IET Nanobiotechnology

Research Article

IET Journals
The Institution of Engineering and Technology

Biosynthesis of Silver Nanoparticles by *Streptomyces griseorubens* isolated from Soil and Their Antioxidant Activity

ISSN 1751-8741
Received on 31st December 2015
Revised 15th June 2016
Accepted on 25th July 2016
E-First on 20th September 2016
doi: 10.1049/iet-nbt.2015.0127
www.ietdl.org

Tuba Baygar[1], *Aysel Ugur*[2] ✉

[1]*Research Laboratories Center, Mugla Sitki Kocman University, 48000 Mugla, Turkey*
[2]*Section of Medical Microbiology, Department of Basic Sciences, Faculty of Dentistry, Gazi University, 06500 Ankara, Turkey*
✉ *E-mail: ayselugur@hotmail.com*

Abstract: Microbial mediated biological synthesis of metallic nanoparticles was carried out ecofriendly in the present study. Silver nanoparticles (AgNPs) were extracellularly biosynthesised from *Streptomyces griseorubens* AU2 and extensively characterised by ultraviolet–visible (UV–vis) and Fourier transform infrared spectroscopy, high-resolution transmission electron microscopy, scanning electron microscopy and X-ray diffraction analysis. Elemental analysis of nanoparticles was also carried out using energy dispersive X-ray spectroscopy. The biosynthesised AgNPs showed the characteristic absorption spectra in UV–vis at 422 nm which confirmed the presence of metallic AgNPs. According to the further characterisation analysis, the biosynthesised AgNPs were found to be spherical and crystalline particles with 5–20 nm average size. Antioxidant properties of the biosynthesised AgNPs were determined by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay and was found to increase in a dose-dependent matter. The identification of the strain was determined by molecular characterisation method using 16s rDNA sequencing. The present study is the first report on the microbial biosynthesis of AgNPs using *S. griseorubens* isolated from soil and provides that the active biological components found in the cell-free culture supernatant of *S. griseorubens* AU2 enable the synthesis of AgNPs.

1 Introduction

Owing to their strong characteristic features, research have been focusing their attention on different application facilities of silver nanoparticles (AgNPs). Materials developed with AgNP incorporation have great optical, electrical, catalytic and antimicrobial properties with unique physicochemical and biochemical characteristics. Various techniques are being used to synthesise metal nanoparticles many of which are expensive and expose toxic chemical wastes [1].

Biological methods for green nanosynthetic routes are known to be environmentally safe, inexpensive and time saving applications thus they provide appreciable results for nanotechnology. Novel green synthesis strategies using various biological organisms have been recognised as a promising source for reducing metals by specific metabolic pathways [2].

Microorganisms of different taxonomic categories from fungi and prokaryotes such as *Fusarium oxysporum* [3], *Aspergillus flavus* [4], *Bacillus licheniformis* [5] and *Klebsiella pneumoniae* [6] have been used for AgNP biosynthesis through their cell mass or extracellular components.

Members of Actinobacteria isolated from various ecological environments were recently investigated as potential synthesisers of metallic nanoparticles [7]. Among the class Actinobacteria, the biosynthesis of AgNPs intra/extracellularly has been reported for *Thermomonospora* sp. [8], *Rhodococcus* sp. [9] and *Streptomyces* sp. [1, 10–17].

Streptomyces is a genus of Gram-positive aerobic bacteria that grows in various environments, with a filamentous form similar to fungi [18]. The Streptomyces species, members of the bacterial order Actinomycetales, are found worldwide in soil. Organisms from the genus *Streptomyces* produce effective bioactive compounds and are intensively used in the agricultural, pharmaceutical and enzyme industries. Therefore, they are well known for industrial manufacturing purposes [10, 19]. Though there is only one study about the AgNP biosynthesis from *Streptomyces griseorubens* isolated from marine sediment [17], the present investigation is the first report of the extracellular AgNP biosynthesis by using *S. griseorubens* isolated from soil.

This paper indicated that *S. griseorubens* AU2 isolated from soil have an excellent reducing capacity for the synthesis of AgNPs. The formation and morphology of the biosynthesised AgNPs were analysed and extensively characterised using spectral techniques such as ultraviolet–visible (UV–vis) and Fourier transform infrared spectroscopy (FT-IR) spectroscopies, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. Elemental analysis of the biosynthesised AgNPs were also performed via TEM and SEM equipped with energy dispersive X-ray spectroscopy (EDS). The *in vitro* antioxidative potential of the biosynthesised AgNPs was performed by free radical scavenging activity.

2 Materials and methods

2.1 Isolation and molecular identification of Streptomyces griseorubens AU2

The actinomycete strain was isolated from soil at an orange farm located in Koycegiz district of Mugla Province in the Aegean region of Turkey. Soil sample was taken according to the method of Brown [20]. International Streptomyces Project 2 (ISP-2) medium (10 g l^{-1} malt extract, 4 g l^{-1} yeast extract, 4 g l^{-1} glucose and 20 g l^{-1} agar, pH 7.3) with 2 g/l calcium carbonate addition was used to isolate the strain. The isolated *S. griseorubens* AU2 strain were maintained in 30% glycerol (v/v) at −20°C in the Mugla Sitki Kocman University Collection of Microorganisms (MU). For biosynthesis, pure *S. griseorubens* AU2 culture were maintained at 4°C in ISP-2 agar plates and sub-cultured continuously at regular intervals.

