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Extracellular Biosynthesis and Antimicrobial Activity of Silver Nanoparticles

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The extracellular synthesis of silver nanoparticles using the cell-free filtrate of *Penicilium sp.* is reported. Our results suggested that the product could be used as an effective antimicrobial agent.

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1. Introduction

Among various metal nanoparticles, the silver nanoparticles have various and important applications, including materials used in electrical batteries and conponents, polarizing filters, staining pigments in glasses and ceramics, etc. [1]. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents [2]. In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses [3]. Bactericidal properties of metallic silver are associated with their slow oxidative properties and the liberation of silver ions to the environment, consequently it seems promising to incorporate the specified nanosilver drugs as a special class of biocidal agents.

It is known that a large number of organisms, both unicellular or multicellular, are able to produce inorganic nanomaterials, either intracellularly or extracellularly [4]. It seems that, especially the filamentous fungi are very good candidates for the synthesis of silver nanoparticles because these types of biomasses are easly handled.

Extracellular synthesis of silver nanoparticles by the wild strain of *Penicillium sp.* Pen2 is reported in this paper. The synthesis process was quick and nanosilver was formed within hours of silver ions coming in contact with the cell filtrate. Also, these nanoparticles are evaluated for their antimicrobial acivity against both the gram-positive, and the gram-negative bacteria.

2. Experimental section

2.1. Materials

All chemical agents including $AgNO_3$ were obtained from POCH (Poland).

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2.2. Synthesis of silver nanoparticles

The *Penicillium sp.* Pen2 strain isolated from soil was studied. Fungal biomass used for the biosynthetic experiments was grown aerobically in a liquid medium containing (g/l): KH_2PO_4 7.0; K_2HPO_4 2.0; $MgSO_4{\cdot}7H_2O$ 0.1; $(NH_4)_2SO_4$ 1.0; yeast extract 0.6; glucose 10.0. After the incubation, the biomass was filtered (Whatman filter paper No. 1), and later extensively washed with distilled water to remove any medium components. The resulting fresh and clean biomass was taken into the Erlenmeyer flasks, containing 100 ml of Milli-Q deionized water. The flasks were agitated at 25 °C with shaking at 150 rpm for 72 h. Then the biomass was filtered again (Whatman filter paper No. 1) and the cell-free filtrate was used in the following experiments. $AgNO_3$ at the final concentration of 1 mM was added to the cell-free filtrate and agitated at 25 °C in dark. Control (without the silver ions) was also run along with the experimental flasks. The concurrent studies include time dependent formation of silver nanoparticles employing UV-Vis spectrophotometer in the range of 200–800 nm (HELIOS λ, ThermoElectron Corp.).

2.3. Electron microscopy (TEM)

TEM samples of the silver nanoparticles synthesized using cell filtrate were being prepared by placing 2 μl of the product solution onto the carbon-coated copper grids, allowing the solvent to evaporate in air.

2.4. Antibacterial activity

The effect of silver nanoparticles on the gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and the gram-positive bacteria (*Staphyloccocus aureus*) was also investigated by culturing the organisms in a liquid broth (Trypcase Soy Broth, bioMarieux) supplemented with doses of silver nanoparticles. Control broths were used without any nanoparticles. The growth rate was determined by measuring the optical density (OD) at 550 nm.

