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Pathobiochemistry

Biosynthesis of selenium nanoparticles using *Enterococcus faecalis* and evaluation of their antibacterial activities

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ABSTRACT

Microorganisms are capable of synthesizing metal nanoparticles, and specifically *Enterococcus faecalis* bacteria were tested for its ability to synthesize selenium nanoparticles (Se-NPs) from sodium selenite. The biosynthesized Se-NPs were spherical in shape with the size range of 29–195 nm. Also, the TEM microscopy showed the accumulation of nano-structures as extracellular deposits. The ability of the bacteria to tolerate high levels of toxic selenite was studied by changing with different concentrations of sodium selenite (0.19 mM–2.97 mM). Also, the effect of Se-NPs was studied on the growth profile of number of pathogenic Gram-positive and -negative bacteria. High concentrations of sodium selenite in the medium led to the production of small amounts of selenium nanostructures by bacteria. In addition, Se-NPs can be used as an anti-staphylococcal element to effectively prevent and treat *S. aureus* infections.
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1. Introduction

Selenium is a functional element with unique physical characteristics such as high optical conductivity, anisotropy of thermo conductivity and X-ray sensing responses. Due to these special features, selenium is considered as a functional element in semiconductor devices, solar cells and photographic exposure meters [1].

To date selenium nanoparticles (Se-NPs) have wide applications in medical diagnostics. Since Se-NPs show a much lower risk compared to selenium, has found widespread use as an antioxidant [2] and a dietary supplement [3]. Most methods that are used in industry for the synthesis of selenium nanomaterials require complex mechanisms with very high temperature and pressure that these processes can be associated with risks to the environment [1]. Recently, there are many reports about the synthesis of metal nanoparticles via "green chemistry" method (e.g., in bioorganic polymers or/and biological systems) through the development of variety of size and morphology of nanoparticles [4,5]. Some microorganisms such as bacteria, fungi, and yeasts are able to survive and grow in the designated concentrations of metal ions and reduce toxic ions into well-defined nanoscale particles [2,1]. For example, Natarajan et al. have described the formation of silver nanoparticles using *Escherichia coli* [6]. Zare et al. have reported an approach to synthesize spherical selenium particles using the fungus strain *Aspergillus terreus* in just during 60 min of the start of the reaction [7]. Fesharaki et al. have described a method to recover Se-NPs produced by *Klebsiella pneumonia* [8]. Studies show that there are significant differences in terms of the toxicity of Se-NPs and selenium oxyanions such as selenite [9]. Selenite was able to prevent the growth and even cause liver toxicity, despite its antioxidant properties [10]. Thus Se-NPs with an effective antioxidant properties and extremely low toxicity can be effective to maintain the proper functioning of the body and health.

In addition to the numerous reports that show anticarcinogenic activity of Se-NPs against several types of cancers [11,12], selenium nanomaterials also have antibacterial properties, especially in the biofilm-forming bacteria [13,14]. A bacterial biofilm is an aggregate of several types of bacteria in a hydrated polymeric matrix (or a polymeric *exo*-polysaccharide composition in formed *S. areus* biofilms) that cause bacterial attachment to the surface. Biofilms cause persistent infections and work as a shield to prevent drugs penetrating into the matrix that this is a problem in the medical field. Therefore, it is important to apply active molecules, such nanoparticles which have higher penetration ability and stronger effects than microparticles [13]. The objective of the present study was to evaluate the possible formation of Se-NPs by selenium reducing bacteria *Enterococcus faecalis*.

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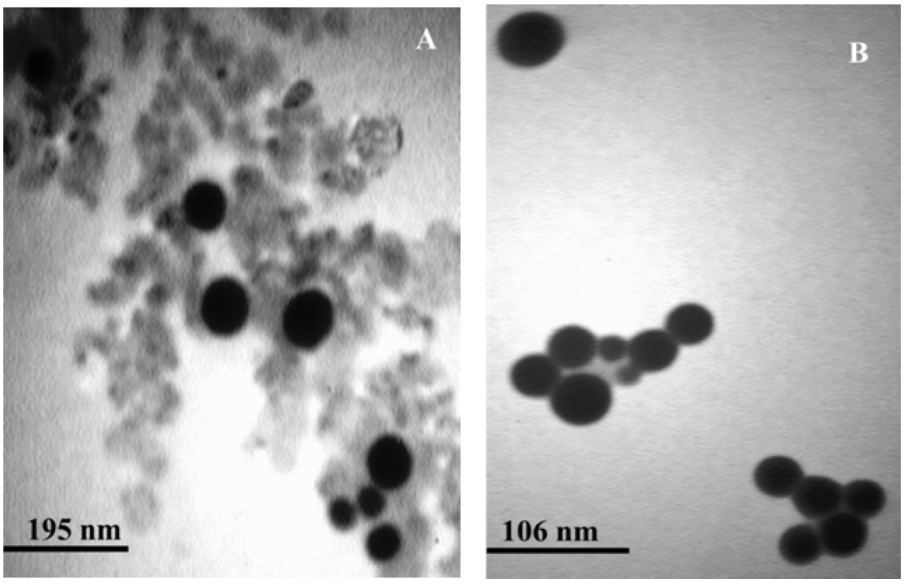

Fig. 1. TEM micrographs of Se-NPs synthesized using *E. faecalis* at 195 nm scale selenium nanospheres and bacteria around them (A) and 106 nm scale selenium nanospheres (B).

