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Biosynthesis of Silver Nanoparticles by Cyanobacterium *Gloeocapsa sp.*

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ABSTRACT

Aims: Synthesis of silver nanoparticles by a blue- green approach. Cyanobacteria strains, commonly known as blue green algae that known as cheap ecofriendly tool for nanometal formation. The cyanobacterium Gloeocapsa sp. was found to be effective microorganism for nana silver production. The extracellular synthesis of AgNPs was initially detected by visual inspection for color changing of cultured flasks solutions from transparent to brown then black, as well as nanoparticles was characterization by UV-Vis spectrophotometer and Fourier Transform Infrared spectroscopy(FTIR) .The cyanobacterium characterized by genetic approach beside the morphological methods, by isolation of DNA that followed with Polymerase Chain Reaction (PCR) with electrophoresis run and finally sequencing technique to accurate identification of the cyanobacterium strain of our study.

Materials and Methods: In this study the cyanobacterium was isolated from fresh water then inoculated on solid Chu 10 medium ,after chain of titrations , by streak plate method[(1)]. The cyanobacterium strain primarily identified depending on species level morphological characterizes with using key books [(2,3,4)]. Then further identification was done by 16S rDNA gene sequencing .The genomic DNA was isolated as a template for PCR that performed with universal primers (8F and 1462R). Sequence of 16S rDNA gene was compared with NCBI sequence database (GeneBank) through BLAST (www.ncbi.nlm.nih.gov/BLAST) .We used the identified cyanobacterium Gloeocapsa sp. as bio-reagent for AgNPs production with addition of silver nitrate ($AgNO_3$) in different concentrations under light and dark conditions. The UV-Vis spectrophotometer and FTIR techniques used for characterization of AgNPs that produced [(5)].

The results: The cyanobacterium strain was identified morphologically and genetically was Gloeocapsa sp. PCC 7428. This cyanobacterium strain was capable for AgNPs biosynthesis and that nanoparticles characterized by UV-Vis spectrophotometer and FTIR techniques. In UV-Vis analysis, the results showed characteristic surface Plasmon absorption peaks at range 400-450 nm and the best production was under light condition. The FTIR spectrum data confirmed the presence of specific functional groups such as proteins that have the main role as capping and stabilizing agent in biosynthesis of AgNPs.

Conclusion: The results of study have demonstrated the efficiency of the Gloeocapsa sp. PCC 7428 for biosynthesis of silver with offering the merits of ecofriendly method , time saving for large –scale production and was found to be much faster as well as it done at ambient temperature and pressure conditions thus it require less amounts of chemicals. Therefore it low cost technique, nontoxic and is more of a greener approach.

Key words: Gloeocapsa sp. PCC 7428, Silver nanoparticles AgNPs, Biosynthesis.

INTRODUCTION

Nanostructure materials have unique physical and chemical characteristics and are applicable in various fields such as biomedical sciences, optics, magnetic, mechanics, catalysis, and energy sciences. Different aspects of this novel technologycomprise the production , characterization ,and manipulation of nanoscale structures [(6,7)]. Nanoparticles are gaining reputation as multifaceted materials exhibiting novel or advanced characteristics compared to larger particles [(8,9)]. Nano biotechnology is a multidisciplinary fields of science like biotechnology, nanotechnology, physics, chemistry, and material science. Nanoparticles are metal particles with size1-100 nm and exhibit different shapes like spherical, triangular, rod, etc. Research on synthesis of nanoparticles is the current area of interest due to the unique visible properties (chemical, physical, optical, etc.) of nanoparticles compared with the bulk material. Bio nanotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterial's[(5, 10)]. Synthesis of nanoparticles has attracted a lot of attention due to their unusual optical , photo electrochemical and electronic properties .Both unicellular and

multicellular organism have been known to produce inorganic materials either intra- or extra- cellular. Microorganisms ranging from bacteria to fungi have been used in recent years to develop non-toxic and environment friendly methods to synthesize nanoparticles as well as nanoparticles synthesized by microorganisms tend to be stabilized by peptides, such as phytochelatins, thus preventing aggregation [11].The biological agents in the form of algae, plants and microbes have emerged as an efficient candidate for the synthesis of nanoparticles [12]. This integration of nanoparticles with biological molecules has led to the development of diagnostic devices, contrast agents, and important tools in cancer therapy [13].The silver nanoparticles have anticipated wide applications for commercial applications, in the field of agriculture applications using bacteria,cyanobacteria,algae, plant that minimizes hazard and waste will be essential for the transition of nanoscience discoveries to commercial product of nanotechnology [12]. The AgNPs have excellent antimicrobial property compared to other salts due to their extremely large surface area which provides better contact with microorganisms. Silver ions and nanoparticles are highly toxic and hazardous to microorganisms. The AgNPs have many other applications; for example, they might be used spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling and as antimicrobials.

The nanoparticles use for biomedical applications, such as drug and gene delivery, biosensors, cancer treatment and diagnostic tools, which have extensively studied throughout the past decade. Recently, nanoparticles have gained significance in the field of biomedicine [14]. Microphytes or Microalgae are microscopic algae, typically species which exist individually, or in chains, or groups. Microalgae, capable of performing photosynthesis, are important for life on earth. The chemical composition of microalgae is not an intrinsic constant factor but varies over a wide range, both depending on species and on cultivation conditions [15]. Research has seriously focused on prokaryotes that means of synthesis of metallic nanoparticles [16]. Cyanophycophyta division includes the blue –green algae which are the only known oxygen producing prokaryotes, all of it species are included in a single class Cyanophyceae or Myxophyceae, and the members of this class are considered the simplest, living autotrophic plants. Individually all the blue- greens are microscopic, widely spread in the aquatic environment with some terrestrial species. The aquatic form mostly occur in fresh water and few are marine [17]. The cyanobacteria are prokaryotes, which have nomembrane bound organelles, including chloroplasts.

