Biosynthesis and Characterization of Silver Nanoparticles produced by *Phormidium ambiguum* and *Desertifilum tharense*

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Biosynthesis and Characterization of Silver Nanoparticles produced by *Phormidium ambiguum* and *Desertifilum tharense*

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Abstract

The world faces a challenge with pervasion of multidrug resistant bacteria which encouraged the scientists to develop and discover alternative ecofriendly and easy to produce new antibacterial agents. Two Egyptian cyanobacteria were isolated and identified according to 16S rRNA gene sequencing as *Phormidium ambiguum* and *Desertifilum tharense*. The sequences were deposited in the GenBank with accession numbers of MW762709 and MW762710 for *Desertifilum tharense* and *Phormidium ambiguum*, respectively. These isolates have the ability to produce silver nanoparticles (Ag-NPs) extra- and intracellularly under light and dark conditions. The results of UV-Vis analysis showed promising extracellular Ag-NPs synthesis by *Desertifilum tharense* and *Phormidium ambiguum* under light conditions. Therefore, these Ag-NPs were characterized and evaluated for antibacterial and antioxidant activity. TEM, SEM and XRD analyses revealed the spherical crystals with face-centered cubic structures and size range of 6.24–11.4 nm and 6.46–12.2 nm for Ag-NPs of *Desertifilum tharense* and *Phormidium ambiguum*, respectively. XRD and EDX results clearly confirmed the successful synthesis of Ag-NPs in its oxide form or chloride form. The FTIR spectrum data confirmed the presence of hydroxyl and amide groups. *Desertifilum tharense* Ag-NPs displayed the largest inhibition zone ranged from 9 mm against *Micrococcus luteus* ATCC 10240 to 25 mm against methicillin resistant *S. aureus* (MRSA) ATCC 43300. For *Phormidium ambiguum* Ag-NPs, the inhibition zone diameter was in a range of 9–18 mm. The Ag-NPs of *Phormidium ambiguum* exhibited the highest scavenging activity of 48.7% comparing with that of *Desertifilum tharense* which displayed 43.753%.

Keywords: *Phormidium ambiguum*, *Desertifilum tharense*, Ag-NPs, biosynthesis, characterization, antibacterial, antioxidant.

1. Introduction

Nanotechnology is a sonorous area of science, engineering, and technology concerning the design and manufacture of materials at the nanoscale. The products of nanotechnology are nanomaterials or nanoparticles (NPs) having dimensions less than 100 nm. Comparing to larger particles, smaller sized nanoparticles exhibit larger surface-to-volume ratio which is an influential feature to improve physical, chemical, and biological properties of the original material. These properties include catalytic reactivity, thermal conductivity, antimicrobial activity, chemical steadiness, *etc.* According to the effectual crystallographic and physiochemical properties of NPs, the nanoparticles have substantial applications in industrial, medical and biotechnological sectors (Rajeshkumar et al., 2014 and Patel et al., 2015).

Generally, the nanoparticles could be synthesized by applying different chemical and physical methods. The chemical methods include precipitation, solvothermal/hydrothermal and sol - gel methods using different reducing and stabilizing agents. These methods are not eco-friendly as using of solvents, reducing and stabilizing agents leads to release of considerable amounts of hazards and toxic materials as non-biodegradable compounds (Zak et al., 2011 and Li et al., 2015). In the physical biosynthesis, attrition and pyrolysis are applied to produce oxidized NPs. The physical methods are very expensive and time consuming. The demerits of chemical and physical methods, and integration of NPs in health and industrial products for human utilization confirming the application of natural and safe methods for nanoparticles synthesis. Therefore, the biosynthesis of nanoparticles using different biological sources including plants, bacteria, fungi and algae has great attention. The biological methods are cost effective, non-toxic, pollutant-free and eco-friendly methods (Malhotra et al., 2013, Keskin et al., 2016, Kumar et al., 2018 and Kashyap et al., 2019).

Cyanobacteria or blue-green algae have a greatest scientific attention for nanoparticles production, not only for their high biomass productivity, but also for they have the ability to bioremediate toxic metals altering them to more adjustable forms. Cyanobacteria have the ability to produce different organic (quaternary ammonium compounds, N-halamine compounds, chitosan, etc.) and inorganic metal and metal oxide NPs (as gold, silver, platinum, zinc oxide and copper oxide, aluminium oxide, etc.). The cyanobacterial synthesis of NPs is accomplished extra or intracellularly. The extracellular biosynthesis involves secretion of extracellular substances to mediate through electrostatic interactions or production of extracellular reductase enzymes. Alternatively, the intracellular production of NPs associates with the activity of reductase enzymes and substance exchange processes (Mahdiah *et al.*, 2012).

