Digest Journal of Nanomaterials and Biostructures Vol. 6, No 2, April-June 2011, p. 385-390

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING MARINE CYANOBACTERIUM, *OSCILLATORIA WILLEI* NTDM01

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Pioneering of reliable and eco-friendly process for synthesis of metallic nanoparticles biologically is an important step in the field of application of nanobiotechnology. This paper reports the extracellular biosynthesis of silver nanoparticles using marine cyanobacterium, *Oscillatoria willei* NTDM01 which reduces silver ions and stabilizes the silver nanoparticles by a secreted protein. The silver nitrate solution incubated with washed marine cyanobacteria changed to a yellow color from 72h onwards, indicating the formation of silver nanoparticles. The characteristics of the protein shell at 265nm were observed in Ultra violet spectrum for the silver nanoparticles in solution. While Fourier Transform Infra Red (FTIR) confirmed the presence of a protein shell which are responsible for the nanoparticles biosynthesis. Scanning Electron Microscopy (SEM) studies showed that the formation of agglomerated silver nanoparticles due to the capping agent in the range of 100 - 200nm. EDS spectrum of the silver nanoparticles was confirmed the presence of elemental silver signal in high percentage. Apart from Eco-friendliness and easy availability and low cost cyanobacterial biomass production will be more advantageous when compared to other classes of micro organism. Biosynthesis nanoparticles would have greater commercial viability if the nanoparticles could be synthesized more rapidly in the reaction vessel.

(Received January 10, 2011; accepted February 2, 2011)

Keywords: Marine cyanobacteria, silver nanoparticles, *Oscillatoria willei* NTDM01, FTIR, EDS

1. Introduction

Nanotechnology is enabling technology that deals with nano-meter sized objects. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. Synthesis of nanoparticles using biological entities has great interest due to their unique shape dependent optical, electrical and chemical properties have potential application in nanobiotechnology. The synthesis and assembly of nanoparticle would benefit from the development of clean, nontoxic and environmentally acceptable "green chemistry" approaches for nanoparticles.

The biosynthesis of silver nanoparticles of different sizes, ranging from 1- 70nm, and shapes, including spherical, triangular and hexagonal has been conducted using bacteria, fungi, plant extracts (1-4). The mechanisms for the bioreduction of silver by bacteria involve reducing and other proteins in which sulphur and carboxylate group from cell wall (2-4). The silver nitrate caused the reduction of silver through a nitrate dependent reductase and synthesized the nanosized materials in *Fusarium oxysporum* (3). In this study, the formation of silver nanoparticles was investigated using silver nitrate in the presence of the marine cyanobacterium, *Oscillatoria willei* NTDM01. Marine cyanobacteria are one of the largest, photoautotrophic bacteria in marine ecosystem and are known to have high affinity transport system for nitrate.

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2. Materials and methods

The marine cyanobacterial samples was collected and isolated from Kurusadai Island (Lat. 9° 18'N & Long 79°10'E) at Gulf of Mannar, Tamilnadu, India. The culture was grown in MN- III medium at pH 8 (6) and made them axenic using different combination of antibiotics. The culture was transferred and grown for approximately 4-6 weeks to reach a stationary 'growth' phase. It was then centrifuged and washed several times with distilled water, deionized water to remove the trace metals from the surface of the cyanobacteria.

Table 1. FTIR analysis of O. willei NTDM01, which shows the functional group of the protein

Frequency cm^{-1}	Type	Vibration Mode
3437.05	Sec.amides, freeNH(trans)	N-H Structure
2926.52	Alkanes(-CH_2-)	C-H Structure
2857.58	Alkanes(-CH_2-)	C-H Structure
2378.14	Charged amines(C=NH^+)	NH^- Structure
2096.19	Organo-silicon compounds	Si-H Structure
1639.24	Alkenes(CHR_1=CHR_1-cis)	C=C Structure
1394.59	Alkanes, tery.butyl	C-H deformation
1224.56	Aliphatic esters	CH_3COOR
1102.71	Secondary alcohols	C-OH Structure

To initiate the experiments, 5mL of silver solution (~560mg/L) was added to 5mL of washed cyanobacterial cultures. The experiment were incubated at 25° C for 28 days and maintained in the dark. Abiotic experiments consisting of ~560mg/L silver were conducted using silver nitrate solution without the presence of cyanobacteria. The pH and cell viability were monitored during the course of the study. The sample have been characterized for the development of silver nanoparticles by using Ultra Violet –Visible Spectrum (*Uv*-Vis) , Fourier Transform Infra Red (FTIR), Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopic (EDS) investigation (3,7).

