Screening of the Collection Cultures for Biosynthesis of Copper Nanoparticles

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ABSTRACT

Screening among different groups of microorganisms isolated from various polluted areas was conducted to assess their synthesizing capacity of the copper nanoparticles. Strains of microscopic fungi from genera*Penicillium* and *Fusarium* and bacteria from genus*Pseudomonas* were the most active among all studied cultures. These microbial strains expressed high biosynthesizing activity for copper nanoparticles after 48-72 h of cultivation. The formation of copper nanoparticles was revealed with use of UV-spectroscopy and AFM methods. Selected microbial strains may be used for development of the new biotechnology.

Key words: microorganisms, culture collection, copper nanoparticles, silver nanoparticles

INTRODUCTION

One of the earliest studies on production of the metal nanoparticles by means of microorganisms (bacteria) was reported in 1964 [1]. But the electrochemical method was supposed as the most efficient for synthesis of large amounts of copper nanoparticles (CuNPs) within short period of time. Nevertheless, nowadays production of metal nanoparticles by living organisms has certain advantages compared to electrochemical one, such as environmental safety and conformity of size and shape [2, 3].Metal nanoparticles possess different useful properties and are widely used in certain areas. There are many studies reported on antimicrobial activity of nanoparticles, including bacteriocidic activity of copper ions upon wide spectrum of microorganisms.Recently several reviews were published, which stipulated receipt of metal nanoparticles with use of microorganisms isolated from different ecological niches [4-8]. A great number of organisms are reported as producers of different metal nanoparticles: Ag, Au, Cd, Cu, Fe, Pb, Pd, Pt, Se, Zn, Zr andso on. Among those organisms are bacteria [5], fungi [9], yeasts [6], viruses [10], microalga [11], alga [12] and plant extracts [13].

Copper nanoparticles represent certain interest due to their antimicrobial, catalytic, electrical, optical and other properties. Varshney with colleagues have reported application of nonpathogenic strain of *Pseudomonas stutzeri* for the fast method of biological synthesis for production of 8-15 nm spherical CuNPs [14]. The innovative approach for synthesis of CuNPs by bacteria *Pseudomonas stutzeri*, earlier isolated from soil, resulted in receipt of 50-150 nm cubic shaped CuNPs[15]. Nowadays research activities on application of living organisms for production of metal nanoparticles, including copper, become extremely interesting. Thus, the aim of this work was screening of the culture collection of industrially important microorganisms of the Institute of Microbiology (Tashkent, Uzbekistan) for efficient producers of CuNPs.

MATERIALS AND METHODS

Strains of microorganisms preserved at the culture collection of industrially important microorganisms of the Institute of Microbiology (Tashkent, Uzbekistan) were used.

Cultivation of fungi and bacteria was conducted on two times diluted Czapek-Dox, Mandels and beef extract peptone (BEP) nutrient media, respectively. Standard solutions of $CuSO_4$ of different concentration (from 25 to 100 mg/l by Cu^{++}) were prepared. Aliquots of copper nitrate were added to the cultural liquid of microorganisms. Mixture of cells and copper ions were incubated at the rotary shaker at 180 rpm and 28°C for 48-72 h. Formation of the copper nanoparticles was visually monitored by change of the color of the solutions. Strains were selected according to their resistance towards different pollutants, including heavy metals, and ability to biosorption of metals as well, since the ability to produce metal nanoparticles is considered as a protective function of the microorganisms [16].

UV-spectroscopic studies were conducted on a spectrophotometer SPECORD 210 (Germany) within range 190–1000 nm. Accuracy of UV photometry with potassium dichromate was according to Ph.Eur. ± 0.01. The morphology of copper nanoparticles was studied using an atomic force microscope Agilent 5500 (USA) at the room temperature.

RESULTS AND DISCUSSION

Modern nanotechnologies are based on development of the reliable, non-toxic, ecologically safe technologies for the production of metal nanoparticles of the wide range of chemical composition, size, synchronized monodispersity, which is mainly possible to achieve by use of biological resources. Several reviews provided in details an information about isolation of the suitable microorganisms from different sources (soil, water, sewages, culture collections) [4, 8].

The culture collection of industrially important microorganisms of the Institute of Microbiology comprises a number of different strains both bacteria and fungi isolated from mines, flotation tails, industrial and household wastes and sewages, polluted soils and waters and so on. Based on analysis of available data several cultures were selected for the screening of ability to produce CuNPs.Filamentous fungi are more resistant towards mutations and possess ability to synthesize different nanoparticles. Selected fungal strains were preliminarily cultivated on nutrient medium containing copper salt. Analysis of selected collection cultures revealed among them several fungal strains capable to produce CuNPs (table 1). The maximum activity among screened cultures was observed at *Penicillium* sp.1, *Fusariumoxysporum*1 and *Fusariumoxysporum* 2.