The strain *S. griseorubens* AU2 was identified with the sequence of 16S rDNA which was amplified by three primer couples as 925F ---->5'-AAA CTY AAA KGA ATT GAC GG-3', 1491R ----> 5'-ACG GCT ACC TTG TTA CGA CTT-3', 529F---->5'-GTG CCA GCM GCC GCG G-3', 1099R---->5'-GGG TTG CGC TCG TTG-3', 27F---->5'-AGA GTT TGA TCC TGG CTC AG-3', 780R---->5'- TAC CAG GGT ATC TAA TCC TGT T-3'. The polymerase chain reaction (PCR) condition was adjusted

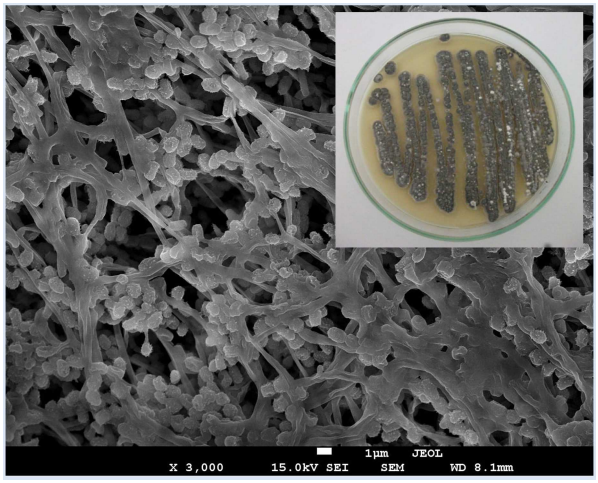


Fig. 1 *Colony morphology of the S. griseorubens AU2*

accordingly initial denaturing for 2 min at 94°C, 30 s at 94°C, 30 cycles of 30 s at 53°C, 40 s at 72°C and a final 10 min extension at 72°C. Amplicons were purified and sequenced at BM Labosis Company (Ankara, Turkey). After sequencing all the fragments were aligned by using the software MEGA6 and 1484 bp of 16S rDNA was searched at Basic Local Alignment Search Tool algorithm in GenBank (http://www.blast.ncbi.nlm.nih.gov), and the bacteria were identified on the basis of their similarity to the 16S rDNA sequence.

2.1 Biosynthesis of AgNPs

The method described by Zonooz and Salouti [10] was slightly modified for microbial mediated synthesis of AgNPs. The pure *S. griseorubens* AU2 culture was inoculated on ISP-2 broth under aseptic conditions and the flasks were incubated at 28°C and 130 rpm for 7 days. After the incubation period of 7 days completed, the culture was centrifuged at 4000 rpm for 20 min and the biomass was used as the main stock for the biosynthesis of AgNPs. For that purpose, the biomass was washed with dH_2O for three times. Then, the biomass was suspended in dH_2O and incubated at 28°C and 130 rpm for 48 h. At the end of the incubation period, the cell-free culture supernatant that would be used for biosynthesis was obtained by centrifugation at 4000 rpm. About 10 ml of cell-free supernatant and 50 ml Ag nitrate ($AgNO_3$) solution (1 mM) (filtered through a 0.22 µm filter) were incubated at 28°C and 130 rpm for 48 h. After the incubation period, the solution was observed for visual colour change. The flasks turned from opaque white to brownish yellow indicated the presence of AgNPs.

2.2 UV–vis spectral analysis

Microbiologically synthesised AgNPs by *S. griseorubens* AU2 cell-free culture supernatant was monitored by Multiskan GO UV–vis Microplate Spectrophotometer (Thermo Scientific, USA) within a range of 300–700 nm operated at a resolution of 1 nm. About 1 mM $AgNO_3$ solution was used as control.

2.3 FT-IR spectral analysis

Bioreduction of the Ag ion (Ag^+) ions using the cell-free culture supernatant of *S. griseorubens* AU2 was monitored by FT-IR (Thermo Scientific Nicolet iS10-ATR, USA) at a resolution of 4 cm^{-1} in potassium bromide (KBr) pellets and the spectra were recorded in the wavelength interval of 4000 and 400 nm^{-1}.

2.4 Transmission electron microscopy

The size and morphology of the biosynthesised AgNPs were studied by high-resolution TEM (HR-TEM) using a JEM 2100F electron microscope at an accelerating voltage of 200 kV (JEOL, Japan). For preparing the TEM specimens, a drop of the aqueous suspension of AgNPs was placed onto a carbon-coated copper grid and allowed to dry at room temperature. Images of the AgNPs were taken with a HR charge-coupled device camera and the presence of Ag metals in the solution was analysed by EDS system (Oxford Instruments, UK) combined with HR-TEM.

2.5 Scanning electron microscopy

For taking the SEM images of the biosynthesised AgNPs, a drop of suspension was placed on specimen stubs with double-sided adhesive carbon tape and allowed to air dry at room temperature. SEM study was performed on a JSM 7600F field emission scanning electron microscope (JEOL, Japan) at an accelerating voltage of 15 kV. Similar to TEM, the presence of metallic Ag in the solution was analysed by EDS system (Oxford Instruments, UK) combined with SEM.

