Extracellular biosynthesis of Selenium nanoparticles using some species of Lactobacillus

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Selenium (Se), a non-metallic chemical element exhibits many properties like relatively high thermoconductivity, superconductivity, catalytic activities. In addition, Se is one of the key elements for maintaining the health of mammalian animals because of its anti-oxidative and pro-oxidative effects. In the present study, we describe the biosynthesis of highly stable selenium nanoparticles using three species of non-pathogenic, eco-friendly and easily available Lactic acid bacteria (LAB): Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus rhamnosus was reported. Lactic acid bacteria can reduce selenium ions to elemental selenium nanoparticles (SeNPs) and deposit them in intracellular spaces. Reduction of Se\(^{+}\) ions in metal nanoparticles was investigated virtually by tracing the solution color which changed red after 48 hrs. Biosynthesized selenium nanoparticles were spherical (by TEM) in shape with size range of 20nm to 150 nm (UV spectrum). Moreover, as inferred from the FTIR spectrum, the presences of highly stable carbonyl group from the aminoacids have the strong ability to bind with the metal. Nanoparticles were further analysed for antimicrobial activity against pathogenic fungi, and they exhibited significant microbial activity.

[Keywords: Selenium nanoparticles, Lactobacillus sp, Aspergillus niger, Candida albicans, antifungal activity]

Introduction

There is a great need for microbe mediated clean, nontoxic and eco-friendly method of nanoparticle synthesis. The unique size-dependent characteristics of nanoparticles make them indispensable in many areas of human life ranging from industries like aerospace engineering to natural science, such as medicine, biology and food technology. Selenium (Se), a metalloid chalcogen, attracts more attention because of its special physical properties, such as the anisotropy of thermo conductivity, superconductivity, catalytic activities to hydration and oxidation reactions. Others are high piezoelectric, thermoelectric, and nonlinear optical responses. As an important semiconductor, it finds use in electronics, glass ceramics, steel and pigment manufacturing. It also plays a vital role in photocells, photographic exposure meters, and solar cells to semiconductor rectifiers. In addition, Se is one of the key elements for maintaining the health of mammalian animals because it exerts anti-oxidative and pro-oxidative effects. Moreover, several studies indicated that some organic forms of Se could show anticarcinogenic properties against certain types of cancer and possible therapy. In medicine, SeNPs have been reported to demonstrate high biological activity and low toxicity.

Biologically synthesized nanoparticles have wide application viz., biosensors, biolabelling, in cancer therapeutics and in coating of medical appliances. Although SeNPs preparations have been reported using 16 diverse species of bacteria including Klebsilla pneumonia and Bacillus subtilis have been discovered to reduce selenium oxyanions to the red, amorphous or monoclinic allotropes of SeO\(^{14}\). Herein, we report a facile, economical and biological route for the synthesis of Selenium nanoparticles using Lacidophilus, L.plantarum, L. rhamnosus, holding a promising alternative for the large-scale commercial synthesis of SeNPs.

Materials and Methods

Lacidophilus, L.plantarum, L. rhamnosus
cultures were obtained from Microbial Type Culture collection, Chandigarh, India. Sodium selenite was of A.R. grade and was obtained from Sigma Aldrich. Culture broths were prepared, sterilized and inoculated with a fresh batch of test strains. The composition of media is as follows:

LB broth was prepared at pH-7.5 by dissolving 10g of Tryptone, 5g yeast extract and 5g NaCl in 1000 ml distilled water. Enrichment medium was prepared by dissolving 0.5g Sodium nitrate, 5g Sodium chloride, 0.1g Ammonium chloride, 2.7g di-potassium hydrogen phosphate, 3g Tryptone, Beef extract 1g, 0.5g Yeast extract and 3g Glucose in 1000 ml distilled water.

100 ml each of Luria-Bertani (LB) media were freshly inoculated using a sterile loop. The flasks were then incubated at 35°C in a rotary shaker at 170 rpm. (Scigenic, Orbitek) The bacterial strains were allowed to grow. After 12 hours of growth, all the three bacterial samples were collected for further experiments.

**Synthesis of Se nanoparticles using Lactobacillus spp. and characterization**

100 ml each of Enrichment media were prepared and sterilized. To the prepared medium 4mM of sodium selenite and the activated cultures were added to it. The cultures were maintained at 35°C until the nanoparticles were formed on a rotary shaker at 170 rpm. With the reaction, the color of solution changed from light yellow to bright red, indicating the presence Se nanoparticles in the solution after 2days. Selenium nanoparticles formed were then harvested by centrifugation at 10,000rpm for 6 min, followed by washing with distilled water and ethanol repeatedly. Samples were further analyzed by different characterization techniques. In our experiments, it is found that different concentrations of sodium selenite (1-4mM) did not affect the morphology of the final products (Table-1). In the control experiment, 4mM sodium selenite was added to 100 ml EM medium allowed to react completely for 48 h at 35°C on a rotary shaker (170 rpm), no change in the color of the reaction solution was observed.

