

Biosynthesis of gold nanoparticles using marine *Streptomyces griseus* isolate (M8) and evaluating its antimicrobial and anticancer activity

Moaz M. Hamed* and Lamis S. Abdelftah

National Institute of Oceanography and Fisheries, Egypt.

* Corresponding author: moaz-micro@hotmail.com

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ABSTRACT

The implementation of actinomycetes for the biosynthesis of metal nanoparticles as a nature-friendly, safe and hopeful way is welcome due to its non-toxicity and naturalness. Out of nine actinomycetes isolates was isolated from sediment of the Suez Gulf, Egypt, only one isolate (M8) showed ability for biosynthesis of gold nanoparticles using extracellular supernatant. Isolate (M8) was selected and identified as *Streptomyces griseus* on the basis of cultural, morphological, and physiological properties, additionally 16S rRNA sequence. The gold nanoparticles were confirmed using by Visible UV spectrophotometer, Fourier Transform Infrared (FTIR) Spectroscopy and Transmission Electron Microscopy (TEM). Au-Nps ranged from 19 to 28 nm in size and hexagonal in shape. Au-NPs synthesized by *Streptomyces griseus* isolate (M8) showed displayed a significant antimicrobial activity against gram positive bacteria *Enterococcus faecalis* 29212 (10 mm), gram negative bacteria *Escherichia coli* 19404, *Pseudomonas aeruginosa* 9027, *Salmonella typhimurium* 14028, *Vibrio fluvialis*, and *Vibrio damsela* (10, 20, 28, 25 and 18 mm respectively), and yeast *Candida albicans* (18 mm) in well diffusion method. Moreover, gold nanoparticles exhibited a significant degree of anticancer activity against two different cancer cell lines Colon carcinoma cells (HCT-116) using 61.9 ug/well and breast carcinoma cells (MCF-7) using 46.6 ug/well.

INTRODUCTION

Nanobiotechnology is one of the most hopeful areas in newfangled Nano-science and technology. This emerging area of research interweave various specialties of science such as chemistry, biology and physics (Narayanan and Sakthivel, 2011) The nanoparticles have nonsuch properties of showing larger surface area to volume ratio, size, shape like spherical or rod, etc. due to which they are being used in the different fields of diagnostic biological realization, optoelectronics, show devices , catalysis, biological sensors, diagnosis of diseases like tumor cells, drug detection, detecting environmental toxic metals or reagents and in curative applications (Honary *et al.*, 2012). In recent years, the biological entities like microorganisms, enzymes and plant extracts has generated a great interesting for using it to synthesis of nanoparticles, because itsunusual opticalproperties, chemical, electrochemical and electronic properties, in addition, its eco-friendly, safe, reliable

and cleanly compared with chemical and physical methods (Narayanan and Sakthivel, 2011).

The actinomycetes have been recognized as prokaryotes and can be readily modified genetically for the accomplishment of better size and poly-dispersed nanoparticles (Ahmad *et al.*, 2003). The actinomycetes have a high similitude with the fungi and the characteristics of prokaryotes such as bacteria. They are presently being used in the nanotechnology as they can be produce secondary metabolites such as antibiotics (Zhang *et al.*, 2011). The biosynthesis methods are ordinarily applied for any compound production from microorganisms, which may be produced sufficient amount in terms of quantity. Few research are available on the biosynthesis of nanoparticles using extracellular supernatant by the actinomycetes (Sastry *et al.*, 2003) and intracellular (Ahmad *et al.*, 2003b).

The aim of this study is gold nanoparticles biosynthesis from marine actinomycetes extracts isolated from Suez gulf, Egypt, and studying their potentialities as antimicrobial and anticancer agents.

MATERIALS AND METHODS

Sample collection

The marine sediment sample was collected from Suez gulf, Egypt at 10 m length and 5 m depth using piston corer in clean plastic bags. The collected sediment samples was stocked in ice bag and then transported to the laboratory for bacteriological analysis within 2 hours.

Isolation and purification actinomycetes isolates from marine sediment

The medium used for the isolation and cultivation of marine actinomycetes was starch nitrate medium comprise 20 starch, 0.5 K₂HPO₄, 1 KNO₃, 0.5 MgSO₄ 7H₂O, 0.01 FeSO₄, 15 agar, sea water 50ml and distilled water 50ml, pH 7 and incubation was performed at 30-32°C for seven days. (Abou-Elela *et al.*, 2009). After autoclaving and before solidification, the medium was supplemented with 20 and 50 µg of nystatin and tetracycline respectively as antifungal and antibacterial agents to inhibit the fungal and bacterial contamination. 10 grams of marine sediment was transferred into 250 ml Erlenmeyer conical flask containing 100 ml of sterilized saline solution, and were shaken for 20 min and plates of starch nitrate agar medium were inoculated with 1 ml of the samples. To purify the marine actinomycetes colonies, Streak Plate method was using by repeated streaking pure colony on Starch nitrate medium by using separate petri plates and then sub cultured to ensure for their axenicity. (Williams and Cross, 2003) Pure culture was transferred on slants and preserved at 4°C for further analysis (Kokare *et al.*, 2004).

