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Synthesis of Gold Nanoparticles by Some Strains of Arthrobacter Genera

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Abstract: The synthesis of gold nanoparticles by two novel strains of *Arthrobacter* sp. 61B and *Arthrobacter globiformis* 151B isolated from basalt rocks in Georgia was studied. The complex of optical and analytical methods was applied for investigation of experimental samples after exposure to gold chloroaurate ($HAuCl_4$) for different time intervals. To characterize formed gold nanoparticles UV-Vis spectrometry, Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Energy-dispersive analysis of X-rays (EDAX), and X-ray diffraction (XRD) were used. It was shown that after 1.5-2 days the extracellular formation of nanoparticles of spherical form with size in the range of 8-40 nm (on average of 20 nm) took place. To determine gold concentrations in the bacterial biomass neutron activation analysis (NAA) and atomic absorption spectrometry (AAS) were applied. The results obtained evidence that the concentration of gold accumulated by bacterial biomass is rapidly growing in the beginning followed by an insignificant increase during the next few days.

Key words: Gold nanoparticles, microorganisms, biotechnology, optical and analytical methods.

1. Introduction

In past two decades the synthesis of metal nanoparticles using microorganisms has received great interest due to their optical, chemical, photoelectrical and electronic properties. The microorganisms are used as possible "nanofactories" for development of clean, nontoxic and environmentally friendly methods for producing nanoparticles [1]. Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the elemental metal through enzymes generated by the cell activities. It can be intracellular and extracellular synthesis according to the location

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where nanoparticles are formed.

The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing them in the presence of enzymes, while in intracellular synthesis ions are transported into the microbial cell to form nanoparticles in the presence of enzymes. The biosynthesized nanoparticles have been used in a variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, separation science, and magnetic resonance imaging [2].

Gold nanoparticles have potential applications in catalysis, chemical sensing, biosensing and photonics. However, the current technology of producing gold nanoparticles causes environmental pollution due to the toxicity of the reagents used. Therefore, there is a great need to develop new alternative, easy and eco-friendly methods to produce gold nanoparticles [3].

In 1980 Beveridge and Murray first demonstrated that *Bacillus subtilis* 168 is able to reduce Au^{3+} ions to produce octahedral gold particles of nanoscale dimensions (5-25 nm) within bacterial cells by incubation of the cells with gold chloride [4].

Prokaryote bacteria *Rhodopseudomonas capsulata*, recognized as one of the ecologically and environmentally important microorganisms, commonly existing in the natural environment, were investigated for reducing Au^{3+} ions at room temperature. It has been demonstrated that the bacteria *R. capsulata* are capable of producing gold nanoparticles extracellular, and that these gold nanoparticles are quite stable in solutions [5].

Bacteria *Desulfovibrio desulphuricans* and *Escherichia coli* play an important role in fabrication of Au(0) particles of 20-25 nm size [6, 7].

Monodispersed spherical gold nanoparticles of 1.9 ± 0.8 nm size have been synthesized extracellular by the reaction of aqueous $AuCl_4^-$ ions with *Bacillus megatherium* D01 [8].

Konishi et al. observed that Fe(III) reducing bacteria *Shewanella algae* can reduce Au(III) ions in anaerobic environments [9].

Pseudomonas stutzeri NCIMB 13420, *Bacillus subtilis* DSM 10, *Pseudomonas putida* DSM 291 tend to synthesize small, relatively uniform-sized gold nanoparticles intracellular [10].

The detailed study on extracellular biosynthesis of gold nanoparticles by the *Pseudomonas aeruginosa* was carried out in [11].

Kalishwaralal et al. have reported synthesis of gold nanoparticles using *Bacillus licheniformis*. The average particle size of the nanocubes was found to be 10-100 nm [12].

Bioreduction of gold by *Rhizoclonium riparium*, *Navicula minima*, and *Nitzschia obtusa* have been reported by Nayak et al. and Pal et al. [13, 14]. In Chakraborty's work, *Lyngbya majuscula*, *Spirulina subsalsa* and *Rhizoclonium hieroglyphicum* were exposed to radioactive and stable gold solutions to study the absorption, recovery and the formation of gold nanoparticles [15].

The actinomycetes-mediated "green chemistry" approach towards the synthesis of nanoparticles has many advantages [16]. The ability of biosorption and bioremediation shown by actinomycetes could be useful in heavy metal removal and helpful in describing the phenomena caused by the valence change of metal ions. Specifically actinomycetes are known to secrete much higher amounts of proteins, thereby increasing significantly the productivity of this biosynthetic approach.

Besides, actinomycetes are classified as prokaryotes and can be manipulated genetically without much difficulty in the future in order to achieve better control over the size and polydispersity of the nanoparticles. Sastry and co-workers have observed that the extremophilic actinomycete, *Thermomonospora* sp., when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity [16]. Ahmad with co-workers have shown that

exposure of alkalotolerant actinomycete, *Rhodococcus* sp., to $AuCl_4^-$ ions results in the rapid reduction of the gold ions and formation of fairly monodisperse intracellular nanoparticles [17].

Basalt-inhabiting *Arthrobacter* species are common indigenous soil bacteria. They are member of the high mol % G + C actinomycete-coryneform bacteria. The isolation and investigation of novel strains of these species for practical purposes is of great interest. Cr(VI)-reducing ability of these species was earlier described in our work [18].