The absorption bands, differing by intensity, were formed at λ_{max}=260 nm in both samples, regardless of the used nutrient medium. UV-spectrum of the strain cultivated on Czapek-Dox medium (sample 6) revealed a small shoulder at λ_{max}=700 nm, testifying presence of Cu^{2+} traces; whereas cultivation on Mandels medium (sample 3) presented no such change.

Morphology of the test samples was studied by AFM method. It was established that systems medium+fungus+Cu^{2+} have both nano- and microstructure, the shape of nanoparticles is cubic. Sample 3 is characterized by formation of needle structures with diameter 800 nm and length 40 micron, which are produced as result of aggregation of cubic nanoparticles with sized 300-400 nm (figure 3).

Visual observation revealed that light blue color characteristic for the ions of Cu^{2+} disappears in the cultural liquid of *Penicillium sp*. 1, and solution acquires light green-yellowish color. Formation of CuNPs by *Penicillium sp*. 1 was determined by UV-spectroscopy methods at the initial concentration of copper ions 100 mg/l (figure 2).

Table 1. Screeningfor CuNPs producing ability among selected fungal strains

№	Culture	Cultivation time, h		
		24	48	72
1.	*Asperegillusniger*	-	-	-
2.	*Aspergillus terreus 33*	-	+	+
3.	*Aspergillus terreus 4*	-	-	+
4.	*Aspergillus terreus 11*	-	-	-
5.	*Aspergillus glaucus*	-	-	-
6.	*Aspergillus flavus*	-	-	-
7.	*Aspergillus versicolor*	-	-	+
8.	*Aspergillus albus*	-	-	-
9.	*Aspergillus oryzae*	+	+	+
10.	*Acremonium sp.*	-	+	+
11.	*Cladosporiumcladosporioides*	-	-	-
12.	*Cladosporium sp.*	-	-	-
13.	*Alternaria sp.*	-	-	-
14.	*Penicillium sp. 1*	+	+	+
15.	*Trichodermaharzianum*	+	+	+
16.	*Alternariapluriseptata*	+	+	+
17.	*Penicillium sp. 2*	-	+	+
18.	*Trichurusterrophilus*	+	-	-
19.	*Nocardia sp.*	-	-	-
20.	*Fusariumoxysporum 1*	+	+	+
21.	*Fusariumoxysporum 2*	+	+	+

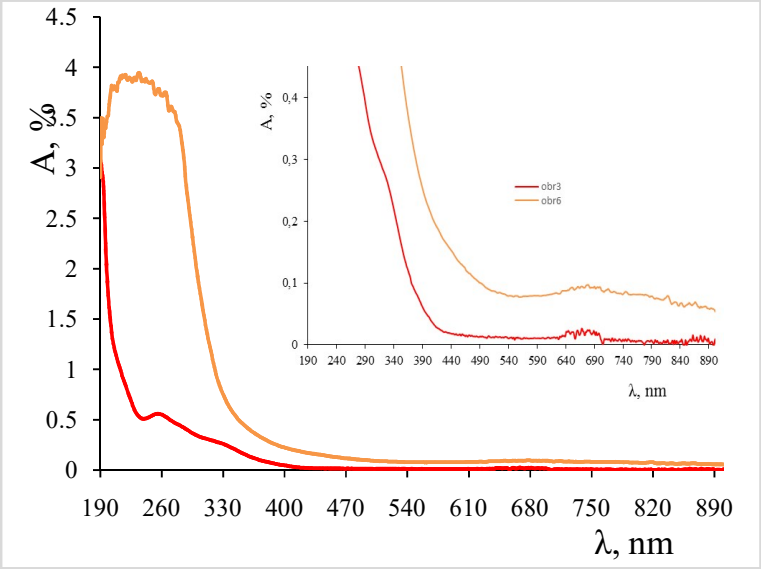
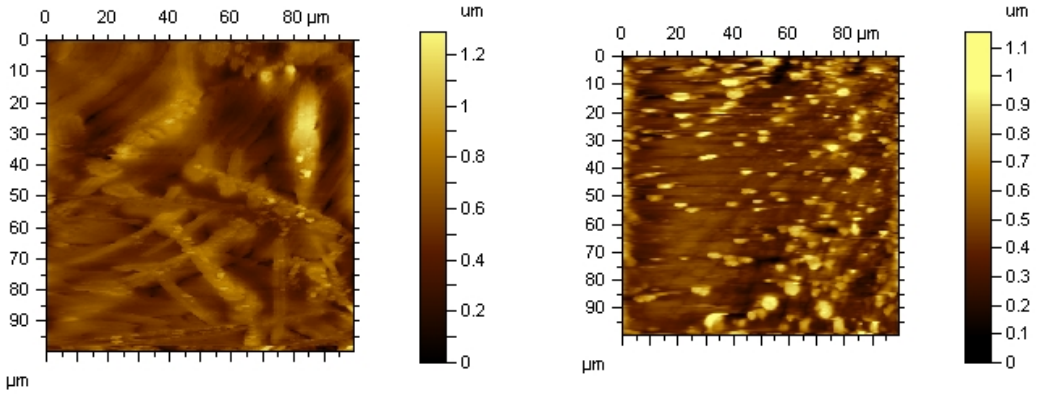