2.6 XRD measurement

To examine the crystal structure of biosynthesised AgNPs, XRD patterns were collected using Cu Kα monochromatic radiation (λ_{Cu} = 1.54018 Å) at 40 kV/20 mA using continuous scanning 2θ mode on an X-ray diffractometer (Rigaku-SmartLab, Japan).

2.7 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The DPPH free radical scavenging assay was carried out to determine the antioxidant properties of the biosynthesised AgNPs [21]. The percentage of inhibition was calculated by the following equation; % DPPH scavenging effect = $[(control_{OD}–sample_{OD})/control_{OD}] \times 100$, where control was prepared without AgNPs. A strong antioxidant, ascorbic acid, was used as positive control.

3 Results and discussion

According to the morphological characterisation of the strain, it was an aerobic Gram-positive actinomycete which grew well on ISP-2 medium that the colour of aerial mycelium was white and the reverse side of the colony was grey (Fig. 1). Sequencing analysis of the strain *S. griseorubens* AU2 was performed on an ∼1484 bp PCR product and identified as *S. griseorubens* on the basis of 16S rDNA sequencing, which revealed 99% homology (GenBank Accession Number: KX156364).

In the present paper, microbial mediated biosynthesis of AgNPs were achieved extracellularly using the cell-free supernatant of *S. griseorubens* AU2. Similar to the previous reports [1, 15, 22, 23], the formation of brown colour in the $AgNO_3$ treated flasks indicated the Ag nanoparticle formation. There was no colour change in the control flasks (cell-free supernatant without $AgNO_3$ and the $AgNO_3$ solution). The visual colour change may be due to the Ag^+ reduction by secondary metabolites present in the cell-free supernatant of the *S. griseorubens* AU2. Using the supernatant formed from the autolysed *S. griseorubens* AU2 biomass caused the formation of nanoparticles to occur in a clear solution, so there were not any mycelial materials, bacteria cell or medium in the reaction mixture [19].

Fig. 2 shows the UV–vis spectrum of the AgNPs following incubation for 24 h. UV–vis spectral analysis indicated that the AgNP solution has a strong and broad absorption peak at 422 nm, which is related to the surface plasmon resonance (SPR) band of AgNPs, as metal AgNPs have free electrons, which give rise to an SPR absorption band [24, 25]. The absorption peak at 422 nm is probably due to the excitation of longitudinal plasmon vibrations and formation of quasi-linear superstructures of nanoparticles [26].

FT-IR sprectroscopy were performed to define the biomolecules that may be responsible for reduction of the Ag^+ by cell-free supernatant of *S. griseorubens* AU2. The FT-IR spectra of the biosynthesised AgNPs peaks at 3408, 2892, 2360, 1577, 1383, 1020, 637 and 514 cm^{-1} are shown in Fig. 3. The presence of bands were due to O–H stretching (around 3408 cm^{-1}), aldehydic C–H stretching (2892 cm^{-1}), C = O group (1577 cm^{-1}), C–C and

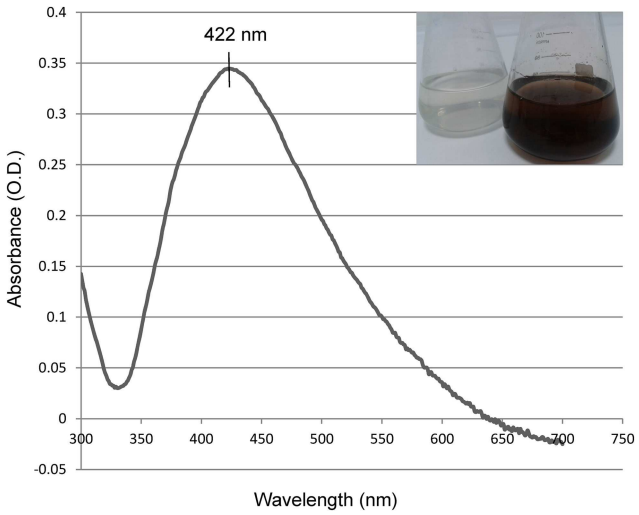


Fig. 2 *UV–vis spectrum of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

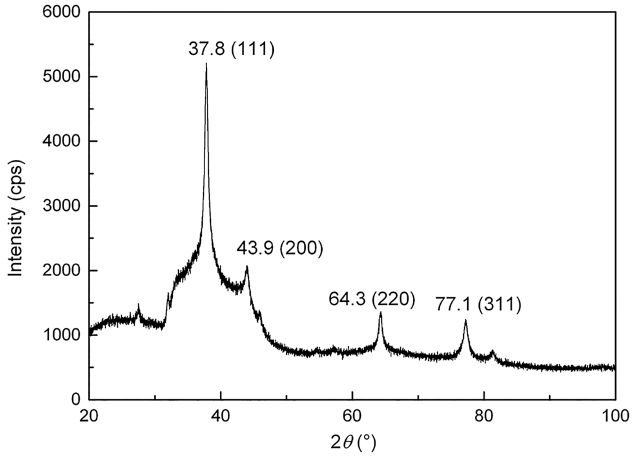


Fig. 4 *XRD pattern of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

C–N stretching (1383 cm^{-1}) and O–H stretch (1020 cm^{-1}). This FT-IR result of synthesised AgNPs were quite similar to previous reports by Mohanta and Behera [22] and Karthik *et al.* [27]. When the metal nanoparticles are formed in the solution, they must be stabilised against the Van der Waals forces of attraction which may otherwise cause coagulation. FT-IR analysis data confirms the presence of O–H stretching (around 3408 cm^{-1}) which may have a key role of reducing metal ions to their respective nanoparticles [22, 28]. Our results are also in agreement with the results of Shanmugasundaram *et al.* [15] and Gopinath *et al.* [28] who suggested that proteins can bind to nanoparticles and stabilise AgNPs.