They have unstacked thylakoids, phycobiliprotein pigments, and cyanophycean starch and peptidoglycan matrices. Or walls. Cyanobacteria represent an ancient, but diverse and abundant group of microorganisms that possess prokaryotic (bacterial) cell structure and predominantly CO_2- dependent oxygen- evolving photosynthesis. Unlike all other algae, cyanobacteria are classified within the Eubacteria due to their simple, prokaryotic cells and gram-negative (peptidoglycan) cell walls. Cells contain chlorophyll-a and several phycobilin –protein complexes [4]. Gloeocapsa, it is a member of the family Chroococcaceae, there are numerous lamellate sheaths in Gloeocapsa and the cells occur in extensive gelatinous masses. The cells in the mass are arranged in families. Each cell is more or less spherical in form and the daughter cells may be held together by the sheath of the mother cell. Alga reproduces only by cell division in three planes at right angles to one another [14,17]. Inadequate culture conditions leading to the loss of various morphological characteristics. Both classification systems for the cyanobacteria- the bacteriological approach as well as the traditional botanical approach- rely primarily on norphological characteristics of cells and colonies and do not necessarily lead to the identification of phylogenetically coherent taxa.

At all taxonomic levels above species, the sequence analysis of genes encoding small- subunit ribosomal RNA (16S rRNA) is currently the most promising approach for the phylogenetic classification of cyanobacteria. S sequence of 16S rRNA genes are independent from cultivation or growth condition and can be retrieved by PCR from small amounts of DNA extracted from laboratory cultures or natural environments [18] . Traditional techniques for identification and systematic of cyanobacteria have relied primarily on observed morphological characteristics. However, morphology may change depending on environmental conditions and the diversity of strains within a culture may be suppressed by selective culturing condition. Photosynthetic pigment content, lipid composition, differentiated cell structures and other characters may also be subject to change because of the variable expression of cyanobacterial gene products in culture. The limitations of such methods have prompted the development of molecular biological techniques for identification of cyanobacteria [19].

The 16S rDNA – based polymerase chain reaction (PCR) detection method is one of the most widely used techniques and can be successfully performed for identifying the isolates in laboratories. Identification of the genus and species of selected isolates using the 16S rDNA method should be carried out on a pure culture of microorganisms. The 16S rDNA gene is amplified by PCR using universal primers. After sequencing of the amplified segment, its sequence should be compared with other sequences in the gene bank using the Basic Local Alignment Search Tool (BLAST). The comparison of rDNA sequence is a powerfultool for study of phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eukaryotic organisms (from Weisburg et al. 1991)[5].

There are some researches that interest in biosynthesis of metal nanoparticles such as Ag, Au, Zn, Cu, and other metals by using cyanobacteria as biogenic reagent. There are sporadic reports available regarding filamentous cyanobacteria based AgNPs production like, Plectonema boryanum[20], Leptolyngbya valderianum[21], Oscillatoria willei[22],

Spirulina platensis[23], and most of cyanobacteria with microalgae tested to biosynthesis of AgNPs [24] both unicellular and multicellular organisms have been known to produce intracellular or extracellular inorganic materials. Among the algae, microalgae are considered as cell factories for nanoscala particle, due to high growth rate and high biomass productivity with less cultibation time. Beside they have been proved as potential organismfor heavy metal detoxification and production of commercially important metabolites. Algal biomass can be easily harvested and the intracellular NP can be resealed by disrupting the cell by commercially available processing equipment [25]. The AuNPs (goldnanoparticles) was synthesized by the cyanobacterium Gloeocapsasp[26]. The cyanobacterium Plactonema boryanum UTEX 485 was produced AuNPs [5].

In this context, the purpose of this study was to isolate, and characterize by complete 16S rDNA gene sequencing, and use the isolated strain of cyanobacterium as bio reagent in biosynthesis of silver nanoparticles (AgNPs) with characterized it by UV- Vis analysis and FTIR methods, as well as study the effect of light in this biosynthesis. This work reports the first detailed experimental results of the effect of light condition in biosynthesis of AgNPs by using the Gloeocapsa sp. with $AgNO_3$ salt with varied concentrations.

MATERIALS AND METHODS

Isolation and Culture of Cyanobacteria: The unicellular, colonial freshwater blue-green algae Gloeocapsa sp. sample was collected from Mosul freshwater, Iraq. It was cultured and maintained in Chu 10 broth medium [27, 28]. An axenic culture of cyanobacterium can be get by wash the sample many times in sterile distilled water with centrifuged them and then made a diluted solution from which we culture the sample on Chu 10 solid medium by using steak method to get an axenic culture from a single pure colony. After that, the colony of algae transferred by sterile loop to a liquid media to obtain broth culture. The cultures were grown at temperature of 25± 2º C under photoperiod light (16:8 –light: dark).

Identification of cyanobacteria: The first and primarily method for identification it , was morphological approach by using light microscope (OPTICA , Microscopes, ITALY)with cyanobacteria identification key books [2,3,4]. The second, was genetically approach by used molecular – based tools like 16S rDNA gene sequencing [29].

Isolation of Cyanobacteria Genomic DNA: The DNA extraction was carried out by using cyanobacterial culture in 14 days age, we take 10 ml of the culture to plane tube and cells harvested by centrifugation at 6,000 rpm for 10 min and the pellet was easily frozen with liquid N_2 and then ground to a fine powder with a mortar and pestle [30]. This pellet of cyanobacterial cells was used for DNA extraction with Wizard ® Genomic DNA Purification Kit protocol (Promega). This Genomic Kit contain: A) Nucleilysis solution. B)RNase solution. C) Protein precipitation solution. D) DNA rehydration solution. The isolation of genomic DNA was done as gram- negative protocol. The steps are:

1- Use 1 ml of pellet cells (prepared previously), that precipitate (13,000-16,000 rpm*) for 2 min by cooled micro centrifuge (Nuve nf 800R /Turkey) and the supernatant was discard.
2- Adding 600µl of Nucleilysis solution to the pellet in eppendorf tube with well shaking.
3- Incubation of this eppendorf tubes in water bath at 80º C for 5 min (Shaking incubator, Electro.mag/ Germany), and then cooling it to room temperature.
4- 3 µl of RNase solution was added to eppendorf components with slight vortexing (Vortex, Labnet/ USA).
5- Incubation the tubes at 37º C for 15-60 min and leave it cooling to room temperature.
6- Adding 200 µl of protein precipitation solution to tubes with mixing by vortex for 20 sec. and incubation it in ice for 5 min.
7- Centrifugation at (13,000-16,000 rpm) for 3 min. , and transfer the upper layer that contain DNA to a new eppendorf tube (1.5 ml) containing 600 µl of isopropanol at room temperature.
8- Shaking the tube gently to observe the DNA pellet.
9- Centrifugation (13,000-16,000 rpm) for 2 min.
10- 10- Pullthe supernatant slowly by micropipette and discard it.
11- Adding 600 µl of room temperature 70%ethanol to that tube and then gently mixing by inverting end to end to wash the DNA pellet.
12- Centrifugation the tube (13,000- 16,000 rpm) for 2 min.
13- The supernatant was removed gently and the pellet was air dried for 10-15 min.
14- Rehydrate the DNA pellet in 100 µl of Rehydration solution for 1 hour at 65º C or overnight at 4 ºC.