3. Results

The axenic strain was identified as *Oscillatoria willei* NTDM01 (Fig.1). In cyanobacterial experiment, soluble silver was completely precipitated from solutions within 28days. A grayish-black silver precipitate on cyanobacteria was observed in experiment macroscopically. The pH was constant at 5.0 and 4.7. In abiotic experiments using silver nitrate, total soluble silver concentration and pH were constant until the completion of experiment.

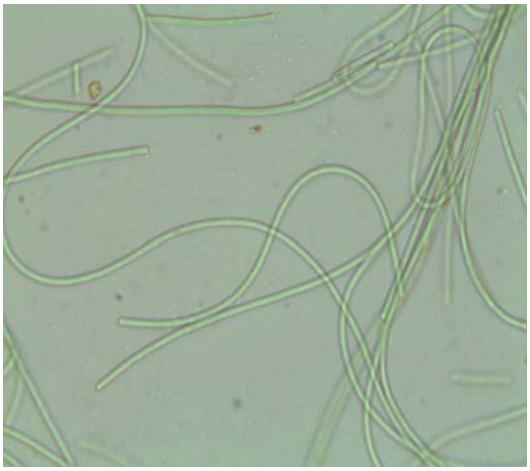
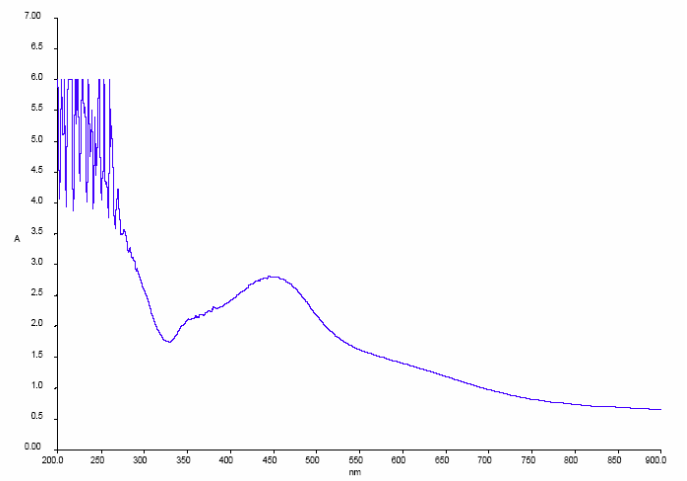
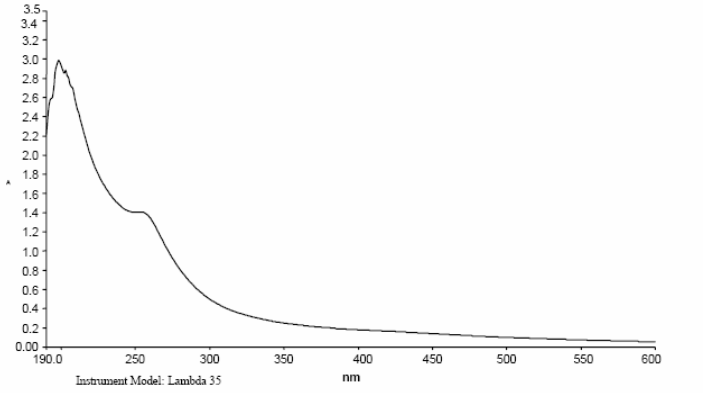


Fig. 1. Microphotography of marine cyanobacterium, Oscillatoria willei NTDM01.

The UV-vis Spectrum reveals that the band observed at 450 nm due to the plasmon resonance of the silver nanoparticles and low wavelength region recorded from the reaction medium exhibited an absorption band at ca.265 nm and it was attributed to aromatic amino acids of proteins (Fig.2). It is well known that the absorption band at ca 265 nm arises due to electronic excitation in tryptophan and tyrosine residue in the protein.



a



b

Fig. 2. UV-Vis spectrum of plasmon resonance of silver nanoparticles synthesized by O.willei NTDM01 (2a), low wavelength region recorded, where the reducing protein present in O. willei NTDM01.