The physicochemical properties of silver nanoparticles (Ag-NPs) and their use extensively in the medical sector as antimicrobial and anticancer agents are the foremost reasons for considerable attention for their production. The silver nanoparticles display efficient antibacterial activity through multiple mechanisms including formation of free radicals stimulating membrane damage (Ninganagouda *et al.*, 2014), increasing the cell membrane permeability through formation of holes or channels through it (Beyth *et al.*, 2010) and disruption of cell proteins through binding with thiol and amino groups (Choi *et al.*, 2008).

Based on the promising features and applications of silver nanoparticles, the intended objective of present study was screening of some Egyptian cyanobacterial isolates, as model biological system, to synthesize the Ag-NPs. Furthermore, the physical properties and potential applications of Ag-NPs as antibacterial and antioxidant agents were evaluated.

2. Materials and Methods

2.1. Green earth crust samples collection

Two natural earth crust samples were collected from Giza Governorate, El-Haraneya near the ring road in locations between longitude of 31° 10' 15" and latitude of 29° 58' 20" for the first sample, and longitude of 31° 9' 59" and latitude of 29° 57' 57" for the second sample.

2.2. Cyanobacteria isolation and purification

The enrichment culture technique was applied to isolate cyanobacteria, in which 1 gm of each green dispersed earth crust samples were aseptically added to 25 ml of liquid BG-11 medium (Rippka et al., 1979) but inside horizontal falcon tube not inside glass flask. The inoculated medium was incubated under continuous illumination using Philips Fluorescent white lamps, at a relatively low light intensity (400 -500 lux) at 30°C for 10 days.

Different methods should be used to obtain cyanobacteria monocultures/pure culture, depending on the type of cyanobacterial: coccoid or filamentous, cell size and the degree of motility. Pure culture is not always possible because cyanobacteria may have such relationships with other microorganisms.

The single filament technique was used to obtain axenic cultures of filamentous cyanobacteria. Whole trichomes or hormogonia will glide on the wall of the tube through few days. This technique was applied through picking these individual migrating trichomes using a Pasteur pipette, and then the individual filament was cleaned with sterile liquid medium in a petri dish. For more purification, the motile filamentous cyanobacteria was dragged inside BG-11 agar medium to allowing them to glide away and remove any attached contaminations as described by Waterbury (2006). Some of the rapidly gliding filamentous can clean themselves of bacterial contaminants within hours of inoculation. Then, the purified isolates were transferred in flasks containing 250 ml of liquid BG-11 (pH 7). All pure isolates were maintained under photoautotrophic growth conditions as aforementioned.

2.3. Morphological characterization of cyanobacteria

The pure isolates were characterized morphologically through preparation of a wet portion which was examined microscopically (Rippka *et al.*, 1979). The filamentous nature, size, shape of vegetative cells, presence of heterocyst and akinetes were identified.

2.4. Molecular identification of cyanobacteria

The cyanobacterial isolates were identified according to 16S rRNA sequencing. The isolates were cultivated in BG-11 broth for 30 days. After centrifugation of the broth culture at 6000 g for 10 min, the harvested biomass was washed three times using 0.85% NaCl saline solution, and genomic DNA was extracted using GeneJET Genomic DNA purification Kit (ThermoFisher Scientific, Republic of Lithuania). The yield and purity of extracted DNA were checked using both UV-Vis NanoDrop spectrophotometer (NanoDrop 2000, ThermoFisher Scientific, Germany) and agarose gel electrophoresis (Bio-rad, USA).

The 16S rRNA gene fragments were amplified using the cyanobacteria specific primers CYA106F (5'-CGGACGGGTGAGTAACGCGTGA-3') and CYA781R (5'-GACTACWGGGGTATCTAATCCCWTT-3') (Koo et al., 2019). The amplification step was performed using Thermal cycler PCR (Bio - Rad T100, USA). The PCR conditions for 16S rRNA gene were: initial denaturation step at 95°C for 12 min, followed by 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 2 min, and one extension step at 72°C for 10 min. The PCR products were checked via agarose gel electrophoresis, purified using gel extraction kit and sequenced by Macrogen, Inc., Seoul, South Korea using automatic ABI 370×1 DNA Sequencer (Applied Biosystem, USA). The sequences were analyzed applying BLAST V2.0 software (<http://www.ncbi.nlm.nih.gov/BLAST/>).

2.5. Phylogenetic analysis of cyanobacteria isolates

The Neighbor-Joining method was used to conclude the evolutionary history (Saitou and Nei, 1987). The tree was computed using the maximum composite likelihood method

(Tamura et al., 2004). The analysis involved 13 nucleotide sequences of which 2 sequences of 16S rRNA gene amplified from cyanobacterial isolates while 11 sequences representing the most similar hits were obtained from the NCBI gene bank database. Evolutionary analyses were conducted in MEGA5 software.