* Maximum speed on a micro centrifuge.
(www.Promega.com).

Determination of concentration and purity for DNA: We use Bionanodrop 2000 spectrophotometer (Cambridge CB England) for measure the quantity and quality of DNA by analyzing the ratio of absorbance at wavelength 260/280 nm by taking 1 µl from isolated genomic DNA , using micropipette , and putting it in sample place then read the concentration in (ng/µl).

Agarose gel Electrophoresis: The extracted genomic DNA was electrophorised to observe its bands and determined molecular size (1.2% agarose) by compared with 1 Kbp DNA Lambda Ladder (Promega, USA) as well as, This approach used after doing PCR for genomic DNA in a concentration (2% agarose). Solutions in this approach:

1: TBE buffers (10X): (gm / 100ml)

Tris – base ….10.8

Boric acid …...5.5

Na_2EDTA……7.44

D. W. ……...70 ml

Then complete to 100 ml by D. W. (pH = 7.8)

2: to prepare TBE buffer (1X):

Taking 10 ml of (10X) TBE buffer and adding 90 ml D. W.

3: Ethidium bromides dye solution:

Preparation of it by solving 0.1 gm of Ethidium bromide in 10 ml D. W. to obtainconcentration 10 mg/ ml, and then take 80 ml from it to add it to I L of D. W. in dark can to use it later.

4: DNA ladder:

1 Kb DNA Ladder is ideal for determining of double- stranded DNA from 250- 10,000 base pairs. The ladder consist of 13 double- stranded (250/253 , 500 , 750 , 1000 , 1500, 2000,2500,3000,4000,5000,6000,8000,and 10000). The concentration of 1 Kb DNA Ladder is 100 µg/ ml. Five microliters (500 ng) of the 1 Kb DNA ladder are mixed with 1 µl of blue/orange 6X Loading Dye and subjected to electrophoresis in a single lane on a 1.2% agarose gel with TBE 1 X buffer[31].

5: Blue/orange 6X Loading Dye(G 190 A)(Promega): This supplied dye was used for loading DNA samples into gel electrophoresis wells and tracking migration during electrophoresis. Recommended usage is on part loading dye to every five parts of DNA solution (Sambrook etal.1989) ([31].

Electrophoresis steps [5]:

1-Gel preparation: Weight agarose (1.2 gm) and add it to 100 ml TBE buffer (1 X). Then heat the flask in a microwave oven for 10 sec. intervals until all agarose crystals are meltedand dissolved. Wait until its temperature reaches 50ºC. Select an appropriate gel casting tray and insert a sample comb around which molten agarose is poured to form sample wells in the gel. After the gel has solidified, the comb is removed, using care not to rip the bottom of the wells. Thegel, still in its plastic tray, is inserted horizontally into the electrophoresis camper and just barely covered with Tris Borate EDTA (1 X- TBE) buffer (Sambrook and Russel 2001).

2- Electrophoresis Run:

Mix reagents containing DNA Ladder (4 µl), 6X loading dye solution (2 µl). This DNA Ladder contain DNA with different lengths that should be used as a reference for determination of genomic DNA or PCR products length. We mixed (5-7 µl)of PCR products with (1 µl) of 6X loading dye separately, and transfer them into individual comb places (wells).The lid and power leads are attached to the apparatus. Turn on the power supply using 50 volt at 75 min., observing bubbles coming out of the electrodes confirms that current is flowing. DNA will migrate towards the positive electrode (Sambrook and Russell 2001) ([5]. Turn off the power supply and transfer the electrophoresis gel cast to a tray containing Ethidium bromide dye and leave it for (30-60 min). Then, the gel was washed with distilled water to remove the overflow dye from the gel. After that, transfer the electrophoresis gel onto the ultraviolet Trans illuminator that set to wavelength 320 nm. Amplified DNA or genomic DNA bands are visualized and then take a photo from the gel. The gel at last transfer to a specific waste box, and clean the surface of Trans illuminator with 70% ethanol.

Polymerase Chain Reaction (PCR):

Classification of primers is based on the ordinary method in which F is known as Forward primer and R is known as Reverse primer. We using extracted genomic DNA (40-45 ng) for specific PCR by using AccuPower ® PCR PreMix (Bioneer, USA) Kit, it is the powerful technology for convenient and easy to perform DNA amplification. It contain DNA polymerase,dNTP, a tracking dye, and reaction buffer in a premixed format, freeze- dried into a pellet.

PCR Primers: Bacterial specific primers were purchased from Alpha DNA (Montereal, Quebec H3COJ7):

Forward Primer (8F) 5´-AGAGGTTTGATCCTGGCTCAG-3´

Reverse Primer (1462)5´-CAGCAGAGGCCTCAGTTCTT-3´

These primers resolved with deionized distilled water and diluted (10 µl primer with 90 µl D.W.) to reach final concentration 10 mole / µl.

PCR Reaction: To prepare samples for PCR reaction; 1- add 11µl of deionized water to each premix tube with well mix.

2- Add 1 µl from diluted two primers (F and R)

3- Add 4 µl of template DNA (25-45 ng / µl) and gently mix

4- For well mixing, centrifugation of eppendorf tube was done by spin in micro centrifuge for (3-5 sec.)

