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Biosynthesis of silver chloride nanoparticles using the cyanobacterium *Anabaena variabilis*

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Abstract: Silver chloride nanoparticles (AgCl-NPs) is a powerful antimicrobial agent. A possible source of biological material for the green biosynthesis of AgCl–NPs was represented by microalgae. In this study rapid biosynthesis of stable AgCl–NPs was achieved by using ethanol extract of cyanobacterium, *Anabaena variabilis* v. Kashiensis. The results were verified using UV-Vis spectroscopy, High Resolution Transmission Electron Microscopy (TEM) and X-ray diffraction (XRD). Bioreduction of Ag^+ ions showed a gradual change in the colour of the extract to brown. Peaks of UV-Vis spectra showed surface plasmon resonance (SPR) at (409 nm). TEM micrograph analyses confirmed formation of homogenous spherical AgCl–NPs, ranged in size from (12-20 nm). The crystalline nature of the synthesized AgCl–NPs was assigned by their remarkable peaks in the XRD patterns corresponding to (111, 200, 220, 311, 222, 400 and 420) planes. Fourier Transform Infrared Spectroscopy (FTIR) of purified nanoparticle fractions suggested that proteins were the main molecular entities involved in AgCl–NPs formation and stabilization. This study provides a new report for the cyanobacterial species, *Anabaena variabilis* that, could serve as good, cheap substrate for biogenic of homogenous spherical AgCl–NPs.

Key words: Silver chloride nanoparticles; Green biosynthesis; *Anabaena variabilis*;

1. Introduction

Biosynthesis of nanoparticles is an important area in the field of nanotechnology which has economic and eco-friendly benefits over chemical and physical methods of synthesis (AL-Katib et al., 2015; Ferreira et al., 2016). The green biosynthesis of nanoparticles can be achieved by the selection of an environmentally acceptable solvent with eco-friendly reducing and stabilizing agents (Jegadeeswaran et al., 2012; Ibraheem et al., 2016). Silver nanoparticles have recently attracted great attention because of their wide range of applications in many fields of science including agriculture (Khota et al. 2012; Krishnaraj et al., 2012), medicine (Elechiguerra et al., 2005), industry (Crooks et al., 2001; Chen et al., 2007; Abdel-Raouf et al., 2017). Silver chloride is perhaps the most widely recognized nanoparticles and it has been extensively used as: photographic material, promising photo catalysis at low cost method for the removal of hazardous materials and organic pollutant, powerful antibacterial agent, antifungal agent, antioxidant (Gopinath et al., 2013a, b; Abdel-Raouf et al. 2013; Kumar et al., 2015; Kang et al., 2016; Ibraheem et al., 2017) and catalysis (Crooks et al., 2001). Microalgae are considered as nano-factories for nanoparticle productions, due to their rapid growth rates and high biomass production in a short time. Cyanobacteria, such as *Anabaena* sp., *Calothrix* sp., have been reported to biosynthesize intracellular gold, silver, palladium and platinum NPs (Brayner et al., 2007). Meanwhile, the cyanobacterium, *A. variabilis* has antibacterial potential against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* (Kaushik et al., 2009) and contribute significantly in maintenance of soil fertility in flooded rice fields (Swarnalakshmi et al., 2013; Datta et al., 2014). It is also able to do photosynthesis, fix atmospheric nitrogen, which is a critical limiting element for plant growth and production (Knoche et al., 2017) however; the efficacy of *A. variabilis* to biosynthesize AgCl–NPs has not been identified yet. The present study provides a biological green route for the synthesis of AgCl–NPs, using the biomass extract of terrestrial cyanobacterial species, *A. variabilis* v. Kashiensis and investigates their physicochemical properties.

2. Material and Methods

2.1. Algae species

Anabaena variabilis v. Kashiensis (soil cyanobacterium) was obtained from the culture collection of Agric. Microbiol. Res. Dept.; Soils, Water and Environment Res. Institute, Agricultural Research Center, Giza, Egypt.

