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Nanosilica synthesis mediated by *Aspergillus parasiticus* strain

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ABSTRACT

Rice husks (RHs) are plant waste materials abundant in phytoliths silica bodies. These were used as starting material for fungal-mediated biotransformation leading to the synthesis of a high-value added product. A strain of *Aspergillus parasiticus* was capable of transforming the amorphous silica conglomerates into structured nanoparticles (NPs) in the process of RHs biotransformation. Silica NPs were produced extracellularly and their size ranged from 3 to 400 nm depending on the biotransformation conditions and the post-biotransformation supernatant processing. To characterize the NP's structure and dimension, SEM, STEM, EDX and FTIR technics were applied. These demonstrated and confirmed that pyramid (400 nm), cubical (85 nm) and spherical (3 nm and 24 ± 8 nm) forms of silica NPs were obtained.

1. Introduction

In recent years, the synthesis of nanoparticles (NPs) of various elements has focused attention to the path of bio-production, however, still the most commonly applied methods in the large-scale processes are physical and chemical techniques. It seems to be reasonable to develop alternative biological methods, which apart from important ecological impact, would be financially competitive to currently applied technologies. The scientific interest of many research teams lies in the elaboration of protocols allowing effective NPs biosynthesis (Amerasan et al., 2016) (Mazumdar and Haloi, 2017) (Ottoni et al., 2017) (Sarkar and Acharya, 2017) (Uddandarao and Balakrishnan, 2017) (Vijayan et al., 2016). In literature data, various methods of NPs biosynthesis are reported, in which bacterial or fungal enzymatic capacities are essential. Bioprocesses mediated by fungi might appear attractive for NPs production in larger scale because of the fact that they exhibit adequate robustness to perturbations occurred during process carried out in bioreactors. They withstand the pressure of the flow and mixing conditions due to their filamentous nature (Gupta et al., 2012) (Kar et al., 2014). Furthermore, they are widely reported to use both biological and physicochemical mechanisms in process of NPs synthesis (Barabadi et al., 2017) (Castro-Longoria, 2016) (Ramalingmam et al., 2015) (Quester et al., 2016).

Fungal mycelium is an excellent candidate for being a biocatalyst, successfully used in the biosynthesis of NPs. Nanosilica particles are an example of NPs, which are of huge application potential and can be manufactured using biological approaches. They are used inter alia in the paper (Julkapli and Bagheri, 2016) and food industry (Peters et al., 2016), agriculture (Majumder et al., 2006), construction materials (Palla et al., 2017), nanobiosensors (Singha et al., 2017), medicine (Liu et al., 2016), electronics and environmental technology (Zielonka and Klimek-Ochab, 2017).

Nanosilica represents the material composed of SiO_2 molecules, that are able to polymerize and appear in many shapes and sizes, depending on synthesis conditions. In nature, nanosilica is deposited in *phytoliths*, which are siliceous bodies produced by plant cells and accumulated both extra- and intracellularly, or mineralized silica which is located in plant residues. *Phytoliths* are structural support and also help to survive the plant exposed to abiotic factors

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like high and low temperatures, ultraviolet radiation, dry weather, disturbance in the amount of nutrients and toxic metals. They may deter both large and small herbivores such as insects, arachnids, gastropods and mammals, by making plant tissues less accessible and/or digestible (Stromberg et al., 2016). They do not form regular hierarchical structures of a certain size and shape (Neethirajan et al., 2009). Silica bodies are interesting research tools, because they are extremely stable in plant tissues even after the organic parts are completely degraded (Dabney et al., 2016).

Annually, huge amounts of rice husks (RHs), about 120 million tons, remain after rice processing as waste. RHs have no commercial value and are enormously underutilized, because of their low nutritional importance and resistance to degradation. For this reason, RHs are not further exploited and in most cases they are stored and burnt later on. This causes environmental pollution, greenhouse gas emission and waste of energy (Mahmud et al., 2016). On the other hand, RHs have such a high content of silica, that presumably they may be substrates for obtaining this precious nanocompound *via* biological way. The silica present in rice husk is in a hydrated amorphous form. A schematic representation of the locations of silica in the RH are shown in Fig. 1.

The idea of silica particles obtained using RHs was introduced by Bogeshwaran et al. (2014), Carmona et al. (2013), Della et al. (2002), Jung et al. (2013), Liou (2004), Liou and Yang (2011), Liu et al. (2011), Madrid et al. (2012), Mahmud et al. (2016), Thuadaij and Nuntiya (2008), Yalcin and Sevinc (2001), Yuvakkumar et al. (2014) and others. Surprisingly, all of mentioned researchers used chemical or physical methods of obtaining nanosilica particles from RHs. Biological processes have been used much less frequently until the breakthrough, performed by Bansal et al. or Pineda-Vasquez et al. work, who used fungus, *Fusarium oxysporum*, to synthesize nanocrystalline silica (2006), (2014) starting from RHs and RHs ash.

