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Biogenic Synthesis of Selenium Nanoparticles using *Streptococcus thermophilus* Bacteria and its Structural Characterization

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A B S T R A C T

Selenium is a trace element which has multiple vital biological functions in the system. Hence, it is being widely used in various forms for preventive and therapeutic use however compared to existing sources, selenium in its nano scale is highly advangtageous owing to its higher bioavailability and lower toxicity. Hence, selenium nanoparticles using the probiotic *Streptococcus thermophilus* bacteria were synthesized using a ecofriendly biological approach. The synthesized selenium nanoparticles were characterized using UV spectrometer, X ray diffraction and transmission electron microscopy. The results of X ray diffraction and transmission electron microscopic studies showed that the nanoparticles were in the uniform size range of 10-30 nm. The present study showed that no chemical changes occurred in selenium nanoparticles during the wet sterilization process and therefore, the wet sterilization method can effectively used to recover the elemental selenium from bacterial cells.

Introduction

Selenium is an essential nutrient having a wide range of physiological activities. Owing to its vital role such as free radical scavenger and immune modulator, it is being regularly used for various biomedical applications in human and as well as in livestock. The low bioavailability and long term toxicity of the existing selenium sources have advocated the use of bioonanotechnology for the development of reliable eco-friendly methodology for the synthesis of materials in nanoscale using biological sources.

Nanoselenium has attracted widespread attention for use in livestock supplementation. Due to its high bioavailability and low toxicity because nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability and low toxicity of routine Se0 (Wang *et al.,* 2007; Zhang *et al.,* 2008). Since surface area-to-volume ratio increases with decreasing particle size, selenium nanoparticles have high biological activity (Zhang *et al.,* 2005), including anti-hydroxyl radical property (Gao *et al.,* 2002) and a protective action against the oxidation of DNA (Huang *et al.,* 2003). Nanosized particles posses unique properties due to their larger surface to volume ratio and higher number of functional sites. Production of nanomaterials can be achieved through conventional physical and chemical methods. Physical methods employ cumbersome high cost equipments and techniques involving elevated temperatures and high pressures. While chemical approaches are the most popular methods for the production of nanoparticles some chemical synthesis protocols involve the use of toxic chemicals which are hazardous to both the environment and the biological system in which they are used. Biosynthetic routes provide nanoparticles of a better defined size and morphology than some of the physicochemical methods of production (Raveendran *et al.,* 2003). Biological methods of nanoparticles synthesis using microorganisms, serve as nontoxic and eco-friendly alternatives to chemical and physical methods especially if they are intended for invasive applications in biological systems and medicine.

These microbial methodologies provide easy, inexpensive, and nontoxic routes to yield nanoparticles that exhibit a diversity of sizes, shapes, and composition along with unique physicochemical attributes and outstanding biological properties. The main benefit is that microorganisms are effective tools that act as nanofactories avoiding thus the use of and/or generation of harsh, toxic chemicals.

They also have the ability to accumulate and detoxify heavy metals due to various reductase enzymes that reduce metal salts to metallic nanoparticles with a narrow size distribution and, therefore, less polydispersity (Xia *et al.,* 2018; Rajasree *et al.,* 2015). Biological processes usually occur at mild conditions, i.e., ambient temperature and atmospheric pressure, require short cultivation period, compatible with the use of the product for medical applications and do not require highly skilled professionals or sophisticated equipment making them amenable to controlled and scaleup procedures. Among the biological methods of synthesis, the methods based on microorganisms have been widely reported (Dhillon *et al.,* 2012; Kaler *et al.,* 2011; Li *et al.,* 2011; Sanghi and Verma, 2010). Zhang *et al.,* (2011) reported a very simple, clean and ecofriendly biological method to synthesize monoclinic selenium nanoparticles with well defined dimensions (200 nm) and disparity using *Pseudomonas alcaliphila* under 28°C with ambient pressure.

Dwivedi *et al.,* (2013) synthesized selenium nanoparticles involving a biological reduction process by the selenium oxide tolerant bacteria *Pseudomonas aeruginosa* strain JS-11 grown in Luria-Bertani broth finally yielding predominantly monodispersed and spherical selenium nanoparticles of an average size of 21 nm.

In this paper, we report a facile, economical and green protocol to synthesize Se nanoparticles (SeNPs) using *Streptococcus thermophilus* bacteria using wet sterilization process**,** which, holds promising alternative for the large-scale commercial synthesis of selenium nanoparticles.