2.2. Preparation of algae extract.

The algal cells were collected at late exponential phase by centrifugation at 5000 rpm for 10 min using (Hettich centrifuge, Germany) the supernatant removed and the pellets were washed by deionized water to remove the trace salts of the media. The fresh

biomass was air dried in clean glass plates. Dried algal biomass was crushed by porcelain mortar. Algae extract was prepared by soaking 1gm of algal powder in 19 ml of 95% ethanol. Mixture was shaken thoroughly at 225 rpm for 30 minutes at 40^0C. The pure ethanol extract was separated by centrifugation and used as a substrate for biosynthesis of AgCl–NPs.

2.3. Biosynthesis of AgCl–NPs.

The biosynthesis of AgCl–NPs was achieved by mixing 9 ml of crude cell extract with 1ml $AgNO_3$ (1mM) in 50 ml conical flask (Abdel-raouf et al., 2013). Cell free extract was used as blank. All solutions were shaken for 30 minutes in orbital shaker at 200rpm.

2.4. Characterization of AgCl–NPs.

2.4.1 Visual characterization

Biosynthesis of AgCl–NPs were confirmed by visual observations of the developed colour in the reaction mixture flask, of the algal extract with 1mM $AgNO_3$ solution, as compared to control. The colour change from pale yellow to brown indicated the extracellular synthesis of AgCl–NPs. The biosynthesized AgCl–NPs were obtained by centrifugation at 12000 rpm for 10 min, washing the pellets with double distilled water and repeating the protocol thrice. The obtained AgCl–NPs pellets were then lyophilized for further studies.

2.4.2. UV–Visible spectroscopy analysis

The sample was monitored by sampling of aliquots 3–5 ml and subsequently measuring UV–Vis spectra of the solution at 300–700 nm in 1cm path length quartz cuvette. UV–Vis spectra of these aliquots were monitored on a (UV-2600, SHIMADZU, Japan) spectrophotometer. All the measurements were carried out at room temperature.

2.4.3. TEM analysis of AgCl–NPs

The morphological analysis of the nanoparticles was achieved by transmission electron microcopy (TEM). The TEM micrograph images were recorded by dipping an aqueous solution of sample on carbon coated grid with an accelerating voltage of 80 kV. Samples were examined by (JEOL JEM- 2100 electron microscope, Japan). The clear microscopic views were observed and documented in different ranges of magnifications.

2.4.4. X-ray diffraction analysis

The crystallinity and elemental composition of the developed AgCl–NPs were assessed and identified by X-Ray powder diffractometer (202964 Panalytical Empyrean) with CuKá1 radiation, the voltage and the current of the X-ray source were 40 KV and 30 mA, respectively. The sample was drop-coated onto silica plate by applying many layers of small amount of the sample on the plate with intermittent drying, this lead to a thick coat of the sample.

2.4.5. Fourier Transform Infra Red analysis (FTIR)

FTIR was used to identify the possible biomolecules responsible for the reduction of the Ag ions and capping of the bioreduced AgCl–NPs, synthesized by *A. variabilis* extract. In order to determine the functional groups and their possible involvement in the synthesis of silver chloride nanoparticles, the freeze-dried synthesized nanoparticles were grinded with potassium bromide (KBr) and the spectrum was recorded on using FTIR spectroscopy (VERTEX 70 Spectroscopy, Japan).

3. Results and discussion

The phenotypic examination of cells of *A. variabilis* species, under light microscope, at the magnification power of 1000 X is shown in (Fig.1).

3.1. Visual characterization

Before adding the silver nitrate solution to the algal extracts, it was observed that, the colour *A. variabilis* ethanol extract was light-yellow. The formations of AgCl–NPs were visually checked by the change in colour of the algal extract-inoculated flask before and after the biosynthesis process. The colour was changed from light yellow to brown in the flask containing *A. variabilis* ethanoic extract and 1mM $AgNO_3$ (Fig.2A). This may be attributed to excitation of surface plasmon resonance (SPR) of the synthesized AgCl–NPs (Mulvaney 1996). No change was recorded in the control flask (Figs 2B). Similar observations were recorded in some studies (Shivaji et al., 2009; Gopinath et al., 2013a). The produced brown colour indicated the synthesis AgCl–NPs and reduction of Ag^+ ions.

