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CONTENT

PHYSICAL CHEMISTRY 2022

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PRODUCTION AND CHARACTERISATION OF SELENIUM NANOPARTICLES BY MYCELIUM OF FUNGUS PHYCOMYCES BLAKESLEEANUS

M. Žižić [1], M. Stanić [1], I. Rodić [1], T. Cvetić Antić [2],
M. Živić [2] and J. Zakrzewska[3]

[1] *University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11030 Belgrade, Serbia*
[2] *University of Belgrade, Faculty of Biology, Studentski trg 12-16, 11000 Belgrade, Serbia*
[3] *Institute of General and Physical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia (jzakrzewska@iofh.bg.ac.rs)*

ABSTRACT

In this study, mycelium of fungus *Phycomyces blakesleeanus* was exposed to soluble toxic form of selenium, selenite (Se^{+4}), to examine its ability to reduce it to nanoparticles. Red coloration appeared after only a few hours of incubation with 10 mM Se^{+4} indicating formation of selenium nanoparticles (SeNPs). SEM-EDS confirmed pure selenium NPs with an average diameter of 57 nm, which indicates to potentially very good medical, optical and photoelectric characteristics. Raman spectroscopy showed several structural forms of SeNPs formed in the extracellular space with a monoclinic Se_8 chain as the most represented, and the other observed forms were trigonal Se polymer chain, Se_8 ring and Se_6 chain structures.

INTRODUCTION

For many organisms, selenium (Se), which can exist in several oxidation states (-2, 0, +4 and +6) is an essential microelement. Elemental selenium (Se^0) is water insoluble, inert and nontoxic in low concentrations [1], while selenite (Se^{+4}) and selenate (Se^{+6}) represent soluble and toxic forms [2, 3]. Many bacteria, some fungi, plants and microalgae have the ability to reduce soluble anionic forms of Se to Se^0 in the form of elemental selenium nanoparticles (SeNPs), which are of great interest from technological and biotechnological point of view. SeNPs exibit strong antibacterial, antimicrobial and anticancer properties, with efficiencies increasing with decreasing diameter [3, 4]. They also have a broad spectrum of industrial applications, especially in electronics and optics due to their enhanced semiconducting, photoconducting, photoelectrical, and catalytic properties [3].

Chemical methods of SeNPs synthesis are highly efficient and controllable and can be used to produce stable nanoparticles in large quantities with defined sizes and shapes in a short period of time, but these methods are energy consuming, employ toxic chemicals and produce hazardous wastes that are major risk to environment [5]. Therefore, the focus of SeNPs production is shifting towards ecologically friendly biological synthesis (BioSeNPs), mainly by bacteria and to smaller extent by fungi, algae and plant extracts [6]. Green biotechnological methods have gained popularity due to their cost-effectiveness, lower toxicity of prepared BioSeNPs and their enhanced stability and biological activity due to natural coating by high molecular mass biomolecules [7].

Fungi are one of main pathways for Se entrance into ecosystems either directly, by accumulating it in their mycelia and fruit bodies [8], or indirectly by stimulating its absorption by plant roots as mycorrhizal symbionts. *P. blakesleeanus* is a non-pathogenic filamentous fungus easy to cultivate, characterized by short life cycle and rich yield of mycelium in a short time. Because of these features it was chosen in this study for investigation of SeNPs production and characterization.

METHODS

Mycelium of the fungus *P. blakesleeanus* (Burgeff) (NRRL 1555(-)) was grown to mid-exponential growth stage (28 h), filtered, washed, and incubated in fresh medium with 10 mM Na_2SeO_3 (Se^{+4}) for 24 h. Medium and mycelium were separated by centrifugation and analyzed separately.

For SEM-EDS, mycelium was fixed with 3% glutaraldehyde, dehydrated in ethanol series, dried in Critical Point Dryer K850 CPD (Quorum Technologies, UK), sputter coated with gold and examined by SEM (JSM-6390LV, JEOL USA, Inc.). Elementary composition of samples was obtained with energy dispersive spectroscopy (EDS, Oxford Aztec X-max).

The particle size distribution pattern in supernatant was done by Dynamic Light Scattering (DLS) spectroscopy by application of laser light-scattering particle size analyzer (PSA) (Mastersizer 2000; Malvern Instruments Ltd., Malvern, Worchestershire, U.K.).

The Raman spectra of mycelium were recorded at the Thermo DXR Raman microscope, using the 532 nm laser excitation line, with a constant power of 10 mW. The exposition time was 30 s, with 10 exposures, with 900 lines/mm and spectrograph aperture of 50 μm slit.

