NP formation is heavily affected by conditions of cultivation and composition of nutrient medium. E.g., the yield of NP at addition of biomass to the solution with silver ions was smaller compared to this in experiments with cultural broth. Obviously, it is linked with that extracellular active compounds restoring silver ions are secreted by cells and are accumulated in the medium during cultivation, and thus promote to the higher yield of silver NP. Use of different media revealed that for the culture *Pseudomonas stutzeri* on BPB medium the NP synthesis is observed after 24 h. Whereas its cultivation on poor synthetic medium resulted in extended length of the process – active formation of NP was observed on 3rd day, which is obviously related to deceleration of the microorganism growth. Nevertheless, at it the more intensive coloring was observed, which testifies increased potential of bacteria in regards to their capacity to NP synthesis (figure 1).


1 2


1 2

**Figure 1.** Impact of composition of the nutrient medium on synthesis of silver NP by *Pseudomonas stutzeri* (upper row – control, lower row – NP formation) (1 – BPB, 2 – poor synthetic medium).

Conducted comparative UV-study of solutions of nutrient medium with and without microorganisms revealed that there is entire intensive band of absorption within range 200-300 ηm linked with presence of $-NO_3^-$, $HPO_4^{2-}$ and $-SO_4^{2-}$ groups within the system (figure 2).

According to obtained results, doubtlessly expressed bands of absorption appear in the spectrum at presence of microorganisms and their intensity grows. It testifies change of the chemical composition of the medium linked with activity of microorganisms, which in its turn leads to formation of silver nanoparticles.

It is necessary to note that NP of silver synthesized by bacteria possessed better stability. In fact, the percent of silver NP synthesized by filamentous fungi after 5-7 days decreased considerably, whereas silver NP synthesized by bacteria preserved higher stability. Silver transfers from nanosize state into micro- and macro-particles because different microorganisms possess different capacity to production of proteins that stabilize nanoparticles. Thus, NP formed at the first stage of restoration of silver ions keep growing unlimited and form aggregates that precipitate later.

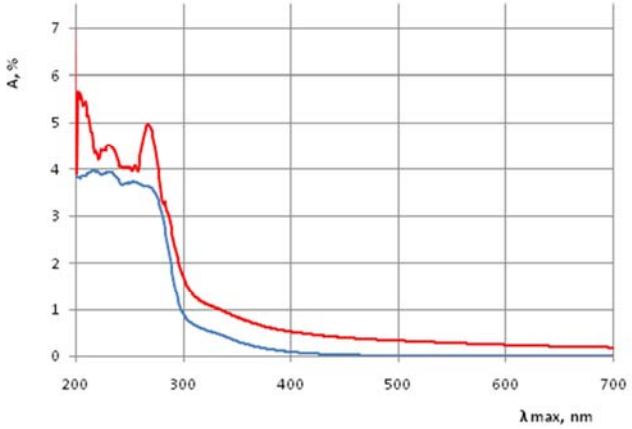



**Figure 2.** UV-spectra of initial solutions: blue line – solution of the nutrient medium (BPB); red line – solution of the nutrient medium (BPB) with microorganisms.

The fact of formation of NP synthesized at presence of different microorganisms was studied by UV-spectroscopy and atomic-force microscopy methods.

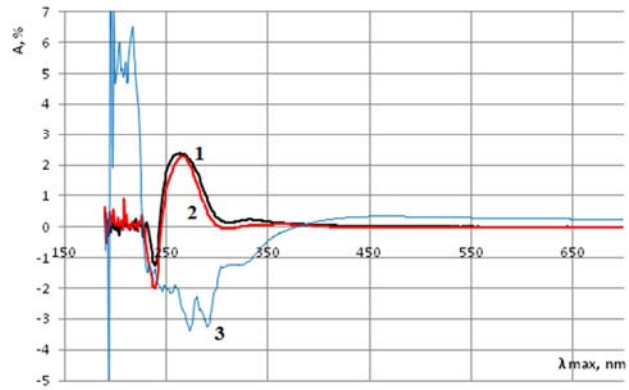
It was established that there are absorption bands of functional amino- and acetamide groups at 250-300 nm in UV-spectra of chitosan stabilized NP of silver. It is necessary to note that the spectra reveal presence of NP of silver in the system. At λ max=325, 375 и 450-600 nm there appear absorption bands characteristic for NP Ag respectively for *Acremonium sp.*, *Penicillium sp.* and *Pseudomonas sp.* (samples № 1-3, figure 3 a and b). However, the spectrum of sample №3 (*Pseudomonas sp.*) differs from the spectra of samples №1 and 2 (*Acremonium sp.* and *Penicillium sp.*),

which possible is related to complexity of the reactional mixture.

