

# Development of Biotechnology for Microbial Synthesis of Gold and Silver Nanoparticles

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**Abstract:** Several bacterial strains of *Actinomycetes* belonging to *Streptomyces* and *Arthrobacter* genera for the first time were used to study the biotechnology of synthesis of gold and silver nanoparticles. The experimental conditions of gold and silver nanoparticles production by the cells of studied strains in aqueous chloroauric acid (HAuCl<sub>4</sub>) and in silver nitrate (AgNO<sub>3</sub>) solutions, respectively, were determined. Concentration and time-dependences of nanoparticle formation were investigated. The complex of optical and analytical methods was used for testing the gold and silver nanoparticles in the bacterial biomass. The TEM (Transmission Electron Microscopy) and XRD (X-ray Diffraction) data in all cases demonstrated the presence of crystals with fcc (face centered cubic) structure. The results obtained show that the *Actinomycetes* are capable of producing gold and silver nanoparticles of spherical shape extracellularly when exposed to suitable compounds. The particle size distribution shows that the sizes of nanoparticles are in the range of 5 nm to 80 nm. The biomass obtained may be used for industrial as well as medical and pharmaceutical purposes.

**Key words:** Microbial synthesis, nanoparticles, gold, silver, biotechnology.

## 1. Introduction

In recent years, the microbial technologies of metal nanoparticles production have received great attention in materials science and industry [1-3]. A large number of microorganisms are characterized by their affinity with metal ions and their tolerance to high metal concentrations. Microorganisms often exhibit

defense mechanisms that contribute to their survival in aggressive environments containing harmful metallic compounds. The same mechanism must be responsible for their ability to produce metallic nanoparticles when exposed to such environments [4].

Nanoparticles produced in the microbial biomass have ultra small size, high surface area to mass ratio and reactivity, which determines their unique physical and chemical properties [5]. Microbial cells have developed specific mechanisms for surface functional groups (peptides, proteins, nucleic acids) interacting

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with metal ions in the aqueous solutions which result in extracellular inorganic precipitation [6, 7]. Anionic bacterial surfaces interact with metal cations, which produce a negative charge density throughout the wall and then intracellular binding of metals [8].

Various microorganisms (bacteria, yeast, fungi) are known for their effectiveness and flexibility in producing gold [9-14] and silver [8, 9, 15, 16] nanoparticles. Gold and silver nanoparticles have potential applications in electronics, information technology, catalysis, medicine, pharmacology, chemical sensing, biosensing and photonics. In medicine they have shown therapeutic potential in oncology, cardiology, immunology, neurology and endocrinology [4, 17, 18].

Gold nanoparticles possess stability, oxidation resistance and biocompatibility. Due to their optical and chemical properties and high infrared phototherapy potential, they may be used for early diagnosis and treatment of cancer. The use of precisely engineered gold nanoparticles as anticancer agents allows direct treatment of diseased cells with reduced damage to healthy cells [19, 20].

Silver nanoparticles are characterized by excellent biocompatibility and low toxicity for man, but at the same time silver at low concentrations is well known to exhibit toxicity to a wide range of bacteria, virus and other (about 650) eukaryotic microorganisms [21, 22]. Silver is also an effective antimicrobial agent. High synergistic activity of silver nanoparticles and antibiotics has been observed.

Thus both gold and silver nanoparticles have a large field of application for many purposes. However, current conventional nanotechnology causes environmental pollution due to the toxicity of the reagents used [1]. Therefore, there is a great need to develop new alternative, easy and eco-friendly methods of producing gold and silver nanoparticles using bacteria and to search for new effective microbial strains.

The actinomycetes are a widespread group of

Gram-positive bacteria. Among microorganisms, actinomycetes (actinobacteria) appear to be a very specific taxonomic group responsible for the biosynthesis of a number of biologically active compounds (enzymes, amino acids, vitamins, antibiotics, etc.). Actinobacteria are distinguished from other bacteria by their morphology and by their G + C rich genome. Though some actinobacteria display pleomorphic or even coccoid elements, characteristically they form filamentous mycelium and may produce spores that are easily detached and may become airborne when disturbed [23].

The ability of biosorption and bioremediation shown by actinomycetes could be useful in heavy metal removal by the valence change of metal ions. They are classified as prokaryotes and may be easily manipulated genetically to control sizes and polydispersity of the resulting nanoparticles [7, 8].

Actinomycetes-mediated chemistry has many advantages for the synthesis of gold and silver nanoparticles [11, 12, 24, 25]. Terrestrial actinobacteria, especially *Streptomyces* genera are rich sources of biologically active products and are being extensively used for industrial production of pharmaceuticals. Numerous actinomycetes have been isolated from different environments—soils, compost, marine sediments, rocks, water etc. Antimicrobial and antifungal activity of actinomycetes is well established—two-third of microbial-produced antibiotics belongs to actinomycetes. They are effective in the treatment of many antibiotic-resistant pathogens. Antibiotics produced by actinobacteria are widely used in human and veterinary medicine, and in agriculture. This group of microorganisms also contributes to soil fertility regeneration and polluted soil cleanup by degrading the carbon skeleton of toxic organic compounds.