2.6. Biosynthesis of silver nanoparticles (Ag-NPs) by cyanobacteria

The cyanobacterial isolates were screened for synthesis of Ag-NPs extra- and intracellularly under light and dark conditions. According to the method of Patel et al. (2015), after 15 days incubation, the broth cyanobacterial cultures were centrifuged (Universal 16r, Hettich) at 6000 rpm/10 min at 10°C.

For extracellular synthesis of Ag-NPs, the supernatant was added to AgNO₃ solution to reach the final concentration of 1 mM AgNO₃. To evaluate the synthesis of Ag-NPs intracellularly, the harvested biomass was washed twice at least with sterile distilled water, kept at -20°C overnight to facilitate complete lysis of cells (Mahamuni et al., 2019), then 0.5 gram of wet weight biomass was suspended in 10 ml of 1mM AgNO₃ solution, pH 7. Both experiments were incubated at 30°C/48 h, either under direct light provided from fluorescent white lamps or in dark conditions provided through wrapping the tubes with aluminum foil. As a control, the AgNO₃ solution, supernatant and 0.5 gram of wet weight biomass suspended in 10 ml of sterile water were incubated under the same conditions.

2.7. Characterization of Ag-NPs

To apply some characterization tests, the dried nanoparticles are used. To prepare the dried Ag-NPs synthesized by isolated cyanobacteria, the Ag-NPs suspension obtained after incubation for 72 h was centrifuged at 6000 rpm for 15 min at 10°C. The pellet was washed 3 times with sterile distilled water, spread in Petri plates and dried at 30°C for 24 h. The dried nanoparticles were scraped by a scalpel, harvested, weighted and stored in sterile microtube for quantitative estimation (Hamida *et al.*, 2020).

The following characterization tests were applied to confirm the formation of Ag-NPs by isolated cyanobacteria.

2.7.1. Visual Color Change Test

Formation of silver nanoparticles was detected by the visual color change from pale yellow to brownish color within the incubation time.

2.7.2. Ultraviolet – Visible (UV–Vis) spectroscopic analysis

In time intervals of 1 and 24 h, 1 ml from each sample was centrifuged at 6000 rpm for 5 min. The absorption measurements in the wavelength range of 300-550 nm were estimated using UV-Vis spectroscopy (Specord 210 plus, Analytic Jena, Germany). The isolates showed an absorption peak in the range between 400 and 450 nm were considered as silver nanoparticle-producing cyanobacteria (Sadowski, 2010).

2.7.3. Scanning electron microscopy (SEM)

After 48h, SEM was used to characterize the surface morphology of nanoparticles at an accelerating voltage of 30 kV. The Ag-NPs solution was centrifuged, and the pellet was allowed to dry. The dried nanoparticles were examined through coated with gold by a coater to prevent building-up the electrical charges (Keskin et al., 2016). Also, Ag-NPs were characterized in a particle suspension.

2.7.4. Transmission electron microscopy (TEM)

TEM gives 1,000 fold higher morphological resolution for both size and shape compared to SEM (Sadowski, 2010). Two-dimensional and high-resolution Ag-NPs images were captured with TEM, Jeol, JEM-1400, Japan. A drop of Ag-NPs suspension was placed on copper-grid carbon coated with 300 mesh palladium and carbon, and allowed to dry. The Ag-NPs morphology was observed by TEM processed at an operating voltage of 80 kV.

2.7.5. X-ray diffraction (XRD) analysis.

The crystal structure of Ag-NPs was characterized using "x-ray-dx pert pro panalytical diffractometer". The powdered Ag-NPs were penetrated by X-rays and scanned across the region of 2θ , from range 0° to 80° .

2.7.6. Energy dispersive X-ray (EDX)

EDX was used to determine elemental composition of metal nanoparticles. Energy dispersive X-ray spectroscopy uses nature advantages of light photons. Generally, EDX operates by recording X-ray signals, energy and intensity distribution which are generated using a focused electron beam (Sadowski, 2010 and Gowramma et al., 2015). Analysis was processed with EDAX FEI INSPECT instrument across layout: kv: 25.00; take-off: 34.58; tilt: 0.00; ampt: 102.4; resolution: 129.66; detector type: sutw-sapphire; edax zaf quantification standardless sec.