3. Results and discussion

A variety of fungal cultures isolated from soil have been screened due to their important ability to extracellular reduction of silver ions to form nanoparticles. The most promising results were obtained using the fungal culture denoted Pen2, characterized as *Penicillium sp.* The silver nanoparticles were synthesized from Ag^+ ions using the cell-free filtrate of this type of strain. The cell--free filtrates obtained from biomass grown for 4, 7 or 11 days, respectively, were tested to evaluate their effects on the age of the culture combined with their ability to produce silver nanoparticles. These nanoparticles were characterized using UV-Vis spectroscopy (Fig. 1). It was shown that the age of the culture had a strong effect on the shape and size of nanoparticles. As illustrated in Fig. 1A, B, when we used the cell-filtrates from the already 4 or 7 day old biomass, the reduction of silver ions started quickly (after 2 h). The UV-Vis spectra showed the appearance of a single and strong band absorption peaks centered at about 439 nm or 431 nm respectively, thus indicating that the nanoparticles are isotropic in shape and uniform in size. This band is called the surface plasmon resonance (SPR) [5]. When older cells were used, the formation of silver nanoparticles started after 48 h (Fig. 1C), and a long tailing on the side of high--wavelength was observed, presumably due to the small particle aggregation. The silver surface plasmon resonance band occurs at *ca.* 403 nm and increased in intensity as a function of the reaction time. This significantly shifted the silver surface plasmon resonance peak maximum wavelength from 431–439 nm to 403 nm, suggesting the formation of smaller silver nanoparticles.

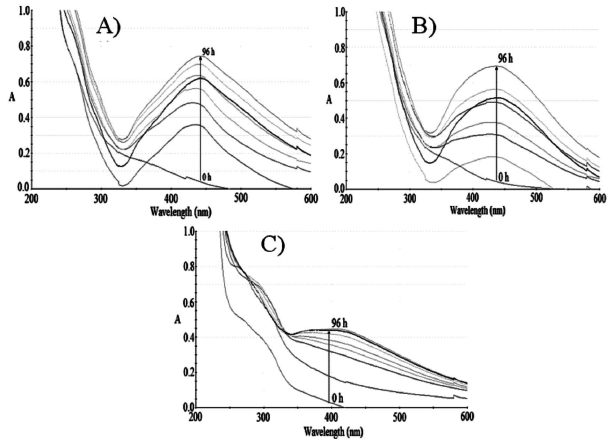

Fig. 1. UV-Vis spectra recorded as a function of reaction time of 1 mM $AgNO_3$ solution with the cell-free filtrate obtained from: (A) 4-days old biomass, (B) 7-days old biomass, (C) 11-days old biomass.

Apart from this, the absorption peak at 210 nm was assigned to the strong absorption of peptide bonds in filtrate. The absorption at 280 nm indicated the presence of aromatic acids residues in the protein. This observation indicates the release of proteins into filtrate that suggests possible mechanisms for the reduction of silver ions present in the solution. Most probably the reduction of the Ag^+ ions occurs due the reductases released by the fungus into the solution. Previous studies [6, 7] have indicated that nicotinamide adenine dinucleotide, reduced form (NADH) and NADH-dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles. However, further experiments should be performed to elucidate the mechanisms involved in the synthesis of silver nanoparticles by *Penicillium sp.* Pen2 strain.

Figure 2 shows representative transmission electron micrpscopy (TEM) images of the silver nanoparticles that were synthesized using cell-free filtrates obtained from 4 and 11 old days biomass. The most dominating morphology shape was the spherical one. The size depended on the age of culture used, and was in the range of 30–40 nm (Fig. 2A) or 5–10 nm (Fig. 2B).

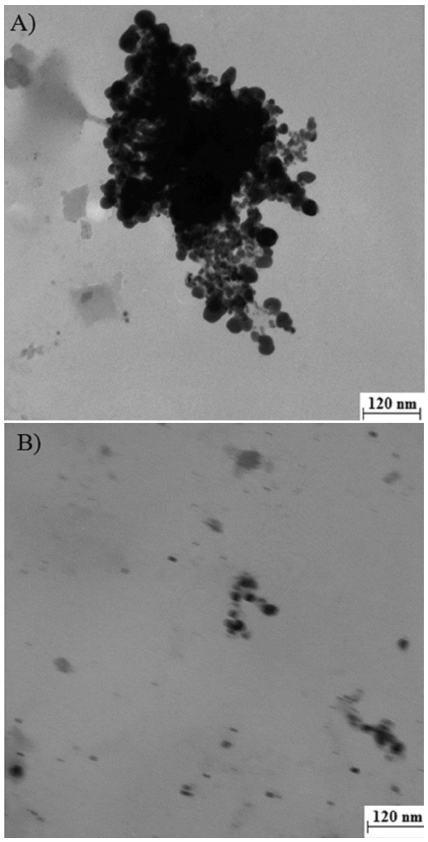

Fig. 2. TEM images; the aging effect of culture on the production of nanoparticles by the cell-free filtrate of *Penicillium sp.* Pen2 obtained from: (A) 4-days old biomass, (B) 11-days old biomass.