Synthesis of gold nanoparticle from actinomycetes isolate

50 ml aqueous solution of 2.0 mM from chloroauric acid solution (HAuCl₄) were separately treated with 50 ml of actinomycetes isolate supernatant solution in a 250 mL Erlenmeyer flask (pH 4, rpm 130 and 40°C) (Prakash *et al.*, 2013). The reaction mixtures were incubated at room temperature for 4 h in dark condition. Control experiments were conducted with un-inoculated media, to check for the role of actinomycetes in the synthesis of nanoparticles. After incubation period colour change was observed.

Characterization of metal nanoparticles

UV-Visible spectroscopy analysis

The bioreduction of gold ions was observed by the changing of color from yellow to red. Moreover, it was assured by presence of sharp peaks observed by the

absorption spectrum of the sample by using UV–Vis spectrophotometer (Double Beam Spectrophotometer 6800 JENWAY). 2ml of the sample was taken in a quartz cuvette and measured in the range of 200 to 800 nm. (Soltani *et al.*, 2015).

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform infrared (Bruker Tensor37) analysis was done. All measurements were carried out in the range of 500 –3500 cm⁻¹ at a resolution of 4 cm. It was carried out at the Central Laboratory, Faculty of Science Alexandria University.

Transmission Electron Microscopy (TEM) analysis

Transmission Electron Microscopic (TEM) was used to observe the size, shape and morphology of the resultant nanoparticles, type JEOL JEM 2100 High Resolution Transmission Electron Microscope, operating at 200kV. It was carried out at Electron Microscope Unit, Faculty of Science, Alexandria University.

Identification of the most potent actinomycetes isolate

The selected actinomycetes isolate was identified according to its morphological and physiological characteristics subordinate the methods of Shirling and Gottlieb (1966). The isolate was then identified according to 16S rRNA gene sequencing studies.

Genotypic characterization

DNA of the selected actinomycetes isolate was extracted and purified. The region of 16S rDNA was amplified using either species universal primers. Genotypic characterization was carried out by using 16S sequence analysis. Finally, the multiple alignments with sequences of most closely related members and the levels of sequence similarity were performed using Bioedit (Hall, 1999). For comparison, Sequences of rRNA genes were detected from the database of NCBI. The DNA sequences were aligned and phylogenetic tree was constructed by neighbor joining method using ClustalW software (Saitou and Nei 1987).

Antimicrobial activity of the biosynthesized AuNPs

Screening for antibacterial activity of biosynthesized AuNPs was carried out by well diffusion method on nutrient agar plates against selected pathogenic bacterial indicators (*Pseudomonas aeruginosa* 9027, *Escherichia coli* 19404, *Staphylococcus aureus* 25923, *Enterococcus faecalis* 29212, *Salmonella typhimurium* 14028, *Bacillus cereus*, *Vibrio fluvialis*, and *Vibrio damsela*) were obtained by the research members of the National Institute of Oceanography and Fisheries (NIOF) , Alexandria branch, Alexandria, Egypt. The wells were loaded with 100 µl of biosynthesized AuNPs nanoparticles solution on and 100 µl of culture broth from actinomycetes isolate cell free supernatant without HAuCl₄ as a control. The dishes were incubated at 37 °C for 24 h and were then examined for the presence of zones of inhibition. The diameter of such zones of inhibition was measured and the mean value for each organism was recorded and expressed in millimeters (El-Naggar *et al.*, 2014a).

Anticancer activity

To estimate the anticancer activity of AuNPs produced by the marine actinomycetes isolate, several steps were carried out: lyophilization, cytotoxicity test (measured by MTT assay) and effect of the median inhibitory dose (IC₅₀) (Abd-Elhaby *et al.*, 2016) on four different cancer cell lines: Hepatocellular carcinoma cells (HepG-2), Breast carcinoma cells (MCF-7), Colon carcinoma cells (HCT-116) and Prostate carcinoma cells (PC-3).

RESULTS AND DISCUSSIONS

Nanotechnology is a vastly improve field due to its prolonged range of applications in various areas of technology and science. Several kinds of processes are used for synthesis of nanoparticles due to their enormous applications. The traditional chemical processes for syntheses nanoparticles have proven restriction with them either in the form of chemical contaminants during their synthesis steps or in later applications and use of higher energy. The biosynthesis of nanoparticles is straightforward, elementary step, eco-friendly and a green approach. The several biological factors like plants, fungi, bacteria, actinomycetes etc. are used for biosynthesis for metal nanoparticles. (Shakeel *et al.*, 2016). One of hopeful bacterial systems for the biosynthesis of nanoparticles is *Streptomyces* sp. which is notify to synthesize Au-NP (Balagurunathan *et al.*, 2011).