The FTIR spectrum was recorded from the film of silver nanoparticles, formed after several days of incubation with the bacteria (Fig.3). The bands seen at 3280 cm−1 and 2924 cm−1 were assigned to the stretching vibrations of primary and secondary amines respectively. The corresponding bending vibrations were seen at 1651 cm−1 and 1548 cm−1, respectively. The two bands observed at 1379 cm−1 and 1033 cm−1 can be assigned to the C–N stretching vibrations of aromatic and aliphatic amines, respectively. The presence of protein as the stabilizing agent surrounded the silver nanoparticles (7). The silver ions were reduced in the presence of nitrate reductase, leading to the formation of a stable silver hydrosol 10-25 nm in diameter and stabilized by the capping peptide.

SEM observation on products of marine cyanobacterium, *Oscillatoria willei* NTDM01 in $AgNO_3$ experiment is presented (Fig.4). At room temperature, the addition of $AgNO_3$ to the cyanobacteria caused the precipitation of silver nanoparticles at cell surfaces (Fig.4a). Small spherical silver nanoparticles with size ranging from 100nm to 200nm (extracellularly) were also precipitated in solution silver nanoparticles were deposited at cell surfaces (Fig.4b). EDS showed the occurrence of silver particle in higher amount with trace of magnesium, calcium and chloride. In this analysis by EDS of the silver nanoparticles was confirmed the presence of elemental silver signal (Fig.4c).

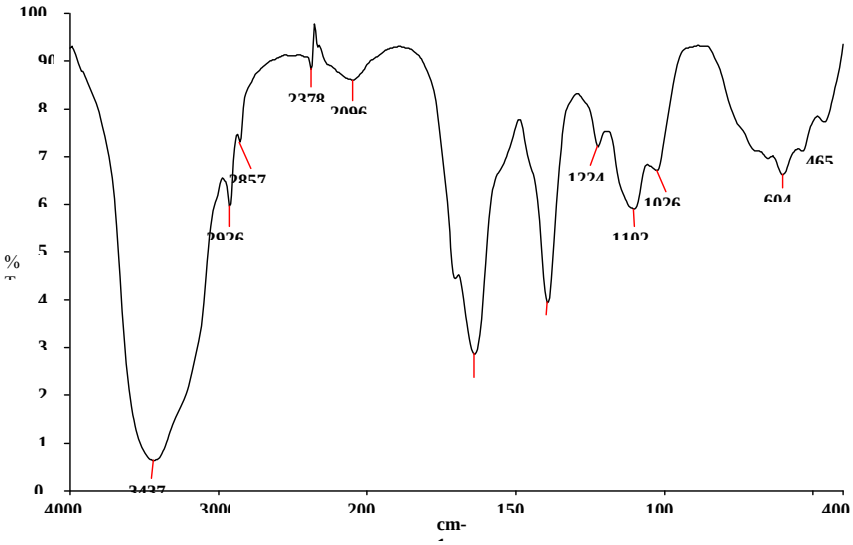
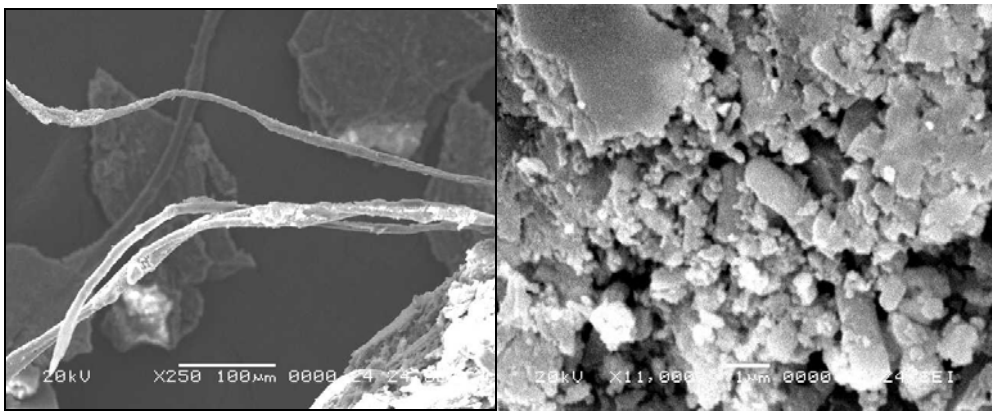
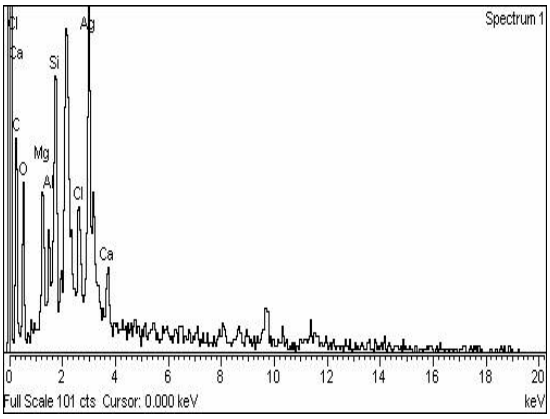