2. Materials and methods

2.1. Materials and reagents

Enterococcus faecalis (ATCC NO.: 29212) was purchased from clinical and American Type Culture Collection. Sodium selenite (Na_2SeO_3) was purchased from Sigma (Germany) and bacterial growth medium Luria-Bertani (LB) broth including 10 g tryptophan, 5 g yeast extract, and 10 g NaCl was purchased from Liofilchem, Italy.

2.2. Synthesis of Se-NPs using E. faecalis

The pure isolate was routinely cultured on LB broths containing different concentrations of sodium selenite (Na_2SeO_3). The concentrations of 0.19, 0.47, 0.66, 0.95, 1.42 and 2.97 mM of Na_2SeO_3 [5,15,16] was added into the culture medium at 37 °C and 42 °C on a rotary shaker (150 rpm) for 24 h and 48 h. Na_2SeO_3 was prepared as 0.1 M stock solution and sterilized by filtration.

The fresh bacterium culture (500 μL) and different concentrations of Na_2SeO_3 (0.1 M) was added into 50 mL sterilized broth medium, and then the reaction started. It is very important to set the PH of the culture medium about 7 at the start of reaction, because it is decreased to 3 or 4 at the end of the fermentation process. To the end of the reaction the LB medium become red because of the produced elemental selenium (Se^0). The formation mechanism of elemental selenium is mainly extracellular in *E. faecalis* (Fig. 1). The samples were analyzed by UV visible (PG instrument, China T60U) spectrophotometer. These nanomaterials were isolated from reaction mixture by centrifugation at 10000 rpm for 10 min and washed with distilled water several times. All harvested samples were stored at 4 °C (Fig. 2). Samples were incubated without Na_2SeO_3 as a control in the UV spectrophotometry analysis.

2.3. Characterization of Se-NPs

The dimension and morphology of the synthesized Se-NPs were analyzed using a transmission electron microscopy (TEM, Zeiss-EM10C) at accelerating voltage of 80 kv on a glass substrate. Absorption spectra were collected on an Ultraviolet visible (UV–vis) spectrophotometer at a wave length range of 200–1000 nm at a resolution of 2 nm.

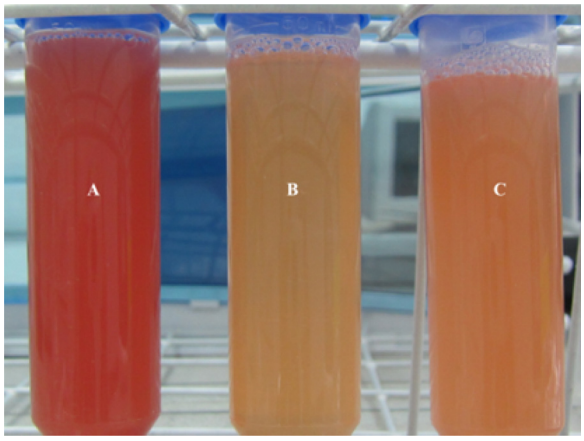

Fig. 2. selenium suspensions in different concentrations of Na_2SeO_3 into 50 mL medium culture, 2.97 mM at 37 °C (A), 0.95 mM at 37 °C (B) and 0.95 mM at 42 °C (C).

2.4. Determining the antibacterial activity

Antibacterial effect of Se-NPs was evaluated using disk diffusion method against common Gram positive bacteria, ie, *Staphylococcus aureus* (PTCC 1431), *Bacillus subtilis* (PTCC 1420) and Gram-negative bacteria, ie, *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1074) according to standard methods [1]. Overnight culture of bacterial suspension was adjusted to 0.5 McFarland equivalents to 1.5×10^8 colony-forming units/mL. Dried surface of Muller–Hinton agar (MHA) inoculated with above bacterial suspension by streaking the swab three times and then 6 mm discs impregnated with 15 μL of Se-NPs solution were placed on inoculated agars [17]. Besides discs impregnated with distilled water (0.1% v/v) considered as negative control. All plates were incubated at 37 °C for 24 h. Gentamicin and Streptomycin were used as antibacterial standards against all pathogens. The zone of inhibition was measured and numbers reported in average (Table 1). Experiments were performed in triplicate.

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