Materials and Methods

Preparation of selenium nanoparticles

A primary stock culture of *Streptococcus thermophilus* (NCDC 74) bacteria was procured from National Dairy Research Institute, Karnal and fresh subcultures were prepared for subsequent use. The fresh subcultures of *S. thermophilus* were used to prepare nanoselenium following the method of Eszenyi *et al.*, (2011).

The nutrient broth (1.36 g) was dissolved in 1000 ml of double distilled water and boiled for 30 min at 120°C. After cooling down to 25°C, 20 mg of sodium selenite dissolved in 20 ml of distilled water was added to 980 ml of broth. Ten millilitre of fresh S. thermophilus bacterial culture was added to 1000 ml of broth containing sodium selenite solution.

The fermentation bottle was placed in shaking incubator for 48 h at 37°C. At the end of the fermentation process the culture medium turned red, indicating the production of nanoselenium.

The medium was centrifuged at 6,000 rpm for 15 min and then the supernatant was discarded. The bacterial culture which formed a pellet at the bottom was taken in 50 ml of distilled water. The culture medium was autoclaved at 121°C for 20 min to disrupt the bacterial cell wall and release the red nanoselenium particles.

The medium was centrifuged at 14,000 rpm for 15 min and washed thrice with distilled water and then the sample was ultrasonicated for 15 min. Finally, the nanoselenium containing solution was passed through vacuum filter, dried at 70°C and stored in sealed tubes for further characterization (Fig.3).

Sample characterization

X Ray Diffraction Analysis

Compositional analysis of the samples were studied based on the energy dispersive analysis of X-Rays using PANalytical X-Ray diffractometer (JEOL Model JED-2300).

Transmission Electron Microscopic Analysis

Samples for transmission electron microscopy (TEM) analysis were prepared by drop-coating selenium nanoparticles solution on to carbon-coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min. The extra solution was removed using a blotting paper and the grid was dried prior to measurement.

Transmission electron micrographs were obtained on JEM- 2100F (JEOL Inc., Japan) instrument with an accelerating voltage of 80 Kv.

UV Spectroscopic Analysis

Absorption spectra of the synthesized nanoparticles were studied using a UV–VIS spectrophotometer (Systronics, Model 2202, India) at a wavelength range of 200-800 nm.

Results and Discussion

The X-ray diffraction pattern of nanoselenium is shown in Fig.1. The diffraction peaks at 2θ (degrees) of 23.26°, 25.01°and 29.88° were indexed as the (211, 202 and 311) planes of Se respectively. All the diffraction peaks in the 2θ range measured corresponded to the trigonal structure of Se with lattice constants a = 4.354Å and c = 4.933Å and were in good agreement with those on the standard data card.

Fig.1 XRD pattern of nanoselenium synthesized using *Streptococcus thermophilus*

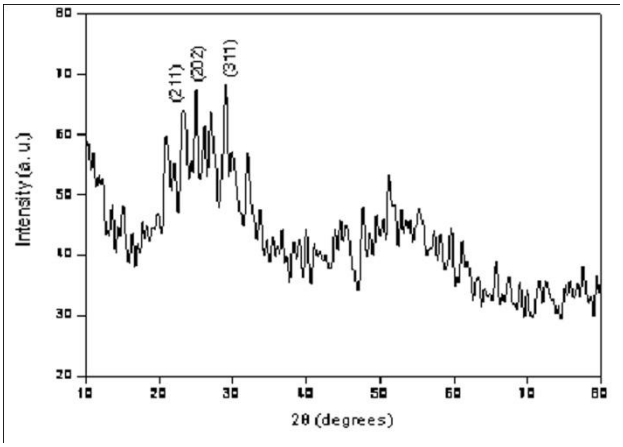


Fig.2 Transmission electron microscope image of nanoselenium synthsised using *Streptococcus thermophilus*

