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APPLICATION OF SOME MICROORGANISMS FOR SYNTHESIS OF GOLD AND SILVER NANOPARTICLES

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Фронтасьева М. В. и др. E14-2012-4

Применение некоторых видов микроорганизмов
для синтеза наночастиц золота и серебра

В последние годы большое внимание уделяется синтезу наночастиц с помощью микроорганизмов. В данной работе для синтеза наночастиц золота и серебра использовались новые штаммы актиномицетов *Streptomyces glaucus* 71MD, *Streptomyces* spp. 211A, бактерии рода arthrobacter — *Arthrobacter globiformis* 151B и *Arthrobacter oxydans* 61B и сине-зеленая микроводоросль *Spirulina platensis*. Исследования проводились с помощью сканирующего и просвечивающего электронных микроскопов, энергодисперсионного анализа, рентгеновской дифракции, атомно-адсорбционной спектрометрии и нейтронного активационного анализа.

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Application of Some Microorganisms
for Synthesis of Gold and Silver Nanoparticles

In recent years, much attention has been paid to microbial technologies of nanoparticle production. Novel strains of actinomycetes *Streptomyces glaucus* 71MD, *Streptomyces* spp. 211A, arthrobacter genera — *Arthrobacter globiformis* 151B and *Arthrobacter oxydans* 61B and blue-green microalga *Spirulina platensis* were used for synthesis of silver and gold nanoparticles. The studies were carried out using scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), transmission electron microscopy (TEM), X-ray diffraction (XRD), atomic absorption spectrometry (AAS), and neutron activation analysis (NAA).

The investigation was performed at the Frank Laboratory of Neutron Physics, JINR, at the E. Andronikashvili Institute of Physics of the Georgian Academy of Sciences, Tbilisi, and in the South African Nuclear Energy Corporation (NECSA), Pretoria, South Africa.

Communication of the Joint Institute for Nuclear Research. Dubna, 2012

INTRODUCTION

Nanobiotechnology is a rapidly advancing area of scientific and technological opportunity that applies the tools and processes of nano/microfabrication to build devices for studying biosystems. The microorganisms (bacteria, microalgae, yeasts, fungi) are often used as possible «nanofactories» for the development of clean, nontoxic and environmentally friendly methods of producing silver and gold nanoparticles [1–4]. Successful collaborative studies of the Sector of NAA and Applied Research of the Division of Nuclear Physics of FLNP with the Institute of Physics, Georgia, in the interaction of metals with microorganisms [5, 6] were extended to nanobiotechnology [7, 8].

1. MATERIALS AND METHODS

Neutron activation analysis (NAA) is used among the variety of analytical and spectral methods: UV-vis spectrometry, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) with energy-dispersive analysis of X-ray (EDAX), atomic absorption spectrometry (AAS) for investigation of the obtained nanomaterials. The few bacterial strains of actinomycetes *Streptomyces glaucus* 71MD and *Streptomyces* spp. 211A (isolated from the rhizosphere of soybeans grown in Georgia), arthrobacter genera — *Arthrobacter globiformis* 151B and *Arthrobacter oxydans* 61B (isolated from the basalt rocks collected from the Kazreti region of Georgia) and blue-green algae *Spirulina platensis* (strain IPPAS B-256 from the algeological collection of the Timiryazev Institute of Plant Physiology, Russian Academy of Sciences) were used for gold and silver nanoparticles synthesis. Cells of actinomycetes and *Spirulina platensis* were grown as described elsewhere [7, 8]. The harvested mycelial mass was then resuspended in 250-ml Erlenmeyer flasks in 100 ml of 10^{-3} M aqueous $HAuCl_4$ (chloroauric acid) solution in the synthesis of gold nanoparticles, and in aqueous $AgNO_3$ (argentum nitrate) solution in the synthesis of silver nanoparticles. Time-dependence of nanoparticle formation was studied in different time intervals (several days).