2.7.7. Fourier transform infrared spectrometer (FT-IR) analysis

The chemistry and variations of functional groups attached to Ag-NPs surface were identified with FT-IR spectrometer (NICOLET 380, China). The dried nanoparticles were mixed with potassium bromide in a ratio of 1:100. The sample of 100 μ l was placed in the attenuated total reflectance (BRUKER) analyzer. The silver nanoparticle solution was analyzed by ATR-FT-IR. The IR ray's spectrum is scanned across 4000 - 400/ cm^{-1} with diffuse reflectance mode (DRS-800) within 4cm^{-1} resolution (Sadowski, 2010) (<https://www IR Spectrum Table & Chart, Sigma-Aldrich>).

2.8. Antibacterial efficiency of Ag-NPs

The antibacterial activity of Ag-NPs synthesized by isolated cyanobacteria was assessed applying well diffusion assay (Jena et al., 2013) against 6 bacterial strains including G^+ and G^- bacteria. These bacterial strains were *Bacillus cereus* ATCC 10876, Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Micrococcus luteus* ATCC 10240, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* O157:H7 wild type strain 93111 as a representative enterohaemorrhagic *E. coli* (EHEC). The broth culture of each bacterial strain was prepared in soyabean casein digest broth (OXOID) with incubation at the optimum temperature/24h. The optical density (OD_{600}) of each culture was adjusted to 0.5 ± 0.1 using the UV/Vis spectrophotometer. Using sterile cotton swabs, each individual culture was spread on the surface of nutrient agar, in which the wells of 10 mm diameter were prepared using a sterile cork borer. Each well was filled with 100 μ l either from vancomycin hydrochloride (100 mg/ml) as a positive control, 100 μ l Ag-NPs resulting solution (1 mM) or AgNO_3 solution (1 mM) or. The plates were incubated for 24 h at the optimum temperature for each microbe. The inhibitory effect of Ag-NPs was estimated through measuring the inhibition zone diameter. Also, the relative inhibition percentage was calculated according to the next formula (Kumar et al., 2010):
Relative percentage inhibition of Ag-NPs = $(100 \times (y-x)) / (z-y)$ as:

x: total inhibition area of Ag-NPs; y: total inhibition area of AgNO_3 solution 1mM and z: total inhibition area of the standard drug.

2.9. Antioxidant activity of Ag-NPs

The capability of cyanobacterial Ag-NPs to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed through mixing 100 μ l of tested Ag-NPs resulting solution with 1 ml from methanolic solution of 0.1 mM DPPH. The mixture was vortexed and incubated at 37°C for 30 min in dark. The absorbance of the samples was measured at 515 nm. The free radical scavenging activity was calculated using the following equation: DPPH scavenging activity (%) (antioxidant activity, %) = $((A_0 - A_1) / A_0) \times 100$ where: A_0 is the absorbance of the control reaction and A_1 is the absorbance of reaction mixture (Hamouda et al., 2017).

3. Results and Discussion

3.1. Cyanobacteria identification by Light microscope examination

Two isolates of cyanobacteria (AM5E and AM6E) were purified and characterized morphologically according to Rippka et al. (1979). The AM5E and AM6E isolates were characterized as filamentous and non heterocystous cyanobacteria (Fig. 1A – 1D). The filaments were unbranched. No gas vacuoles were observed within the cells. The filaments of AM5E were long, thin, straight or slightly curvy, and non-constructed. Also, they were motile and had sharp apical terminals. The filaments may exist in a separate state or in bundles. The filaments of AM6E isolate were thicker, and exist as separate filaments (Fig. 1C and D). The filaments were slightly motile and had round ends.

Fig. 1. Light micrographs of cyanobacterial isolates AM5E (A: 10x, B: 40x) and AM6E (C: 10x, D: 40x).

3.2. Molecular identification and phylogenetic analysis of cyanobacterial isolates

According to 16S rRNA gene sequence of the two isolates, AM5E showed 100% similarity to *Desertifilum tharense* and *Desertifilum dzianense*, while AM6E showed 99.6% similarity to *Phormidium ambiguum*. The phylogenetic relatedness was confirmed in the neighbor joining tree (Fig. 2). Sequences were deposited in the NCBI GenBank as *Desertifilum tharense* AM5E (accession number: MW762709) and *Phormidium ambiguum* AM6E (accession number: MW762710).

Fig. 2. A neighbor-joining phylogenetic tree (dark circles) with the closest hits obtained from the NCBI gene bank.

3.3. Characterization of biosynthesized silver nanoparticles (Ag-NPs) by cyanobacteria

Generally, silver nanoparticles are synthesized through two pathways comprising enzymatic and non-enzymatic reduction (Otari et al., 2015). The nitrate reductase enzyme, which involves in enzymatic synthesis of silver nanoparticles, converts nitrate to nitrite and an electron shuttle is induced thus reducing the silver ions to silver nanoparticles. The enzymatic production of silver nanoparticles is fast and non-toxic approach. Conversely, the non-enzymatic synthesis of Ag-NPs depends on chemical reduction of silver atoms using reducing and stabilizing compounds produced by plants or microorganisms.