In the present study two novel arthrobacter strains: *Arthrobacter globiformis* 151B and *Arthrobacter* sp. 61B were isolated from the basalt rocks in Georgia for synthesis of gold nanoparticles. A variety of spectral and analytical methods was used to characterize the synthesized gold nanoparticles: UV-Vis spectrometry, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) with energy-dispersive analysis of X-rays (EDAX), neutron activation analysis (NAA), and atomic absorption spectrometry (AAS).

2. Experiment

2.1 Materials

Two Gram-positive aerobic bacterial strains belonging to Arthrobacter genera, *Arthrobacter globiformis* 151B and *Arthrobacter* sp. 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. Pure colonies were tested to investigate their morphological and physiological characteristics. Growth ability of the bacteria was studied in different synthetic and organic media. As the carbon source glucose, fructose, galactose, arabinose, xylose, ramnose, saccharose, lactose, maltose and starch were tested. Nitrogen-containing inorganic compounds: $NaNO_3$, KNO_3, $Ca(NO_3)_2 \cdot H_2O$ and organic compounds: peptone, β-alanin, L-asparagin, glycerin, thyrosin, tryptophan etc. was used. Antagonistic properties of the tested bacteria against 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 liter of distilled water. All chemicals used in the experiment were ACS-reagent grade, produced by Sigma (St. Louis, MO, USA). Bacterial cells were grown in 250 mL Erlenmeyer flasks as a suspension. The medium was inoculated with 0.1 mL of overnight broth and incubated at 21 °C being shaken continuously for 5 days. After cultivation, mycelia were separated from the culture broth by centrifugation and washed thrice with distilled water. The harvested mycelial mass (2 g of wet mycelia) was then resuspended in 100 mL of 10^{-3} M aqueous gold chloroaurate ($HAuCl_4$) solution in 250 mL Erlenmeyer flasks. *Arthrobacter globiformis* 151B was exposed to the action of gold chloroaurate for 40 hours, 3, 6, 8, and 10 days; and *Arthrobacter* sp. 61B was exposed for 40 hours, 5, 7, 9, and 12 days being shaken continuously.

For UV-Vis spectral analysis and TEM, 5 mL samples of the suspension were taken after different time intervals. For SEM, X-ray, AAS and NAA, the bacterial cells were harvested by centrifugation at 12,000 g for 20 min. The wet biomass was placed in an adsorption-condensation lyophilizer and dried [19] until constant weight.

2.2 Methods

2.2.1 UV-vis Spectrometry

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

2.2.2 X-ray Diffraction (XRD)

XRD measurements of the bacterial biomass were made at a Dron-2.0 diffractometer. The BCV-23

X-ray tube with the Cu anode (CuK_α: λ = 1.54178 Å) was used as a source of radiation; the Ni grid with a width of 20 μm was used for filtration of the radiation; the rate of the detector was 2^o/min, the interval of intensity was 1,000 pulses/min and the time constant was 5 s.

2.2.3 Transmission Electron Microscopy (TEM)

TEM was performed using the JEOL SX-100 equipment (Japan) operating at 100 kV. The TEM studies were done at 50 000x magnification. 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 Scanning Electron Microscopy (SEM)

The FEI Quanta 3D FEG, USA/ Systems for Microscopy and Analysis, (Moscow, Russia) high-performance instrument with three modes was used for sample visualization.

SEM was carried out using the SDB (small dual-beam) FEI Quanta 3D FEG with the EDAX Genesis system with the resolution 1.2 nm. Operational features of the microscope used in the experiment: magnification 5000-150000x; voltage 1-30 kV.

2.2.5 Energy-Dispersive Analysis of X-Rays (EDAX)

To identify different elements associated with the studied sample specimen the "built-in" spectrometer called an Energy Dispersive X-ray Analysis (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 [20]. Microprobe analysis of gold nanoparticles clusters was conducted with the energy-dispersive X-ray analysis spectrometer (EDAX, USA). The acquisition time ranged from 60 to 100 s, and the accelerating voltage was 20 kV.

2.2.6 Atomic Absorption Spectrometry (AAS)

A flame AAS with a "Beckman-495" spectrometer was used for gold determination in the bacterial samples. Measurement was carried out at the wavelength of the gold resonance line λ = 242.8 nm.

2.2.7 Neutron Activation Analysis (NAA)

The gold concentration in bacterial samples was determined by neutron activation analysis (NAA) at the nuclear research reactor SAFARI-1 of the NECSA (Nuclear Energy Corporation of South Africa), Pelindaba, South Africa. The samples were irradiated for 8 s by a neutron flux density of approximately 10^{14} n $cm^{-2}\cdot s^{-1}$. Their activities were measured three times, after cooling for 3 and 30 h and 7 days, respectively. The gold content was determined on the 411.8 keV γ-line of ^{198}Au. The NAA data processing and determination of element concentrations were performed using Genie 2000 software.

3. Results and Discussion

Addition of the gold chloroaurate solution to arthrobacter biomass led to the appearance of a grey-violet color in suspension of *Arthrobacter globiformis* 151B and also a violet color in *Arthrobacter* sp. 61B after 40 h, indicating formation of gold nanoparticles. Thus, both strains could synthesize gold nanoparticles by incubating with $HAuCl_4$ aqueous solution and the reduction of $AuCl_4^-$ ions occurs extracellular.