XRD pattern of the cell-free supernatant of *S. griseorubens* AU2 treated with $AgNO_3$ (Fig. 4) corresponded four intense peaks located over the range of 2θ values (i.e. 30–80°). A comparison of our XRD spectrum with the reported reference values of International Centre for Diffraction Data card number 01-714-613 provided by the instrument's library matched well and AgNPs were present as nanocrystals. The peaks at the 2θ values of 37.88°, 43.94°, 64.31° and 77.19° corresponded to the 1 1 1, 2 0 0, 2 2 0 and 3 1 1 cubic crystal lattice planes of Ag, respectively [10, 15, 28].

TEM and SEM are powerful methods to determine the size and morphology of nanostructures. Representative TEM images (Fig. 5) exhibit well-dispersed AgNPs biosynthesised by cell-free supernatant of *S. griseorubens* AU2. The images recorded at higher magnification displayed the detailed morphology and size of the biosynthesised AgNPs. The AgNPs were found to be spherical and exhibit particle sizes ranging from 5 to 20 nm.

Elemental analysis of the biosynthesised AgNPs was investigated by EDS (Fig. 6) combined with HR-TEM. The EDS linescan spectrum recorded a strong signal for Ag. The peaks corresponding to C, O, Si and Cu were most likely caused by X-ray emission from the carbon-coated copper grid.

Fig. 7 shows representative SEM of the AgNPs synthesised by *S. griseorubens* AU2. As it was confirmed using the TEM images, SEM also showed the presence of homogenous spherical AgNPs that are relatively uniform.

The EDS analysis applied over the SEM showed a strong Ag signal which confirms the presence and crystalline structure of metallic Ag (Fig. 8). Other signals of C, O and N atoms are maybe due to X-ray emission from the remaining media [29].

TEM and SEM images of biosynthesised AgNPs are similar to the reports of Sadhasivam *et al.* [1] and Shanmugasundaram *et al.* [15] who biosynthesised AgNPs via *Streptomyces hygroscopicus* and *Streptomyces naganishii* MA7, respectively.

DPPH is a stable free radical which has a characteristic absorbance at 517 nm, but reduction by an antioxidant or a radical species decreases its absorption. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity [30]. In this paper, various concentrations of 20, 40, 60, 80 and 100 µg/ml of biosynthesised AgNPs exhibited 9.66, 14.27, 15.59,

