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Biosynthesis of gold Nanoparticles by *Lyngbya sp.* Clone Zen / Mira 16S ribosomal RNA gene and its antibacterial activity

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Abstract---Different methods to biosynthesis of Gold nanoparticles was done with using cyanobacterium Lyngbya sp., and salt gold ($HAuCL_4$) and evaluate their antibacterial activity. Gold nanoparticles were prepared in different ways, dry and fresh weight of this cyanobacterium to obtain aqueous extract. The synthesized nanoparticles were characterized using analytical techniques such as UV-Visible spectroscopy, SEM (Scanning Electron Microscopy), FTIR (Fourier Transform Infrared) and XRD (X-Ray Diffraction). UV-visible spectroscopy show a Surface Plasmon Resonance (SPR) peak at (532-552) nm; confirmed by (SEM) image revealed the spherical shape and the size range from (39.1-61.9) and (59.4-84.0) nm for dry, wet aqueous extract respectively. FTIR result showed containing of (phenol, protein and carbonyl) as afunctional group. The crystalline nature of the nanoparticles was evident from Peaks in the (XRD) pattern. The prepared gold - nanoparticles show antibacterial activity was carried against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Kliebsella pneumonia* using turbidity methods, aqueous extract from fresh weight showed the highest effect as,(0.004 , 0.001 , 0.005 , 0.001) for *Escherichia coli , Kliebsella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus ,* respectively , and the effect of the aqueous extract prepared by dry weight the highest value was (, 0.048 , 0.022, 0.028 , 0.000) for *Escherichia coli ,*

Kliebsella pneumonia, Pseudomonas aeruginosa and *Staphylococcus aureus,* respectively.

Keywords---biosynthesis, gold nanoparticles, ribosomal RNA gene, antibacterial.

Introduction

Cyanobacteria are smallest, simplest and ancient members of photosynthetic organisms on earth, some are found in thermal condition, or in hot springs others are observed in low temperature regions (1). Cyanobacteria include more than 2000 species in 150 genera, they are different in size and shape. They are (photoautotrophic) gram-negative prokaryotes microorganisms, which show connective link between eukaryotes and prokaryotes. They are source of peptides, fatty acids , vitamin , minerals , amino acids and pigments like chlorophylls , carotenoids which have different color (2). Cyanobacteria are good source for synthesis of NPs due to their potential to produce metabolites (3) various nanoparticles are fabricated through metabolites of cyanobacteria silver Ag , gold Au , platinum ….etc. (4)

Nanotechnology is a science producing materials at the nano-scale size (1-100) nm (5). Nanoparticle can be synthesized by different methods chemical, physical and biological methods (6) interesting increased in biological methods (green) nanotechnology which using various microorganisms such as bacteria, fungi, plants, cyanobacteria and macro-algae (7). Green methods eco-friendly, effectiveness and they offer promising alternative antimicrobial and anti- cancer (8). Lyngbya is used in this study for biosynthesis of gold nanoparticles it is filamentous, unbranching, filament are inter woven to form an expander, they reproduce asexually, it belong to oscillatoreales order and oscillatoriaceae family (9).

Material and Methods

- Preparation of metallic salt solution:
  Aqueous solution of chloro auric chloride acid ($HAuCl_4$) (1) mM was prepared by dissolving (0.170) gm of chloro auric chloride in deionized water and used for the synthesis of gold nanoparticles
- Collection and identification for *Lyngbya* :
  Lyngbya cyanobacterium was detected morphologically as well as genetically using pcr technique. The pure culture is maintain on solid medium and grow in liquid Chu10 media that incubated at 25±2 with light period density 2500 lux (10)
- DNA extraction
  DNA was extracted from cyanobacterium *Lyngbya* using commercial purification system Genomic DNA mini kit (Geneaid, Taiwan)
  - Take cyanobacterial precipitate in liquid media about 1×10^9 cell to eppendrof tube 1.5 ml.
  - Add 200 microliter of Lysozyme enzyme concentrated 0.8mg/200 ml mixing with vortex.