5- Transfer that tubes to thermo cycler apparatus (Sens Quest, Germany) and use suitable program for doing PCR [29]:

Num.	Steps		Temp. ºC	Time
1	Initial denaturation		94	5 min.
2	denaturation	30 cycle	94	30 sec.
3	annealing		54	1 min.
4	elongation		72	2 min.
5	Terminal elongation		72	1 min.
6	Terminal incubation		4	…

A 5 µl of the PCR product was electrophoresed in 2 % agarose using 50 Volt at 75 min.The bands position of 16S rDNA were observed.

DNA Sequence analysis:
In sequencing, sufficient PCR product for DNA sequencing analysis should be prepared. From previously PCR described method, we must select higher amounts of PCR ingredients for amplification. The amplified DNA products contain some impurities such as nucleotides and PCR primer. Sequencing of 16 S rDNA gene in cyanoacterium of our study was performed by Macrogen Company, USA, by sending them DNA sample (PCR product, 20 µl) with primers. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online:

1- Enter this web site, http://www.ncbi.nlm.gov/BLAST(Claverie and Noterdam 2007)[5]
2- In section Basic Blast , please select " Nucleotide BLAST " item
3- Copy the final sequence of the 16 S rDNA gene to the " Enter Query Sequence " box
4- In section " Choose Search Set at Database Port", click other option item
5- Enter the BLAST option.

The result consist of different parts , including Accession number, Description, Max score, Query coverage, E value, and Max identity, which can be used for identification of an isolated bacterium with alignment of its amplified 16S rDNA sequence and other submitted gene sequences published in the gene bank .

Synthesis of silver nanoparticles: We used three separately experiments in biosynthesis of AgNPs, the first one , by using silver nitrate salt($AgNO_3$) to make three concentrations of solutions with (20 ml) double distilled water (1,3, and 5 Mm), then we add (2 ml) of Gloeocapsa sp. culture at (14 days old).The second, by made two concentrations (25 ml) of $AgNO_3$ solutions (1 and 9 Mm) and add (3 ml) of cyanobacterium culture as inoculums. The third, by adding Gloeocapsa sp. as fresh weight cells approximate(0.5gm) obtained from centrifuge of 10 ml liquid culture, and then adding 10 ml of deionized water to make with $AgNO_3$ salt to make that solutions [24]. Log phase culture of microalgae were harvested by centrifugation at 5000 rpm for 10 min. (Cypress Diagnostics, 3201 Langdorp, Belgium, CL008 Centrifuge) at 20 º C and was washed three times with sterile distilled water. All sets of experiments were incubated at 25 ± 2 ºC either under cool white fluorescent light or in the dark conditions for (24, 48 , and 72 h) , as wellas it shaking handily twice a day . As a control, fresh Chu10 medium with cyanobacterium – without $AgNO_3$ – as positive control, and silver nitrate solutions – without cyanobacterium – as negative control ,in the later analyses (UV-Vis and FTIR).

CHARACTERIZATION OF AGNPS:

1-Change of solutions color and optical microscopy [32]: biosynthesis of AgNPs was followed by change of color of solutions that inoculums with Gloeocapsa sp., the darkening of the brownish color was time- dependent. Thus, scientists have identified many species ofbacteria, yeast mold, algae, and plant can synthesize metal nanoparticles [32, 33]. The use of microorganisms in the synthesis of nanoparticles is a relatively new and exciting area of nanotechnology. That operation was identified through the addition of metal salts to the growth media or the cell extract followed by monitoring cells or the medium for the presence of nanoparticles. Thus, that formation could easily be monitored by visual observation of the solution [5].

2- UV-Vis Spectrometry: This tool has proven to be very useful for analyzing of different nanoparticles such as gold and silver nanoparticles (Ramezaniet al. 2008). Observation of strong broad surface Plasmon peaks at visible region (400-600 nm) has been well documented for various metal nanoparticles, with size ranging from 2 to 100 nm. A UV-Visible spectrophotometer consist of two devices, namely a spectrometer for producing light with different wavelengths, and a photometer for measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer [5]. The bio reduction of precursor silver ions was monitored by sampling of aliquots (3ml) at different time intervals. Absorption measurement were carried out on UV-Visible spectrophotometer at wavelength range 190 and 1100 nm [24]. We used UV-1800,SHIMADZU,JAPAN,and spectrophotometer.

FTIR spectroscopy analysis: For Fourier transform infrared spectroscopy (FTIR), air dried biomass was used, and measured using (BRUKER, 10033394, Model ALPHA-P, Germany, OPUS/ATR-Diamond, Laser classic1, ZnSe source IR). A small amount of biomass was grinded and put on a place consist of a sample. All measurements were carried out in the range of 400- 4000 cm^{-1}.

RESULTS AND DISCUTION

IDENTIFICATION OF THE CYANOBACTERIA:

Morphological characters: The cyanobacterium strain was identified under light microscope. Morphological characteristics of it was similar to Gloeocapsa sp. (Fig.1).

A B

Fig (1): Morphological shape of Gloeocapsasp.cells-A and its culture- B

Genetically: analysis of cyanobacterium isolates by 16S rDNA sequencing. The concentration of extracted DNA from cyanobactrium ranged between (120- 46 ng/µl). These samples isolates by wizard Genomic kit. The samples of genomic DNA ware observed when we used gel electrophoresis and determine the molecular size to it when compare its bands with 1 Kb ladder DNA (Fig.2).

A B

Fig (2): A) Genomic DNA isolation from cyanobactrium, B) 16S rDNA amplification by PCR.