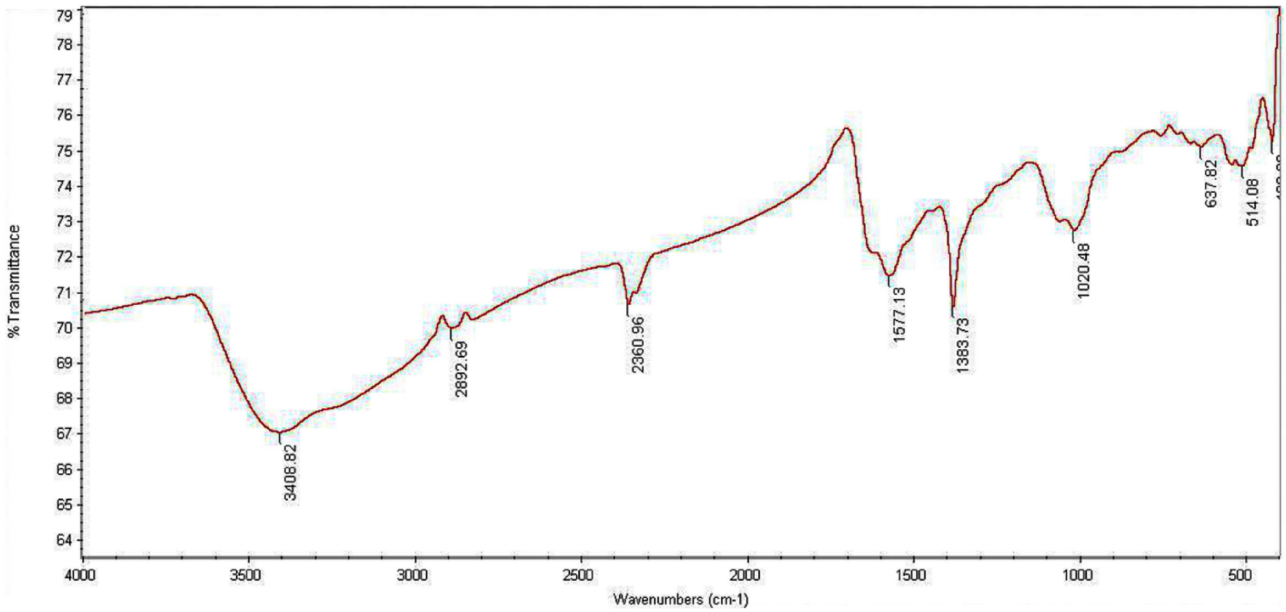


Fig. 3 *FT-IR spectrum of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

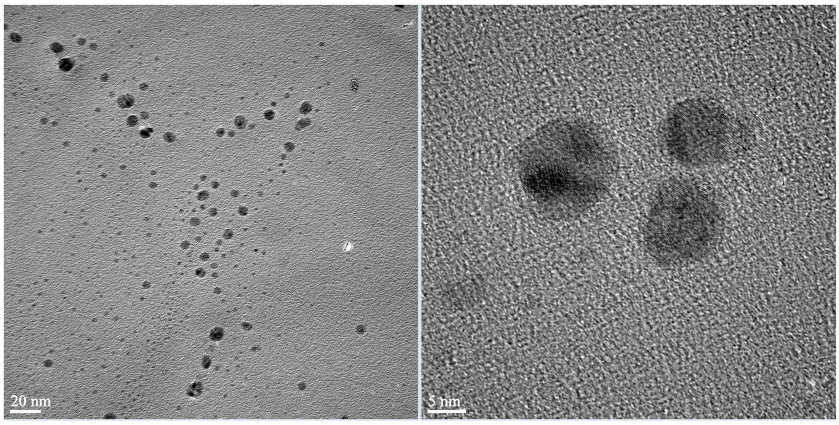


Fig. 5 *TEM images of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2. The scale bar corresponds* ***(a)*** 20 nm, ***(b)*** 5 nm

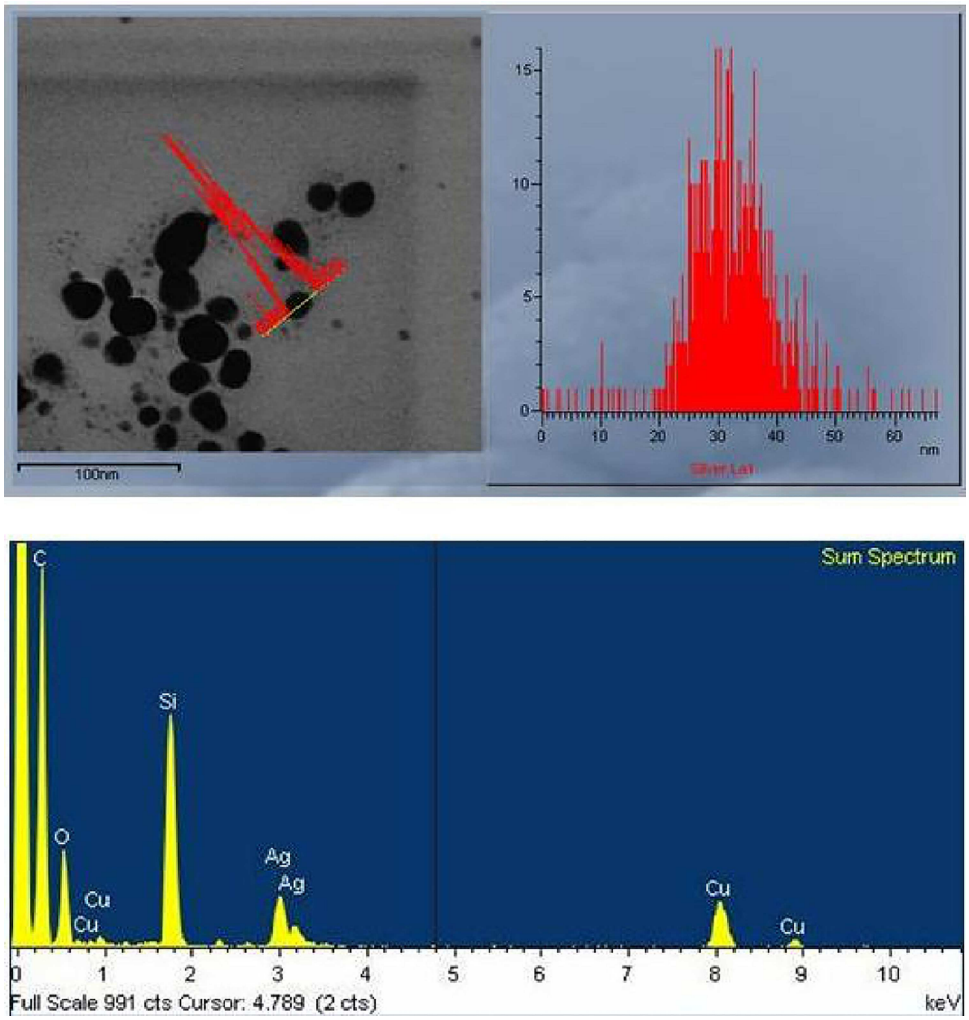
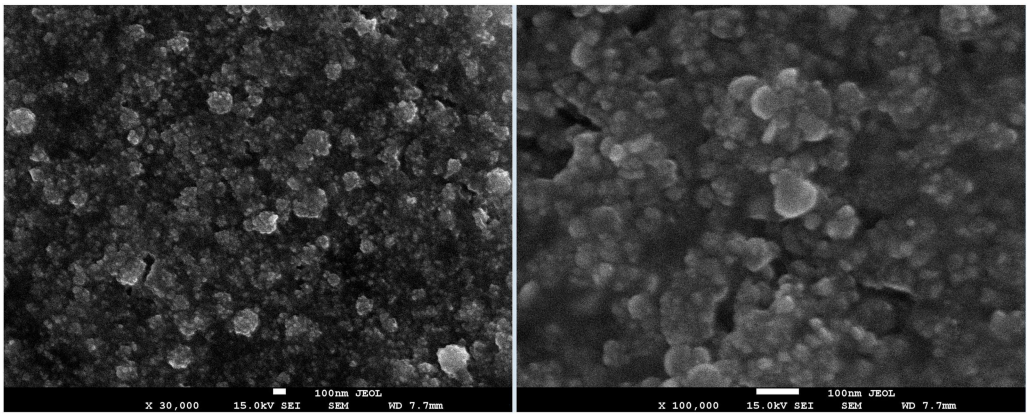