Thus, it is important to screen new classes of actinomycetes to synthesize gold and silver nanoparticles with technologically important properties. In the present work, the results of the

studies of several new strains of actinomycetes for developing methods of producing gold and silver nanoparticles are presented. Some results of earlier investigations are presented in Refs. [26, 27]. Strains of microorganisms belonging to *Streptomyces* and *Arthrobacter* genera have been studied as possible “nanofactories” for the development of clean and nontoxic methods of the synthesis of silver and gold nanoparticles. A few different analytical and spectral methods have been used for examining gold and silver nanoparticles. The obtained results are discussed to evaluate the ability of the studied strains to produce gold and silver nanoparticles.

## 2. Material and Methods

### 2.1 Materials

Among many geographical regions of the planet, the South Caucasus has particularly attracted the attention of bioprospectors. There are 14 different soil-climatic zones on the southern slopes of the Caucasus with an average annual temperature between 5-25 °C. Georgia is a country covering 69,000 km<sup>2</sup>, occupying more than 80% of the South Caucasus. This area is characterized by extreme microbial and plant biodiversity. The study of biodiversity of the microorganisms, their distribution and classification into definite groups and species is performed on the bases of an in-depth study of their biological properties. Special interest is focused on adaptation mechanisms of the microorganisms to extreme environment. Among extremophilic microorganisms actinomycetes-actinobacteria in particular, widely distributed in nature, are species of a great interest [28]. The distribution of extremophilic actinomycetes in various types of soils, rocks and rhizosphere of Georgia has been studied. Among extremophilic actinomycetes isolated from various types of soils of Georgia, there were 49% of halophiles, 27% of alkaliphiles and 24% of thermophiles [29, 30].

The new strain *Streptomyces* spp. 211A (alkaliphilic

actinomycetes) was isolated from the Cinnamonic calcareous soil of Sagarejo region in Georgia using the method of dilution [30]. Incubation was performed in a thermostat at 26-28 °C for 14 days. Pure colonies were characterized for their morphological and physiological characteristics by various biochemical tests. The growth ability of the culture was studied in different synthetic and organic nutrient media. Pridham’s method was used to study the carbon source uptake ability [31]. Fedorov’s [32] nutrient medium was used to establish the uptake of different sources of nitrogen. The hydrocarbon absorption ability of actinomycetes was determined according to their growth intensity. Antagonistic properties were studied by the agar block method [33]. The protease activity was determined by Anson’s method, modified by Petrova [34]. Actinomycete identification was performed according to Krasilnikov and Bergey’s Manuals [35]. The strain *Streptomyces* spp. 211A develops long branched straight hyphae. Aerial mycelium is white-violet coloured, colonies—dark violet, nutrient medium—brownish. The culture is extremophilic, specifically, alkaliphilic. In experiments the cells were grown aerobically at pH 7-8, 28-30 °C in 500 mL Erlenmeyer flasks in the liquid medium Gauze-1 [29]: K<sub>2</sub>HPO<sub>4</sub> (0.05%), MgSO<sub>4</sub> (0.05%), NaCl (0.05%), KNO<sub>3</sub> (0.1%), FeSO<sub>4</sub>·7H<sub>2</sub>O (0.001%), starch (2%), east extract (0.03%). The culture was grown with continuous shaking on a shaker (200 g) at 30 °C for 10 days.

All chemicals used in the experiment were ACS-reagent grade, produced by Sigma (St. Louis, MO, USA).

The bacterial strain *Streptomyces glaucus* 71MD (mezophilic actinomycetes) isolated from the rhizosphere of soybeans in Georgia creates spirally twisted sporophores. The aerial mycelium is bluish-green. It develops a melanoid pigment. On a synthetic nutrient medium, the bacteria grow well. Their colonies and nutrient medium are colorless. The contents of the medium for optimal growth of

actinomycete *Streptomyces glaucus* 71MD is:  $K_2HPO_4$  (0.05%),  $MgSO_4$  (0.05%), NaCl (0.05%),  $KNO_3$  (0.1%),  $FeSO_4 \cdot 7H_2O$  (0.001%), glucose (2%), yeast extract (0.03%), pH 7.5. In this nutrient medium the bacteria were grown in 250 mL Erlenmeyer flasks under aerobic conditions with permanent shaking of the suspension and at the temperature of 28-30 °C, pH = 7-8. The biomass was harvested by centrifugation. Two Gram-positive aerobic bacterial strains belonging to *Arthrobacter* genera—*Arthrobacter globiformis* 151B and *Arthrobacter oxydans* 61B were isolated from the basalt rocks collected from the Kazreti region in the Republic of Georgia. In both cases incubation was carried out at 20-30 °C, pH 7-12 for 15-17 days. The pure colonies were tested to examine their morphological and physiological characteristics. The growth ability of the bacteria was studied in different synthetic and organic media. Antagonistic properties towards other Gram-positive bacteria, fungi and yeasts were not observed. The bacteria were grown aerobically in the following nutrient medium: 10 g of glucose, 10 g of peptone, 1 g of yeast extract, 2 g of caseic acid hydrolysate, 5 g of NaCl, and 1 L of distilled water. Bacterial cells were grown in 250 mL Erlenmeyer flasks as a suspension at 21 °C being shaken continuously for 5 days.