- Incubated at 37 °C for 30 minutes , invert the tube every 3 minuets.
- Add 20 microliter of proteinase K and mix with vortex.
- Incubate the mixture at 60 °C for 10 minutes.
- Add 200 microliter from GB buffer solution and mixed by vortex then incubate at 70 °C.
- Add 200 microliter of absolute ethanol mixing with shake then transfer the mixture to the GD column that placed in collection tube.
- The mixture was centrifuge at 16000 g for 30 second ,the supernatant then get rid then add 600 microliter of washing solution and centrifuge at the same speed and time and remove the precipitate then centrifuge for 3 minute to remove all the wash solution residue .
- The GD column transfer to the tube(1.5 ml) and add 100 microliter from dissolving solution then left for 3 minutes and then centrifuge at 16000 g for 30 second and then DNA kept until use .

DNA Electrophoresis

DNA samples were prepared by mixing (5 ml) of it with (3 ml) of loading solution, the electrophoresis process was done in electric current (5 volt / cm) for 1.5– 2 hour then we photographed the gel under UV- ray using Gel Documintation for DNA bands viewing. Molecular diagnosis of cyanobacteria based on 16 srRNA region The presence of 16S rRNA region was detected by adding (4) microliter (100) nanogram of template DNAand (1) microliter(10 picompl) of each gene – specific primer were added to the content of the premix

primer	Sequence
Forward	5 -ACGGGCGGTGTG-3
Revers	5 -TTGGGCGTAAAGCGT-3

PCR Thermocycler quantities: PCR Thermo cycler quantities were perform by using conventional PCR Thermo cycler described in (11)

Preparation of aqueous extract of cyanobacteria :

To prepare the aqueous extract by wet weight method the biomass was collected and crushed by a sterile ceramic mortar until It was smooth , to obtain the cell extract (10) gm of fresh biomass dissolved in (100) ml of deionized water using stirrer for (1) hour (12) , to prepare the aqueous extract of dry weight the biomass was collected and dried in oven at (40)C for 24 hour in the next step the dried biomass was crushed by sterile ceramic mortar until it was smooth and to obtain the cell extract add (1) gm of dry weight and dissolved in (100) ml of deionized water and in (100) ml of methanol using stirrer for 24 hour at room temperature (13) and then the solution in to casses was filtered through whatman no. 1 filter paper and the bacterial cell extract was obtained, and the extract kept in refrigerators at (4) C until used.

Synthesis of gold nanoparticles

The synthesis of nanoparticles was done by using the ratio 1:1 of cyanobacterial extract : chloro auric chloride (HAucl4) (1) mM (13) using magnetic stirrer at (80) C for (15) min until color of the reaction mixture change from pale yellow to purple (Fig 3) .

Characterization of gold nanoparticles:

The synthesized gold nanoparticle was detected by using UV-vis spectrophotometer at 200-800 nm length. As well as the FTIR technic was used to determine the functional groups that contributed to the preparation of the gold-nanoparticles SEM (scanning electron microscopy) (Inspect F 50 FE – SEM) from FEI. American company, was used to image and evaluate the morphology and size of the gold nanoparticles and to study the structural properties. Additiontheally XRD (x –ray Diffraction) was used to determine the crystalline nature of the AuNps after interacting with frequently and regularly distributed crystalline materials.

Antibacterial activity assay

The synthesized gold nanoparticles as antibacterial activity of synthesized AuNps were studied using the turbidity methods (15) .