Fig. 6 *TEM-EDS spectrum analysis of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

23.46 and 54.99% free radical scavenging activity, respectively (Fig. 9). When compared with standard ascorbic acid, the antioxidant activity of the biosynthesised nanoparticles was found to increase in a dose-dependent matter. Similar results were also reported by Shanmugasundaram *et al.* [15]. Researchers who studied about plant-mediated AgNP synthesis have also indicated that the antioxidant activity increases gradually with the increase in the treatment doses [31–33].

Among the scientific papers about the biogenic nanoparticle synthesis, it is suggested that DNA [34], sulphur-containing proteins [35] and nicotinamide adenine dinucleotide (NADH)-dependent nitrate reductase [3, 9, 36] are involved in the synthesis of AgNPs by the bioreduction of Ag^+ to metallic Ag. Ahmad *et al.* [9] have indicated that NADH and NADH-dependent nitrate reductase enzymes are important factors in the biosynthesis of metal nanoparticles. The mechanism of the biosynthesised nanoparticles involves the reduction of Ag^+ by the electron shuttle enzymatic metal reduction process [36, 37]. In an AgNP biosynthesis study done by using extremophilic actinobacterium S. naganishii (MA7), Shanmugasundaram *et al.* [15] figured out that the partially purified enzyme (nitrate reductase) from the cell biomass of *F. oxysporum* was determined to be ~44 kDa and their results showed that this enzyme was responsible for the synthesis of AgNPs. Chun *et al.* [38] reported that P450 (cytochrome P450)



a *b*

Fig. 7 *SEM images of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2. The scale bar corresponds* ***(a)*** ×30,000, ***(b)*** ×100,000

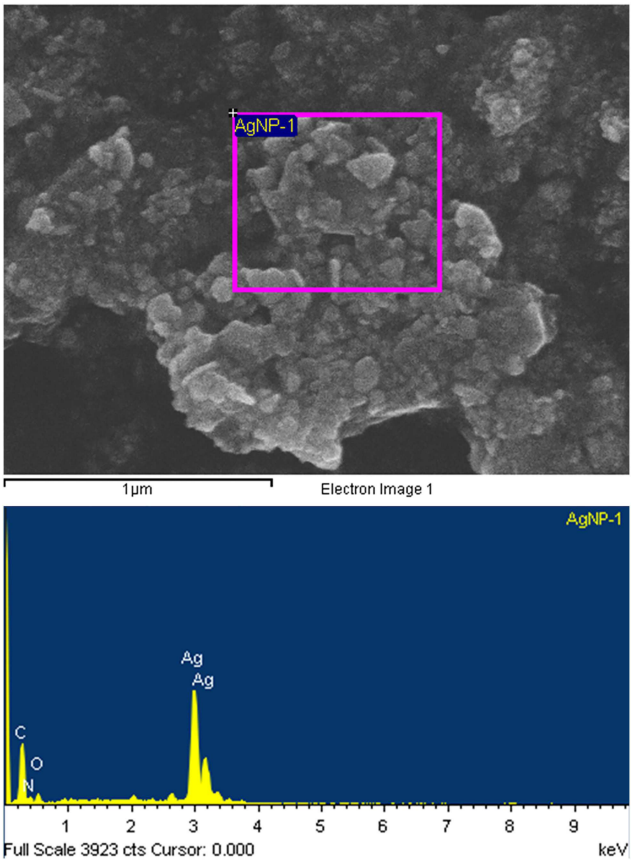


Fig. 8 *SEM-EDS spectrum analysis of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

enzymes of *Streptomyces* genus may have key roles in biosynthesis and biotransformation reactions. Vaidyanathan *et al.* [39] explained that α-nicotinamide adenine dinucleotide phosphate-dependent nitrate reductase in the microorganisms has been found to be responsible for the synthesis of metallic Ag and this enzyme is induced by nitrate ions and reduces Ag^+ to metallic Ag. Alani *et al.* [19] suggest that different NADH-dependent reductases may also be produced by *Streptomyces* sp. In the present paper, cell-free supernatant of *S. griseorubens* AU2 could induce the synthesis of AgNPs from $AgNO_3$. Thus, it is stated that the factors which took part in the biosynthesis of Ag nanoparticles are present in the cell-free supernatant of *S. griseorubens* AU2.