Isolation and screening of actinobacteria for the synthesis of gold nanoparticles

The current study was focused on the extracellular synthesis of Au-NPs from marine actinobacteria supernatant. A total of nine actinomycetes isolates were isolated from collected sediment sample from Suez gulf, Egypt checked for their ability to produce gold nanoparticles. The screening revealed that only one isolates (M8) showed the ability to synthesis Au-NPs. The Au⁺ ions reduction was evidently noticeable when HAuCl₄ was added to the marine actinobacteria supernatant, and the color changed from yellow to purplish pink and in control there was no color development (Fig. 1). The similar reports of color change during extracellular biosynthesis of Au-NPs were also reported in other studies (Shivaji *et al.*, 2014) and (Balagurunathan *et al.*, 2011). A purplish-pink colour was observed with 2 mM chlorauric acid which is similar to that reported earlier (Bankar *et al.*, 2010).

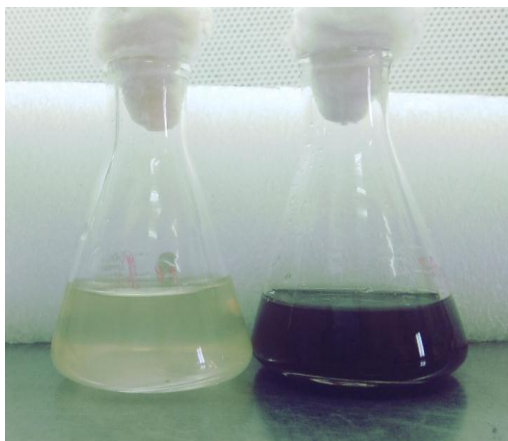


Fig 1: Biosynthesized gold nanoparticles by actinomycetes supernatant isolate (M8) before (left) and after (right) exposed to HAuCl₄ after 4 h.

UV-Visible spectroscopy analysis

The biosynthesis of gold nanoparticles by marine actinomycetes isolate was completed in this study. Metallic nanoparticles exhibited peculiar optical absorption spectra in the UV-Vis area due to collective oscillation of conduction band electrons around the nanoparticle surface. The convergent vibration of the conduction band electrons on absorption of visible light on the surface of nanoparticles was recognized as surface plasmon resonance (Pasqua *et al.*, 2009).

The surface plasmon resonance indicated the specific vibration mode according to size and shape of nanoparticles. The synthesis of gold nanoparticles was ocular

identified by observing the change in the yellow color of gold hydrous solution with gold cations into pink color colloidal gold. This visual change in color due to surface plasmon resonance was minutely studied with UV-Vis absorption spectrophotometer analysis of colloidal gold solution (Nishant and Mausumi, 2014). In this research, we use UV-Vis spectroscopy to follow up with the reaction operation. After 4 h of incubation, pink color formed which has absorption maxima of 534 nm which obviously indicate the forming of gold nanoparticles, (Fig. 2).

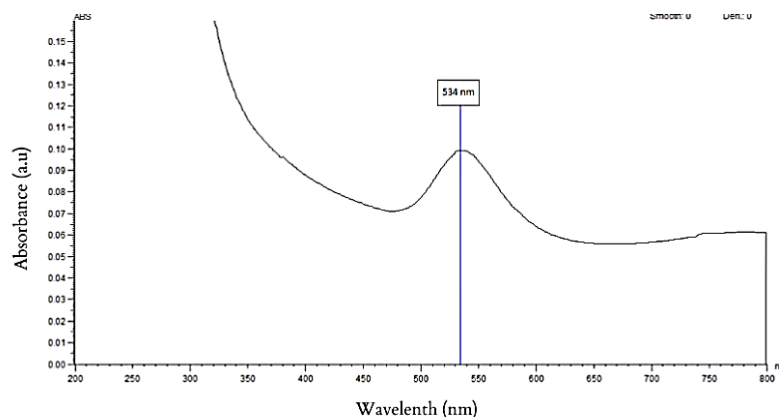


Fig. 2: The UV-vis spectrometer was used to record surface plasmon resonance of gold nanoparticle. After reactions with the supernatant of marine actinomycetes isolate (M8) for 4h. Presence of a strong peak with maximum absorbance at 534 nm.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectrum clearly illustrates the bio fabrication of gold nanoparticles mediated by the marine actinomycetes extracts and the FTIR spectrum (Fig.3.) has showed the presence of three bands at 3429 cm^{-1} , 2925 cm^{-1} and 2854 cm^{-1} which can be assigned to the stretching vibrational frequency of primary and secondary amines. The presence of bands at $1,056\text{ cm}^{-1}$ in the FTIR spectrum suggests the capping agent of biosynthesized nanoparticles possesses an aromatic amine group (El-Naggar *et al.*, 2014a).