3.3.1. Visual color change

Reduction of silver atoms into silver nanoparticles extra- and intracellularly using isolated cyanobacteria could be observed visually through the color change from pale yellow to brown. Generally, this process is time dependent as the intensity of brown color is directly proportional to reaction time (Khan et al., 2019). Saifuddin et al. (2009) reported that the changing in color was due to the excitation of surface plasmon vibrations in the resultant nanoparticle. The Ag-NPs produced by cyanobacteria were observed to be highly stable for a variant time ranged from 1 to 120 h (Govindaraju et al., 2009).

In this study, the brown color was observed after 1 hr, either for *Desertifilum tharense* (Fig. 3) or *Phormidium ambiguum* (Fig. 4) indicating their ability to synthesize Ag-NPs extra- and intracellularly under both light and dark conditions. For the both isolates, after 48 hours, the darkest brown color was perceived under light conditions for extra- and intracellularly produced Ag-NPs. Comparing the intra- and extracellular biosynthesis of Ag-NPs, the dark brown color was evidence for promising extracellular biosynthesis by *Desertifilum tharense* or *Phormidium ambiguum*. Therefore, further characterization testes, antibacterial and antioxidant activity were assessed for Ag-NPs synthesized extracellularly under light conditions.

Fig. 3. Color change during the reduction process of silver to Ag-NPs extra and intracellularly using *Desertifilum tharense*. A, 1mM silver nitrate solution (control 1). B, cell suspension in water (control 2). C, formation of Ag-NPs intracellularly under dark conditions after 48 h. D, formation of Ag-NPs intracellularly under light conditions after 48 h. E, cell free supernatant (control 3). F, formation of Ag-NPs extracellularly under dark conditions after 48 h. G, formation of Ag-NPs extracellularly under light conditions after 48 h.

Fig. 4. Color change during the reduction process of silver to Ag-NPs extra and intracellularly using *Phormidium ambiguum*. H, 1mM silver nitrate solution (control 1). I, cell suspension in water (control 2). J, formation of Ag-NPs intracellularly under dark conditions after 48 h. K, formation of Ag-NPs intracellularly under light conditions after 48 h. L, cell free supernatant (control 3). M, formation of Ag-NPs extracellularly under dark conditions after 48 h. N, formation of Ag-NPs extracellularly under light conditions after 48 h.

3.3.2. UV-Vis spectrophotometer analysis

Formation of Ag-NPs detected by visual observation of color change was further confirmed by sharp peaks given in the visible region from UV-Vis spectrum. Ag-NPs

characterization using UV-visible spectrophotometer is considered the most widely technique (Sun et al., 2001; Gowramma et al., 2015). In this study, UV-Vis spectrophotometer analyses were carried out at absorbance spectrum range of 300 - 550 nm for Ag-NPs synthesized extracellularly under light conditions.

For *Desertifilum tharense*, Ag-NPs gave the sharp SPR (surface plasmon resonance) peak at 450 nm after one hour (Fig. 5A). With increasing reaction time reached 24h, the peak shifted to shorter wavelength region as two peaks appeared at 410 and 435 nm (Fig. 5B). Kathiraven et al. (2014) reported that when SPR band moves towards shorter wavelength that means a decrease in particle size. This phenomenon was not observed for Ag-NPs synthesized by *Phormidium ambiguum* as the SPR peak appeared at 450 nm after 1 and 24 hours (Fig. 6A and 6B).

Fig. 5. UV-Vis absorption spectra of Ag-NPs synthesized extracellularly by *Desertifilum tharense* after 1h (A) and after 24h (B).

Fig. 6. UV-visible absorption spectra of Ag-NPs synthesized extracellularly by *Phormidium ambiguum* after 1h (A) and after 24h (B).

It was known that Ag-NPs give the plasmon resonance peak in absorption spectrum between 410 - 450 nm (Kashyap et al., 2019). Consequently, the results confirmed successful extracellular Ag-NPs synthesis by *Desertifilum tharense* and *Phormidium ambiguum* under light conditions.

3.3.3. Scanning and Transmission electron microscopy

Scanning electron microscopy (SEM) micrographs indicated the presence of Ag-NPs produced by *Desertifilum tharense* (Fig. 7A and 7B) and *Phormidium ambiguum* (Fig. 8A and 8B) as irregular polydisperse clusters of particles.