Figure 2. UV-spectra of systems at different concentrations of CuNPs



#3 #6

d -800 nm, length 40 nm 100-800 nm

Figure 3. Morphology of the test samples*Penicillium* sp. 1

There many reports are available on a wide variety of the fungi-producers of metal nanoparticles [17-21, 3]. Microscopic fungi are less susceptible to mutagenic factors like ions of metals, including heavy ones, and at the same time possess ability to synthesize different compounds, including metal nanoparticles.Nevertheless, there is no consensus on biological mechanism of the metal nanoparticles production. It is general assumption that there is no evidence that some specific type of protein or carbohydrate or lipid or any other molecule is the main factor responsible for production of the metal nanoparticles [22].In these regards, apparently, proteins play fundamental role in production of CuNPs[2].On the other hand, it was established that fungal enzymes affect the production of metal nanoparticles, and not only stability [3].

It is well known that bacteria may serve as perspective producers of metal nanoparticles [23-28]. Metal nanoparticles of the bacteria origin proposed for use in different fields of industry. Therefore, screening of the culture collection for potential bacterial producers of CuNPs was essential. Study on selected collection cultures of bacteria, earlier isolated from the polluted areas, established that maximum CuNPs producing activity was determined among strains related to *Pseudomonas,Bacillus* and *Acinetobacter* species(Table2).*Acinetobacter* is not known as active producer of CuNPs, but it participates in formation of the other nanoparticles. Thus, *Acinetobacter* was reported as an efficient producer of selenium nanoparticles when cell suspension and total cell protein of the strain *Acinetobacter* sp. SW30 was used [24]. In our case, capacity of this microorganisms to produce CuNPs was established (table 2).

Based on preliminary results of the tested bacteria to synthesize nanoparticles, production of the CuNPs in dynamics of the process was studied on example of *Pseudomonas* species. It was established that all studied strains possess the ability to synthesize nanoparticles to one degree or another and the maximum synthesis is observed after 48-72 h(table 3).

Strains *Pseudomonas stutzeri*and *Pseudomonas sp.* 23revealed the best CuNPs synthesizing capacity, moreover nanoparticles synthesized by these strains expressed stability for up to a fortnight (table 3). Its possible reason may be the formation of smaller nanoparticles and synthesis of compounds, which envelop nanoparticles preventing their aggregation and by this, probably, stabilizes them as well. Formation of the CuNPs in the cultural liquid of *Pseudomonas* strains was determined both visually and by UV-spectroscopy (figures 4 and 5).

Table 2. Screening for CuNPs producing ability among selected bacterial strains

№	Culture	Cultivation time, h		
		24	48	72
1.	*Pseudomonas putida*	+	+	-
2.	*Pseudomonas stutzeri*	+	2+	3+
3.	*Pseudomonas fluorescens*	+	+	+
4.	*Arthrobacterglobiformis*	+	+	+
5.	*Bacillus megatherium*	+	2+	2+
6.	*Bacillus subtilis*	-	+	+
7.	*Bacillus sp.*	-	+	+
8.	*Acinetobactersp*	+	+	-+

Table 3. Screening of *Pseudomonas* strains for CuNPs synthesis

Strain	Growth, days					
	1	2	3	5	7	14
Pseudomonas stutzeri	+	3+	3+	3+	3+	3+
Pseudomonas putida 1	+	+	-	-	-	-
Pseudomonas sp 23	+	2+	2+	2+	2+	+
Pseudomonas sp. R	+	+	+	+	+	+