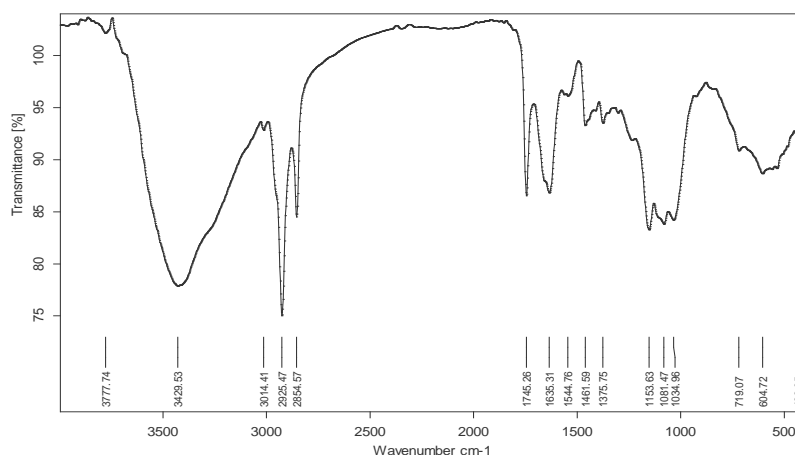


Fig. 3: FT-IR spectra of the solution of the gold nanoparticles

The band at $3250\text{--}3450\text{ cm}^{-1}$ corresponds to primary aliphatic amines (Renugadevi and Gayathri, 2010). Two bands were observed in the FT-IR spectra at 1745 cm^{-1} C=O stretch carbonyls group and 1635 cm^{-1} N-H bend which characteristic indicators of amide I and amide II linkages. The bands at 1461 cm^{-1} and

1375 cm^{-1} may be assigned to methylene scissoring vibrations from the proteins in the solution. VanmathiSelvi and Sivakumar (2012), reported that, the band at 1,380 cm^{-1} assigned to the C–N stretching vibrations of the aromatic amines, suggested the capping agent of biosynthesized nanoparticles possesses an aromatic amine groups. The bands at 1153 cm^{-1} , 1081 cm^{-1} and 1034 cm^{-1} coincide to primary aliphatic amines, while the peaks at 719 cm^{-1} and 604 cm^{-1} indicates C–Cl stretch. These bands altogether clearly indicate that the secondary structure of the proteins remains unaltered in presence of AuCl_4^- ions and during formation of gold nanoparticles.

Transmission Electron Microscopy (TEM)

The TEM image (Fig.4.) disclosed that Au-Nps in the reaction mixture were hexagonal in shape. Au-Nps synthesized by marine actinomycetes isolate (M8) were found to be in the range of 19-28 nm. A study by Prakash and others on synthesis of Au- Nps using *Streptomyces* NK52 reported the average particle size range from 10-100 nm (Prakash *et al.*, 2013) and also Soltani and his team reported the size of Au-Nps produced from *streptomyces fulvissimus* ranged from 20 to 50 nm (Soltani *et al.*, 2015).

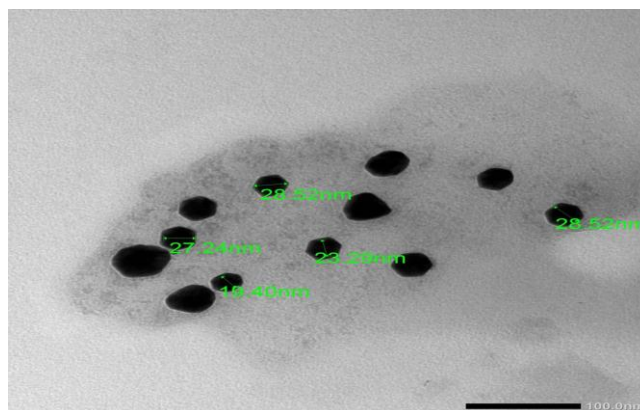


Fig. 4: TEM micrograph of gold nanoparticles synthesized by actinomycetes isolate (M8).

Identification of the most potent actinomycetes isolate (M8)

The marine actinomycete isolate (M8) that produced gold nanoparticles was identified by phenotypic characterization and molecular phylogenetic analysis. The isolate branching and filamentous bacteria, it had yellow substrate mycelium with gray aerial mycelium. Grown well on glucose yeast extract malt extract agar, oat meal agar, starch nitrate agar and Glycerol-nitrate agar but not grown on Czapek - Dox agar medium. It grown at 25-35 °C and there is no growth at 40-50°C also, it can grow at pH 5-10 and tolerated up to 10 % of NaCl. It can be utilized starch, lactose, dextrose and glucose as carbon sources. The organism degraded methyl red, produced urease, glucose, gelatinase and protease. It hydrolyzed arginine and decarboxylation of ornithine but negative for lysine hydrolysis, production of hydrogen sulfide Voges-Proskauer test, and indole test. Positive for citrate production and esculin hydrolysis as shown in Table 1. Fig. 5, shows scanning electron micrograph of spore chains of this isolate. The isolate was then identified according to 16S rRNA sequence. The complete 16S rRNA gene sequence of marine actinomycete isolate (M8) was determined. The similarity and homology of the 16 S rRNA partial gene sequence was analyzed with the similar existing sequences available in the data bank of (NCBI) using BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The DNA sequences were aligned and phylogenetic tree was constructed by neighbor joining (Saitou and Nei, 1987) method with the software package MEGA 6. Comparison of

the near full length 16S rDNA sequence of isolate (M8) to GenBank sequences, showed that it was most similar to *Streptomyces griseus* strain SYA.E50 (Evalue=0.0 and max. identity = 99 %). Fig. 6 represents the phylogenetic relationships between experimental strain and the most closely related species.