The Ag-NPs were further characterized for shape and size determination by transmission electron microscopy (TEM). The TEM micrographs (7C) and (8C) showed spherical shape of Ag-NPs produced by *Desertifilum tharense* and *Phormidium ambiguum*,

respectively with good particles dispersal without agglomeration. Also, TEM micrographs displayed the size of particles in a range of 6.24 – 11.7 nm and 6.46 – 12.2 nm for Ag-NPs produced by *Desertifilum tharense* and *Phormidium ambiguum*, correspondingly. The SPR peak at 435 nm of Ag-NPs synthesized by *Desertifilum tharense* confirmed the spherical shape of these particles as reported by Govindaraju et al. (2009).

Generally, the cyanobacterial silver nanoparticles are found in different shapes. Patel et al. (2015) studied the shape of Ag-NPs produced by different cyanobacterial strains. They found that the Ag-NPs produced by *Anabaena* sp. and *Limnothrix* sp. appeared as elongated particles, while Ag-NPs of *Coelastrum* sp. and *Botryococcus braunii* appeared with spherical shape. The particles formed by *Synechocystis* sp. were irregular clusters.

The size of Ag-NPs produced by *Desertifilum tharense* and *Phormidium ambiguum* was in a range reported by Singh et al. (2014). In other studies, the size of particles produced by cyanobacteria was larger. Rashed et al. (2018) produced Ag-NPs with an average size of 60 nm from *Convolvulus arvensis* extract. The average size of Ag-NPs from *Chlorella* sp. was 90.6 nm (Kashyap et al., 2019). Some cyanobacterial cultures synthesized silver particles with a size larger than 100 nm. Keskin et al. (2016) and Kashyap et al. (2019) produced particles with average size of 140 nm from *Synechococcus* sp., and size of 136.2 and 241.8 nm from *Scenedesmus vacuolatus* and *Lyngbya putealis*, respectively.

Fig. 7. SEM micrographs of suspended Ag-NPs (A) and dried Ag-NPs (B) obtained from *Desertifilum tharense*. TEM micrographs of Ag-NPs (C) showed the spherical shape of Ag-NPs with a size of 6.24-11.7 nm.

Fig. 8. SEM micrographs of suspended Ag-NPs (A) and powder Ag-NPs (B) obtained from *Phormidium ambiguum*. TEM micrographs of Ag-NPs (C) showed the spherical shape of Ag-NPs with a size of 6.46-12.2 nm.

3.3.4. X-ray diffraction (XRD) analysis

X-ray diffraction analysis (XRD) is a technique used to determine the crystallographic structure of a material through irradiating it with X-rays and measuring the intensities and

scattering angles of the X-rays that leave the material. XRD pattern was investigated in the range of 0° to 80° at diffraction angle of Pos (°2Th.).

The diffractogram of *Desertifilum tharense* Ag-NPs (Fig. 9A) showed five main peaks of 32.19°, 38.089°, 44.2567°, 64.4547° and 77.4727° corresponding to (101), (111), (200), (220) and (311) lattice planes, respectively which were indexed in Joint Committee on Powder Diffraction Standards (JCPDS) file no. 84-0713 and 04-0783 as reported by Mahiuddin et al. (2020). Similar diffraction peaks were identified for *Phormidium ambiguum* Ag-NPs (Fig. 9B).

Fig. 9. XRD pattern of Ag-NPs synthesized extracellularly by *Desertifilum tharense* (A) and *Phormidium ambiguum* (B).

The results of XRD analysis confirmed that the biosynthesized Ag-NPs have face - centered cubic (fcc) structure.

it was noticed that there are additional peaks at 54.82° and 57.5° in the diffractogram of *Desertifilum tharense* Ag-NPs, and at 54.78° and 57.47° in that of *Phormidium ambiguum* Ag-NPs. These peaks could be attributed to the presence of unreduced AgNO₃ as stated by Mehta et al., (2017).

3.3.5. EDX analysis

The purity, elemental constituents and relative abundance of Ag-NPs synthesized by *Desertifilum tharense* and *Phormidium ambiguum* were analyzed by means of Energy Dispersive X-ray (EDX) as presented in Figures (10A) and (10B), respectively.

In Figures (10A) and (10B), two peaks were observed at 3.0 and 3.2 keV for silver nanoparticles revealing the presence of pure metallic Ag-NPs (Magudapathy et al., 2001; Patel et al., 2015).