<table>
<thead>
<tr>
<th>Conc. of sodium selenite (in mM)</th>
<th>L. acidophilus</th>
<th>L. plantarum</th>
<th>L. rhamnosus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>40-50</td>
<td>40-60</td>
<td>60-80</td>
</tr>
<tr>
<td>4</td>
<td>40-60</td>
<td>20-60</td>
<td>60-80</td>
</tr>
<tr>
<td>6</td>
<td>40-60</td>
<td>40-60</td>
<td>80</td>
</tr>
</tbody>
</table>

**Characterization of nanoparticle**

The UV- visible spectrum of this solution was recorded in UV-Vis spectrophotometer UV-2450 (Shimadzu). The particles wavelength ranges from 200 to 700nm. Lactobacillus sp. treated with selenium nanoparticle was air dried and used for analysis. Morphology and particle sizes were determined by Transmission Electron Microscope (TEM) was performed on a JEOL Model 1200EX instrument operated at an accelerating voltage at 80 kV by focusing on nanoparticles. TEM sample of the Selenium nanoparticle was recorded by placing a drop of the suspension on carbon coated copper grids and allowed to stand for 2 min, following which the extract solution was removed using a blotting paper and the grid allowed drying for overnight prior to measurement.

FTIR measurement was done by the removal of any free biomass residue or compound that is not the capping ligand of the nanoparticles. Residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was re-dispersed in 10 ml sterile distilled water. Centrifuging and re-dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR on a Perkin-Elmer Spectrum-One instrument in the diffuse reflectance mode.

**Antifungal activity of synthesized SeNPs**

The selenium nanoparticles synthesized
using *Lactobacillus* spp. was tested for antifungal activity by agar well-diffusion method against *Aspergillus niger* and *Candida albicans*. The pure cultures fungal pathogens were sub cultured on Potato Dextrose Agar (PDA). Wells of 10 mm diameter were made on PDA plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl and 50 µl) was poured onto each well on all plates. After incubation at 37°C for 48 hrs, the different levels of zone of inhibition were measured.

**Results and Discussion**

After the incubation of *Lactobacillus* strains separately with sodium selenite, the media displayed a time-dependent color change indicating the reduction of sodium selenite

![Fig: 1 Control (1-a) L.acidophilus (1-b), L.plantarum (1-c), L .rhamnosus (1-d)](image)

The synthesized selenium nanoparticle was primarily characterized by UV spectrophotometer. The UV-visible spectra recorded at different time intervals showed increased absorbance with increasing time of incubation. The absorbance scan showed a sharp Plasmon...
peak at 300nm in all the three Lactobacillus species which indicates the formation of SeNPs.

**Fig: 2** UV-Visible Spectrophotometry of the NPs synthesized by *L. acidophilus* (3-a), *L. plantarum* (3-b), *L. rhamnosus* (3-c)

**TEM and FT-IR analysis**

The dimension and morphology of SeNPs collected from the reaction solution of 3 *Lactobacillus* spp. were examined by HRTEM and shown [Fig 3]. TEM images of nanoparticles that were synthesized by *L. acidophilus* indicated that the nanoparticles were in the size range of 10-20nm [Fig 3a] where as in *L. rhamnosus* and *L. plantarum* the particle were 60-80nm size range.

Further, the sample showed particles had a spherical morphology with smooth surface.

To identify the possible biomolecules responsible for the reduction of the Se\(^+\) ions and excapping of the bioreduced selenium nanoparticles synthesized using microbes, FTIR studies were carried out and the representative spectrum of the nanoparticles obtained in the present study is presented in [Fig 4].
Fig: 3  TEM images of NPs synthesized by *L. acidophilus* (3-a), *L. plantarum* (3-b), *L. rhamnosus* (3-c)
The FTIR spectrum recorded for the purified SeNP powder reveals bands at 3430 cm\(^{-1}\) and 3417 cm\(^{-1}\) is attributed to the O-H stretching mode and the N-H stretch in amine group respectively. The broad peak at 747 cm\(^{-1}\), 783 cm\(^{-1}\) and 791 cm\(^{-1}\) corresponds to C-H stretching motion and the narrow peak at 1645 cm\(^{-1}\) and 1651 cm\(^{-1}\) corresponds to C-C stretching. An earlier FTIR spectroscopic study confirmed that the carbonyl groups form amino acid residues and peptides of proteins have the strong ability to bind metal. Hence, the proteins could most possibly form a coat covering the metal nanoparticles to prevent agglomeration of the particles and stabilizing in the medium\(^{20}\). The amide linkages between the amino acid residues in polypeptides and proteins could give rise to the well-known signatures in the infrared region of the electromagnetic spectrum. It has been reported earlier that proteins can bind to gold nanoparticles through either free amine groups or cysteine residues in the proteins\(^{21}\).