Table 1: Phenotypic characteristics of actinomycetes isolate (M8) under investigation

| Character | Result |
|--|--------|
| Growth on | |
| -Glucose yeast extract malt extract agar | + |
| -Czapex- Dox agar | - |
| -Oat meal agar | + |
| - Starch nitrate agar | + |
| - Glycerol nitrate agar | + |
| Morphological characters | |
| Substrate mycelium | Yellow |
| Aerial mycelium | Gray |
| Growth at (°C) | |
| 25-35 | + |
| 40 | - |
| Growth at pH | |
| 5-10 | + |
| 11-12 | - |
| Utilization of | |
| Starch | + |
| Lactose | + |
| Dextrose | + |
| Glucose | + |
| Growth in presence of NaCl (%) | |
| 0-10 | + |
| 11-13 | - |
| Biochemical test | |
| Urea Hydrolysis | + |
| Gelatin Hydrolysis | + |
| Ornithine dihydrolase | + |
| Lysine hydrolysis | - |
| Protease production | + |
| H ₂ S production | - |
| MR | + |
| VP | - |
| Indole production | - |
| Glucose production | + |
| Citrate production | + |
| Arginine dihydrolase | + |
| Esculin | + |

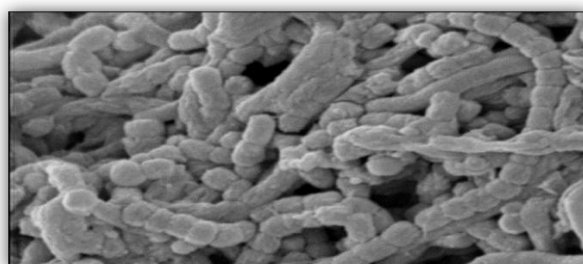


Fig. 5: Scanning electron micrograph of *Streptomyces griseus* strain (M8) after growth for 14 days on starch nitrate agar medium.

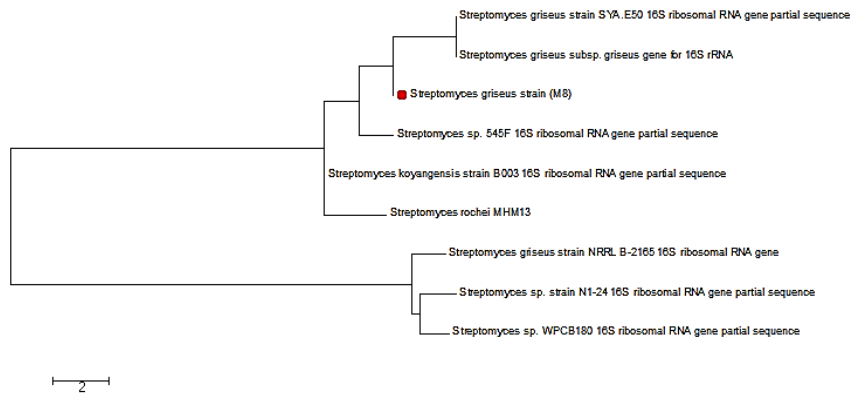


Fig. 6: Phylogenetic analysis of *Streptomyces griseus* strain (M8) based on partial sequencing of 16S rDNA.

Antimicrobial analysis of gold nanoparticles against bacterial pathogens

The antimicrobial activity where the interaction between the microorganisms and nanoparticles exploited, due to the size or shape of the nanoparticles, it have ability to make changes the permeability of the cell membrane of the microorganisms by make gaps or bore, thus can be creating inhibiting to the enzymatic activity of the respiration leading to apoptosis of the cells (BakerandSatish, 2015).

In this study, the antimicrobial activity of biosynthetic Au-NPs by *Streptomyces griseus* strain (M8) was estimated against Gram-positive (*Staphylococcus aureus* 25923, *Enterococcus faecalis* 29212 and *Bacillus cereus*), Gram negative (*Escherichia coli* 19404, *Pseudomonas aeruginosa* 9027, *Salmonella typhimurium* 14028, *Vibrio fluvialis*, and *Vibrio damsela*) bacterial strains and yeast (*Candida albicans*) by the well diffusion method (Fig. 7). A control (cell free supernatant broth without H₂AuCl₄ addition) was also take part in each test plate. The resulted inhibition zones were measured in terms of mean diameter of inhibition zones (mm) as recorded in Table 2.

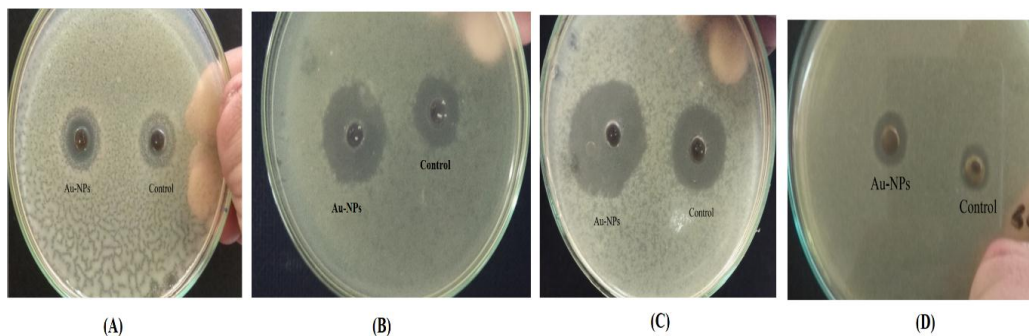


Fig. 7: Antimicrobial activity of gold nanoparticles produced by *Streptomyces griseus* strain (M8) against bacterial species: A) *Candida albicans*, B) *Pseudomonas aeruginosa* 9027, C) *Salmonella typhimurium* 14028 and D) *Escherichia coli* 19404