After cultivation in all cases mycelia (cells) were separated from the culture broth by centrifugation (12,000 g) for 20 min, and then the biomass was washed three times with sterile distilled water. The harvested mycelial biomass (2-3 g of wet mycelia) was resuspended in 250 mL Erlenmeyer flasks with 100 mL of  $10^{-3}$  M aqueous chloroauric acid ( $HAuCl_4$ ) solution for synthesis of gold nanoparticles and aqueous silver nitrate ( $AgNO_3$ ) solution for synthesis of silver nanoparticles. The resulted mixtures were put again into the shaker at 28-30 °C (200 g) for different periods of time (1-12 days).

For UV-Vis (Ultraviolet-Visual) spectral analysis and TEM (Transmission Electron Microscopy) of the suspension samples were taken after different time

intervals. For these studies, both the suspension and supernatant obtained after centrifugation were used. For SEM (Scanning Electron Microscopy), X-ray diffraction, equilibrium dialysis, AAS (Atomic Absorption Spectrometry) and NAA (Neutron Activation Analysis) analysis, the bacterial cells were harvested by centrifugation at 12,000 g for 20 min and this wet biomass was placed in an adsorption-condensation lyophilizer and dried [36].

## 2.2 Methods

### 2.2.1 UV-Vis Spectrometry

The UV-Vis (Ultraviolet-Visual) spectra of the samples were recorded by a spectrophotometer “Cintra 10” (GBC Scientific Equipment Pty Ltd., Australia) with a wavelength range of 190-1,100 nm.

### 2.2.2 XRD (X-ray Diffraction)

XRD measurements were made with a Dron-2.0 diffractometer. The BCV-23 X-ray tube with the Cu anode ( $CuK_{\alpha}$ :  $\lambda = 1.54178 \text{ \AA}$ ) was used as a source of radiation; the Ni grid with a width of 20  $\mu\text{m}$  was used for filtration of the radiation; the rate of the detector was 2°/min; the interval of intensity was 1,000 pulses/min and the time constant was 5 s.

### 2.2.3 TEM (Transmission Electron Microscopy)

TEM (Transmission Electron Microscopy) was performed using the JEOL SX-100 equipment (Japan) operating at 100 kV. The TEM studies were done at 50,000 $\times$  magnification. The samples were prepared by placing a drop of solution with the gold nanoparticles on carbon-coated TEM grids. The films on the TEM grids were allowed to dry at room temperature before analysis.

### 2.2.4 SEM (Scanning Electron Microscopy)

SEM (Scanning Electron Microscopy) was carried out using the SDB (small dual-beam) FEI Quanta 3D FEG with the EDAX Genesis EDX system with the resolution 1.2 nm. Operational features of the microscope used in the experiment: magnification 5,000-150,000 $\times$ ; voltage 1-30 kV. Microprobe analysis of gold nanoparticles clusters was conducted

with the energy-dispersive X-ray spectrometer (EDAX, USA). The acquisition time ranged from 60 s to 100 s, and the accelerating voltage was 20 kV [37].

#### 2.2.5 EDAX (Energy-Dispersive X-ray Analysis)

To identify different elements associated with the sample specimen the “built-in” spectrometer called an EDAX spectrometer was used. EDAX is an analytical technique which utilizes X-rays that are emitted from a specimen when bombarded by an electron beam to identify the elemental composition of the specimen. The EDAX X-ray detector measures the number of emitted X-rays versus their energy. Microprobe analysis of gold nanoparticles clusters was conducted with EDAX spectrometer, USA. The acquisition time ranged from 60 s to 100 s, and the accelerating voltage was 20 kV.

#### 2.2.6 AAS (Atomic Absorption Spectrometry)

Flame AAS with a “Beckman-495” spectrometer was used for gold and silver determination in the experimental samples. Measurement was carried out at the wavelength of the gold resonance line = 242.8 nm.

#### 2.2.7 NAA (Neutron Activation Analysis)

The gold and silver concentrations as well as the elemental content of samples were determined using NAA at the reactor IBR-2 of the Frank Laboratory of Neutron Physics of the Joint Institute for Nuclear Research (Dubna, Russia). The experimental equipment and irradiation conditions of samples are described elsewhere [38]. The concentrations of elements based on short half-life radionuclides were determined by irradiation for 60 s under a thermal neutron fluency rate of approximately  $1.6 \times 10^{13} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ . The long half-live isotopes were determined using a cadmium-screened irradiation channel under a resonance neutron fluency rate of approximately  $3.31 \times 10^{12} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ . The samples were irradiated for 5 days, repacked and then measured twice after decays of 4 days and 20 days.

The counting time varied from 30 min to 1.5 h. The gold content was determined with the 411.8 keV

$\gamma$ -line of  $^{198}\text{Au}$ . The silver content was determined using the 657.7 keV  $\gamma$ -line of  $^{110\text{m}}\text{Ag}$ . The NAA data processing and determination of element concentrations were performed using Genie 2000 software [39].

### 3. Results and Discussion

At first the UV-Vis spectrometry was used for testing of bacterial samples with gold and silver nanoparticles. The gold SPR (surface plasmon resonance) peak at  $\sim 530 \text{ nm}$  in UV-Vis absorption spectra corresponds to aggregation in the solutions of the gold nanoparticles which are formed by means of the gold ion reduction from Au (III) to Au (0) by biomolecules, proteins and enzymes on the surface of bacteria cells. The spectra in silver cases exhibit the appearance of an absorption peak at 425 nm, which is characteristic of silver nanoparticles and corresponds to silver reduction from Ag (I) to Ag (0) [32].