The UV-Vis absorption spectra of the bacteria studied are shown in Fig. 1. The presence of the gold surface plasmon resonance (SPR) peak at ~ 530 nm confirms the gold ion reduction from Au(III) to Au(0) and aggregation of the gold nanoparticles in the solutions. The intensity of the peak increased as a function of the reaction time. As it is known, the position of the plasmon adsorption of gold nanoclusters strongly depends on the particles size, dielectric constant of the medium and the surface-adsorbed species. According to Mie´s theory, only a single SPR band is expected in the adsorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands

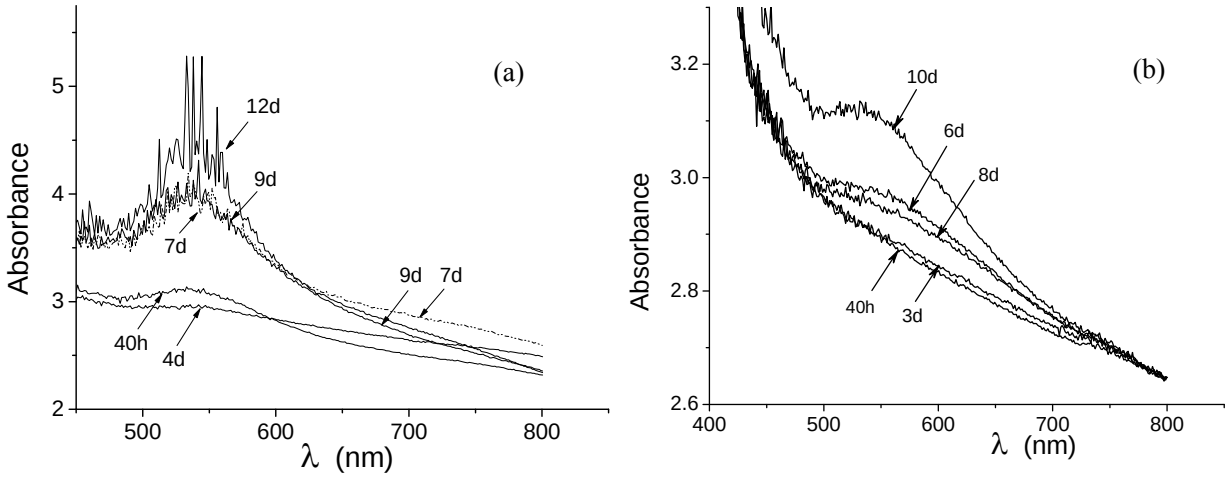


Fig. 1 UV-vis spectra of *Arthrobacter* sp. 61B (a) and *Arthrobacter globiformis* 151B (b).

depending on the shape of the particles [21]. In the present case a single band was observed that gives evidence for spherical shape of gold nanoparticles, which was confirmed by TEM and SEM images.

Fig. 2 shows the TEM image recorded from the drop-cast film of gold nanoparticles synthesized after reaction of the chloroauric acid solution with *Arthrobacter* sp. 61B biomass for 12 days. Here the patterns correspond to the face centered cubic (fcc) structure of gold nanoparticles. The particle size histogram shows that the size of the gold nanoparticles ranges from 8 nm to 40 nm with an average size of 20 nm.

In Fig. 3, as an example, the XRD pattern of gold nanoparticles synthesized by treating *Arthrobacter* sp. 61B with chloroauric acid aqueous solution for 7 days is presented. The diffraction patterns correspond to the amorphic structure of gold particles. However, a number of Bragg's reflections corresponding to the fcc structure of gold are also seen here: four characteristic peaks (111), (200), (220) and (311). The results obtained clearly show that gold nanoparticles formed, by reduction of Au(III) ions by *Arthrobacter* sp. 61B, are crystalline in nature and they are generally produced extracellular.

Fig. 4 presents a SEM image of *Arthrobacter* sp. 61B cells (after interacting with gold for 40 hours and 12 days) and Fig. 5, of *Arthrobacter globiformis* 151B cells (after 40 hours and 10 days). The mode of the natural environment (ESEM) allows studying moist and non-conducting samples. Since actinomycetes cells are non-conducting, they were visualized at this mode. The SEM images illustrate that most of the particles are spherical and do not create big agglomerates. The comparison of the results obtained for the two tested strains arthrobacter shows that in biomass of *Arthrobacter* sp. 61B gold nanoparticles formed at a larger scale (Figs. 4 and 5).

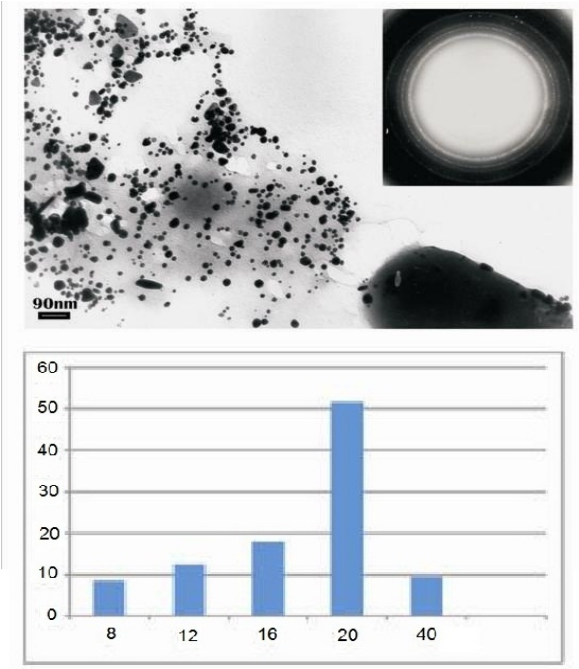


Fig. 2 TEM image and size histogram of Au nanoparticles in biomass of *Arthrobacter* sp. 61B.