The results of 16S rDNA gene sequences showed that , the cyanobacterium belongs to the genus scientific name Gloeocapsa sp. PCC 7428, complete genome with Nucleotide Sequence (581 and 778 letters) , that showed 100% as percentage of similarity , and was submitted to NCBI GenBank under accession number (Sequence ID) CP003646.1

SILVER NANOPARTICLES BIOSYNTHESIS:

Optical observations: The formation of AgNPs was initially observed by the change in color of solutions , that immediately changed from clear transparent with green cells in it to brown then dark (through few hours under light conditions), and changed to dark brown or dark radish (through days under dark conditions) (Fig. 3)

A B

Fig. (3): Color change in different solutions A) under light B) under dark condition

The microscopic view of the cells showed the decrease in chlorophyll by observed yellow color cells compared with healthy cells (in solution without $AgNO_3$ salt) (Fig. 4)

A B

Fig (4): Gloeocapsa sp. cells A) before B) after addition of $AgNO_3$ salts

Previous studies have shown that silver exhibit yellowish –brown color due to the excitation of their Surface Plasmon Response (SPR), when dissolved in water. The solution color change from colorless to brownish yellow, within 50 minutes at 80ºC and dark yellow; this reaction takes up to 2 hour for completion at room temperature [34]. In this study, the change of color done in few hour to solutions under light compared with few days to that under dark conditions .The addition of algal extract to silver nitrate solutions resulted in color change of the solutions from transparent to dark reddish –yellow due to the production of silver nanoparticles. These color changes arise becomes of the excitation of surface Plasmon vibration with the silver nanoparticles. The harvested microalga cell incubated with deionized water (positive control) retained its original colour but the silver nitrate treated cells turned from bright green to dark brown within 24 h. due to the deposit of silver nanoparticles. Algal cells were intact and more or less of the same shape as unexposed cells. In case of silver nitrate solution alone (negative control), no change in colour was observed even after 7 day [25]. The results of our study agreed with that study.Biological synthesis of silver nanoparticles by Lactobacillus sp. VRS2 is primarily confirmed by change of the reaction mixture from pale yellow color indicating the production of silver nanoparticles (Ag^+ to Ag^0). It is reported that reduction of Ag^+ to Ag^0 occurs through nitrate reeducates enzyme. These enzymes released in the solution can reduce the silver nitrate to silver nanoparticles through capping agents such as proteins. The reaction mixture was analyzed using UV-Vis spectrophotometer.

The characteristic brown color due to the excitation of Plasmon vibrations in the nanoparticles provides a convenient signature of their formation [35].Generally, synthesis of nanoparticles is considered to be a result of exposure to toxic substances by secreting extracellular substances to capture the material or mediated through electrostatic interactions. Alternatively, nanoparticles can be formed enzymatically either with extracellular or intracellular enzymes. In the extracellular pathway, the reduction of Ag^+ ions occur through reeducates enzymes and electron shuttle quinines. However, intracellular formation of nanoparticles imparts the nutrient and substance exchange processes. Intracellulary, the ions are reduced by electrons produced by the organisms to avoid damage in the presence of enzymes such as NADH – dependent reeducates. This suggest that, the metabolic status and growth phase of an organism determines its ability to synthesize nanoparticles [24]. In other study, Langke etal. 2007, the formation of extracellular silver nanoparticles by photoautotrophic cyanobacterium Plectonema boryanum, they used silver solution (560 mg/L) with 10 mg dry weight of cyanobacterium at 25, 60 and 100 ºC for up to 28 days in the dark. A grayish – black silver particles adhered to bacterial cells were observed microscopically and the addition of $AgNO_3$ caused the precipitation both inside and outside the microbial cells. The bio reduction of the Ag^+ ions could be associated with metabolic processes utilizing nitrate by reducing nitrate to nitrile and ammonium. Cyanobacteria commonly use nitrate as the major source of nitrogen. Nitrate was reduced by cyanobacteria metabolic process:

$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$
$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O$
It suggest that Ag^+ ions could be reduced by an intracellular electron donor [13].

UV-Vis spectroscopy
Characterization surface Plasmon absorption bands at range 400-450 nm were observed within different concentrations of $AgNO_3$ solutions (Table 1)and Fig(5), for 1-5 Mm and (Table 2) and Fig(6 , 7), for 1 and 9 Mm experiment.

Table 1: O.D. values for Gloeocapsa sp. in different (1-5) mM concentration.

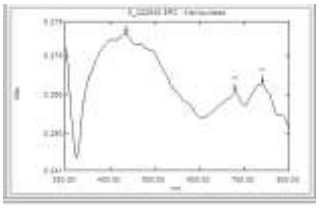
Co.	24 hour				72 hour			
	Light		Dark		Light		Dark	
	Wav.Len.	O.D.	Wav.Len.	O.D.	Wav.Len.	O.D.	Wav.Len.	O.D.
1	740	0.263	-		714	0.232	252	0.637
	680	0.261	-		618	0.231		
	436	0.275	-		554	0.242		
					348	0.287		
3	726	0.323	764	0.178	758	0.234	728	0.141
	438	0.347	724	0.178	674	0.298	688	0.139
	404	0.345	644	0.185	628	0.250	516	0.166

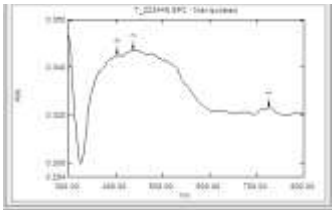
			434	0.237	430	0.267	256	0.360
			410	0.239	370	0.269		
			386	0.241				
5	792	0.277	672	0.194	714	0.275	494	0.174
	708	0.272	418	0.251	616	0.276	258	0.338
	644	0.275			552	0.271		
	616	0.276			350	0.287		
	446	0.282						
	404	0.284						


1 mM


3 mM


5mM





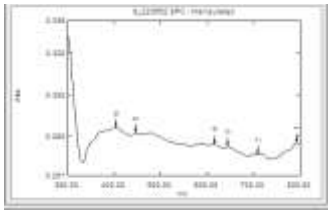
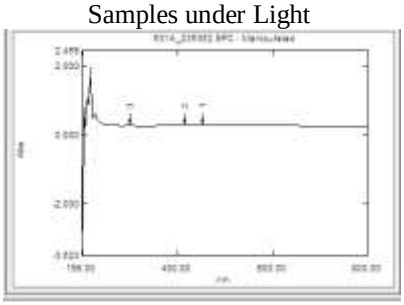


Fig (5): Color changing in different $AgNO_3$ solutions (1, 3, 5 Mm) and their UV-Vis charts

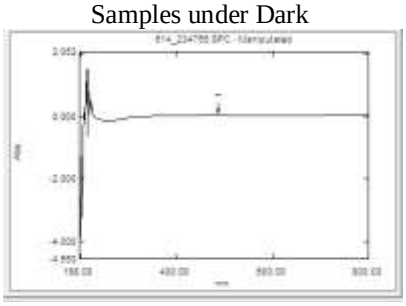
Table 2: O.D. values for Gloeocapsa sp. in (1 and 9 Mm) $AgNO_3$ solutions