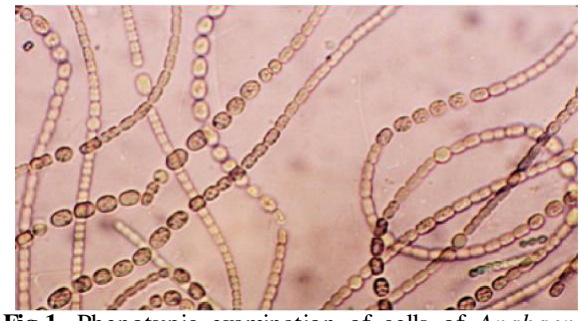

Fig.1. Phenotypic examination of cells of *Anabaena variabilis*, examined under light microscope at magnification power of 1000 X.

3.2. UV–visible spectroscopy analysis

A prominent and sharp absorption band is observed at 409 nm by UV-Visible spectroscopy analysis (Fig. 2). AgCl–NPs have free electrons which give rise to an SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave (Dubey et al., 2010).

Due to the excitation of plasma resonances on inter-band transitions, some metallic nanoparticles dispersions exhibit unique bands or peaks. This absorption band is characteristic of AgCl–NPs as a result of the excitation of surface plasmon vibrations in the nanoparticles (Shankar et al., 2004). Similar finding was reported by AL-Katib et al., (2015) and the recent contribution by Ferreira et al. (2016) indicated to biosynthesis of Ag/AgCl–NPs using *Chlorella vulgaris* with UV-Vis absorption maximum 415 nm. Mie's theory (2014) stated that "only a single SPR band demonstrates small and spherical nanoparicles, while anisotropic particles show two or more SPR bands. Accordingly, the presence of a single band in this study (Fig. 2) indicates formation of spherical and small-sized of biosynthesized AgCl–NPs (Saifuddin et al. 2009; Mie et al. 2014).

3.3 Transmission Electron Microscopy (TEM)

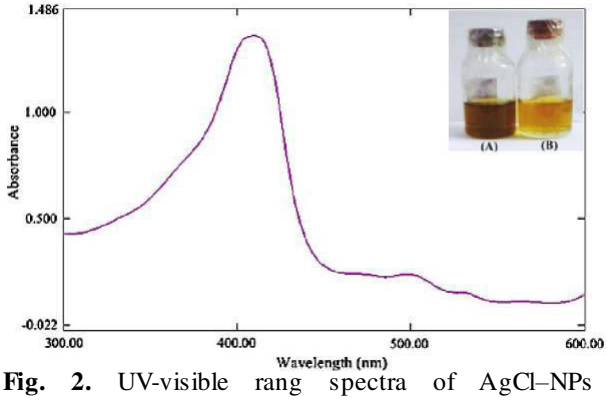

Fig. 2. UV-visible rang spectra of AgCl–NPs synthesized by *Anabaena variabilis* ethanol extract. Visual changes in colour of silver nitrate solution 10^{-3}M; (A) represents the developed colour after adding *A. variabilis* ethanol extract with brown colour and (B) represents the original colour of algal extract (control).

A typical TEM image showing the size and morphology of the biosynthesized AgCl–NPs is given in (Fig. 3). Morphological characterization revealed that, biogenic method generated uniformly homogenous spherical shaped nanoparticles by using *A. variabilis* ethanol extract. The AgCl–NPs size ranged from (12-20nm). Interestingly, the solutions of *A. variabilis* nanoparticles (Fig. 2A) were extremely stable with no evidence of aggregations of the particles. The sharp resonance indicates no flocculation of the particles in solution. It is clear from the above results that, *A. variabilis* contains reducing agents which releases into solutions that are responsible for formation of the AgCl–NPs. Additionally, TEM micrograph analyses (Fig.3) revealed a quiet uniform AgCl–NPs in shape and size. We hypothesize that to the bioactive constituents of the studied micoalga which play a crucial role in reducing and controlling the formation of AgCl–NPs in the solution. These results in agreement with Plaza et al., who reported that macro-marine algae especially, brown algae contain high values of natural functional compounds as terpenes, alkaloids amino acids and fatty acids which act as a stabilizers and prevent the formation of nanoparticles aggregations (Plaza et al., 2010).