RESULTS AND DISCUSSION

Elemental selenium can be produced intra- or extracellularly, with extracellular formation being especially interesting as the particles can be collected without the additional steps of cell lysis [10], while intracellular synthesis of SeNPs prevents migration of Se through water and soil [11]. SEM micrographs showed presence of extracellular SeNPs (Fig 1A), and EDS microanalysis of both mycelium suspension and supernatant showed characteristic Se signals at 1.4 (SeLα), 11.2 (SeKα) and 12.5 (SeKβ) keV [12] (Fig 1B). The signal of selenium in supernatant is practically devoid of any other signals belonging to cellular components (not shown), indicating pure SeNPs. This methodology, however, cannot show whether any intracellular nanoparticles were formed.

Diameters of SeNPs in supernatant were 32-95 nm, averaging at 57 nm (Fig 1D). The size of nanoparticles plays a significant role in their biological activity [13] as the likelihood of interaction with the target system increases with the surface area, so SeNPs produced by *P. blakesleeanus* with the size range of 32-95 nm are worthy of further research.

Structural arrangement of Se atoms in SeNPs was studied by Raman spectroscopy (Fig 1C). The most intensive band at 255 cm^{-1} (low-wavenumber spectral region, characteristic for vibrational bands of SeNPs) [14, 15] corresponds to symmetric stretching vibration of Se-Se bond, implying production of monoclinic eight-membered single-chain selenium (Se_8) [14, 15]. However, deviation from regular Lorentzian shape and broadening points to overlapping of more than one band in this region, implying existence of amorphous form of SeNPs [16]. Deconvolution of a group of signals around 255 cm^{-1} was performed and showed that the most represented form was monoclinic Se_8 chain, accompanied by trigonal Se polymer chain, Se_8 ring and Se_6 chain structures.

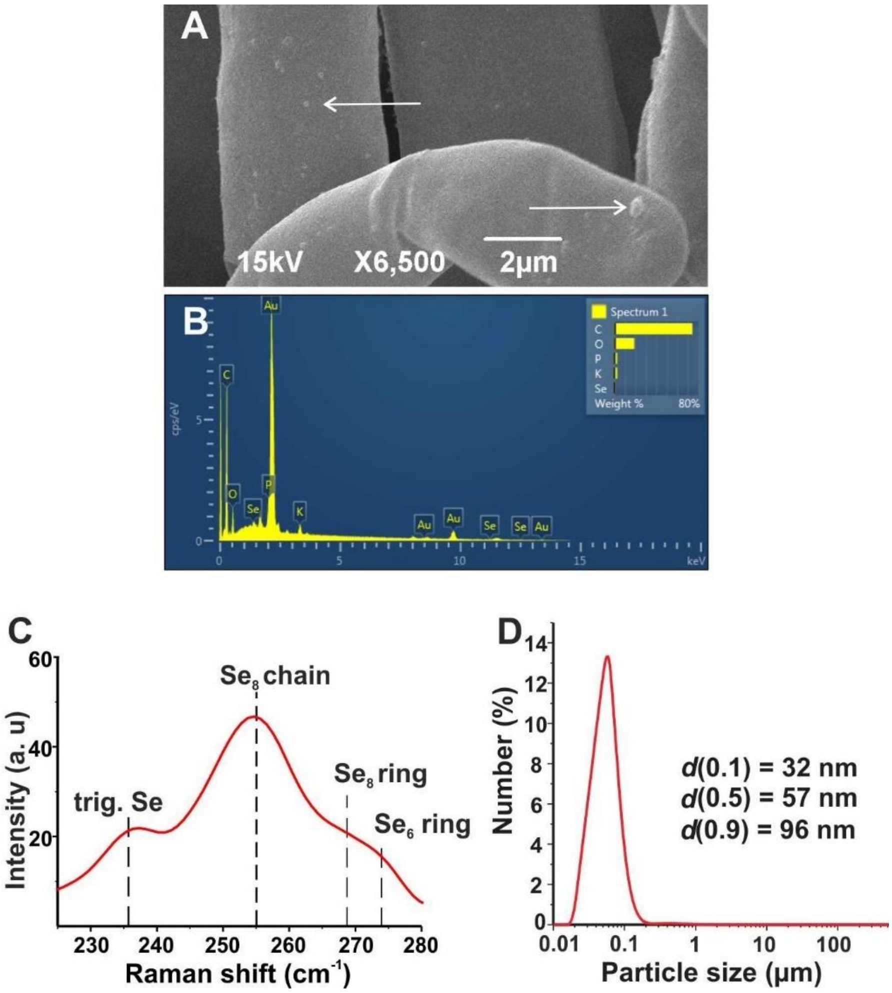

Figure 1. Characterization of SeNPs produced by *P. blakesleeanus*. **A.** SEM of mycelium Arrows point to SeNPs **B**. EDS of mycelium. **C.** Raman spectra of SeNPs in mycelium. **D.** DLS of SeNPs in supernatant.

CONCLUSION

Zygomycetous fungus *P. blakesleeanus* can transform soluble toxic selenite to insoluble innocuous SeNPs [17]. Synthesized SeNPs are present extracellularly and are composed of Se only [17]. The dimensions of the obtained nanoparticles promise great biological potential and are therefore worth further research.

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