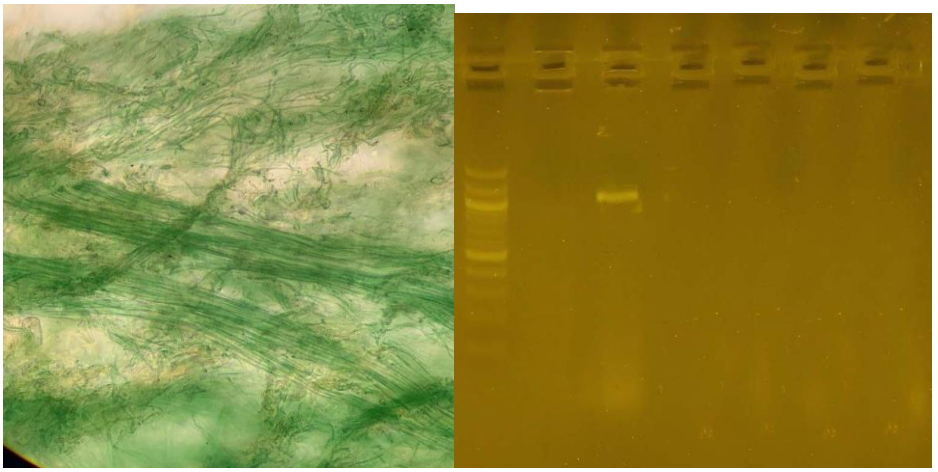
Statistical analysis

Because our samples are known parametric the statistical methods used to analyze the result was the Kruskal and the result show significant difference in the antibacterial activity.

Result and Discussion

Morphological identification

Filament are straight or slightly undulating several species are finely screw like or coiled (a few free floating species)solitary mainly arranged in thin or thick flat compact large layered ,leathery prostrate mats on the sub straight and very false branch , they are wider than 6mm, sheathes are always present and attached to trichome , distant thin or thick , colorless ,yellow – brown or reddish ,some time lamellate containing one motile trichome ,which is cylindrical and may constricted at cross walls . cell are shorter and with out aero topes , apical cell have thickened outer wall , reproduction via trichome disintegration in short motile hormogonia , some species are terrestrial or subaerial occurring on wet rocks , several are cosmopolitan .(17)



Microscopic photo Genome extracted

Fig 1. Morphological and Genetical identification of *Lyngbya*

Genetical identification

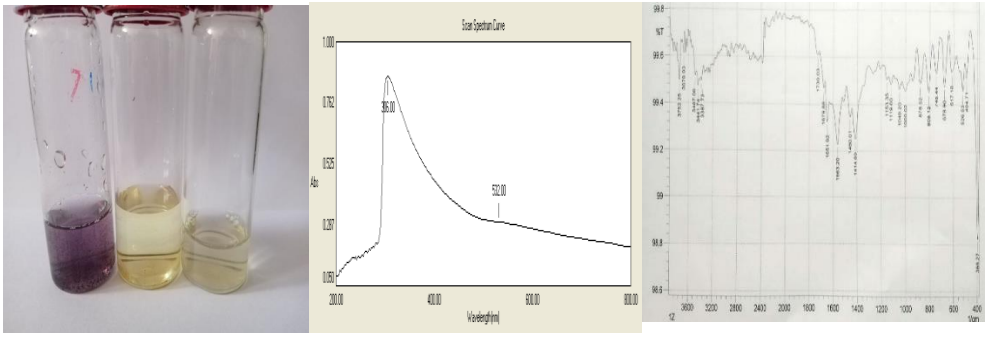
GCCTGAGATGGAGCTCGCGTCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAA
GGCAACGATCAGTAGCTGTTCTGAGAGGAAGAGCAGCCACACTGGGACTGAGACA
CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAAG
CCTGACGGAGCAATACCGCGTGAGGGAGGAAGGCTTGTGGGTTGTAAACCTCTTTT
CTCACGGAAGAAGATCTGACCGGACCAAAGGAATCAGCATCGGCTAACTCCGTGC
CCCCAGCCGCGGTAAACGGAGGATGCAAGCTTTATCCTGAATAATTGGGCGTAAAG
CGTCCTTAGGTGGTACTTCAAGTCCGTTGTAAAAAACGAGGGCTTAACTCTGGACA
GGCACTGAAAACTGATGAACTACAGTAGGGTTGGGGTAGAGGGAATTCCTAGTGTA
CCGGTGAAATGCTTAGATTTTAGGAAGAACATCATGGCGAAGGCGCTTTACTGGCA
CTGAACTGACCCTGAGGGACGAAAC