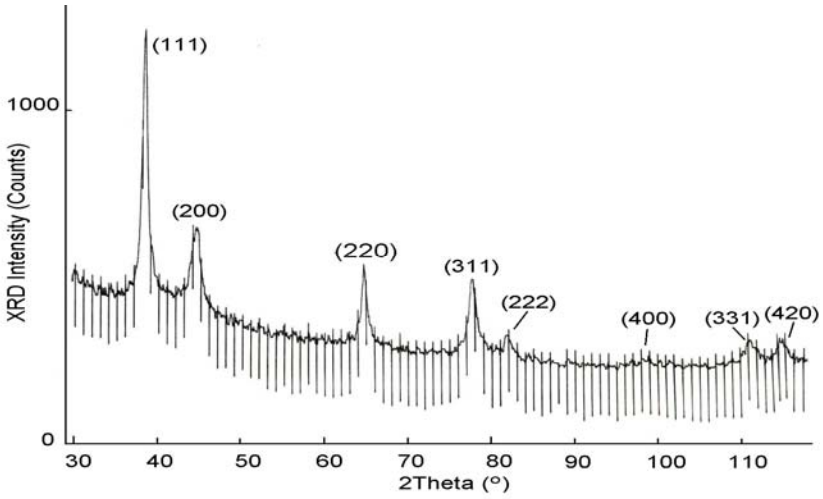


Fig. 3 XRD spectrum of Au nanoparticles in biomass of Arthrobacter 61B.

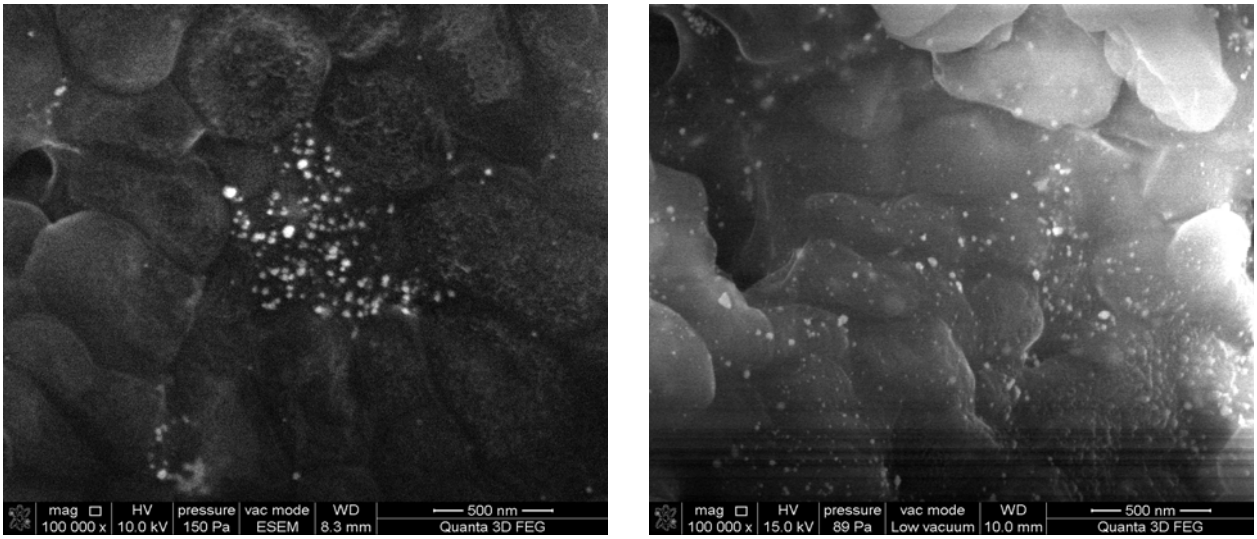


Fig. 4 SEM pictures of gold nanoparticles in *Arthrobacter* sp. 61B.