The highest antimicrobial activity was observed against *Salmonella typhimurium* 14028 and *Vibrio fluvialis*, whereas a moderated activity was found against *Vibrio damsela* and *Candida albicans*, whilst a lower activity was found against *Enterococcus faecalis* 29212 and *Escherichia coli* 19404, lastly no activity showed against *Staphylococcus aureus* 25923 and *Bacillus cereus*. These results are in concord with those of the previous studies which studied the antimicrobial activity of biosynthetic gold nanoparticles against pathogenic microorganisms (Neveen *et al.*,

2017). Balagurunathan *et al.* (2013) reported that, the nanoparticles synthesized by *Streptomyces viridiogens* strain H10 found to be highly effective against *S. aureus* and *E. coli* about (20mm). Antibacterial activity of gold nanoparticles against Gram-positive and Gram-negative bacteria has been notified also via (Ramamurthy *et al.*, 2012).

Table2: Antimicrobial activity of gold nanoparticles produced by *Streptomyces griseus* strain (M8) against tested pathogenic bacteria

| Pathogens | Inhibition zone (mm) |
|-------------------------------------|----------------------|
| <i>Enterococcus faecalis</i> 29212 | 10.0 |
| <i>Staphylococcus aureus</i> 25923 | 0.0 |
| <i>Bacillus cereus</i> | 0.0 |
| <i>Escherichia coli</i> 19404 | 10.0 |
| <i>Pseudomonas aeruginosa</i> 9027 | 20.0 |
| <i>Salmonella typhimurium</i> 14028 | 28.0 |
| <i>Vibrio fluvialis</i> | 25.0 |
| <i>Vibrio damsela</i> | 18.0 |
| <i>Candida albicans</i> | 18.0 |

The mode of action of metal nanoparticles against bacteria is not well known. There are some suggested bactericidal mechanisms of metal nanoparticles, which at most have been described for gold nanoparticles. The interaction between SH groups of proteins and gold nanoparticles ions play major role in bacterial inactivation (Guzman *et al.*, 2012). The presence of gold NPs in the electron dense granules observed after gold NP treatment in the cytoplasm of bacterial cells suggests an interaction with nucleic acids that probably results in the impairment of DNA replication. Thus it is reasonable to infer that the biosynthesized gold NPs can be used to manage the disease. Lately, the proteomic analysis expose that even a short exposure of gold nanoparticles to *E. coli* cells resulted in modification in the expression of a panel of envelope and heat shock protein (Eom *et al.*, 2012). Therefore gold nanoparticles can penetrate and can damage the bacterial membranes. A large loss of the potassium which founded in intracellular was induced by Au-NPs and above the Nano-gold decreased the ATP grade. The mode of action behind the efficacy of Au-NPs on bacteria are not yet fully explained. Three most common mechanisms were suggested on the activity of AgNPs on bacteria up to now: (1) uptake free silver ions followed by disruption of ATP production and DNA replication, (2) formation of Reactive Oxygen species (ROS) and (3) direct damage to cell membranes (Sahayaraj and Rajesh, 2011). It may presume to be the same action by Au-NPs. (Neveen *et al.*, 2017).

Anticancer activity of gold nanoparticles

As obviously shown in Tables 3 and 4, the effect of gold nanoparticles on cancer cell lines showed reduction in viability of cell lines activity. Gold nanoparticles from *Streptomyces griseus* strain (M8) exhibited a plausible degree of anticancer activity after a 24 h exposure to different concentrations of Au-NPs. where HCT-116 and MCF-7 cell lines were the most sensitive cell lines towards the cytotoxic activity of the tested Au-NPs, while the HepG-2 and PC-3 cell lines were the most resistant cell lines towards the cytotoxic activity.

Table 3: IC50 determination cell viability was measured by MTT assays on different cancer cell lines after 24 h exposure to doses of Au-NPs produced by *Streptomyces griseus* strain (M8).

| Cell line | IC50 (ug/Well) |
|-----------|----------------|
| HCT-116 | 61.9 |
| MCF-7 | 46.6 |
| HepG-2 | 107 |
| PC3 | 121 |

Table 4: Anticancer activity for different concentrations of Au-NPs which produced by *Streptomyces griseus* strain (M8) against different cancer cell lines

| Au-NPs conc. (ug/Well) | HCT-116 | MCF-7 | HepG-2 | PC3 |
|---------------------------|------------|-------|--------|-------|
| | Viability% | | | |
| 500 | 9.72 | 7.45 | 13.96 | 21.88 |
| 250 | 24.38 | 18.64 | 30.75 | 35.29 |
| 125 | 37.46 | 31.79 | 43.89 | 48.76 |
| 62.5 | 49.73 | 42.86 | 65.20 | 70.41 |
| 31.25 | 64.61 | 56.93 | 87.13 | 89.62 |
| 15.6 | 80.92 | 71.30 | 94.56 | 97.34 |
| 7.8 | 93.27 | 86.92 | 98.18 | 100 |
| 3.9 | 99.64 | 92.48 | 100 | 100 |
| 0 | 100 | 100 | 100 | 100 |

Colon carcinoma cells (HCT-116), breast carcinoma cells (MCF-7), Hepatocellular carcinoma cells (HepG-2) and prostate carcinoma cells (PC-3).