As is known, absorption bands of nanocluster plasmons strongly depend on the particle sizes, the dielectric constant of the medium and surface-adsorbed species. Using Maxwell’s electromagnetic theory to explain the interaction of light with spherical particles, the size of which is of the order of the light wavelength or smaller, Mie [41] assumes that the shape of plasmon resonance absorption bands depends on the particle nature. According to Mie’s theory, a single SPR band is expected in the adsorption spectra of spherical isotropic nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of particles [42]. The intensity of the peaks for nanoparticles of definite sizes increased as a function of time of reaction.

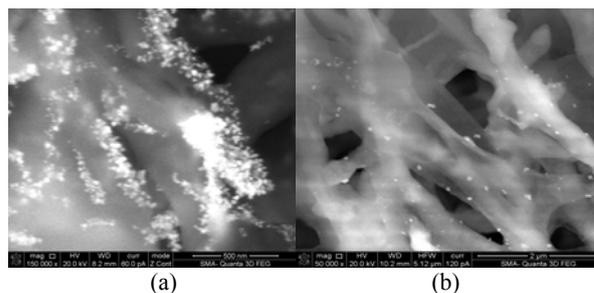
In the preliminary investigations optimal concentrations of gold and silver compounds for synthesis of nanoparticles were determined. Dose-dependence of absorbance versus chloroauric acid and silver nitrate concentrations  $10^{-2}$ - $10^{-4} \text{ M}$  in UV-Vis spectra was studied for different microorganisms. The optimal concentration in all

cases was near  $10^{-3}$  M. At a concentration of  $10^{-2}$  M the sizes of nanoparticles were higher than 100 nm and the peaks were not observed. In Ref. [43], it is hypothesized that the number of active objects on the surface of bacteria cells that were involved in the synthesis was not sufficient for the reduction of metal ions at higher concentrations. Consequently, the synthesis process depended on the metal concentration as well as on the number of the cells in solution. This differential response indicates the possibility of custom designed nanoparticles by varying cell number and metal concentration in solution.

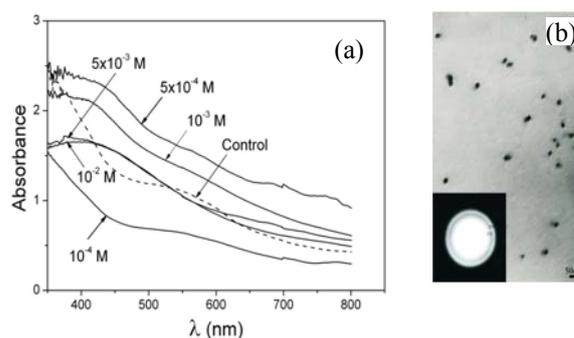
In addition, in nutrient medium, some organic compounds in the cell cultivation may be used for activation of cell growth and synthesis processes. Using NAA it was demonstrated that in the synthesis of silver nanoparticles by actinomycete *Streptomyces glaucus* 71MD the addition of glucose in the nutrient medium increases the production of nanoparticles by 3 times as compared with the starch. The SEM image in Fig. 1 demonstrated intensive production of silver nanoparticles in biomass of *Streptomyces glaucus* 71MD.

UV-Vis spectra of gold nanoparticles for all the bacteria studied have better identified SPR peaks at 530 nm than the peaks of silver nanoparticles at 425 nm. In Fig. 2, (a) the dose-dependence for silver nanoparticles synthesis by actinomycete *Streptomyces* spp. 211A and (b) the TEM image are presented. As can be seen from the image, production of silver nanoparticles at a silver nitrate concentration of  $10^{-3}$  M takes place, but the surface plasmon peak of silver at 425 nm is not well separated which may be caused by the dispersed sizes of the nanoparticles, dielectric properties of the medium and the poor state of surface-adsorbed species because of the antibacterial properties of silver.

The silver nanoparticles were also synthesized using *Arthrobacter globiformis* 151B (Fig. 3). For the measurement of UV-Vis spectra the suspensions in some cases were diluted with distilled water 3-4 times.



**Fig. 1** The SEM images of silver nanoparticles formed in *Streptomyces glaucus* 71MD cells after 5 days treatment by silver nitrate (a) with glucose and (b) with starch.



**Fig. 2** (a) The dose-dependence for silver nanoparticles synthesis by actinomycete *Streptomyces* spp. 211A and (b) the TEM image of silver nanoparticles in biomass *Streptomyces* spp. 211A.

The spectra were observed (a) in the suspension that was diluted 4 times as well as (b) in supernatant obtained after centrifugation of the suspension interacted with silver nitrate for 5 days. The UV-Vis spectra in Fig. 3 show that silver nanoparticles formed in the supernatant have a good monodispersity, but their number is 2.5 times less. So, in some cases, the supernatant may be successfully used for the production of nanoparticles.