2. RESULTS AND DISCUSSION

The gold and silver surface plasmon resonances (SPR) were observed in the UV-vis absorption spectra at $\sim$ 530 nm for gold and at 425 nm for silver, respectively. The presence of SPRs indicates the gold and silver ion reduction and the subsequent aggregation of nanoparticles in the solutions. The intensity of the peaks increased as a function of the reaction time. A single band in all spectra gives evidences for the spherical shape of gold and silver nanoparticles, which is also confirmed by TEM images (Fig. 1).

In all TEM images the diffraction patterns correspond to the face centered cubic (fcc) structure of gold and silver nanoparticles. The particle size histogram for studied samples shows that the size of gold and silver nanoparticles is in the

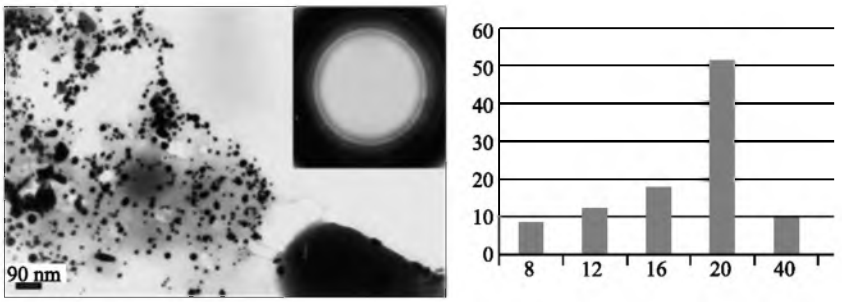

Fig. 1. TEM image and size histogram of Au nanoparticles in biomass of *Arthrobacter* 61B

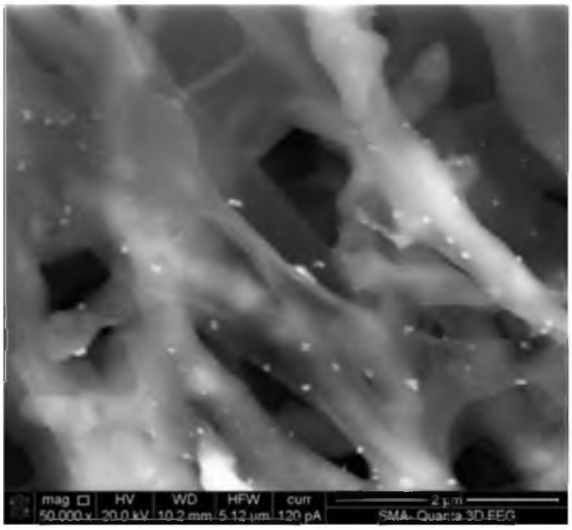

Fig. 2. SEM image of Ag nanoparticles in biomass of *Streptomyces glaucus* 71MD

range of 5 to 80 nm, with an average of 25 nm. The XRD data for gold and silver nanoparticles confirm the presence of the fcc structure. As an example, the SEM image of *Streptomyces glaucus* 71MD cells (after interacting with $AgNO_3$ solution for seven days) is given (Fig. 2). The SEM images illustrate that most of the particles are spherical and do not create big agglomerates.

The EDAX X-ray spectra were registered proving the presence of gold nanoparticles in *Spirulina platensis* cells treated with $HAuCl_4$ for 5 days (Fig. 3). Along with the Au peaks, the signals from C, O, Cl and Fe were recorded. Neutron activation analysis (NAA) was carried out in collaboration with the South African Nuclear Energy Corporation (Necsa), Pelindaba, Pretoria, South Africa, at the nuclear research reactor SAFARI-1. The samples were irradiated for 8 s at a neutron flux density of $\sim 5 \cdot 10^{14}$ cm$^{-2} \cdot$ s^{-1}. Their activities were measured three times, after cooling for 3 and 30 hours and 7 days, respectively. The gold content was determined on the 411.8 keV γ-line of ^{198}Au. Genie 2000 software was used to process NAA data. The data obtained by NAA illustrate that uptake of metals includes two phases: rapid and slower uptake. In the first «rapid» stage, the metal ions are adsorbed onto the surface of the microorganism. The concentration of gold increases rapidly. In the «slow» stage, the metal ions are transported across the cell membrane into the cytoplasm. The total concentration of gold in the samples (extracellular and intracellular) does not change significantly. The data obtained by AAS (Fig. 4) are confirmed by NAA (Fig. 5).