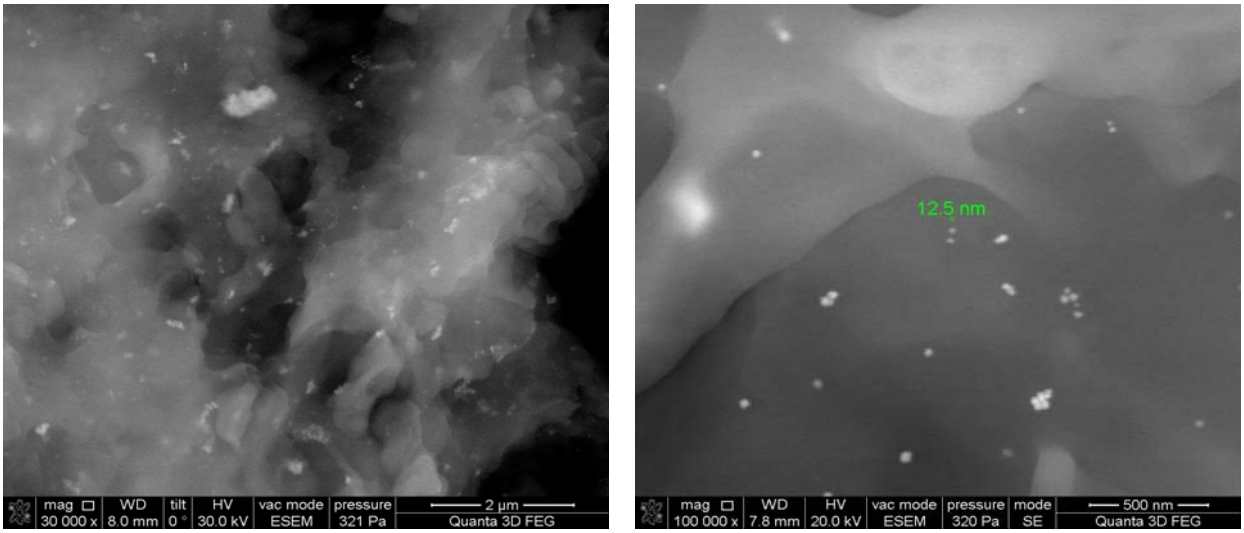
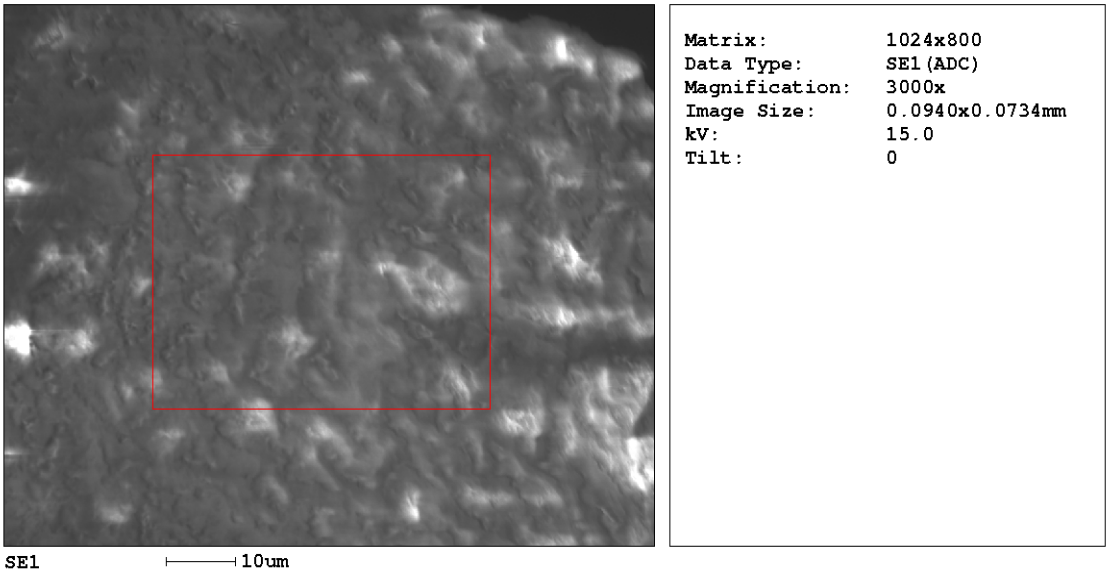