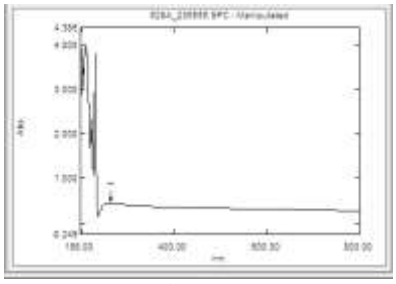
Con.	24 h.				48 h.				120			
	Light		Dark		Light		Dark		Light		Dark	
	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.	W.L.	O.D.
1..1	404	0.474	---	---	586	0.275	314	0.230	260	0.416	212	4.000
	268	0.592			418	0.280						
					308	0.263						
1..2	468	0.463	---	---	452	0.280	486	0.032	260	0.415	---	---
	418	0.463			414	0.280			212	0.354		
					298	0.280						
9..1	268	0.835	258	0.458	324	0.255	262	0.335	262	0.353	260	0.350
					260	0.306						
9..2	274	0.677	262	0.339	264	0.451	262	0.373	212	0.354	258	0.749

Samples under Light

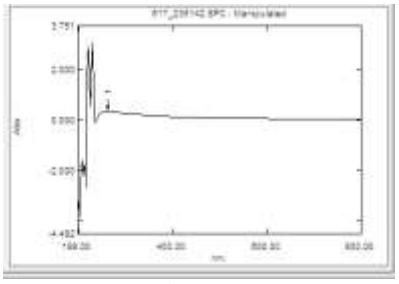

1 mM

Samples under Dark


1 mM



9 mM



9 mM

Fig. (6): UV-Vis spectrophotometer measurement of samples at 48 hours

When Gloeocapsa sp.used to product gold nanoparticles, the study refer to its success of that aim in a few hours and the reduction of gold ions in the sample was due to the reduction of protein present in the algal sample. In Fig. (4), the normal cells have blue-green color but after adding $AgNO_3$, they have pale yellow color. However, silver has shown inhibit algal photosynthesis [(36)]. Also, exposure of intact cells of Spirulina to silver ions (5-15 µM)caused decrease in the phycocyanin absorption. The phycocyanin is the major susceptible pigment protein for silver ion action [(37)]. Another study, used dry thalli that shade dried then ground to powder in a glass mortar to biosynthesis of gold nanoparticles using green alga Parsiola crispa[(38)]. The cyanobacteria Phormidium valderianum, P. tenue and Microcoleus chthonoplastes produced gold nanoparticles intracellular, confirmed by purple coloration of the thallus within 72 h. of treatment at 20 ºC [(39)].

The extracellular biosynthesis of AgNPs by marine cyanobacterium Oscillatoriawillei was studied [(40)]. Reduction of silver ion by culture filtrate of a filamentous cyanobacterium Westiellopsis sp. and maximum absorbance at 420 nm in UV-Vis spectroscopy [(41)]. The cyanobacterium Leptolyngbyavalderianum was found to be an effective bio reagent for Nano silver production after 72 h. of dark exposure in 9 Mm $AgNO_3$ solutions [(21)]. Spirulina platensis also capable for the formation of silver nanoparticles (SNPs) and showed an absorption peak at 430 nm in UV-Vis spectrum [(42)]. Almost of these studies had doing recently that refer to the important of this new field of blue-green approach for biosynthesis of metal nanoparticles. The results in Table (1) showed that, the best concentration from $AgNO_3$ solutions for nanoparticles production was (3mM) because that production happen under light and dark condition at 24 hours and continuous in production to 72 hours under light depending on the samples that showed a peak in the range between 400 and 450 nm in the absorption spectra, were identified as nanoparticles production sample [(21, 24, 33)].

As well as, from the results we observed, the production of nanoparticles was best under light condition than dark once. This strains of cyanobacteria (7strains) and green algae (4strains), all of them were identified as those capable of producing Ag-NPs under light condition. However under dark condition, only (3cyanobacteriastrains) and one green algal strain produced Ag-NPs. When the same experiment done with cell-free culture liquid, in most of the cases Ag-NPs formed in the absence of biomass in light but not in dark condition [(24).] The absence of specific surface Plasmon of silver nanoparticles in results of samples at 72 hours except 3 mM light sample, may be due to further incubation that lead to decrease in intensity indicating complete reduction of silver ions.

The observation of strong but broad surface Plasmon peak has been well known in the case of various metal nanoparticles over a wide size range of 2-100 nm by kowshiket.al. 2003, Shanker et.al. 2003 suggested that the shoulder at 370-390 nm corresponded to the Transverse Plasmon vibration in silver nanoparticles, whereas the peak at 440 nm due to excitation of longitudinal Plasmon vibrations [(43)]. The low wavelength region recorded from the reaction medium exhibited an absorption band at 265 nm and it was attributed to aromatic amino acids of proteins, it was well known that the absorption band at 265 nm arises due to electronic excitation in tryptophan and tyrosine residue in the protein[(40)].

Metal nanoparticles such as silver and gold have free electrons, which gives rise to surface Plasmon response (SPR) resonance band of biogenic AgNPs occurred at 420 nm and 416 nm for reaction carried out at 80 ºC and room temperature, respectively [(34)]. Many studies used this technique in characterization of AgNPs at 430-440 nm with microalgae Chlorococcum[(25)], 450 nm[(12)], 400-450 nm[(24,33)], 420 nm[(35)], 411 nm[(20)], 425-440 nm[(44)], 421 nm[(45)], 427-432 nm[(46)], 420-430 nm[(47)], 440 nm[(48)] and at 386 nm[(43)].Whereas Shanker et al. suggests that shoulder at 370 nm corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 440 nm due to the excitation of longitudinal Plasmon vibrations [(40)]. A unique property of spherical silver nanoparticles is that SPR peak wavelength can be turned from 400 nm (violet light) to 350 nm (green light) by changing the particle size and the local refractive index near the particle surface [(49)].