3.4. Fourier Transform Infra Red analyses

FTIR analysis was employed to identify the presence of possible functional groups within the biomolecules associated with AgCl–NPs that might be responsible for the reduction of silver ions and stabilization of AgCl–NPs in solution (Fig. 4). The major infrared absorption peaks detected in the AgCl– NPs fraction were 3344, 2975, 2893, 1650, 1390, 1326,1142, 1085, 1044, 878 and 639 cm^{-1}. The intense band observed at 3344cm^{-1} was associated with OH functional group in alcohols and phenolic compounds (Gopinath et al., 2013a, b; Gole et al., 2001) while, the side chain vibrations of C-H stretching at 2975 and 2893 cm^{-1} were characteristic of aliphatic and aromatic compounds, respectively. Peak at 1650 cm^{-1} could be assigned to the stretching vibrations of C=O and -N-H- (amide I and amide II) bonds associated with proteins (Giordano et al., 2001). Peaks located at 1326,1142 and 1044 cm^{-1} might be attributed to the presence of stretching vibrations of carboxylic acids and amino groups and 878 cm^{-1} attributed to alkyle halide stretching (Salem et al., 2016). The peak at 1390 cm^{-1} suggested the asymmetric deformation of CH_3 and CH_2 in proteins (Huang et al., 2007; Shanmugam et al., 2012). The peak at 1085 cm^{-1} was attributed to C-O-C bonds in polysaccharides, which are typically found in the region between 1200 and 900 cm^{-1} (Giordano et al., 2001). The broad absorption band at 639 cm^{-1} indicated (C-H bend alkyne). Overall, FTIR data indicated that proteins are key components that drive the bioproduction and stabilization of AgCl– NPs derived from *A. variabilis* ethanol extract.

3.5. X-ray diffraction (XRD)

The XRD pattern of AgCl–NPs is shown in (Fig.5). The crystallinity of the nanoparticles revealed the existence of major 7 peaks at 2θ value (27.48, 31.83, 45.64, 54.10, 56.72, 66.53, and 75.65). This result corresponds to 111, 200, 220, 311, 222, 400 and 420 planes respectively, of cubic crystalline phases of AgCl–NPs (JCPDS card file no:01-075-9577), with one peak at 2 theta value (29.14) corresponds to (110) plane of silver nitrite nanoparticles (JCPDS card file no: 00-006-0349). Chlorine contents in the algal extract, previously obtained from saline BG11media components, $CaCl_2$ and NaCl, could be responsible for the formation of AgCl–NPs through this study. This observation is consistent with contribution of Gopinath et al. (2013a) who suggested that Cl^- ions

existed in the leaf extract of *Cissus quadrangularis* might be responsible for formation of AgCl–NPs. Similar finding was reported by Paulkumar et al. (2013) in their experiment on biosynthesis of AgCl–NPs in *Bacillus subtilis*, supposed binding of Cl^- ions already existed in the culture medium to Ag^+ ion.