Fig 2. Sequencing 16S rDNA of *Lyngbya sp.*

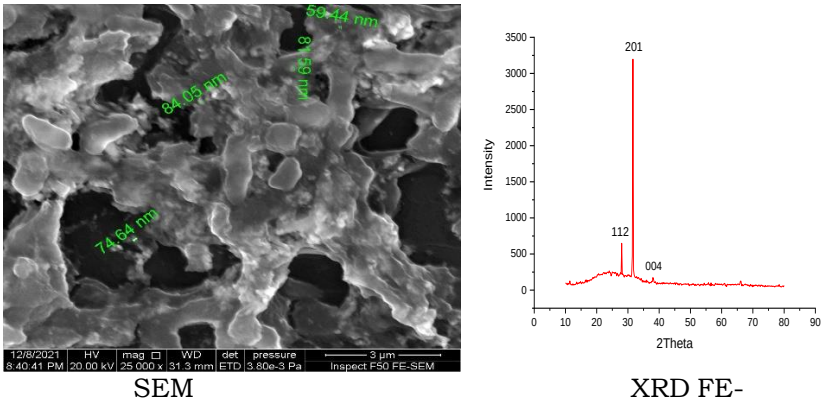
The change of color for AuNps samples was considered as the primary detection of synthesis for AuNps, the final color of extract after (1 h). Was purple at (500) nm. as seen in Fig (3 a and b) The result of UV- visible at various waves length (200 - 800 nm) refer to Plasmon resonance (SPR). This result agree with (13). UV –vis. Spectra of green synthesized AuNps. demonstrated peak at (532 -552) nm for Nano synthesized from wet and dry extract indicating the production of gold nanoparticles as seen in Fig (3) (c and d), the formation of purple color would be due to the reduction of Au^{+3} to Au^{0} (17). The aggregation of gold nanoparticles in the solution which possibly produced by biomolecules protein and enzyme on the surface of cyanobacterial cell (18)

FTIR analysis for AuNps synthesized by dry and wet aqueous extract showed particle obtained from Lyngbya sp. the presence of major peaks at 3453 cm-1 ,3421, 3752, 3679, 3441,3387 cm -1, is due to (O-H) stretching vibration of phenolic compounds (19) peaks a t 2926 cm-1 related to (C-H) aliphatic ,and 2375 cm-1 (C-O) stretching vibration of carbonyl .Whereas the band at (1649 , 1651)cm-1 corresponding to the stretch band of carbonyl groups . and the observed band at (1460, 1414) cm -1 due to (NO_2). Scanning electron microscope (SEM) image of the synthesized gold nanoparticles show spherical nanoparticles of nano size ranged from (39.1 – 61.9) and from (59.4 – 84.0) nm for Nano prepared from wet and dry aqueous extract respectively, Sem image used to determine the size and the shape of nanoparticle and this indicate the ability to synthesized AuNPs from cyanobacteria.

In order to confirm the presence of gold nanoparticles collected in the Crystals form use (XRD) examination was done,(fig ,6) in aqueous extract prepared by fresh weight show the semi crystalline nature of the particles with small shape peak at 20=(38 ,45 , 64) corner at (111, 200 ,220) corresponding to the crystalline levels of the gold Nano particles according to Braque's law (21) dry weight show sharp and narrow peaks of the diffraction which indicate the material has crystalline structure , the peak are obvious at the corner ((27.86 , 31.4 , 38 C) and according to Braque's law (112 , 201 , 004) improved that the component gold has a crystalline nature (18) as seen in Fig (5)