Fig. 3. FTIR analysis of O. willei NTDM01 shows the presence of protein shell for the reduction of silver ions.



a



b

Fig. 4a. Precipitation of silver nanoparticles on the cyanobacterial surfaces.
b. SEM micrograph of cyanobacteria cells with nanoparticles silver deposited outside the cell with size range of 150nm c. EDS of the silver nanoparticles was confirmed the presence of elemental silver signal in high percentage.

The reaction of cyanobacteria with $AgNO_3$ solutions results in the formation of silver nanoparticles. The presence of spherical silver nanoparticles was observed in experiment. The precipitation of silver nanoparticles was not observed for abiotic experiment that were run under similar condition and duration, that suggesting that cyanobacteria were required for silver precipitation and the precipitation processes were not controlled by inorganic chemical reactions.

4. Discussion

A brief overview of the use of micro organisms such as cyanobacteria, yeast, algae, fungi and actinomycetes in the biosynthesis of metal nanoparticles has been described. Our UV-vis observation indicated the release of protein into the culture medium indicting the electronic excitation of aromatic amino acids. A similar observation has been made in *Fusarium oxysporum* and suggests a possible mechanism for the reduction of the metal ions present in the solution **(8)**. The overall peaks from FTIR observation confirm the presence of protein in the samples of silver nanoparticles. It can be assumed that nitrate reducing bacteria to produce nitrate reductase enzyme (protein). This reduces to silver nitrate solution form silver nanoparticles. The synthesis of silver nanoparticles using ß-NADPH-dependent nitrate reductase and phytochelatin *in vitro* has been demonstrated for the first time **(9)**.

The cyanobacterial cells were encrusted with silver nanoparticles and separation of some filaments into their constituent cells were still observed. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by capping agent. This corroborates with the previous observation in *F. oxysporum* (8)**.** Cyanobacteria commonly

use nitrate as the major source of nitrogen for three different purposes including growth, generation of metabolic energy, and redox balancing (10). The presence of silver nanoparticles throughout the cells suggests that Ag^+ and NO_3 entered the cyanobacteria cells through a transport system and degraded in solution.

The presence of silver nanoparticles inside the cytoplasm, Ag^+ is presumably reduced to Ag^o Because $AgNO_3$, a toxic reagent, was used in metabolic processes, it ultimately killed the cells. During the death of cyanobacteria, nanoparticles of silver produced inside the cells were release through the cell membrane into solution, as indicated by the precipitation of silver nanoparticles around the cells. The dead cyanobacteria also released organics (protein, and other biochemical) that caused further precipitation of silver from solution outside the cells. The protein molecules act as reducing agent for silver nanoparticles. The protein molecule made up of different functional group in amino acid sequences such as amino, carboxyl, sulfate groups present in the cyanobacterial protein favor the formation of extremely small-sized silver nanoparticulates with narrow particle size distribution and hydroxyl and sulfonic groups are beneficial to synthesis of silver nanoparticle with a slightly larger particle size in a weak reducing environment. It has already been certified that the silver nanoparticles can be stabilized and are well functional groups, such as carboxyl, hydroxyl, and amido and thiol group, except the steric effect arising from the large molecular structures of the organic modifier (protein) on the surface of the particles (11,12). The micro organisms in the synthesis of nanomaterials as a possible viable alternative to the more popular physical and chemical methods, currently in vogue. The use of cyanobacteria as a source of enzymes that can catalyze specific reactions leading to inorganic nanoparticles is a new and rational biosynthesis strategy that is being developed. Extracellular secretion of enzymes offers the advantages of obtaining large quantities in a relatively pure-state, free from other cellular proteins associated with the organism and can be easily processed by filtering of the cells and isolating the enzyme for nanoparticles synthesis from cell-free filtrate.

Acknowledgments

The authors wish to acknowledge support from University Grant commission (UGC) for their financial assistance.

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