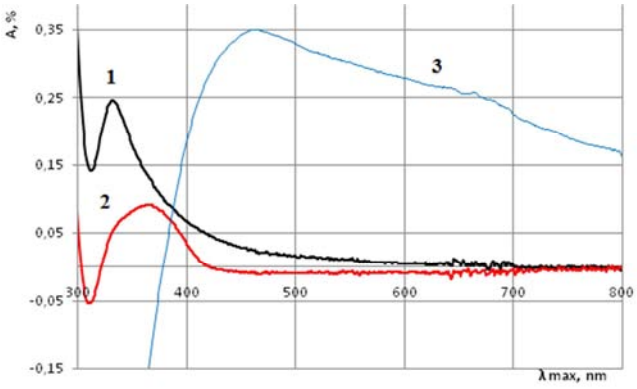
There is an absorption band at λ max = 375 nm in UV-spectrum of the sample №2, which testifies formation of larger NP compared to the sample №1. Decrease in intensity of absorption bands of received samples by the time reveals that synthesized NP of silver are less stable. Solutions of obtained silver NP are stable for 7 days, then agglomeration takes place, which stipulates necessity for their stabilization from agglomeration and oxidation. Earlier for stabilization of metal nanoparticles we used natural polysaccharide – chitosan [3, 16]. In these regards, silver NP were stabilized with chitosan *Bombyx mori* [13]. Molar ratio chitosan (monomer chain)/$Ag^+$ was 2.

The films were obtained by the method of dry formation from the mixture of solutions of chitosan and NP of silver. It was established that in selected conditions of the synthesis the spherical NP of silver are formed (figure 4).

Atomic-force microscopy and histogram of distribution of NP of silver in polymer matrix revealed that NP are formed within the range from 1.5 to 5 nm.

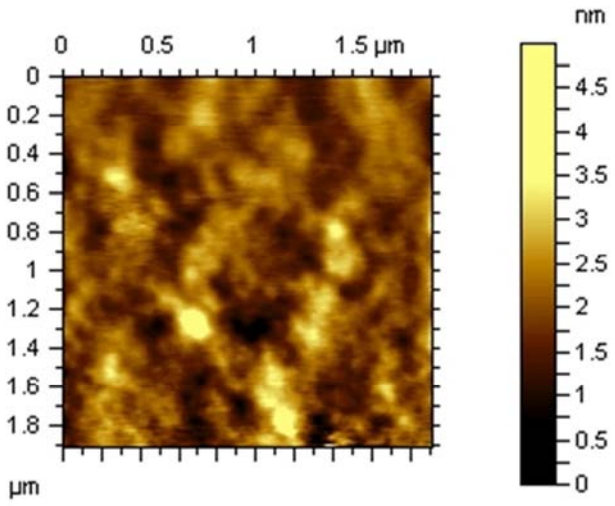



a

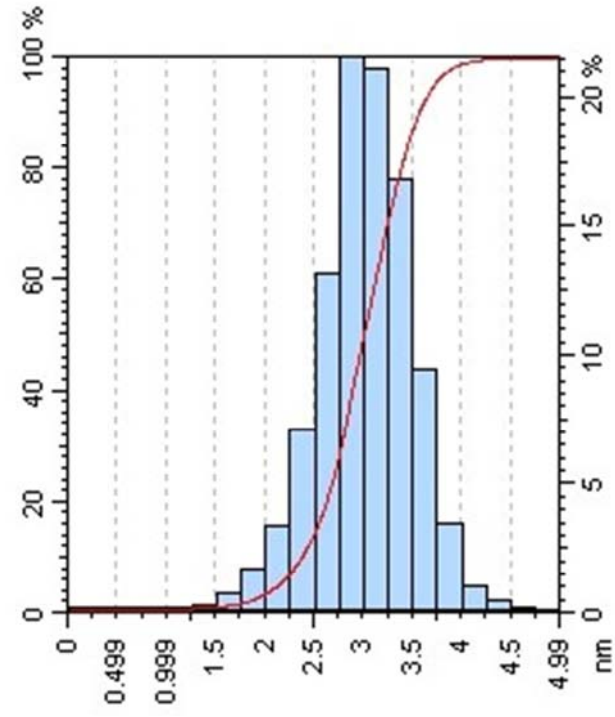



b

**Figure 3.** UV-spectra of samples (on 7th day): 1 – *Acremonium sp.*, 2 – *Penicillium sp.*, 3 – *Pseudomonas sp.*




a




b

**Figure 4.** Atomic-force microscopy (a) and histogram of distribution (b) of NP of silver in polymer matrix. Sample №1 – *Acremonium sp.* (7th day), pH=6, color – dark-brown.