In similar results, Rajeshkumar, (2016) succeeded to biosynthesis of stable gold nanoparticles from marine bacteria *Enterococcus* sp after 2h as spherical in shape with size ranging from 6 to 13 nm and have significant anticancer activity against HepG2 and A549 cells at 100 ug. Abolghasem *et al.*, (2016) reported that gold nanoparticles at different concentration which synthesized biologically showed anticancer activity on the prevalent cancer cell lines including breast (MCF-7), cervical (HeLa) and ovarian (Caov-4). Similarly, the silver nanoparticles synthesized by marine *Streptomyces rochei* MHM13 show good cytotoxic activity against Hep-G2, HCT-116, A-549 and MCF-7 cell lines (Abd-Elnaby *et al.*, 2016). Rajeshkumar *et al.* (2016) synthesized the silver nanoparticles and notify its have ability to inhibit liver and lung cancer cell lines. The cytotoxic effect of AuNPs is the result of active physicochemical interaction of gold atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA (Ahmed *et al.*, 2007). In study of (Sriram *et al.*, 2010) notify that the nanoparticles acquiring anticancer activity are known for their ability to slow down the activities of abnormally expressed signaling proteins, such as Akt and Ras, cytokine-based therapies, DNA- or protein based vaccines against specific tumor markers, and tyrosine kinase inhibitors which exhibit a consistent antitumor effect (Martins *et al.*, 2010).

CONCLUSION

In our investigation, we indicated that biosynthesis of gold nanoparticles can be done by using cell free supernatant from marine *Streptomyces griseus* strain (M8) treated with 2 mM HAuCl₄. Biosynthesized gold nanoparticles were asserted by UV-Visible spectroscopy, FT-IR and TEM. The biosynthesized gold nanoparticles using *Streptomyces griseus* strain (M8) showed satisfied results as antibacterial and anticancer activities.

REFERENCES

- Abolghasem, A. K.; Sayyed, H.; Zarkesh, E.; Abdol-Khalegh, B. and Amir, R. (2016). Gold nanoparticles as potent anticancer agent: green synthesis, characterization, and in vitro study. *The Royal Society of Chemistry*, 6: 63973–63983.
- Ahmad, S. S.; Islam Khan, M.; Kumar, R. and Sastry, M. (2003). Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic Actinomycete, *Thermomonospora* sp, ACS Symp. Ser. Am. Chem. Soc. (19): 3550–3553.
- Ahmad, A.; Senapathi, S.; Khan, M. I.; Kumar, R. and Sastry, M. (2003b). Extracellular Biosynthesis of Monodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete, *Thermomonospora* sp. *Longmuir*, 19: 3550-3553.
- Baker, S. and Satish, S. (2015). Biosynthesis of gold nanoparticles by *Pseudomonas veronii* AS41G inhabiting *Annona squamosa* L, *Spectrochim. Acta A Mol. Biomol. Spec-trosc.*, 150: 691–695.
- Balagurunathan, R.; Radhahrishnan, M.; Rajendran, R.B. and Velmurugan, D. (2011). Biosynthesis of gold nanoparticles by actinomycete *Streptomyces viridogens* strain HM10, *Indian J Biochem Biophys.* (48)331.
- Bankar, A.; Joshi, B.; Ravi Kumar, A. and Zinjarde, S.B.(2010). peel extract mediated synthesis of gold nanoparticles, *Colloids Surf B Biointerfaces*, 80 :45.
- El-Masry, M.H; Khalil, A.I. and Hassouna, M.S. (2002). In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. *World J Microbiol Biotechnol*, 18: 551 558.
- El-Naggar, N. E.; Abdelwahed, N. A. M. and Darwesh, O. M. M. (2014a). Fabrication of biogenic antimicrobial silver nanoparticles by *Streptomyces aegyptia* NEAE 102 as eco-friendly nanofactory. *J. Microbiol. Biotechnol*, 24(4): 453-464.
- Eom, S. H.; Kim, Y. M. and Kim, S. K. (2012). Antimicrobial effect of phlorotannin from marine brown algae. *Food Chem. Toxicol.*, 50: 3251–3255.
- Gamez, G.; Dokken, K.; Herrera, I.; Parsons, J.G.; Tiemann, K. J. and Gardea-Torresdey, J. L. (2000). In: Chemical processes involved in Au (III) binding and bioreduction by Alfalfa biomass, Proceedings of the 2000 Conference on Hazardous Waste Research, Denver, Colorado, pp. 46–53.
- Hanan, M. A.; Gehan, M. A.; Usama, M. A. and Moaz, M. H. (2016). Antibacterial and anticancer activity of extracellular synthesized silver nanoparticles from marine *Streptomyces rochei* MHM13. *Egyptian Journal of Aquatic Research*, 42(3): 301-312.
- Honary, S.; Fathabad, E.G.; Paji, Z.K. and Eslamifar, M. (2012). A novel biological synthesis of gold nanoparticle by enterobacteriaceae family, *Trop. J. Pharm. Res.*, 11 (2012) 887–891.
- Kokare, C.R.; Maadik, K.R. and Kadam, S. S. (2004). Isolation of bioactive marine actinomycetes from sediments isolated from goa and Maharashtra coast lines (West Coast of India). *Ind. J. Marine Sci.*, 33: 248-256.
- Martins, D.; Frungillo, L.; Anazzetti, M.C.; Melo, P.S. and Durán, N. (2010). Antitumoral activity of L-ascorbic acid-poly-D,L-(lactide-co-glycolide) nanoparticles containing violacein. *Int J Nanomedicine*, pp. 77-85
- Muthu, I. S.; Selvaraj, B. M. K.; Kalimuthu, K. and Sangiliyandi, G. (2010). Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. *Int J Nanomedicine*, 5: 753–762.

