Rafidain Journal of Science

https://rsci.uomosul.edu.iq

Vol. 34, No. 2, pp. 14-22, 2025 (June).

Novel Identification Technique of Gold Nanoparticles Biosynthesized by "*Mesobacillus Foraminis* ShAm 98 Iraq" Bacteria Isolated from Hot Spring Water at Hammam A-Aleel in Mosul/ Iraq

Shafaq T. Burhan **Amera Mahmood M. Al-Rawi**

Department of Biology/ College of Sciences/ University of Mosul/ Mosul/ Iraq

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Article information

Received: 22/7/2024
Revised: 9/12/2024
Accepted: 19/12/2024

DOI: 10.33899/rjs.2025.187738

corresponding author:
Shafaq T. Burhan
shasbio108@uomosul.edu.iq

ABSTRACT

A new strain *Mesobacillus foraminis* (*M. foraminis*) locally isolated from hot spring water at Hammam Al-Aleel in the south of Mosul city/ Iraq was identified using phylogenetic analysis. Its ability to synthesize gold nanoparticles has been studied for the first time as a micro-factory for GNPs by observing the colour change. To prove the formation of GNPs, X-diffraction (XRD) analysis was done and the best formation of crystalline GNPs was observed at the (III) crystal plane. Atomic Force Microscope (AFM) technique was used to determine the shape, size, and surface smoothness of nanoparticles, while Transmission and Scanning Electron Microscope (TEM and SEM, respectively) were applied to characterize the size and morphology. Moreover, fluorescent microscope was used for the first time as a novel method in determining the characteristic morphology of GNPs polymers by using acridine orange which used for GNPs staining and examined under a fluorescent microscope. The fluorescent images show amazing GNPs polymers with shiny spots with more details of dendroid structures in the polymers. Since there was no previous study on using a fluorescent microscope and acridine orange stain in the field of nanotechnology, this study is considered the first effort to characterize biosynthesized GNPs using this technique.

Keywords: *Mesobacillus foraminis*, gold NP, fluorescent microscope, acridin orange stain, AFM.

INTRODUCTION

Nanoparticles play a major role in a number of fields, such as biotechnology (e.g. food industry) and medicine. Gold nanoparticles (GNP) are widely used in ecological applications of bioremediations for the removal of toxic chemicals from soil and atmosphere as well as for dye degradation (Menon *et al*., 2017). Moreover, GNPs used in therapeutic applications especially in cancer chemotherapy by controlling the release of chemotherapeutic agents. Also, it can improve local radiotherapy targeting the GNPs to the tumour (Amjad, 2021). Chemical and physical techniques, such as aerosol technology, ultraviolet irradiation, lithography, and ultrasonication, have been used as successful methods for nanoparticles synthesis in large quantities in a short time (Narayanan and Sakthivel, 2010; Patra and Baek, 2015). However, when researchers studied these methods, they found many disadvantages such as the use of toxic, hazardous and expensive chemicals, furthermore, they require high temperature which results in environmental pollution (Sunkar *et al*., 2014). 'Green Chemistry' offers an easier, cleaner, and more eco-friendly approach to obtain nanomaterials using a biological system while also protecting the environment (Sunkar and Nachiyar, 2012).

Microorganisms are examples of possible eco-friendly nano factories which can exert control over the size, composition, morphology, and crystallographic orientation of prepared nanostructured particles (Du *et al*., 2007).

Diverse range morphologies of biosynthesised noble metal nanoparticles (e.g. gold) have been observed, including rods, triangles, spheres, hexagons, flat sheets, icosahedrons, decahedrons, nodous, prisms, dog bones shells, tetrapod, cubes, and several hollow structures (Boisselier and Astruc, 2009; Jazayeri *et al*., 2016; Aljobori and Al-Rawi, 2024).

Bacillus foraminis, is phylogenetically related to species that belongs to the genus *Bacillus*. This bacterium is gram-positive and aerobic with rod-shaped cells. It lives an optimum pH ranging from 7.0 to 8.5 and an optimum growth temperature of 40^{o}C. The strain sp. nov., which was designated CV53 T, was isolated from non-saline, alkaline groundwater (Tiago *et al*., 2006; Abdullah, 2024).

Many researches focused on GNPs synthesis by microorganisms, and some of these studies aimed to isolate active ingredients to explain the mechanisms of GNPs microbial synthesis. (Li *et al*., 2016) found that the cyclic peptide in the cell-free extract was responsible for GNPs biosynthesis by *Bacillus niabensis* 45 through a possible electron transfer process.

The main purpose of the current study is to fabricate GNPs using a novel bacterial species isolated from hot spring water as a safe, easy, cost-effective, and eco-friendly method. Moreover, this study aims at the characterization of GNPs morphology using the new method in this regard by using acridine orange stain and fluorescent microscope and comparing the results with SEM images.

MATERIALS AND METHODS

Detection of GNPs synthesis

One ml of water sample was taken from a hot spring and cultured in 9 ml of Nutrient Broth (NB) containing 1 mM of $HAuCl_4.3H_2O$ (Sigma Aldrich USA) and incubated for 24 hours at 55 ^{o}C then the changing of colour was observed (Honary *et al*., 2012).