In case of *Desertifilum tharense* Ag-NPs, EDX analysis (Fig. 10A) suggested that the powder has high purity and intensity as the two emission peaks of metal silver shows more than 49%. The percentage relative composition of elements was Carbon (C) 23.88%, Oxygen (O) 12.81%, Chlorine (Cl) 6.95%, Sodium (Na) 5.2%, Silicon (Si) 0.9% and Sulfur (S) 0.51%. While in case of *Phormidium ambiguum*, EDX characterization (Fig.10B) exhibited less purity and weight as the two emission peaks of metal silver represents only more than 32%. The percentage relative composition of elements was Carbon (C) 41.6%, Oxygen (O) 12.33%, Chlorine (Cl) 3.7%, Sodium (Na) 3.65%, Iron (F) 2.85, Arsenic (As) 0.95%, Silicon (S) 0.41%, Sulfur (S) 0.98% and Calcium (Ca) 0.68%.

XRD and EDX results clearly confirm the successful synthesis of Ag-NPs in its oxide form or chloride form.

Fig. 10. EDX quantitative analysis and spectra of Ag-NPs synthesized by *Desertifilum tharense* (A) and *Phormidium ambiguum* (B).

3.3.6. Transform Infrared Spectroscopy (FTIR)

The structure of organic compounds on the surface of AgNPs was investigated using FTIR Spectroscopy.

The FTIR spectrum of Ag-NPs synthesized by *Desertifilum tharense* and *Phormidium ambiguum* specified peaks around 3450, 2065, 1634 and 428 cm^{-1} (Fig. 11). Strong absorption peak at 3450 cm^{-1} assigned to O-H stretching vibration of polysaccharides, and N-H stretching vibration of proteins. This indicates the polysaccharides and proteins found in extracellular extract may play role in synthesis of Ag-NPs (Hamida et al., 2020; Mahiuddin et al., 2020). Also, the polysaccharides and proteins, as organic capping agents, may contribute in particles stabilization. Jena et al. (2013) reported that the capping peptide can bind to nanoparticles by free amino groups or by different residues inside the proteins. The peak around 1634 cm^{-1} attributed to stretching vibration of C=O in amides (Mahiuddin et al., 2020). The peak at 428 cm^{-1} is related to binding Ag-NPs with oxygen from hydroxyl groups (AL-Katib et al., 2015).

Fig. 11. FTIR spectra of Ag-NPs synthesized extracellularly from *Desertifilum tharense* (A) and *Phormidium ambiguum* (B).

3.4. Antibacterial activity

Currently, using nanoparticles as antimicrobial agents are gaining excessive interest. Generally, the nanoparticles are evaluated as antimicrobials alone or in combination with antibiotics. The nanoparticles can increase antimicrobial effect of some antibiotics against G+ and G- bacteria (Li et al., 2011).

In this study, 6 bacterial pathogens were selected as indicator microorganisms for screening the antibacterial activity of Ag-NPs synthesized extracellularly under light conditions by the two isolated cyanobacteria. The results were compared with vancomycin hydrochloride (100 mg/ml) and AgNO_3 (1 mM) for evaluating the relative percentage inhibition of Ag-NPs.

Desertifilum tharense Ag-NPs displayed the largest inhibition zone ranged from 9 mm against *Micrococcus luteus* ATCC 10240 to 25 mm against methicillin resistant *S. aureus* (MRSA) ATCC 43300 (Table 1). The greatest relative percentage inhibition was recorded against *S. typhimureum* ATCC 14028 (129.6%), followed by MRSA ATCC 43300 and *E. coli* O157:H7 wild type strain 93111 (100%).

For *Phormidium ambiguum* Ag-NPs, the inhibition zone diameter was in a range of 9 – 18 mm. Maximum relative percentage inhibition of 86.6% was displayed against *Salmonella typhimureum* ATCC 14028, followed by *E. coli* O157:H7 wild type strain 93111 (81.7%) and *P. aeruginosa* ATCC 9027 (75.1%).

Table 1. Inhibition zone diameter (mm) and relative percentage inhibition of Ag-NPs synthesized extracellularly from *Desertifilum tharense* and *Phormidium ambiguum* under light conditions.

Indicator microorganisms	<i>Desertifilum tharense</i> Ag-NPs		<i>Phormidium ambiguum</i> Ag-NPs	
	Inhibition zone (mm)	Relative percentage inhibition (%)	Inhibition zone (mm)	Relative percentage inhibition (%)
<i>B. cereus</i> ATCC 10876	12	84.8	9	58.2
MRSA ATCC 43300	25	100.0	17	44.8
<i>M. luteus</i> ATCC 10240	9	45.0	9	45.0
<i>S. typhimureum</i> ATCC 14028	17	129.6	13	86.6
<i>P. aeruginosa</i> ATCC 9027	14	87.1	13	75.1
<i>E. coli</i> O157:H7 wild type strain 93111	21	100.0	18	81.7