## 5. Summary and Conclusions

Thus, as result of conduced study it was established that majority of selected microorganisms possess capacity to the NP synthesis. It was determined that composition of nutrient medium and conditions of cultivation express significant impact on synthesis of NP. Separately should be noted that in our experiments nanoparticles synthesized by bacteria expressed better stability compared to nanoparticles synthesized by filamentous fungi.

It was determined that chitosan stabilized NP of silver were obtained at the presence of strains of microorganisms. It was established that solutions absorbed emission in the visible range 325-600 nm characteristic for NP of silver. Synthesized NP of silver has spherical form and their size ranges from 1.5 to 5 nm. Obtained data represent certain interest for further development of methods of receipt of NP of silver with application of microbiological approaches.

## References


[1] Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R., & Sastry, M. (2003). Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids and surfaces B: Biointerfaces, 28(4), 313-318.

[2] Baker, S., Harini, B. P., Rakshith, D., & Satish, S. (2013). Marine microbes: invisible nanofactories. Journal of Pharmacy Research, 6(3), 383-388.

[3] Bochek, A. M., Vokhidova, N. R., Saprykina, N. N., Ashurov, N. S., Yugai, S. M., & Rashidova, S. S. (2015). The properties of chitosan-cobalt nanoparticle solutions and related composite films. Polymer Science Series A, 57(4), 460-466.

[4] Ganbarov, Kh. G., Jafarov, M. M., Ramazanov, M. A., Agamaliyev, Z. A., & Eyvazova, G. M. (2017). Biosynthesis of silver nanoparticles using Saccharomyces sp. strain BDU–XR1. Environment, 4(6), 11-13.

[5] Kalimuthu, K., Babu, R. S., Venkataraman, D., Bilal, M., & Gurunathan, S. (2008). Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids and Surfaces B: Biointerfaces, 65(1), 150-153.

[6] Khandel, P., & Shahi, S. K. (2016). Microbes mediated synthesis of metal nanoparticles: current status and future prospects. International Journal of Nanomaterials and Biostructures, 6(1): 1-24.

[7] Klaus, T., Joerger, R., Olsson, E., & Granqvist, C. G. (1999). Silver-based crystalline nanoparticles, microbially fabricated. Proceedings of the National Academy of Sciences, 96(24), 13611-13614.

[8] Klaus-Joerger, T., Joerger, R., Olsson, E., & Granqvist, C. G. (2001). Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. TRENDS in Biotechnology, 19(1), 15-20.

[9] Krutyakov, Yu. A., Kudrinskiy, A. A., Olenin, A. Yu., & Lisichkin, G. V. (2008). Synthesis and properties of silver nanoparticles: advances and prospects. Russ Chem Rev, 77(3), 233–257.

[10] Mandal, D., Bolander, M. E., Mukhopadhyay, D., Sarkar, G., & Mukherjee, P. (2006). The use of microorganisms for the formation of metal nanoparticles and their application. Applied Microbiology and Biotechnology, 69(5), 485-492.

[11] Narayanan, K. B., & Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. Advances in colloid and interface science, 156(1), 1-13.

[12] Pavani, K. V., Kumar, N. S., & Sangameswaran, B. B. (2012). Synthesis of lead nanoparticles by Aspergillus species. Pol J Microbiol, 61(1), 61-63.

[13] Rashidova, S. S., & Milusheva, R. Y. (2009). Chitin and Chitosan Bombyx Mori. FAN, Tashkent.

[14] Revina, A. A., Baranova, E. K., Mulyukin, A. L., & Sorokin, V. V. (2005). Some peculiarities of impact of cluster silver on yeast cells Candida utilis. Electronic scientific journal Studied in Russia, 1403-1409.

[15] Sadowski, Z., Maliszewska, I. H., Grochowalska, B., Polowczyk, I., & Kozlecki, T. (2008). Synthesis of silver nanoparticles using microorganisms. Materials Science-Poland, 26(2), 419-424.

[16] Vokhidova, N. R., & Rashidova, S. S. (2016). Synthesis and Stabilization of Cobalt and Cooper Nanoparticles by Chitosan Bombyx mori. Journal of Inorganic and Organometallic Polymers and Materials, 26(6), 1380-1386.