In the *Streptomyces* genera strains *Streptomyces* spp. 211A and *Streptomyces glaucus* 71MD intensive production of gold nanoparticles was observed (Fig. 4). For example in Fig. 4 the UV-Vis absorption spectra of gold nanoparticles in (a) *Streptomyces glaucus* 71MD suspension treated with  $\text{HAuCl}_4$  for 40 h and (b) in the same suspension diluted 4 times are given. In all TEM images the diffraction patterns correspond to the fcc (face centered cubic) structure of gold and silver nanoparticles. For example, Fig. 5a shows TEM image and diffractogram of Au nanoparticles

synthesized in biomass of *Arthrobacter oxydans* 61B treated by  $\text{HAuCl}_4$  for 12 days. The particle size histograms for the samples studied show that the sizes of gold and silver nanoparticles are in the range of 5 nm to 80 nm, with an average of 20-25 nm. Example

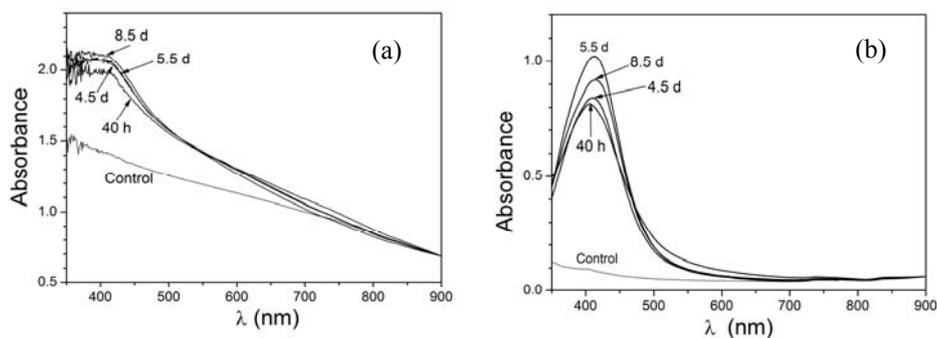


Fig. 3 The UV-Vis absorption spectra for silver nanoparticles synthesized by *Arthrobacter globiformis* 151B (a) in the suspension diluted 4 times and (b) in supernatant.

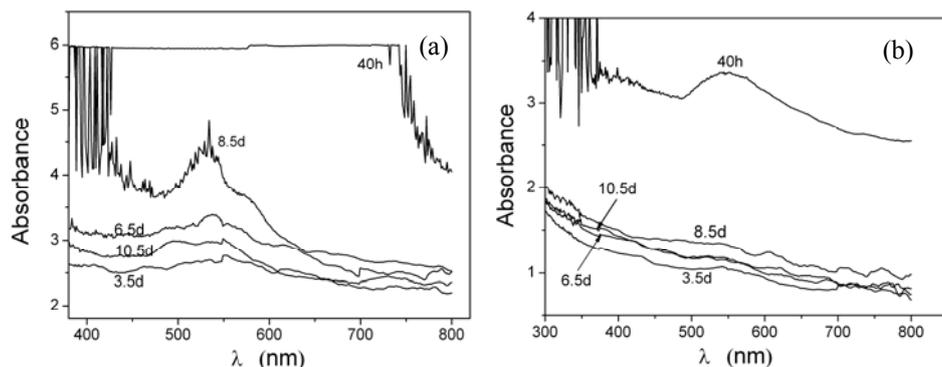


Fig. 4 The UV-Vis absorption spectra for gold nanoparticles (a) in *Streptomyces glaucus* 71MD suspension treated with  $\text{HAuCl}_4$  for 40 h and (b) in the same suspension diluted 4 times.

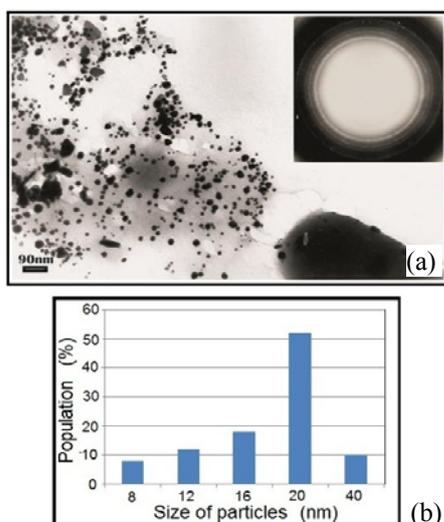


Fig. 5 The TEM image and the size histogram of gold nanoparticles synthesized in *Arthrobacter oxydans* 61B treated by  $\text{HAuCl}_4$  for 12 days.

of the histogram for *Arthrobacter oxydans* 61B is presented in Fig. 5b.

The XRD data for gold nanoparticles synthesized by *Arthrobacter oxydans* 61B treated with chloroauric acid for 12 days confirm the presence of fcc structure

(Fig. 6). The diffraction pattern shows the amorphous structure of gold nanoparticles. However, a number of Bragg reflections corresponding to the fcc structure of gold are also seen here: four characteristic peaks (111), (200), (220) and (311). In cases of silver nanoparticles synthesis the same characteristic peaks corresponding to the fcc structure of elemental silver were observed. The results obtained clearly show that gold and silver nanoparticles, formed by bacterial reduction of ions, are crystalline in nature and they are generally produced extra cellularly.