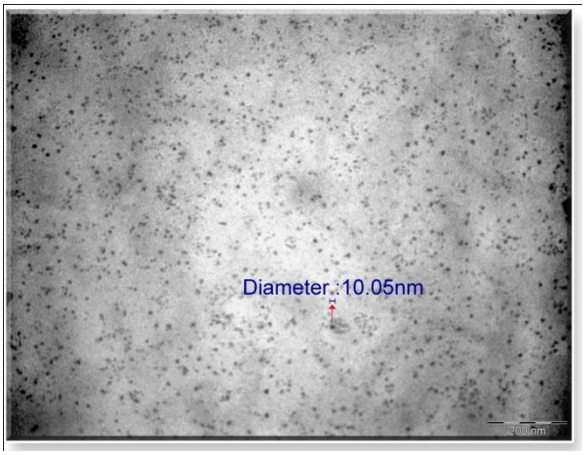
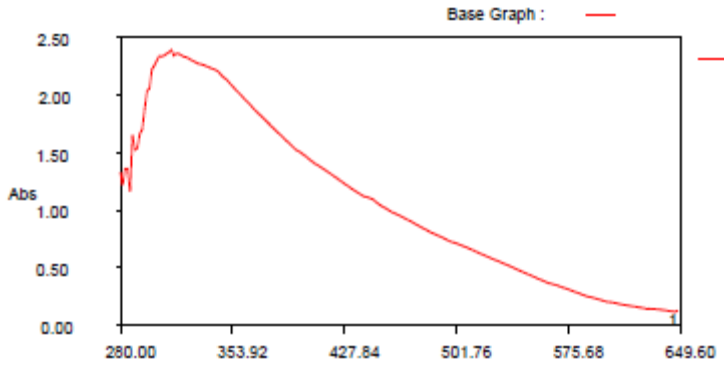


Fig.3 UV spectrogram of nanoselenium synthesized using *Streptococcus thermophilus*



The sharpness of the diffraction peaks suggested that the product was well crystallized. The crystallite size of selenium was calculated using Scherrer's equation.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the grain size, K is the constant taken to be 0.94, λ is the wavelength of the X-ray radiation, β is the line broadening at half the maximum intensity, θ (bragg angle) is the angle of diffraction. The calculated crystallite size of the nanoselenium was found to be in the range of 21 - 28 nm.

The results of XRD pattern analysis of selenium nanoparticles prepared by the above methods were similar to Dwivedi *et al.,* (2011) who prepared selenium nanoparticles using sodium selenosulphate and organic acid, and reported that all the diffraction peaks of nanoselenium corresponded to trigonal phase, with lattice constants a = 4.362Å and c = 4.958Å. Ingole *et al.,* (2010) reported the nanoselenium XRD pattern with calculated lattice constants to be, a = 4.363Å and c = 4.952Å. Similar findings were reported by Chen *et al.,* (2011) who synthesized selenium nanoparticles using glucose as reducing agent.

The morphology of the prepared nanoparticles was investigated by TEM analysis which clearly showed that the particle sizes of spherical selenium prepared were in the range of 10-30 nm (Fig.2) The nanoparticles obtained in the present study were of relatively smaller size than that reported by Eszenyi *et al.,* (2011) who synthesized nanoselenium using *Lactobacillus spp* and obtained nanoparticles with the sizes of 100-200 nm. The reduction in the size of the nanoselenium obtained could be due to the variation in the strain of bacteria used, which differed in their protein characteristics. The bacterial proteins play a major role in controlling the size and shape of nanoparticles (Dobias *et al.,* 2011).

UV spectroscopic analysis

The UV spectroscopic analysis of nanoselenium is presented in Fig.3. The nanoselenium particles prepared showed peak absorption values between 280-353 nm (UV range). This clearly indicated that all the nanoselenium particles had size below 100 nm, as recorded by Lin and Chris Wang (2005) who stated that the particle size could be correlated with the nature of the UV-visible spectra and if the particle size below was 100 nm or less, it showed a clear absorption maximum in the UV range.

The present results concurred with the findings of Fesharaki *et al.,* (2010) who reported the absorption band between 200-300 nm for the nanoselenium synthesized using *Klebsiella pneumoniae*.

Similiar observations were reported by Zhang *et al.,* (2011) and Harikrishnan *et al.,* (2012) who synthesized nanoselenium using *Pseudomonas alcaliphilia* and *Saccharomyces cerevisiae* respectively which exhibited absorption band between 200-300 nm.

Use of microorganisms for the production of nanomaterials is rapidly gaining significance owing to its growing successes, cost effective procedure and simplicity. There are several potential advantages around the microbe's ability to grow in aerobic conditions which include rapid ability to generate more number of bacterial cells within a short time period and less stringent culture conditions.

This green method of biosynthesis of selenium nanospheres is a simple, economically viable and an ecofriendly process resulting in nearly monodispersed highly stable selenium nanoparticles. The nanoselenium particles

synthesized by this method can be well utilized for supplementation in livestock and poultry.

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