

MICROBIAL SYNTHESIS OF SILVER NANOPARTICLES BY STREPTOMYCES GLAUCUS AND SPIRULINA PLATENSIS

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ABSTRACT

Microbial synthesis of nanoparticles has a potential to develop simple, cost-effective and eco-friendly methods for production of technologically important materials. In this study, for the first time a novel actinomycete strain *Streptomyces glaucus* 71 MD isolated from a soy rhizosphere in Georgia is for the first time extensively characterized and utilized for the synthesis of silver nanoparticles. Scanning Electron Microscope (SEM) allowed observing extracellular synthesis of nanoparticles, which has many advantages from the point of view of applications. Production of silver nanoparticles proceeded extracellularly with the participation of another microorganism, blue-green microalgae *Spirulina platensis* (*S. platensis*). In this study it is shown that the production rate of the nanoparticles depends not only on the initial concentration of AgNO_3 but also varies with time in a nonmonotonic way. SEM study of silver nanoparticles remaining on the surface of microalgae revealed that after 1 day of exposure to 1 mM AgNO_3 nanoparticles were arranged as long aggregates along *S. platensis* cells strongly damaged by silver ions. However, after 5 days of exposure to silver *S. platensis* cells looked completely recovered and the nanoparticles were distributed more uniformly on the surface of the cells.

Key words: silver, nanoparticles, *Spirulina platensis*, *Streptomyces glaucus* 71 MD, scanning electron microscope

INTRODUCTION

Silver nanoparticles have a great number of applications, e.g. in nonlinear optics, spectrally selective coating for solar energy absorption, biolabelling,

intercalation materials for electrical batteries, high-sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics, catalysis and micro-electronics.

Various microorganisms (bacteria, yeast, fungi) are known to synthesize silver nanoparticles. The produced nanoparticles have different size and shape. Nanoparticles resulting from some microbial processes are composite materials and consist of inorganic component and special organic matrix (proteins, lipids, or polysaccharides) and they have unique chemical and physical properties different from the properties of conventionally produced nanoparticles and of other microorganisms even when they are incubated in the same medium under the same conditions.

The ability of *Streptomyces glaucus* 71MD, a novel strain of actinomycetes isolated in Georgia, and microalga *S. platensis* to produce silver nanoparticles was studied

METHODS OF SAMPLE MANUFACTURING AND ANALYSIS

Cultivation of Streptomyces glaucus 71MD

Cells were grown aerobically at pH 7–8, 28–30 °C in 500 ml Erlenmeyer flasks. The cells were grown in a liquid medium Gauze-1 [1]: K₂HPO₄ (0.05 %), MgSO₄ (0.05 %), NaCl (0.05 %), KNO₃ (0.1 %), FeSO₄ × 7H₂O (0.001 %), starch (2 %), yeast extract (0.03 %), pH 7.5. The culture was grown with continuous shaking on a shaker (200 rpm) at 30 °C for 9 days. After cultivation, mycelia (cells) were separated from the culture broth by centrifugation (4500 rpm) for 20 min and then the mycelia were washed thrice with sterile distilled water under sterile conditions. The harvested mycelial mass (16 g of wet mycelia) was then resuspended in 100 ml of 10⁻³ M aqueous AgNO₃ solution in 500 ml Erlenmeyer flasks. The whole mixture was put into a shaker at 30 °C (200 rpm).

Cultivation of S. platensis

Cells were cultivated in Zarrouk growth media at constant shaking at 30–31 °C, pH 9 [2]. The bacterial cells were harvested after 5–6 days and then were washed twice in distilled water. Then 1 g of wet biomass was placed in a 250-ml Erlenmeyer flask with 100 ml 10⁻³ M aqueous AgNO₃ and incubated at room temperature for different time intervals (1–5 days). The pH was checked during the course of reaction and it was found to be 5.6.

UV-vis Spectrometry

The UV-vis spectra of the samples were recorded on a “Cintra 10e” spectrophotometer (GBC Scientific Equipment Pty Ltd, Australia, wavelength range 190–1100 nm).

Scanning Electron Microscope

SEM was carried out using the SDB (small dual-beam) FEI Quanta 3D FEG with the EDAX Genesis EDX system with the resolution 1.2 nm. Opera-

tional features of the microscope used in the experiment: magnification 5000 – 150000x; voltage 1–30 kV.

RESULTS AND DISCUSSION

1) Synthesis of silver nanoparticles by a novel actinomycetes strain *Streptomyces glaucus* 71MD

Addition of actinomycetes biomass to a silver nitrate solution led to the appearance of yellowish brown color in the solution after a few days, indicating formation of silver nanoparticles. First, the UV-visible spectroscopy method was used to quantify this process (*Fig. 1*).