4 Conclusion

Biosynthesis methods developed by utilisation of microorganisms have appeared as a non-toxic and ecofriendly alternative to

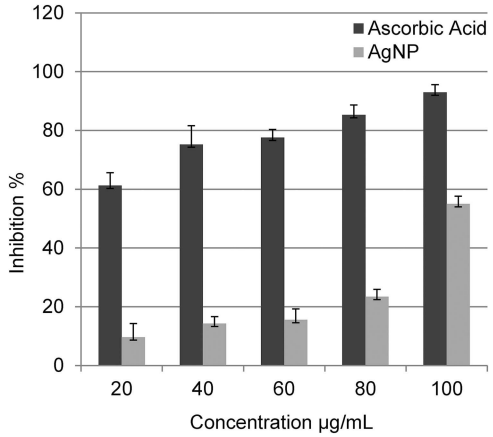


Fig. 9 *Antioxidant activity of AgNPs synthesised by cell-free supernatant of S. griseorubens AU2*

physical and chemical methods. This paper indicated that *S. griseorubens* AU2 has the ability to reduce Ag^+ into Ag nanoparticles extracellularly. AgNPs were ecofriendly synthesised by the active metabolites of *S. griseorubens* AU2. It could be concluded that *S. griseorubens* AU2 cell-free extract has a great potential to be used for antioxidant AgNP synthesis for industrial and pharmaceutical applications effectively. To our knowledge, this is the first report of microbial nanoparticle biosynthesis by *S. griseorubens* isolated from soil. Further research based on the *S. griseorubens* AU2 will involve investigation of a great number of useful bioactive products and diverse nanomaterials besides AgNPs.

5 Acknowledgment

This study is a part of Tuba Baygar's Ph.D. Thesis. Authors thank Assoc. Prof. Dr. Omer Simsek for molecular identification of the bacterium.

6 References

[1] Sadhasivam, S., Shanmugam, P., Yun, K.S.: 'Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganism', *Colloids Surf. B, Biointerferences*, 2010, **81**, (1), pp. 358–362

[2] Ali, M., Kim, B., Belfield, K.D., *et al.*: 'Green synthesis and characterization of silver nanoparticles using *Artemisia absinthium* aqueous extract – a comprehensive study', *Mater. Sci. Eng. C*, 2016, **58**, pp. 359–365

[3] Ahmad, A., Mukherjee, P., Senapati, S., *et al.*: 'Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*', *Colloids Surf. B, Biointerfaces*, 2003a, **28**, (4), pp. 313–318

[4] Vigneshwaran, N., Ashtaputre, N.M., Varadarajan, P.V., *et al.*: 'Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*', *Mater. Lett.*, 2007, **61**, (6), pp. 1413–1418

[5] Kalishwaralal, K., Deepak, V., Ramkumarpandian, S., *et al.*: 'Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*', *Mater. Lett.*, 2008, **62**, (29), pp. 4411–4413

[6] Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R., *et al.*: 'Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*', *Nanomed. Nanotechnol. Biol. Med.*, 2007, **3**, (2), pp. 168–171

[7] Verma, V.C., Kharwar, R.N., Gange, A.C.: 'Biosynthesis of noble metal nanoparticles and their application', *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.*, 2009, **4**, (026), pp. 1–17

[8] Ahmad, A., Senapati, S., Khan, M.I., *et al.*: 'Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, *Thermomonospora* sp.', *Langmuir*, 2003b, **19**, (8), pp. 3550–3553

[9] Ahmad, A., Senapati, S., Khan, M.I., *et al.*: 'Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species', *Nanotechnology*, 2003c, **14**, (7), p. 824

[10] Zonooz, N.F., Salouti, M.: 'Extracellular biosynthesis of silver nanoparticles using cell filtrate of *Streptomyces* sp. ERI-3', *Sci. Iranica*, 2011, **18**, (6), pp. 1631–1635

[11] Kumar, P.S., Balachandran, C., Duraipandiyan, V., *et al.*: 'Extracellular biosynthesis of silver nanoparticle using *Streptomyces* sp. 09 PBT 005 and its antibacterial and cytotoxic properties', *Appl. Nanosci.*, 2015, **5**, (2), pp. 169–180

[12] Mohamedin, A., El-Naggar, N.E.A., Shawqi Hamza, S., *et al.*: 'Green synthesis, characterization and antimicrobial activities of silver nanoparticles by *Streptomyces viridodiastaticus* SSHH-1 as a living nanofactory: statistical optimization of process variables', *Curr. Nanosci.*, 2015, **11**, (5), pp. 640–654

[13] Samundeeswari, A., Dhas, S.P., Nirmala, J., *et al.*: 'Biosynthesis of silver nanoparticles using actinobacterium *Streptomyces albogriseolus* and its antibacterial activity', *Biotechnol. Appl. Biochem.*, 2012, **59**, (6), pp. 503–507

[14] Shetty, P.R., Kumar, Y.S.: 'Characterization of silver nanoparticles synthesized by using marine isolate *Streptomyces albidoflavus*', *J. Microbiol. Biotechnol.*, 2012, **22**, (5), pp. 614–621

[15] Shanmugasundaram, T., Radhakrishnan, M., Gopikrishnan, V., *et al.*: 'A study of the bactericidal, anti-biofouling, cytotoxic and antioxidant properties of actinobacterially synthesised silver nanoparticles', *Colloids Surf. B, Biointerfaces*, 2013, **111**, pp. 680–687

[16] Shanmugaiah, V., Harikrishnan, H., Al-Harbi, N.S., *et al.*: 'Facile synthesis of silver nanoparticles using *Streptomyces* sp. VSMGT1014 and their antimicrobial efficiency', *Dig. J. Nanomater. Biostruct.*, 2015, **10**, pp. 179–187