Fig. 5 SEM pictures of gold nanoparticles in *Arthrobacter globiformis* 151B.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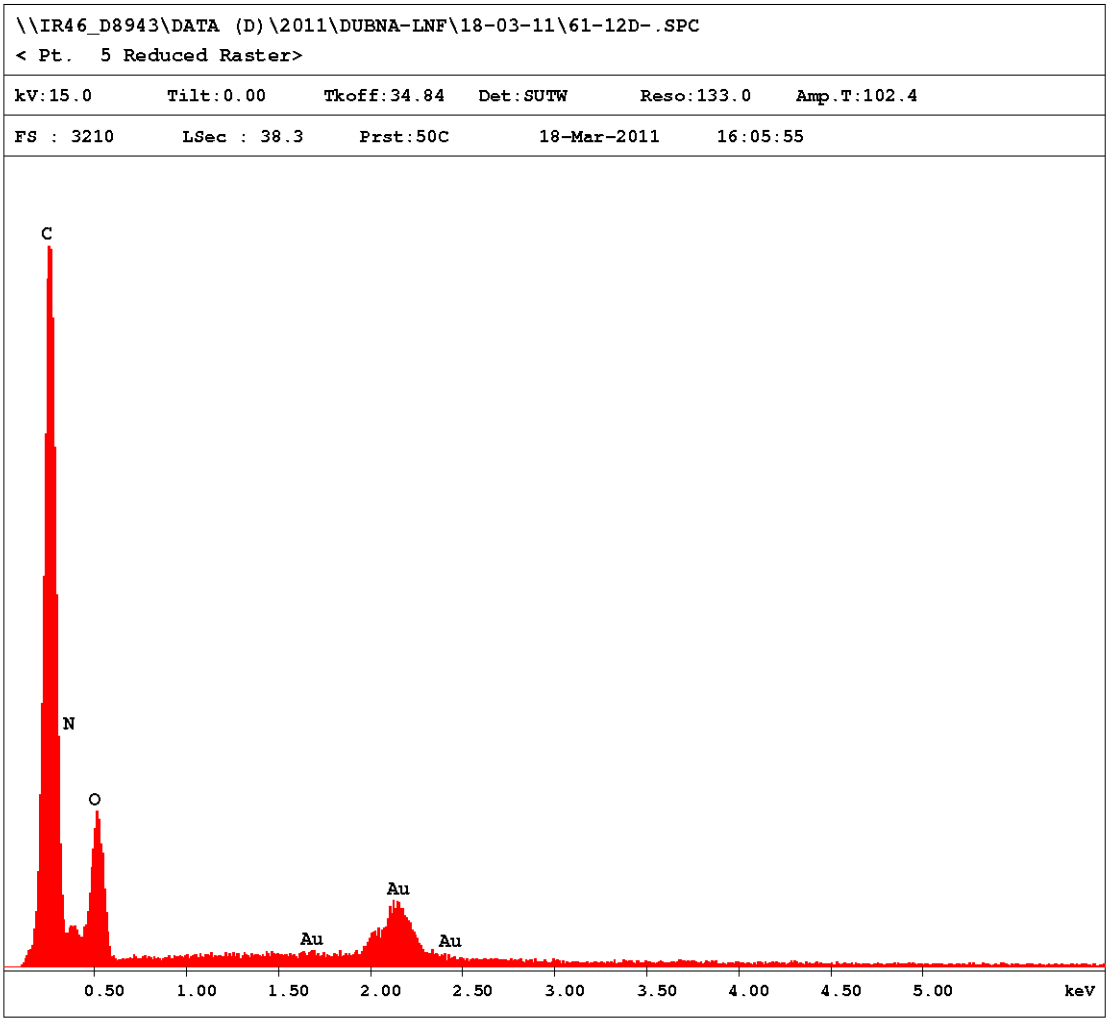
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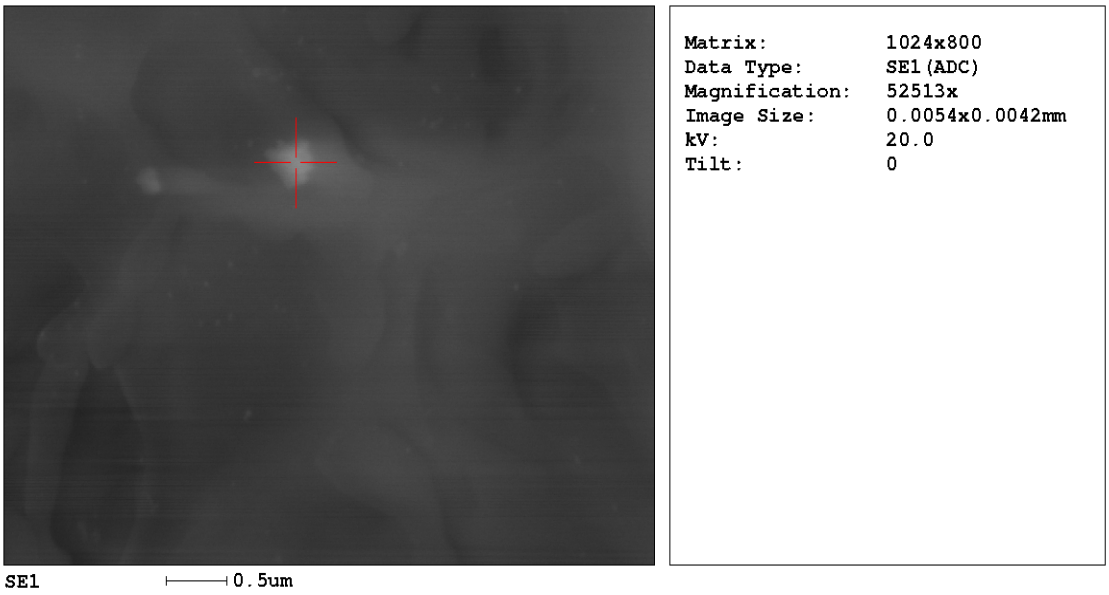