The Scherrer equation was used for an approximate assessment of the sizes of nanoparticles using the broadening of interference peak of gold (111) on the diffractogram (Fig. 6):

$$d = K \lambda / \beta \cos \theta$$

where  $K$  is the shape factor, for cubic crystals it is 0.9-1;  $\lambda$  is x-ray wavelength, for  $\text{CuK}_\alpha$   $\lambda = 1.54178 \text{ \AA}$ ;  $\beta$  is the line broadening at half the maximum intensity in radians;  $\theta$  is the Bragg angle; and  $d$  is the size of nanoparticles in nm. It is important to realize that the Scherrer formula is applicable to grains less than  $0.1 \mu\text{m}$  [44, 45]. For an approximate assessment of the size of nanoparticles, the (111) interfacial maximum was used. In this case  $\theta = 38^\circ$ . The calculations were carried out taking into account only instrumental broadening of  $\beta$  ( $\approx 0.3^\circ$ ) without evaluation of crystal defects on the maximum shape. The results obtained show that the size of gold nanoparticles is about 22 nm, confirming the range of size determined using the TEM image.

Fig. 7 presents the SEM image of gold nanoparticles formed by actinomycetes *Streptosporangium* spp. 94A cells after interacting with chloroauric acid for 3.5 days. The SEM image of silver nanoparticles in the biomass of *Streptomyces glaucus* 71MD was presented in Fig. 1. Since the studied samples are non-conducting, they were visualized in the natural environment (ESEM) mode. The SEM images illustrate that the extra cellular production of gold and silver nanoparticles in all experiments takes place, most of the particles formed are spherical and do not create big agglomerates.

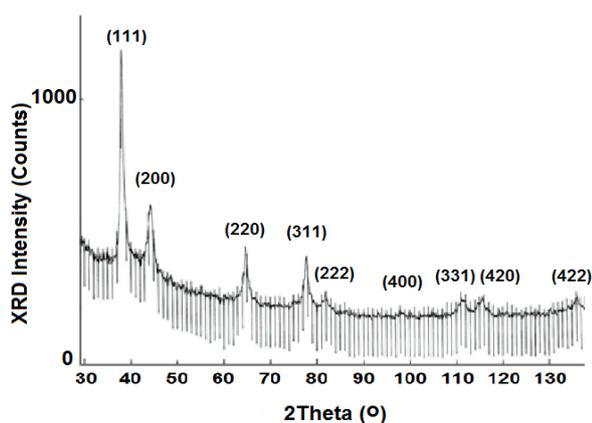


Fig. 6 The XRD diffractogram for gold nanoparticles synthesized by *Arthrobacter oxydans* 61B treated with chloroauric acid for 12 days.

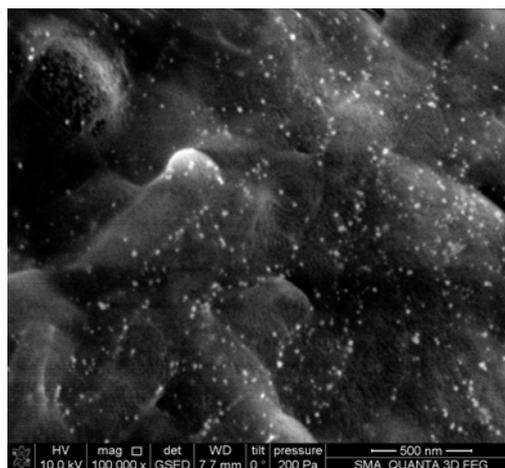


Fig. 7 The SEM image of the actinomycetes *Streptosporangium* spp. 94A cells after interacting with chloroauric acid for 3.5 days.

The EDAX spectra proved the presence of (a) gold nanoparticles in *Arthrobacter globiformis* 151B cells treated with  $\text{HAuCl}_4$  solution for 10 days and (b) silver nanoparticles in *Streptomyces* spp. 211A cells treated with  $\text{AgNO}_3$  solution for 5 days (Fig. 8). Nine peaks of Au were observed for the biomass of *Arthrobacter globiformis* 151B (Fig. 8a). Signals from C, O, K, P, Al and Ca atoms were also recorded. These signals are likely to be due to X-ray emission from the proteins and enzymes present in the cell wall of the biomass. Four peaks of Ag were observed for the biomass of *Streptomyces* spp. 211A (Fig. 8b). The signals from C, O, and P atoms were also recorded.

To study biosorption process on the bacterial cells during nanoparticle production the method of dialysis and atomic-absorption analysis were used. Concentrations of metal sorbed by bacterium in solution at equilibrium obeyed the Freundlich equation, suggesting the presence of heterogeneous sorption sites on bacterium surfaces and in each species of microorganisms, since the sorption depends on the nature and the composition of the cell wall [46]. On the other hand, gram-positive bacteria have a greater sorptive capacity due to their thicker layer of peptidoglycan which contains numerous sorptive sites [47].

