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Biosynthesis and Structural Characteristics of Selenium Nanoparticles using *Lactobacillus Acidophilus* Bacteria by Wet Sterilization Process

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Abstract A facile ecofriendly biological approach for the synthesis of selenium nanoparticles using the probiotic *Lactobacillus acidophilus* bacteria and its characterization is reported in this paper. 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 15-50 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 use to recover the elemental selenium from bacterial cells.

Keywords *Selenium Nanoparticles; Lactobacillus Acidophilus; X-Ray Diffraction; Transmission Electron Microscopy; Wet Sterilization*

1. Introduction

Bio nanotechnology is an emerging important technical tool for the development of reliable eco-friendly methodology for the synthesis of materials in nanoscale using biological sources. Nano sized particles possess unique properties due to their larger surface to volume ratio and higher surface energy. Metal nanoparticles have diverse broad ranging tremendous applications in the field of chemistry, electronics, diagnostics, biomedical and material science. Production of nanomaterials can be achieved through conventional physical and chemical methods. Physical methods employ cumbersome high cost equipment 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.

Biological methods of nanoparticles synthesis using microorganisms, enzymes, and plant or plant extracts deserve merit and have been suggested as possible clean, nontoxic and eco-friendly alternatives to chemical and physical methods especially if they are intended for invasive applications in biological systems and medicine.

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). Microbial synthesis is readily scalable, environmentally benign and compatible with the use of the product for medical applications. Biosynthetic routes provide nanoparticles of a better defined size and morphology than some of the physicochemical methods of production (Raveendran et al., 2003). The application of bacteria strains for bio manufacturing of nanoparticles have advantage over other biological sources such as easy handling and short cultivation period. 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 *Lactobacillus acidophilus* bacteria using wet sterilization process, which, holds promising alternative for the large-scale commercial synthesis of selenium nanoparticles.

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 Se (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).

2. Experimental Methods

2.1. Preparation of Selenium Nanoparticles

A primary stock culture of *Lactobacillus acidophilus* (NCDC 15) bacteria purchased from National Dairy Research Institute, Karnal was revived and fresh subcultures were prepared (Eszenyi et al., 2011). The subcultures of *L. acidophilus* were used to prepare nanoselenium. Lactobacillus MRS broth (55.15 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 (Sigma, USA) dissolved in 20 ml of distilled water was added to the 980 ml of broth. Ten millilitre of fresh L. acidophilus bacterial culture was added to 1000 ml of MRS broth containing sodium selenite solution. The fermentation bottle was placed in shaking incubator for 48 h at 37°C. At the end of 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. 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.

2.2. Sample Characterization

2.2.1. 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).

2.2.2. 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.

2.2.3. 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.

3. Results and Discussion

The X-ray diffraction pattern of nanoselenium is shown in Figure 1. The diffraction peaks at 2θ (degrees) of 23.28°, 25.01°, 27°, 29° and 32.22° were indexed as the (211, 202, 221, 230 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 = 4.352Å and c = 4.929Å and were in good agreement with those on the standard data card. The sharpness of the diffraction peaks revealed 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 23 - 31nm.

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 15-50 nm (Figure 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 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 pneumonia*. Similar observations were reported by Zhang et al. (2011) and Harikrishnan et al. (2012) who synthesized nanoselenium using *Pseudomonas alcaliphila* and *Saccharomyces cerevisiae* respectively which exhibited absorption band between 200-300 nm.

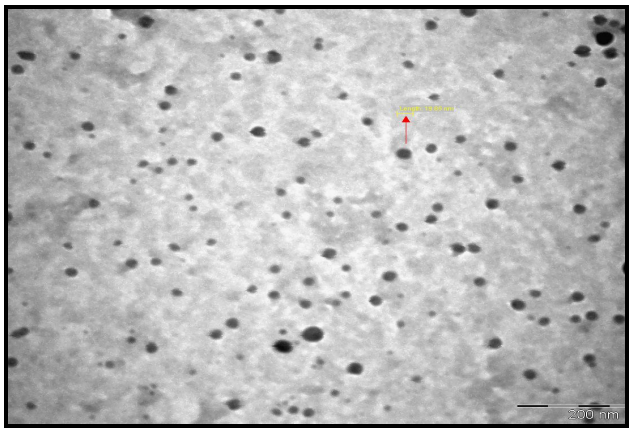

Figure 1: *Transmission Electron Microscope Image of Nanoselenium*

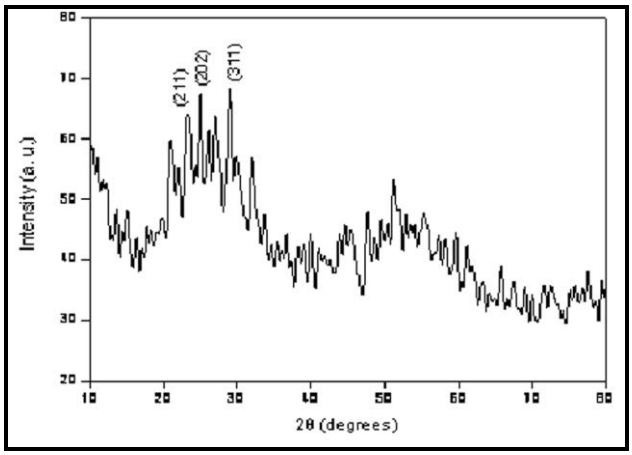

Figure 2: *X-Ray Diffraction Pattern on Nanoselenium*

4. Conclusion

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 route of biosynthesis of selenium nanospheres is a simple, economically viable and an ecofriendly process resulting in nearly monodispersed highly stable selenium nanoparticles. In this process, the proteins secreted by the bacteria play an important role in the stabilizing and determining the size of nanoparticles.

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