Since the instability and aggregation of AgNPs increased with incubation time an optimum reaction time is desirable. Silver nanoparticles tend to agglomerate after certain reaction time which caused the formation of larger nanoparticles. Absorbance of colloidal silver solution improved upon increasing the reaction time and maximum absorption was

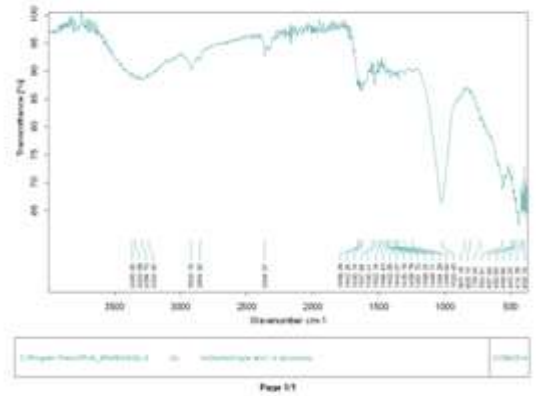
observed after 12 h., aggregation of AgNPs was observed (through broading of absorption spectra) after 24h. reaction, which could be due to the instability of AgNPs [51]. The reduction of Ag ions and the resulting growth of $Ag^{(0)}$ must have driven by some active species in the algal extract, it observed that the graph suggests that the formation of AgNPs is exponential in nature within 48 hours of reaction [25].

FTIR SPECTROSCOPIC ANALYSIS:

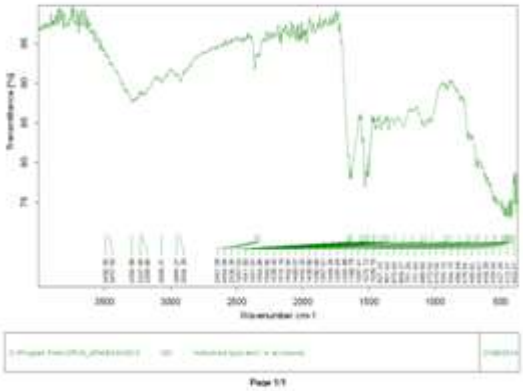
FTIR measurements were carried out to identify the possible biomolecule responsible for the reduction of Ag^+ ions and capping of bio reduced silver nanoparticles by cyanobacterium Gloeocapsa sp.(Fig.7,8), that manifested absorption peaks located from 400 $cm^{-1}$to 4000 $cm^{-1}$and indicated the specific functional groups. For the samples (1 and 9 Mm) $AgNO_3$ and Gloeocapsa sp. without $AgNO_3$ under light and dark conditions were recorded in (Table 3).

Fig. (7): Color changing in (1 Mm) $AgNO_3$ solutions (GD, CD, 1D –on the left, 1L, CL, GL – on the right)

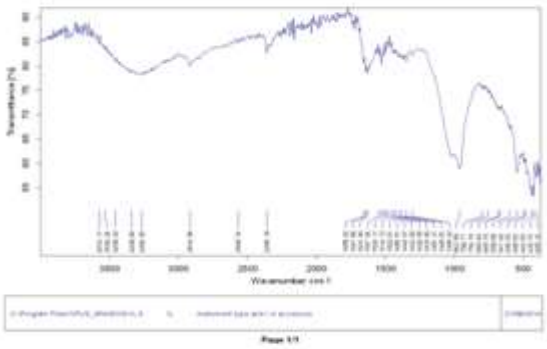
GD=Gloeocapsa without $AgNO_3$+Dark, CD=$AgNO_3$ in Dark, 1D=Gloeocapsa+1$AgNO_3$ in Dark,1L=Gloeocapsa+ 1 $AgNO_3$ in Lightly=$AgNO_3$ in Lightly=Gloeocapsa without $AgNO_3$+ Light.



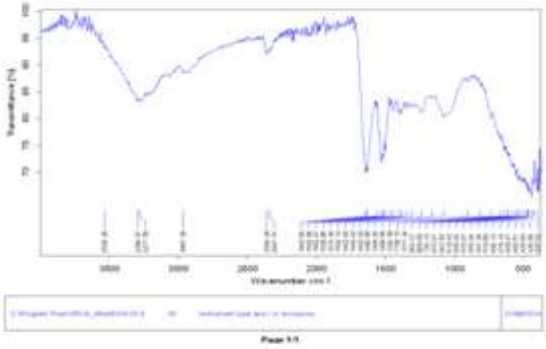
Gloeocapsa sp. without $AgNO_3$ under light



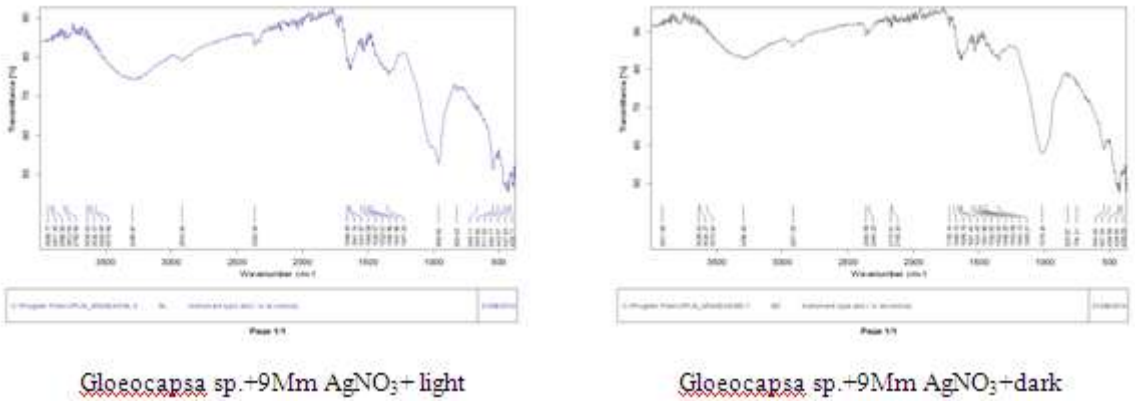
Gloeocapsa sp. without $AgNO_3$ under dark