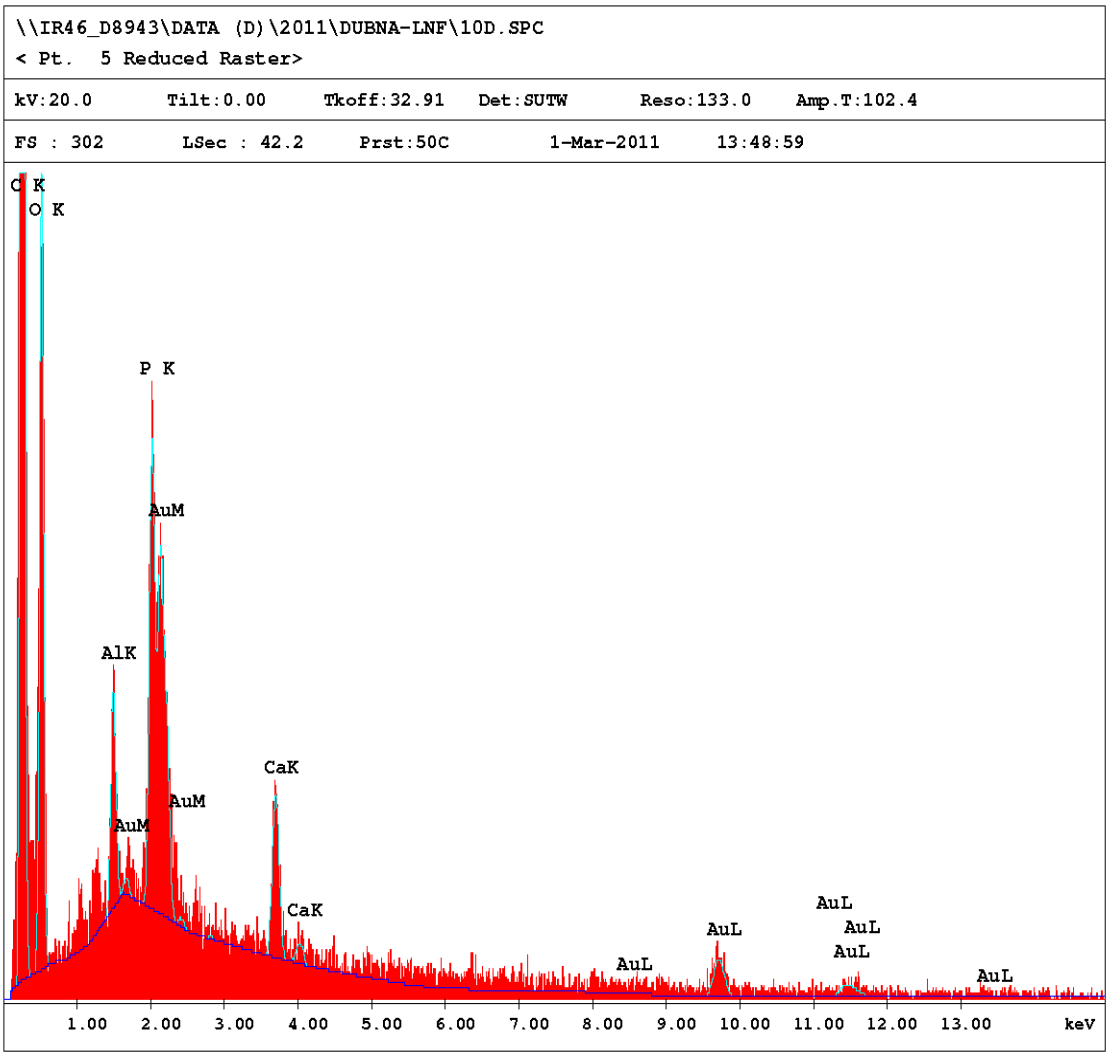

(b)

Fig. 6 EDAX spectra of *Arthrobacter* sp. 61B (a) and *Arthrobacter globiformis* 151B (b).

The EDAX X-ray spectra proved the presence of gold nanoparticles in *Arthrobacter* sp. 61B cells treated with Au in 12 days and *Arthrobacter globiformis* 151B cells treated in 10 days (Fig. 6a, b). The energy of X-rays is characteristic of the element from which these X-rays are emitted. A spectrum of the energy versus relative counts of the detected X-rays is presented in Fig. 6. One peak of Au was observed for *Arthrobacter* sp. 61B. The signals from C, N, and O atoms were also recorded. A few peaks of Au were observed for *Arthrobacter globiformis* 151B and the signals from C, O, K, P, and Ca were recorded as well. These signals are likely to be due to X-ray emission from the proteins and enzymes present in the cell wall of the biomass. As is known, proteins can bind to nanoparticles either through free amino groups or cysteine residues in the protein and/or via the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the actinomycete cell wall [22].

The data obtained for *Arthrobacter globiformis* 151B by AAS are presented in Fig. 7 and by NAA in Fig. 8, which illustrates that uptake of gold includes two phases: rapid and slower. In the first 'rapid' stage, the metal ions are adsorbed onto the surface of microorganism. The concentration of gold increases rapidly. The cell wall of *Arthrobacter* contains a second bilayer of waxy lipids in the form of mycolic acids in addition to the thicker peptidoglycan layer, polysaccharides, acids, and proteins. Functional groups within these biomolecules provide the amino, carboxylic, sulfydryl, phosphate, and thiol groups that can bind metal ions. In the 'slow' stage, the metal ions are transported across the cell membrane into the cytoplasm. The results of NAA show that the total concentration of gold in samples (extracellular and intracellular) does not change significantly. The other elements in the given experiment were not determined.

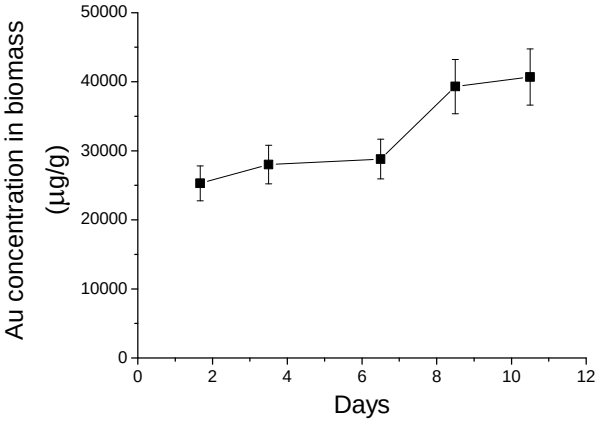

Fig. 7 The gold concentrations in biomass of *Arthrobacter globiformis* 151B versus the time of exposure gold cloroaurat determined by AAS.