**Antimicrobial Activity**

Selenium nanoparticles synthesized via biological route have antifungal activity against two different fungal species by well diffusion method [Figure 5 and 6]. Nanoparticle solution was prepared using DMSO (Dimethyl sulfoxide) and added to the well. Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of selenium nanoparticles that inhibited the visible growth of *Aspergillus niger* and *Candida albicans*. Zone of inhibition ranged from 4 to 10 mm. These results indicated that the selenium nanoparticles synthesized have stronger antimicrobial activity. The diameter of inhibition zones (in Centimeters) around the different selenium nanoparticles against test strain are shown in Table 2. The control plate shows no effect on these organisms.

Bacteria are easy to handle and can be manipulated genetically without much difficulty. Considering these advantages, a bacterial system could prove to be an excellent alternative for synthesis of SeNPs. Selenium possesses several applications in medicine, chemistry, and electronics. In recent years, there has been an increasing interest in synthesizing metal particles using chemical and biological methods. Zhang and coworkers showed that Se NPs has a size dependent effect in directly scavenging free radicals *in vitro*\(^{22}\). The use of “green” synthesis of metal nanoparticles is going to be of considerable importance; thus, appropriate methods should be
developed and tested, especially for the recovery of these nanoparticles from natural resources such as bacterial cells. The efficiency of the antimicrobial drug can be enhanced with addition of these cost effective SeNPs and can reduce the cost of the production of these expensive synthetic drugs. Ever growing antibiotic resistant strains of bacteria constantly forces the scientific community to search for and to develop novel antibiotics and many new antibiotics have been introduced in the last decade and unfortunately none of them was successful in combating the multi-drug resistant strains\textsuperscript{23}. As the nanoparticles have delivered and demonstrated effective antimicrobial activities, the development of novel preparations in this field finds attractive alternative to antibiotics. To authenticate, nanoparticles have been examined for their ability to suppress microbial infections in skin\textsuperscript{24} and burn wounds\textsuperscript{25}, and even in preventing bacterial colonization and the results are promising. Reduction in the size of the particles of the materials is an efficient and reliable tool for reinforcing their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science\textsuperscript{26}. Studies reveal that, selenium nanoparticles were prepared by the reduction of sodium selenite and thus synthesized nanoparticles were stable at room temperature for more than several months. Recently there is an increasing interest to prepare Se NPs for different medical and industrial purposes. The synthesis of Se NPs using a culture supernatant of \textit{Lactobacillus} strains appears to be simple and an appropriate method for synthesis of Se NPs with particle size less than 100 nm. Also this approach would be suitable for developing a biotechnological process for large-scale production of small Se NPs.

<table>
<thead>
<tr>
<th>Strain</th>
<th>L. acidophilus</th>
<th>Zone of Inhibition</th>
<th>L. plantarum</th>
<th>Zone of Inhibition</th>
<th>L. rhamnosus</th>
<th>Zone of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>0.4 cm partial zone</td>
<td>0.8 cm clear zone</td>
<td>1 cm clear zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>-</td>
<td>0.9 cm clear zone</td>
<td>0.9 cm clear zone</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

zone of inhibition of Candida albicans by SeNPs synthesized by \textit{Lactobacillus} acidophilus

zone of inhibition of Candida albicans by SeNPs synthesized by \textit{Lactobacillus} plantarum
**Fig: 5** Antifungal activity of NPs synthesized by *L. acidophilus* (5-a), *L. plantarum* (5-b), *L. rhamnosus* (5-c) against *C. albicans*

**Fig: 6** Antifungal activity of NPs synthesized by *L. plantarum* (6-a), *L. rhamnosus* (6-b) against *A. niger*
References

25. Ulkur E., Oncul O., Karagoz H., Yeniz E. and Celikoz B, Comparison of Silver-Coated Dressing (Acticoat), Chlorhexidine Acetate 0.5% (Bacti-Grass), and Fusidic Acid 2% (Fucidin) for Topical Antibacterial Effect in Methicillin-Resistant Staphylococci-Contaminated, Full-Skin Thickness Rat Burn Wounds, *Burns*, 31(2005) 874-877.