The myconanotechnological attempts to obtain structured silica NPs were definitely insufficient due to their huge application possibilities in medicine, electronics and manufacturing. High-tech applications, such as optical data transmission fibers or precision casting also require nanosilica particles (Lumen, 2014). These kinds of NPs can be also applied as therapeutics carriers in controlled drug delivery systems, which are designed to the drugs from degradation and denaturation (Yuan et al., 2011). Nanosilica is also used in the manufacture of automotive tires and wherever nanosizes are required – paints, fillers, magnetic materials, rubbers, glass, cosmetics, medicines (Giftson Felix and Siva Kumar, 2014) (Napierska et al., 2010).

It should be stressed, that obtaining desired forms of spatial nanoparticles determines their further use. The NPs shapes can be very different, from simple geometric shapes, through tubular, pillar, to completely irregular. Because of their wide applicability and the fact that the chemical and physical production of nanosilica is costly and requires significant energy expenditure, it is important to find an alternative method of obtaining this valuable product.

The aim of present work was to examine the enzymatic potential of *Aspergillus parsiticus* mycelium in bioconversion of RHs in order to obtain silica NPs of various shapes.

2. Materials and methods

2.1. Materials and microorganism

RHs were purchased from PyroGarage company (Poland). All reagents were commercially available and were purchased from Sigma–Aldrich.

A. parasiticus NRRLY 2999 strain (from Anadolu University, Department of Pharmacognosy, Eskisehir, Turkey) was routinely maintained on Potato Dextrose Agar, which provided profuse sporulation suitable for inoculum collection.

2.2. Microorganism cultivation

A. parasiticus was maintained on Potato Dextrose Agar (PDA). To obtain biocatalyst biomass, fungal strain was cultured in Czapek-Dox liquid medium containing per liter: 30 g sucrose, 0.5 g $MgSO_4$ * 7 H_2O, 0.5 g KCl, 2.64 g $(NH_4)_2SO_4$, 0.01 g $FeSO_4$ and 0.5 g K_2HPO_4, pH 7.2. Cultures were grown at 130 rpm in 250 mL Erlenmeyer flasks containing 100 mL of medium, which was inoculated with spores suspension in 0.05 % Triton X-100 to a density of 10 000 spores mL^{-1} and incubated at 27 °C until mid-log phase (4 d). Then, mycelium was separated by filtration, washed twice with distilled water and finally suspended in 100 mL of sterile water. After 24 h incubation under starvation conditions, mycelium was separated by filtration and used as a biocatalyst.

2.3. Biotransformation – general procedure

4 g of raw RHs were suspended in 100 mL of biotransformation medium (distilled water) and after sterilization (121 °C, 15 min, 1.5 atm) or without sterilization, the biocatalyst (10 g of wet fungal biomass) was added. Biotransformation flasks were incubated on a rotary shaker (130 rpm) for period of 16 d. The samples for further analysis were collected daily (1 mL) and placed in freezer (−18 °C). The concentration of silica in such samples was determined by heteropoly blue method (2.4) to define the most effective day of biotransformation. Experiments were done in triplicates. At the same time, corresponding control experiments lacking the biocatalyst were carried out.

After the determination of the most productive day of bioconversion, next experiments were performed according to general procedure. Then the post-biotransformation fluid was separated from the substrate by gravity filtration on a qualitative disc filter with pore size 240 mm. Dried samples of both post-biotransformation fluid and plant residues were analyzed. RHs were incinerated in a muffle furnace (600 °C, 6 h) or dried in a laboratory drier (200 °C, 2 h). Post-biotransformation fluid was

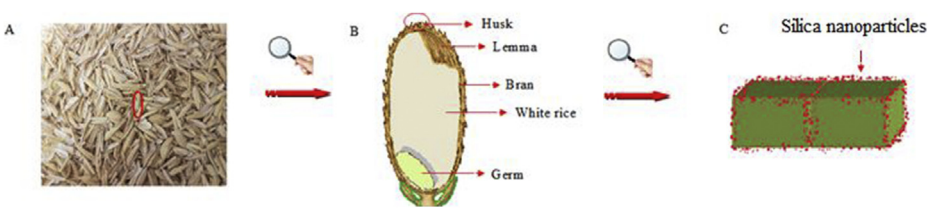

Fig. 1. (A) RHs, (B) structure of rice and its covering, (C) rice husk cells with silica particles.