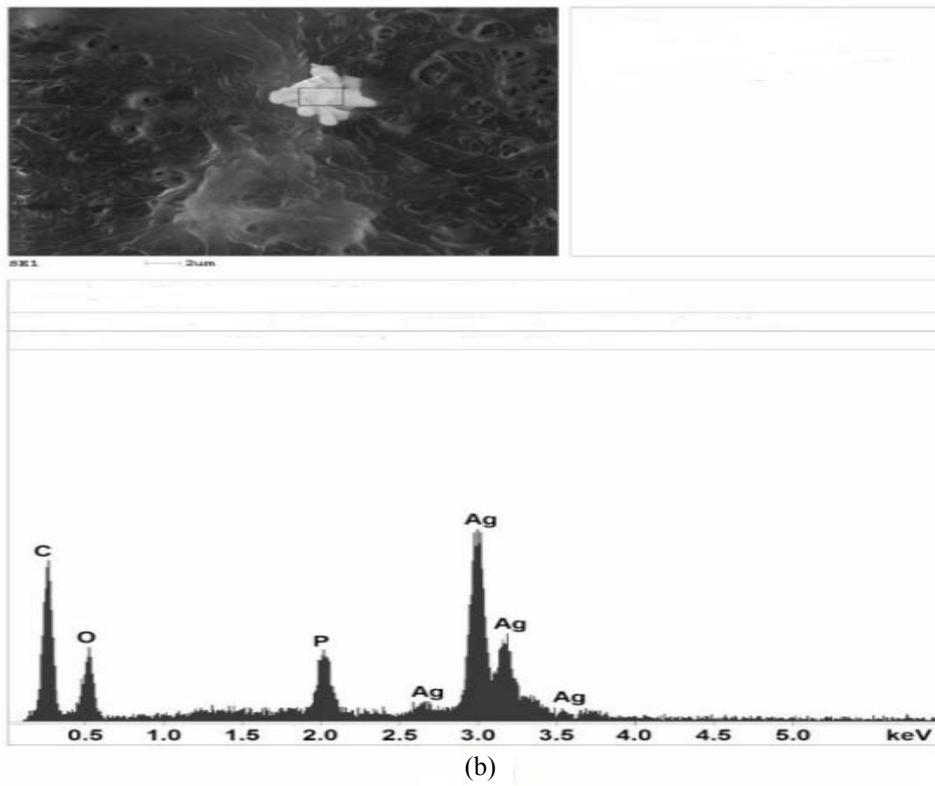
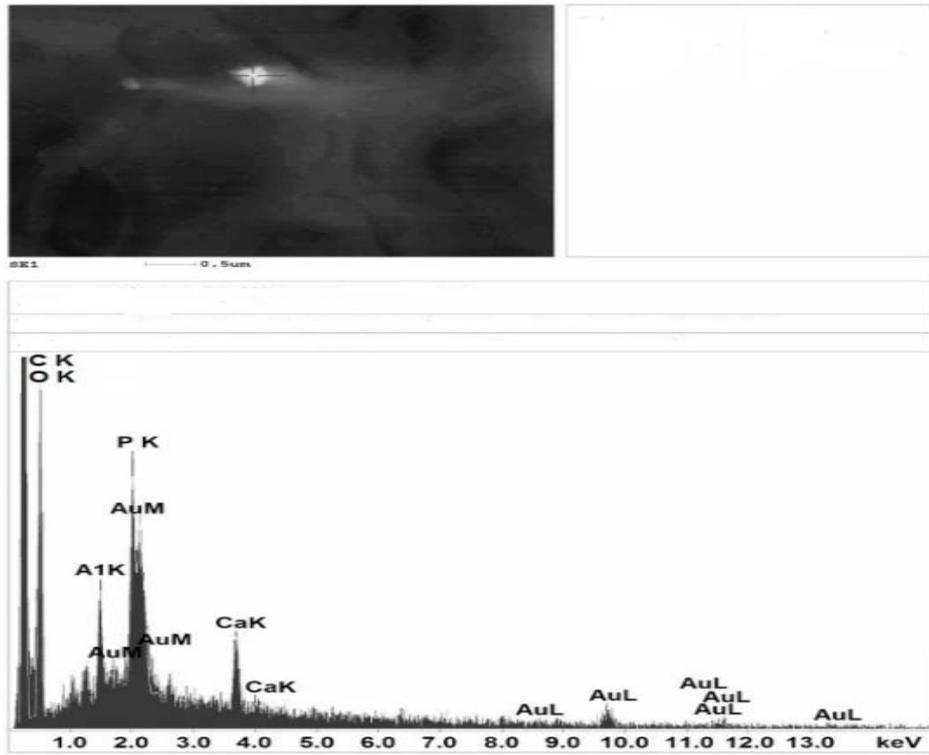


Fig. 8 The EDAX spectra of (a) gold nanoparticles in *Arthrobacter globiformis* 151B and (b) silver nanoparticles in *Streptomyces* spp. 211A.

The capacity of the adsorbent and the equilibrium relationships between adsorbent and adsorbate are described by Freundlich adsorption isotherms:

$$C_b = KC_t^{1/n}$$

where  $C_b$  is the concentration of the metal adsorbed,  $C_t$  is equilibrium concentration of the metal ion in the solution,  $K$  and  $n$  are empirical constants, which may be characterized as the biosorption constant and sorptive capacity, respectively.

In Fig. 9 biosorption isotherms for chloroauric acid solution and *Streptomyces* spp. 211A cells (A—homogenized cells, and B—particulate homogenized cells (insert)) are presented. Each dot is the average of three independent values, and the standard deviation is < 13%.

By means of Freundlich isotherms the biosorption constants ( $K$ ) and the sorptive capacity ( $n$ ) were determined:

(1)  $K = 4.11 \times 10^{-4}$ ,  $n = 3.84$ . (gold—*Streptomyces* spp. 211A—homogenized cells);

(2)  $K = 1.47 \times 10^{-4}$ ,  $n = 4.55$ . (gold—*Streptomyces* spp. 211A—particulate homogenized cells).