The antibacterial effect of silver nanoparticles is size, charge and shape dependent. The great antibacterial activity of *Desertifilum tharense* Ag-NPs against tested pathogenic bacteria could be attributed to their particle size (6.24 – 11.7 nm) which was smaller than that of *Phormidium ambiguum* Ag-NPs (6.46 - 12.2 nm). This is in accord with the findings of Ivask et al. (2014) Patel et al. (2015) who found that the antibacterial activity of Ag-NPs is inversely proportional with their particle size as smaller size means larger surface area which facilitates the interaction between nanoparticles and microbial cells. The particles with size of 10 nm or smaller can easily penetrate the bacterial cell wall and interact with the cytosol biomolecules. It was known that the antibacterial activity of Ag-NPs is not attributed only to the small size but also to their positive charge. Generally, positively charged particles electrostatically bind to lipopolysaccharides on the outer membrane of G⁻ bacteria or lipoteichoic acids on the surfaces of G⁺ bacteria which facilitate the cell penetration (Glinel et al., 2012). Rajeshkumar et al. (2014) and Otari et al. (2015) found that spherical shape with small size of Ag-NPs increase the contact area that ensuring the elimination of bacterial growth.

In general, the mode of action of Ag-NPs as antibacterial mainly includes loss the outer membrane integrity in G⁻ bacteria, and permeabilization of the bacteria cell membrane through formation of pores across it resulting in releasing the cellular material and causing cell death ((Raffi et al., 2008 and Dehkordi et al., 2011). On the other hand, the pores formed in the cell membrane facilitate the Ag-NPs inflowing into the cell to combine with proteins containing sulfur and DNA causing DNA and enzymes damage, and leading to blocking vital metabolic processes (Hamouda et al., 2019).

3.5. Free radical scavenging potential of Ag-NPs

The results confirmed the highest scavenging activity of cyanobacterial Ag-NPs comparing with cyanobacterial extracellular extract. Silver nanoparticles of *Phormidium*

ambiguum exhibited the highest scavenging activity of 48.7% comparing with that of *Desertifilum tharense* which displayed 43.753% (Table 2).

Table 2. Antioxidant capacity (%) of Ag-NPs synthesized extracellularly from *Desertifilum tharense* and *Phormidium ambiguum* under light conditions.

Tested cyanobacterial strains	Absorbance at 515 nm			DPPH scavenging effect (%)	
	Methanolic DPPH	Extracellular extract	Ag-NPs	Extracellular extract	Ag-NPs
<i>Desertifilum tharense</i>	0.770	0.492	0.433	36.143	43.753
<i>Phormidium ambiguum</i>	0.770	0.508	0.395	33.974	48.701

4. Conclusion

In this study, Egyptian cyanobacterial isolates of *Desertifilum tharense* and *Phormidium ambiguum* are found to be potent for the green synthesis of Ag-NPs extra- and intracellularly under light and dark conditions. They can produce smaller spherical particles with face-centered cubic structures. Presence of amides and hydroxyl groups indicate that proteins and polysaccharides could be considered as important factors in the Ag-NPs biosynthesis. Generally, the characterization assay showed that a Novel *Desertifilum tharense* cyanobacteria have superior power in green synthesis of AgNPs. The current findings indicate that Ag-NPs may be potent antibacterial agents against different pathogenic bacteria and could be used as alternatives to antibiotics. Further studies are recommended to define the optimal conditions for Ag-NPs biosynthesis. Also, more biological characterizations and *in vivo* experiments are required to establish the real potential for their application in medical and food sectors.

5. Declarations

List of abbreviations;

NPs, nanoparticles; Ag-NPs silver nanoparticles; AgNO₃, silver nitrate; nm, nanometer; μ L, microliter; mg, milligram; min, minute; h, hour; mL, milliliter; mM, millimolar; μ g, microgram; cm^{-1} , inverse centimeter; rpm, revolutions per minute; PCR, polymerase chain reaction; TEM, transmission electron microscope; SEM, scanning electron microscope; FTIR, Fourier transform infrared spectroscopy; UV-vis, ultraviolet-visible spectroscopy; XRD, X-ray diffraction; kb, kilobase; kV, kilovolt; SPR, surface plasmon resonance; JCPDS, Joint Committee on Powder Diffraction Standards; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Availability of data and materials:

The data supporting this article are available in our Figures and Tables. The data sets analyzed in the present study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate:

Not applicable. The manuscript does not involve any animals, humans, human data, human tissue or plants.

Consent for publication:

Not applicable. The manuscript does not contain any individual person's data in any form.

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Author Contributions:

ALH designed the experiments, carried out the study, interpreted the data and drafted the manuscript. TRE carried out the molecular identification and phylogenetic analysis of cyanobacteria isolates. MWS, HMH and HAG supervised all experiments and reviewed the manuscript.

Conflict of interest: The authors declare that they have no conflict of interest for this study.

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