[17] Vidhyashree, N., Yamini Sudha Lakshmi, S.: 'Screening, isolation, identification, characterisation and applications of silver nanoparticles synthesized from marine actinomycetes (*Streptomyces grieseorubens*)', *World J. Pharma. Res.*, 2015, **4**, (8), pp. 1801–1820

[18] de Lima Procópio, R.E., da Silva, I.R., Martins, M.K., *et al.*: 'Antibiotics produced by Streptomyces', *Braz. J. Infect. Dis.*, 2012, **16**, (5), pp. 466–471

[19] Alani, F., Moo-Young, M., Anderson, W.: 'Biosynthesis of silver nanoparticles by a new strain of *Streptomyces* sp. compared with Aspergillus fumigatus', *World J. Microbiol. Biotechnol.*, 2012, **28**, (3), pp. 1081–1086

[20] Brown, J.C.: 'Soil fungi of some British sand dunes in relation to soil type and succession', *Ecology*, 1958, **46**, pp. 641–664

[21] Blois, M.S.: 'Antioxidant determinations by the use of a stable free radical', *Nature*, 1958, **181**, pp. 1199–1200

[22] Mohanta, Y.K., Behera, S.K.: 'Biosynthesis, characterization and antimicrobial activity of silver nanoparticles by *Streptomyces* sp. SS2', *Bioprocess Biosyst. Eng.*, 2014, **37**, (11), pp. 2263–2269

[23] Singh, P., Kim, Y.J., Singh, H., *et al.*: 'Biosynthesis, characterization, and antimicrobial applications of silver nanoparticles', *Int. J. Nanomed.*, 2015, (10), pp. 2567–2577

[24] Noginov, M.A., Zhu, G., Bahoura, M., *et al.*: 'The effect of gain and absorption on surface plasmons in metal nanoparticles', *Appl. Phys. B*, 2007, **86**, (3), pp. 455–460

[25] Wang, C., Kim, Y.J., Singh, P., *et al.*: 'Green synthesis of silver nanoparticles by Bacillus methylotrophicus, and their antimicrobial activity', *Artif. Cells Nanomed. Biotechnol.*, 2015, **44**, (2), pp. 1127–1132

[26] Shankar, S.S., Ahmad, A., Sastry, M.: 'Geranium leaf assisted biosynthesis of silver nanoparticles', *Biotechnol. Progress*, 2003, **19**, (6), pp. 1627–1631

[27] Karthik, L., Kumar, G., Kirthi, A.V., *et al.*: '*Streptomyces* sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application', *Bioprocess Biosyst. Eng.*, 2014, **37**, (2), pp. 261–267

[28] Gopinath, V., MubarakAli, D., Priyadarshini, S., *et al.*: 'Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach', *Colloids Surf. B, Biointerfaces*, 2012, **96**, pp. 69–74

[29] Zaki, S., El Kady, M.F., Abd-El-Haleem, D.: 'Biosynthesis and structural characterization of silver nanoparticles from bacterial isolates', *Mater. Res. Bull.*, 2011, **46**, (10), pp. 1571–1576

[30] Bhakya, S., Muthukrishnan, S., Sukumaran, M., *et al.*: 'Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity', *Appl. Nanosci.*, 2015, **6**, (5), pp. 755–766

[31] Dipankar, C., Murugan, S.: 'The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts', *Colloids Surf. B, Biointerfaces*, 2012, **98**, pp. 112–119

[32] Swamy, M.K., Sudipta, K.M., Jayanta, K., *et al.*: 'The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract', *Appl. Nanosci.*, 2015a, **5**, (1), pp. 73–81

[33] Swamy, M.K., Akhtar, M.S., Mohanty, S.K., *et al.*: 'Synthesis and characterization of silver nanoparticles using fruit extract of *Momordica cymbalaria* and assessment of their in vitro antimicrobial, antioxidant and cytotoxicity activities', *Spectrochim. Acta A, Mol. Biomol. Spectrosc.*, 2015b, **151**, pp. 939–944

[34] Feng, Q.L., Wu, J., Chen, G.Q., *et al.*: 'A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus', *J. Biomed. Mater. Res.*, 2000, **52**, (4), pp. 662–668

[35] Morones, J.R., Elechiguerra, J.L., Camacho, A., *et al.*: 'The bactericidal effect of silver nanoparticles', *Nanotechnology*, 2005, **16**, (10), p. 2346

[36] Kalimuthu, K., Babu, R.S., Venkataraman, D., *et al.*: 'Biosynthesis of silver nanocrystals by *Bacillus licheniformis*', *Colloids Surf B, Biointerfaces*, 2008, **65**, pp. 150–153

[37] Chauhan, R., Kumar, A., Abraham, J.: 'A biological approach to the synthesis of silver nanoparticles with *Streptomyces* sp JAR1 and its antimicrobial activity', *Sci. Pharm.*, 2013, **81**, (2), p. 607

[38] Chun, Y., Shimada, T., Waterman, M.R., *et al.*: 'Understanding electron transport systems of Streptomyces cytochrome P450', *Biochem. Soc. Trans.*, 2006, **34**, (6), pp. 1183–1185

[39] Vaidyanathan, R., Gopalram, S., Kalishwaralal, K., *et al.*: 'Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity', *Colloids Surf. B, Biointerfaces*, 2010, **75**, (1), pp. 335–341