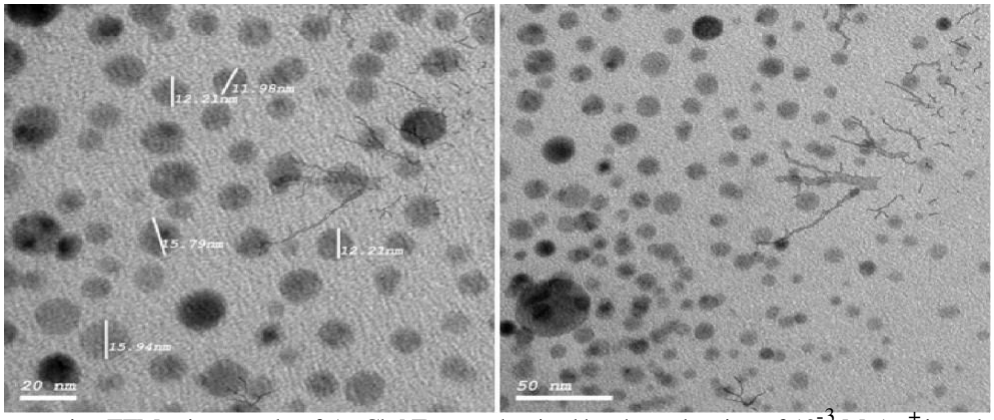

Fig.3. Representative TEM micrograph of AgCl–NPs synthesized by the reduction of 10^{-3} M Ag^+ ions by the ethanol extract of *Anabaena variabilis*.

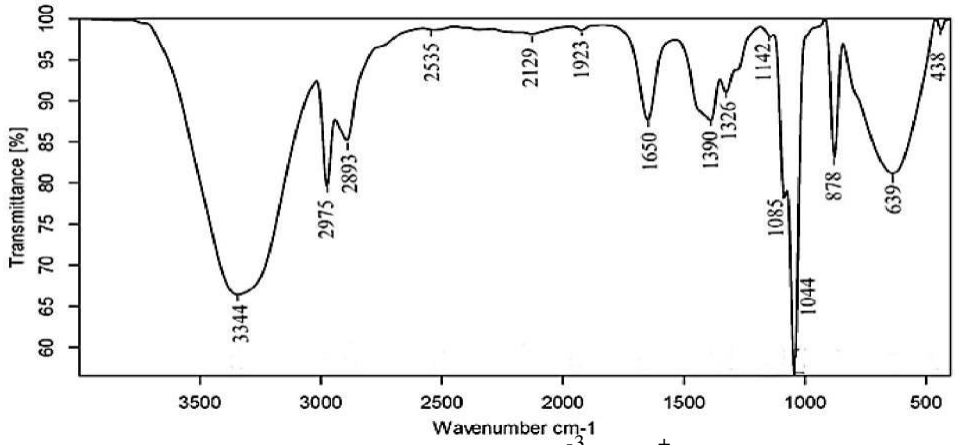

Fig.4. FTIR spectrum of AgCl-NPs synthesized by reduction of 10^{-3} M Ag^+ ions by the ethanol extract of *Anabaena variabilis*.

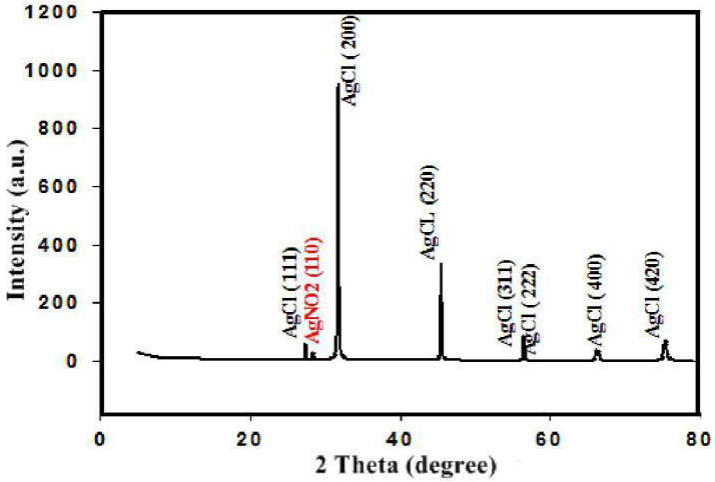

Fig.5. XRD pattern of AgCl–NPs synthesized by reduction of 10^{-3} M Ag^+ ions by the ethanol extract of *Anabaena variabilis*.

4. Conclusion

This study demonstrates the efficiency of *Anabaena variabilis* v. Kashiensis for biosynthesis of homogenous, stable spherical silver chloride nanoparticles with offering the merits of ecofriendly method, time saving for large–scale production and was found to be much faster as well as it was conducted at ambient temperature and pressure conditions. Therefore, it provides low cost technique, nontoxic green approach. This study provides a new report for utilizing the cyanobacterial species; *Anabaena variabilis* in the nanotechnology field, which could provide future possible strategy for application in many fields.

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