- Narayanan, K.B. and Sakthivel, N. (2011). Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. *Adv. Colloid Interface Sci.*, 169: 2–59
- Neveen. Abdel-Raouf ; Nouf Mohammad Al-Enazib and Ibraheem B.M.Ibraheem (2017). Green biosynthesis of gold nanoparticles using *Galaxaura elongata* and characterization of their antibacterial activity. *Arabian Journal of Chemistry*, 10: 3029-S3039.
- Nishant, S. and Mausumi, M.(2014).Biosynthesis and Characterization of Gold Nanoparticles Using *Zooglea ramigera* and Assessment of Its Antibacterial Property. *Journal of Cluster Science*, 26(3):1-18
- Pasqua, A.J.D.; Mishler, R.E.; Ship, Y.L.; Dabrowiak, J.C. and Asefa, T.(2009).Preparation of antibody conjugated gold nanoparticles. *Mater. Lett.*, 63:1876.
- Prakash, D.; Mahale, V.; Bankar, A.; Nawani, N.; Zinjarde, S. and Kapadnis, B. (2013). Biosynthesis of colloidal gold nanoparticles by *Streptomyces* sp. NK52 and its anti-lipid peroxidation activity. *Indian Journal of Experimental Biology*, 51(11): 969-972.
- Rajeshkumar, S. C.; Malarkodi, M.; Vanaja, G. and Annadurai, J. (2016). Anticancer and enhanced antimicrobial activity of biosynthesized silver nanoparticles against clinical pathogens). *Mol. Struct*:165–173.
- Ramamurthy, C.H.1.; Padma,M.; samadanam, I.D.; Mareeswaran, R.; Suyavaran, A.; Kumar, M.S.; Premkumar, K. and Thirunavukkarasu, C.(2012). The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. *Volium 102*. 808-815.
- Renugadevi, T.S. and Gayathri, S.(2010).FTIR and FT-Raman spectral analysis of paclitaxel drugs. *Int. J. Pharm. Sci. Rev. Res.*, 2:106110.
- Sahayaraj, K. and Rajesh, S. (2011). Bionanoparticles: synthesis and antimicrobial applications. In: Mendez–Vilas, A. (Ed.), *Science against microbial pathogens; communicating current research and technological advances*, pp. 228–244.
- Saitou, N. and Nei, M. (1987).The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-425.
- Sastry, M., Ahmad, A., Khan, M.I. and Kumar, R. (2003) Biosynthesis of Metal Nanoparticles Using Fungi and Actinomycete. *Current Science*, 85: 162-170.
- Shakeel, A.;Annu, S. I. and Yudha, S. S. (2016). Biosynthesis of gold nanoparticles: A green approach. *Journal of Photochemistry and Photobiology B: Biology.*, 161: 141-153.
- Shivaji, S. W.; Arvind, M. D. and Zygmunt, S. (2014).Biosynthesis, optimization, purification and characterization of gold nanoparticles. *African Journal of Microbiology Research*, 8(2): 138-146.
- Soltani Nejad, M.; Shahidi Bonjar, G.h. and Khaleghi, N.(2015).Biosynthesis of gold nanoparticles using *Streptomyces fulvissimus* isolate U. *Nanomed J.*, 2(2): 153 -159.
- Williams, S.T. and Cross, T. (2003).Actinomycetes Isolation from soil. In: *Methods in Microbio.* Booth, C. (Ed.), Vol. 4, Academic Press, London, New York, pp: 295-334.
- Zhang, X.; He, X.; Wang, K. and Yang, X.(2011).Different active biomolecules involved in biosynthesis of gold nanoparticles by three fungus species, plant resources conservation and utilization research, *J. Biomed. Nanotechnol*, 7: 53–64.