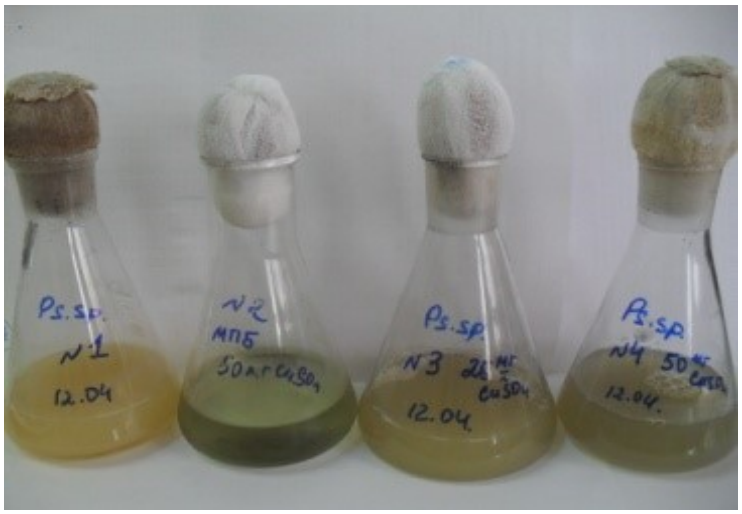


Figure 4. Formation of CuNPs by strain *Pseudomonas* sp.:1 – control (pure culturalliquid of the strain); 2 – BEP broth+ $CuSO_4$; 3 – cultural liquid of *Pseudomonas* sp.with added 25 mg/l of Cu^{2+};*4* – cultural liquid of *Pseudomonas* sp. with added 50 mg/l of Cu^{2+}

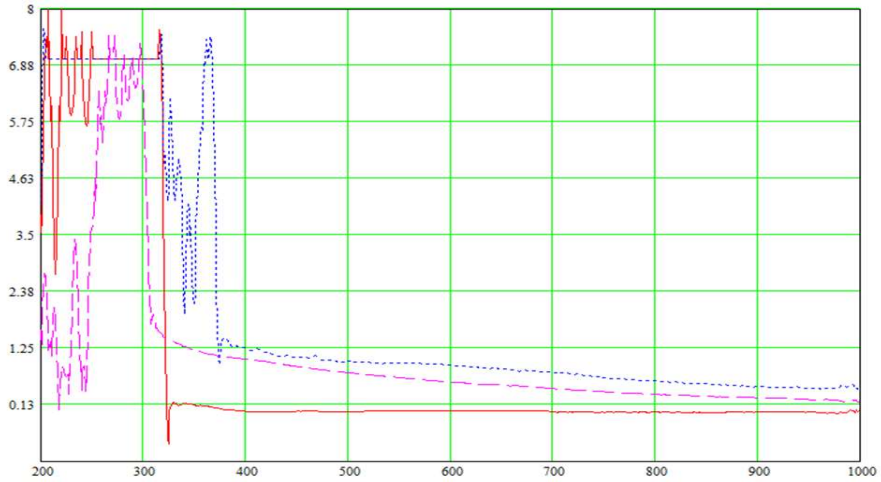


Figure 5. UV-spectra of the systems with different amount of CuNPs:a) beef extract peptone broth (BEPB); b) BEPB+microorganism+Cu^{2+}(25 mg/l); c) BEPB+microorganism+Cu^{2+}(50 mg/l)

UV-spectroscopy analysis revealed that, with an increase in the concentration of copper (II) ions, a bathochromic shift of the absorption wavelength is observed, which is directly proportional to the size of the nanoparticles. It was established that copper nanoparticles are observed in the form spherical particles, elongated particles are observed as well. At the same time, majority of studied microbial cultures, both bacteria and actynomycetes, produced insignificantquantities of copper nanoparticles.

It was established that nanoparticles were observed mostly after 24-48 h depending on certain microorganism and the highest synthesizing activity was observed after 48-72 h for the most of the cultures. More extended contact with copper salt causes aggregation of nanoparticles and precipitation, especially this is true in case of microscopic fungi.

CONCLUSION

Thus, it was established that strains *Penicillium* sp. 1and*Fusariumoxysporum,* and*Pseudomonas*species were the most active producers of copper nanoparticles among all screened culture collection strains. Biogenic production of copper nanoparticles by bacteria and fungi presents certain scientific and applied interest. Based on results of the UV-spectroscopy and AFS microscopy a database of microbial strains synthesizing copper nanoparticles was established, which may be used in future studies.

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