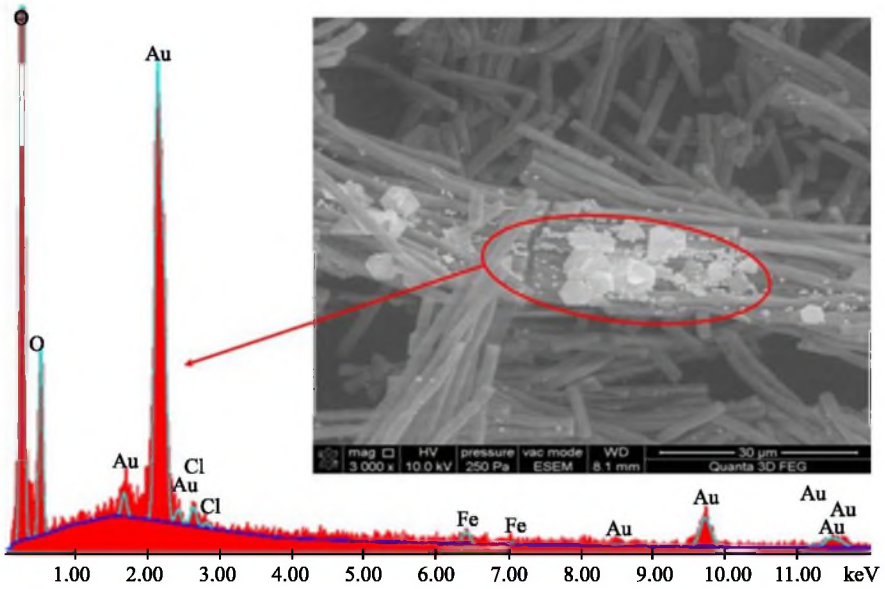

Fig. 3. EDAX spectrum of *Spirulina platensis* exposed to $HAuCl_4$ (10^{-2} M)

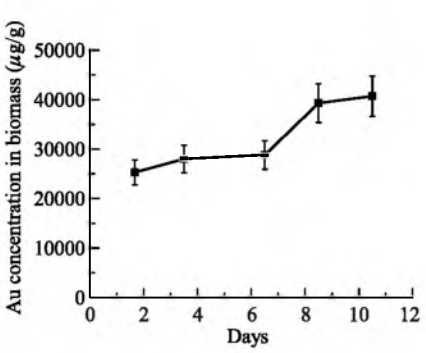

Fig. 4. The gold concentrations in biomass of *Arthrobacter globiformis* 151B versus the time of exposure to gold chloroaurate determined by AAS

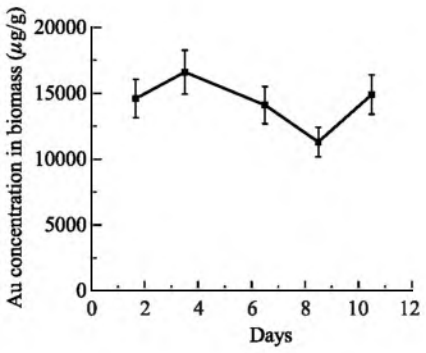

Fig. 5. The gold concentrations in biomass of *Arthrobacter globiformis* 151B versus the time of exposure to gold chloroaurate determined by NAA

CONCLUSIONS

The results of the performed investigations show that the studied microorganisms are capable of producing nanoparticles extracellular when exposed to the gold and silver compounds. The shape of the majority of the nanoparticles is spherical and the average size is 25 nm. The biosynthesis of nanoparticles is simple, economically viable and an eco-friendly process.

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