It is known that silver nanoparticles exhibit a high antibacterial effect due to their well-developed surface which provides the maximum contact with the environment. Furthermore, toxicity is presumed to be size and shape dependent [8] because small size nanoparti-

cles (< 10 nm) may pass through cell membranes. Inside a bacterium, nanoparticles can interact with DNA, thus losing its ability to replicate which may lead to the cell death.

The antimicrobial activity of silver nanoparticles was determined on the basis of their minimum inhibitory concentrations (MICs, at the lowest concentration of silver nanoparticles at which the microorganisms were tested they did not show any visible growth; $OD_{550} = 0.0$). As shown in Fig. 3, the tested nanoparticles were effective against all strains studied.

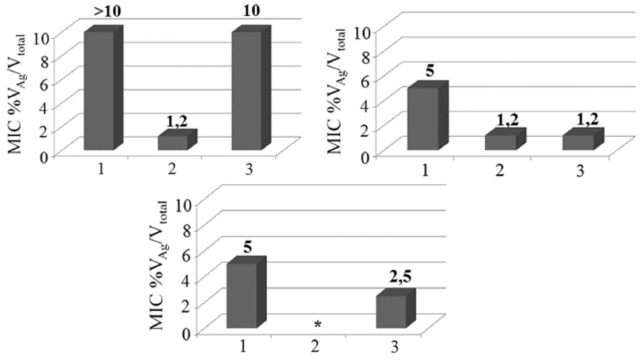

Fig. 3. MIC values for silver nanoparticles produced by cell-free filtrate of *Penicillium sp.* Pen2 obtained from: 4-days old biomass (top left), 7-days old biomass (top right) and 11-days old biomass (bottom). (1) *S. aureus*, (2) *E. coli*, (3) *P. aeruginosa*.

4. Conclusions

The concentration of 5–10% (v/v) has been found to have a strong inhibitory effect on the *S. aureus*. It seems that the gram-negative bacteria (*E. coli, P. aeruginosa*) were the most sensitive. The silver nanoparticles inhibited the growth of these bacteria even when the concentration was at 1.2% (v/v).

Our results support the hypothesis that biological synthesis of silver nanoparticles, using cell-free filtrate of fungi, is a simple and cost-effective method to use new types of bactericidal materials in the production.

References

[1] M. Kowshik, S. Ashtaputre, S. Kharrazi, *Nanotechnol.* **14**, 95 (2003).

[2] Y.A. Krutyakov, A.A. Kudrynskiy, A.Y. Olenin, G.V. Lisichkin, *Russ. Chem. Rev.* **77**, 233 (2008).

[3] V.K. Sharma, R.A. Yngard, Y. Lin, *Adv. Coll. Int. Sci.* **145**, 83 (2009).

[4] D.P.E. Dickson, *J. Magn. Magn. Mater.* **203**, 46 (1999).

[5] P. Mulvaney, *Langmuir* **12**, 788 (1996).

[6] N. Duran, P.D. Marcato, O.L. Alves, G.I.H. De, E. Souza, Esposito, *J. Nanotechnol.* **3**, 477 (2005).

[7] D. Mandal, M.E. Bolandr, D. Mukhopadhyay, G. Sarkar, P. Mukherjee, *Appl. Microbiol. Biotechnol.* **69**, 485 (2006).

[8] O. Choi, K.K. Deng, N.J. Kim, L. Ross Jr., R.Y. Surampalli, Z. Hu, *Water Res.* **42**, 3066 (2008).