Gloeocapsa sp.+1Mm $AgNO_3$+ light



Gloeocapsa sp.+1Mm $AgNO_3$+dark



Gloeocapsa sp.+9Mm $AgNO_3$+ light Gloeocapsa sp.+9Mm $AgNO_3$+dark

Fig. (8): FTIR analysis for samples of Gloeocapsa sp. under different conditions

Table 3: FTIR peaks and their represented functional groups

group	GL	1L	9L	GD	1D	9D
O-H Stretching of H-bonded alcohols and phenols		3458	3573	3458		
	3342*	3339				
		3267*	3296*	3294*	3295*	3295*
				3228		
				3209		
				3069*		
O-H Stretching of carboxylic acids	2922*	2917*	2917*	2924*	2967*	2917*
	2855*					
	2364*	2359*	2363*	2361*	2360*	2360*
				2344	2342	2340
				2326		
N-H Bending of primary amines	1658	1658	1658	1658		1658
	1642*	1642*	1642*	1642*	1643*	1641*
	1631	1631	1631	1632	1633	1631
Related to C-C stretching				1565	1564	
	1549	1548	1548	1545		1548
	1530*	1530*	1531*	1529*	1530*	1530*
				1515	1515	
GROUP	GL	1L	9L	GD	1D	9D
C=O bending amines				1503	1503	1502
C-N Stretching vibration of aromatic and aliphatic amines	1484	1485	1484			1484
	1462	1462	1462	1469	1463	1462
				1452		
	1443	1443			1443	
	1426	1427				
				1404	1402	
	1382	1381		1391	1384	
	1368			1368		
	1348*	1350*	1347*	1348*	1346*	1349*
		1306		1305	1310	
	1250			1234*	1235*	
				1169	1159	
				1097		
				1075*	1077*	
	1023*	1014**	1014**	1030		1019**
		963	960	921		
				902	902	
	845		824	876		825
	809	799		809	810	
		756		751*	773*	754*

	729			732		
		683		686		
		670		670		
	564*	547*	546*			545*
	469		465			
	440*	443*		439	440*	439*
	414	427*	427*			

*Distinct peak, ** peak shoulder, GL=Gloeocapsa sp. +Light, GD= Gloeocapsa sp. +Dark, IL=GL+1Mm $AgNO_3$, 9L=GL+9 Mm, 1D=GD+1Mm, 9D=GD+9Mm

The FTIR spectrum was recorded from the samples with (1 and 9 9Mm) $AgNO_3$ after 2 days of incubation with the cyanobacterium Gloeocapsa sp. (Fig.7). The results showed in (Table 3) refers to the bands in Gloeocapsa sp. without adding $AgNO_3$ under light and under dark condition, as well as after adding $AgNO_3$ to it at 1 and 9 Mm. From the above results we showed that, the disappear of silver nitrate which confirmed the reduction of it to metal Ag. The silver nanoparticle have relatively high energy, because that it try to decrease it by molecular interaction that occur by produce physical linkages with the nitrogen present in cyanobacterum. The evidence on above, was the increase of absorption energy for (O-H, N-H, C-N) Linkage due to the molecular interactions that increase the vibration energy compared with samples without adding $AgNO_3$ (GL, GD).

From Table 3, the distinct peaks of 1L (best sample in UV-Vis) seen at (3339, 3267 and 2917 cm^{-1}) were assigned to the stretching vibration of primary and secondary amines (O-H), respectively [40, 42, 50]. The corresponding bending vibrations were seen at (1658, 1642) and (1548, 1530) cm^{-1}respectively [40]. The peak at 2359 cm^{-1}indicates carbonyl specific absorption [44]. The bands at 1350 and 1014 shoulder of band 956 cm^{-1} can be assigned to the C-N stretching vibration of aromatic and aliphatic amines respectively [40]. The peaks at 799 and 756 cm^{-1} corresponds to C-H stretching of aromatic compounds, and the peaks at 683 and 670 cm^{-1} could be assigned to C=Othe stretching of carbonyl groups. The peaks at 444 and 427 cm^{-1} are related to AgNPs bonding with oxygen from hydroxyl groups. This results very close to that in (45) study. This results agreed with many studied used other species of cyanobacteria such as Oscillatoriawillei [40]. In addition, the presence of bands at that spectral range suggested that, the capping capping agent of biosynthesized nanoparticles possesses an aromatic amines groups with specific signatures of amide linkages between amino acid residues in the proteins in the infrared region of the electromagnetic spectrum.

The presence of a protein type of compound on the surface of biosynthesized nanoparticles, confirming that metabolically produced proteins acted as capping agents during production and prevented the reduced silver particles agglomeration [47]. The silver ions were reduced in the presence of nitrate reeducates, leading to the formation of stable silver and stabilized by the capping peptide [40]. Proteins can bind to nanoparticles either through free amino groups or different residues in the proteins and through the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of protozoa, therefore stabilization of the nanoparticles occurs by proteins[51]. The study used microalgae Chlorococcum[25], FTIR analysis of algal biomass before and after bio reduction of Ag showed bands for cell components like proteins, lipids and carbohydrates. Biomass after bio reduction, showed shift in few peak positions and appearance of new peaks at 1350 and 1598 cm^{-1} .

There are a few studies in biosynthesis of AgNPs by using microalgae especially cyanobacteria Gloeocapsa sp., and most of it used his cellular extract or cell-free liquid but not biomass to formation the nanoparticles.Recently, few of the researches reported that microalgae have been used for the production of metallic nanoparticles [52]. One of these studies, showed that one of microalgae was used,Gloeocapsa sp. biomass (15 days), and it does not formed AgNPs under dark condition [52].

CONCLUSION

It is concluded that the collected cyanobacterium strain from freshwater was Gloeocapsa sp. PCC 7428 and have sequence ID (Accession number) in NCBI GeneBank, cp003646.1, that identified by 16S rDNA gene sequencing. This strain of cyanobacterium showed the ability for biosynthesis of silver nanoparticles under light and dark conditions with exhibit the best production in light comparable with dark condition. This cyanobacterium is good source for eco-friendly and economically low cost for nanomaterial's production. The extracellular producing of AgNPs by Gloeocapsa sp. is an efficient and simple process and the producing AgNPs are quite stable in solution due to capping of silver nanoparticles by proteins secreted from the cyanobacterium.

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