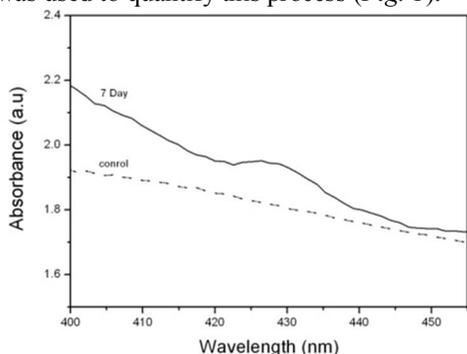


Fig. 1–UV-Vis spectra recorded after one week for the reaction mixture prepared using 1mM silver nitrate and 1 g biomass of *Streptomyces glaucus* 71MD

The cells of *Streptomyces glaucus* 71 MD were imaged by the SEM method after the reaction with the silver nitrate solution for one week. The SEM images (*Fig. 2*) illustrate that most of the particles are spherical-like and do not create big agglomerates.

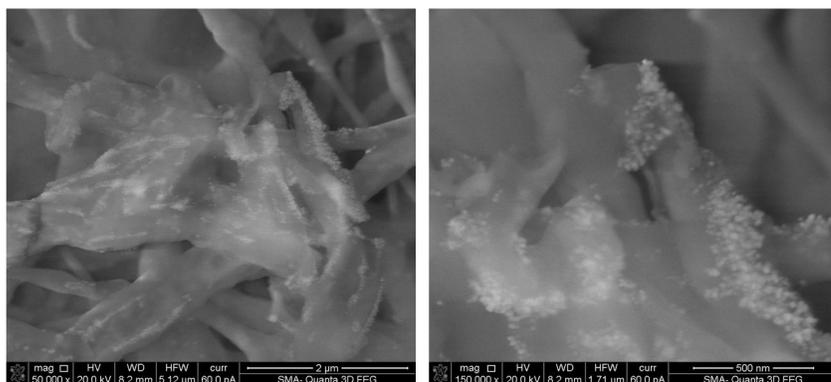


Fig. 2–SEM of *Streptomyces glaucus* 71MD cells with silver nanoparticles

EDAX (Energy dispersive analysis of X-rays) spectra also prove the presence of silver in *Streptomyces glaucus* 71 MD. The particle sizes range from 4 nm to 25 nm with an average of 13 nm.

1) Synthesis of silver nanoparticles by *S. platensis*

Synthesis of silver nanoparticles by *S. platensis* under different experimental conditions was investigated.

The size and the distribution of the silver nanoparticles depend on the time of silver action. After one day of the silver ion action, large agglomerates of nanoparticles could be observed. The mean size of the nanoparticles observed in these agglomerates is about 27 nm (Fig. 3).

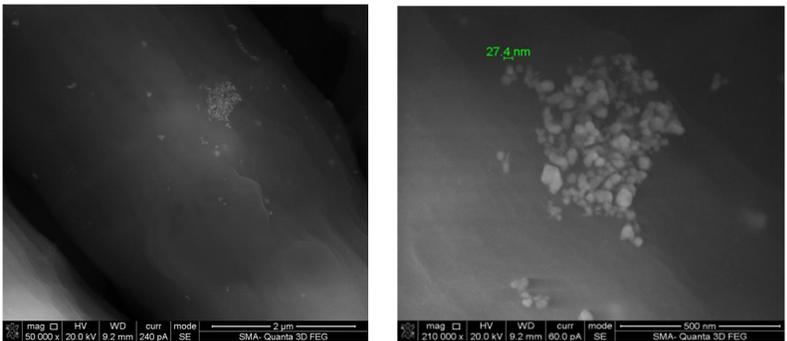


Fig. 3– SEM of *Sp. platensis* cells at different magnifications at 1 mM AgNO_3 for 1 day

After immersion of *S. platensis* cells in the silver nitrate solution for 5 days, the produced silver nanoparticles are relatively uniformly distributed along the surface of the cyanobacterium cells. In this case, the maximum size of the formed agglomerates is about 235 nm and the minimum size is 75 nm (Fig. 4).

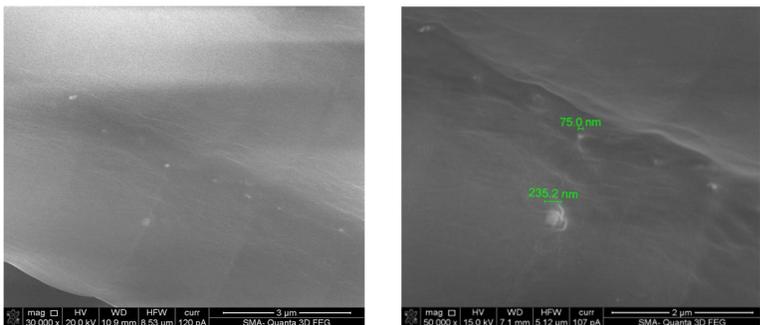


Fig. 4– SEM of *Sp. platensis* cells at different magnifications at 1 mM AgNO_3 for 5 days

CONCLUSIONS

(a) Actinomycetes *Streptomyces glaucus* 71 MD produces silver nanoparticles extracellularly which offers a great advantage over an intracellular process of synthesis from the point of view of applications.

(b) Production of silver nanoparticles proceeded extracellularly by blue-green microalgae *Spirulina platensis*; however, process depends on the experimental conditions

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