As can be seen, the biosorption constant of homogenized cells is greater than that of particulate homogenized cells for which, on the contrary, sorptive capacity is greater.

The results obtained show that cell homogenization increases the surface of bacterial cells that increases the probability of nanoparticle formation.

The roles of processes, which take place on cell surfaces also during the microbial synthesis, are revealed by the results of NAA and AAS. Examples of analytical determination (using AAS and NAA) of gold and silver total concentrations in the bacterial biomass for *Streptomyces* spp. 211A are given in Fig. 10.

The data obtained by NAA (Fig. 10a) illustrate that during the first day the metal concentration increases rapidly and then does not change significantly for a few days. In the first “rapid” phase, the metal ions are mainly adsorbed onto the surface of microorganisms

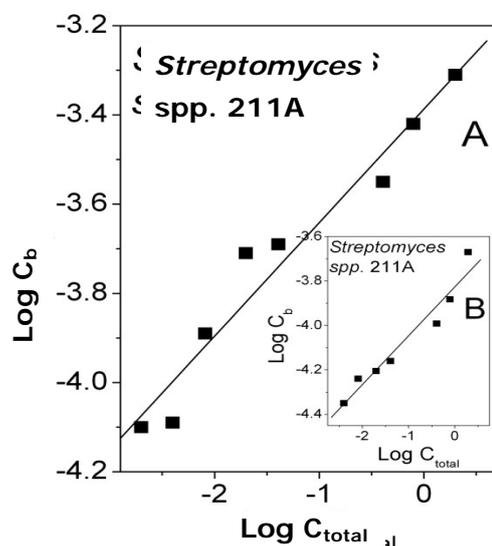


Fig. 9 The linearized Freundlich adsorption isotherms for gold *Streptomyces* spp. 211A cells (A: homogenized cells; and B: particulate homogenized cells).

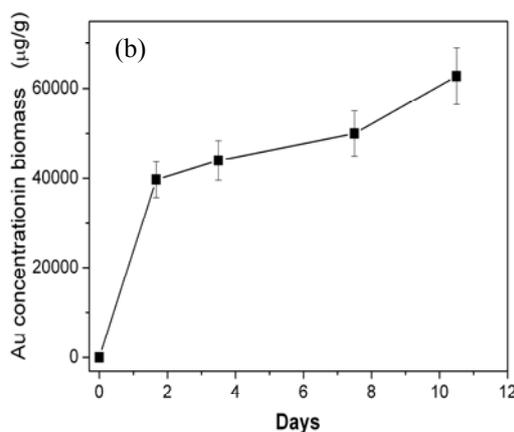
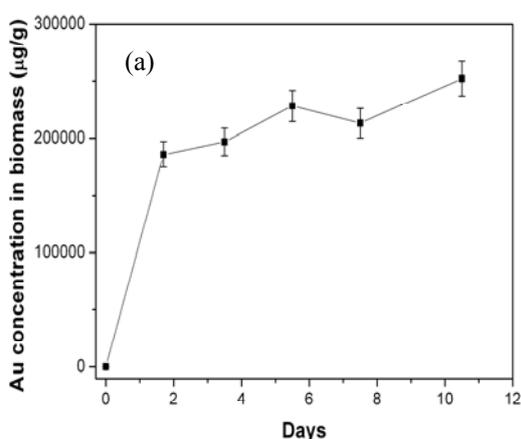


Fig. 10 The total gold concentrations in biomass *Streptomyces* spp. 211A determined by (a) NAA and by (b) AAS.

by the functional amino, carboxylic, sulfhydryl, phosphate and thiol groups that can bind metal ions. In the second “slow” phase, the metal ions are transported across the cell membrane into the cytoplasm and accumulated intra cellularly. The data obtained for *Streptomyces* spp. 211A by AAS (Fig. 10b) are confirmed by data obtained for this bacteria using NAA. NAA was also used to study elemental content of examined samples. The results obtained show that the concentrations of some toxic elements in analyzed biomass do not exceed permissible levels [48]. The biomass of actinomycetes containing gold and silver nanoparticles may be used for medical purposes.

#### 4. Conclusion

The results of performed study show that the new strains of *Actinomycetes* belonging to *Streptomyces* and *Arthrobacter* genera produce gold nanoparticles by interacting with  $10^{-3}$  M aqueous chloroauric acid ( $\text{HAuCl}_4$ ) solution and silver nanoparticles by interacting with  $10^{-3}$  M aqueous silver nitrate ( $\text{AgNO}_3$ ) solution.

The obtained gold and silver nanoparticles formed by bacterial biomass are crystalline in nature and they are generally produced extra cellularly. In general, they have the spherical shapes and sizes in the range of 5-80 nm with the average size of 20-25 nm.

The determination of gold and silver total concentrations in the bacterial biomass shows that in the first day the metal ions are mainly rapidly adsorbed onto the surface of microorganisms and then slowly transported into the bacterial cells.

The concentrations of some toxic elements in biomass of studied *Actinomycetes* do not exceed permissible levels and nanomaterials obtained may have great potential especially in medicine and pharmacology.

The developed microbial methods of nanoparticle biosynthesis are innovative, simple, non toxic and applicable in many branches of science and industry.

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