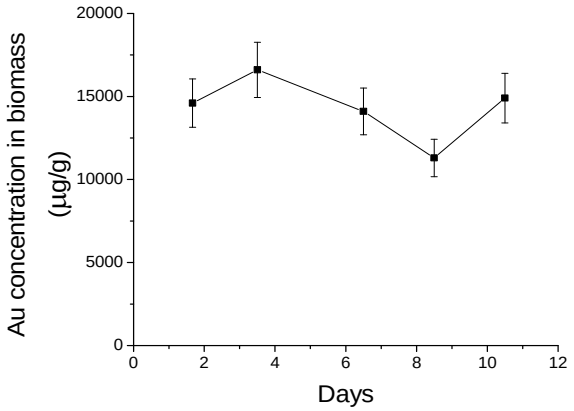

Fig. 8 The gold concentrations in biomass of *Arthrobacter globiformis* 151B versus the time of exposure gold cloroaurat determined by NAA.

4. Conclusions

The results of performed investigations show that *Arthrobacter sp.* 61B and *Arthrobacter globiformis* 151B are capable of producing gold nanoparticles extracellularly when exposed to the gold chloroaurate solution. The shape of the majority of the nanoparticles is spherical and the average size is 20 nm. The "green route" of biosynthesis of gold nanoparticles is simple, economically viable and an eco-friendly process, which offers a great advantage over an intracellular process of synthesis from the point of view of applications in medicine, catalysis and electronics.

Acknowledgments

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References

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

[2] X. Li, H. Xu, Z.S. Chen, G. Chen, Biosynthesis of nanoparticles by microorganisms and their applications, J. Nanomaterials 2011 (2011) 16.

[3] D. Mandal, M.E. Bolander, D. Mukhopadhyay, G. Sarkar, P. Mukherjee, The use of microorganisms for the formation of metal nanoparticles and their application, Appl. Microbiol. Biotechnol. 69 (2006) 485-492.

[4] T.J. Beveridge, R.G.E Murray, Site of metal deposition in the cell wall of Bacillus subtilis, J. Bacteriol. 141 (1980) 876-887.

[5] S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang, N. Gu, Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulate, Materials Letters 61 (2007) 3984-3987.

[6] K. Deplanche, L.E. Macaskie, Biorecovery of Gold by Escherichia coli and Desulfovibrio desulfuricans, Biotechnology and Bioengineering 99 (5) (2008) 1055-1064.

[7] S. Sinha, I. Pan, P. Chanda, S. K Sen, Nanoparticles fabrication using ambient biological resources, J. Appl. Biosci. 19 (2009) 1113-1130.

[8] L. Wen, Z. Lin, P. Gu, J. Zhou, B. Yao, G. Chen, J. Fu, Extracellular biosynthesis of monodispersed gold nanoparticles by a SAM capping route, J. Nanopart. Res. 11 (2009) 279-288.

[9] S. Douglas, T.J. Beveridge, Mineral formation by bacteria in natural microbial communities, FEMS Microbiology Ecology 26 (1998) 79-88.

[10] M. Gericke, A. Pinches, Biological synthesis of metal nanoparticles, Hydrometallurgy 83 (2006) 132-140.

[11] M.I. Husseiny, M. Abd El-Aziz, Y. Badr, M.A. Mahmoud, Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa, Spectrochimica Acta Part A 67 (2007) 1003-1006.

[12] K. Kalishwaralal, V. Deepak, S.R.K. Pandian, S. Gurunathan, Biological synthesis of gold nanocubes from Bacillus licheniformis, Bioresource Technology 100 (2009) 5356-5358.

[13] D. Nayak, M. Nag, S. Banerjee, R. Pal, S. Laskar, S. Lahiri, Preconcentration of ^{198}Au in a green alga, Rhizoclonium, J. Radioanal. Nucl. Chem. 268 (2006) 337-340.

[14] N. Chakraborty, R. Pal, A. Ramaswami, D. Nayak, S. Lahiri, Diatom: a potential bio-accumulator of gold, J. Radioanal. Nucl. Chem. 270 (2006) 645-649.

[15] N. Chakraborty, A. Banerjee, S. Lahiri, A. Panda, A.N. Ghosh, R. Pal, Biorecovery of gold using cyanobacteria and an eukaryotic alga with special reference to nanogold formation, a novel phenomenon, J. Appl. Phycol. 21 (2009) 145-152.

[16] M. Sastry, A. Ahmad, M.I. Khan, R. Kumar, Biosynthesis of metal nanoparticles using fungi and actinomycete, Current science 85 (2) (2003) 162-170.

[17] A. Ahmad, S. Senapati, M.I. Khan, R. Kumar, R. Ramani, V. Srinivas, et al., Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species, Nanotechnology 14 (2003) 824-828.

[18] N. Tsibakhashvili, T. Kalabegishvili, L. Mosulishvili, E. Kirkesali, S. Kerkrnjia, I. Murusidze, et al., Biotechnology of Cr(VI) transformation into Cr(III) complexes, J. Radioanal. Nucl. Chem. 278 (3) (2008) 565-569.

[19] L.M. Mosulishvili, V.S. Nadareishvili, N.E. Kharabadze, A.I. Belokobylsky, Facility for Lyophilization of Biological Preparation. (1980) Patent URSS. No. 779765, Bul. 42.

[20] I.O. Sosa, C. Noguez, P.G. Barrera, Optical properties of metal nanoparticles with arbitrary shapes, J. Phys. Chem. B 107 (2003) 6269-6275.

[21] W.R. Herguth, Applications of scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS) to Practical Tribology Problems.

[22] M.A. Stroscio, M. Dutta, Biological nanostructures and applications of nanostructures in biology: electrical, mechanical, and optical properties, Kluwer Academic Publisher, New York, 2004.