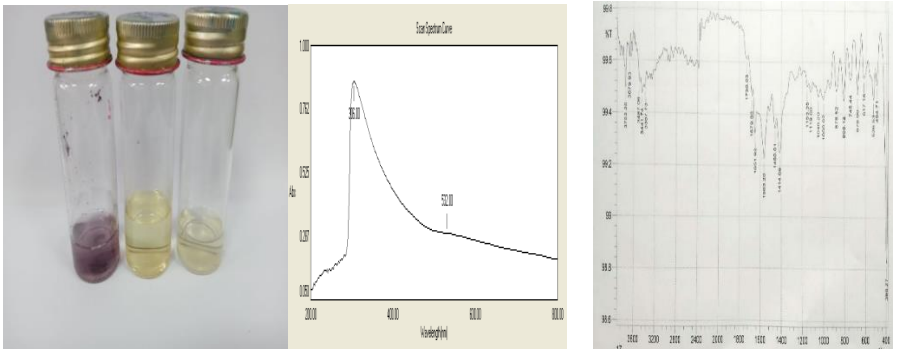


UV-vis purples colored FTIR

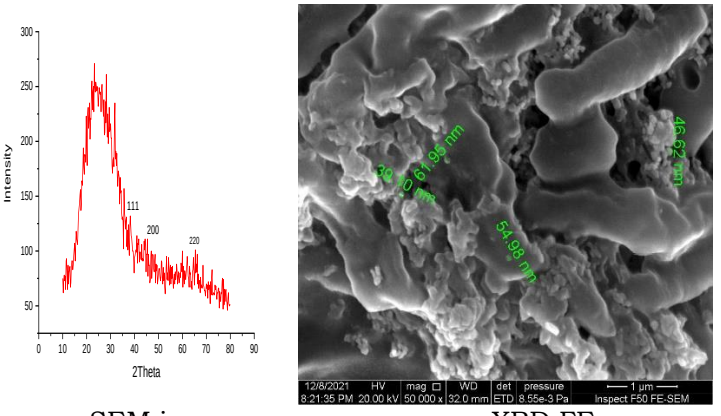


SEM XRD FE-

Fig 3. Characterization of gold nanoparticles of dry *Lyngbya* aqueous extract



FTIR purples colored UV-vis



SEM image XRD FE-

Fig 4. Characterization of gold nanoparticles of fresh *Lyngbya* aqueous extract

The result of our study show that the AuNps synthesized by the aqueous extract have antibacterial activity against gram- positive and gram-negative bacteria and the most sensitive bacteria wa s *Staphylococcus aureus* with an inhibition rate 0.000 when using the nano from aqueous extract prepared by dry weight as well as when we use the nano prepared from freh weight show good effect against pathogenic bacteria with an inhibition rate 0.001 % for *Kliebsella pneumonia* and *Staphylococcus aureus,* the reason was the presence of biologically active compounds in the nano prepared from cyanobacterium (22). or nano penetration of the bacterial cell which will increase the accumulation of nanoparticles inside the cell and therefor the bacterial cell will be destroyed (23).

Table 1
Effect of aqueous extract of cyanobacterium *Lyngbya* on different bacteria

Name of bacteria	Dry weight extract sig*	Fresh weight extract sig*
E-coli	0.048	0.004
Klebsiella	0.022	0.001
Pseudomonas	0.028	0.005
Staphylococcus	0.000	0.001

Sig*: significant at 0.05 %

Conclusions

Cyanobacteria are important source of chemical compounds that are used to reduce metal ions and synthesis nanoparticles. The result of the study showed that the cyanobacterium *Lyngbya sp* . is efficient in the synthesizing of gold nanoparticle by using green synthesis method which is attractive , non -toxic and economical method and the gold nanoparticle which prepared in this study showed good effect on different types of pathogenic bacteria,and the most sensitive bacteria was *Staphylococcus aureus* with an inhibition rate of 100% percent.

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