Isolation of bacterium

After the change in colour in the previous step, 0.1 ml of the same broth was cultured on mineral medium prepared by adding 4.0 gm $NaNO_3$, 3.4 gm KH_2PO_4, 10.5 gm $Na_2 HPO_4$, 0.2 mg $MgSO_4$. $7H_2O$, 0.2g yeast extract, and 2.0 g. Agar to 1000 ml of distilled water (Sun *et al*., 2015).

Identification by phylogenetic analysis

Pure bacterial colonies were collected by centrifugation and chromosomal DNA was isolated by using an i-genomic BYF DNA mini kit. 16S rRNA gene fragment was amplified using forward and reverse primers for molecular characterization. The amplification of the gene was done by using Maxime PCR PreMix (i-taq) 20 M/xrn.

The primers which amplified the gene had a following sequence

F 5´ AGA GTT TGA TCC TGG CTC AG 3‘`
R 5´ GGT TAC CTT GTT ACG ACT T 3`

The amplified DNA fragment was separated and eluted from 1.5% agarose gel and purified. The pure PCR product was then sequenced using the same primers that are used in the biotechnology lab in nicem and using DNA sequencer 373 XL from applied bio system in Korea.

The similarity of the sequence was researched using BLAST and the phylogenetic analysis of the isolated bacteria was done by neighbour-joining (NJ) method (Hall, 2011). The isolated novel strain was phylogenetically characterized and named strain ShAm 98. Iraq.

Screening of microbial biosynthesis of GNPs and its characterisation

The novel strain ShAm 98 was cultured in an NB medium containing 1 mM of $HAuCl_4.3H_2O$ and incubated at 55 ℃ for 24 hrs. The change in color was observed.

Characterisation of GNPs

The initial step after the change in color was to confirm the GNPs synthesis using the XRD technique. The culture containing bacterial biomass and GNPs was centrifuged at 600 rpm for 10 min. A thick smear of the supernatant on a glass slide was done, dried at room temperature, and then analyzed using XRD- 600 Shimadzu diffractometer. The diffracted intensities were obtained for 30° to 80° at 2θ.

Additionally, the size, shapes, and distribution of GNPs were detected using different microscopic analyses SEM, TEM, and AFM. Furthermore, a fluorescent microscope was used as a new method in this field to characterize microscale GNPs, especially the crystalline and polymeric nanoparticles. The smear was prepared from cultured bacteria with $HAuCl_4.3H_2O$. After 24 hours of incubation, it was dried at room temperature, stained with acridine orange stain as described in MacCarthy and Senne (1980), allowed to dry, and then examined by fluorescent microscope (Optika-Italy).

RESULTS AND DISCUSSION

Adding the sample of hot spring water to NB containing $HAuCl_4.3H_2O$, the colour changed from yellow to red in the first few seconds and then turned immediately to clear and colourless with black deposits.

This phenomenon may be due to the combined effect of many different microorganisms which have redox activity of archaea and bacteria that have the ability to actively catalyse the precipitation of toxic Au(III) complexes. For example, sulphate-reducing bacteria (SRB) may contribute to the formation of gold-bearing sulphide, while sulphur-oxidising bacteria (SOB) can have the ability to release the associated gold by breaking down the gold hosting sulphide minerals which is the main step of primary mineralization (Reith *et al*., 2007).

When GNPs absorb and scatter the light, it results in different colours ranging from red and blue to purple and black, and finally to clear and colourless, depending on particles' size, shape, local refractive index, and aggregation stage (Anderson *et al*., 1999).

The result of bacterial isolation on mineral medium showed growth of small colourless colonies after 30 days of incubation at room temperature. Phylogenetic analysis of the partial 16S rRNA (gene sequence, genbank accession number MT 889920) was obtained from the sequence data algination and analysis for identifying of bacteria and their closest neighbours. The results showed that the isolated ShAm 98 belongs to *Mesobacillus foraminis* with closest similarity of 99% to the *Bacillus foraminis* India strain JN 187363 as illustrated in Fig. (1).

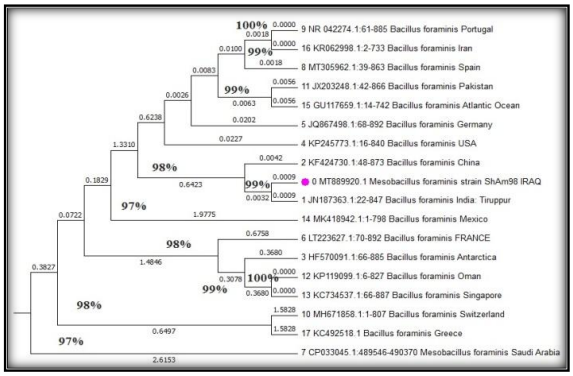


Fig. 1: *Mesobacillus foraminis* ShAm98 and its closest neighbor strain 1 JN187363.

The gene sequence of *Mesobacillus foraminis* ShAm98 Iraq is as bellow:

gatggagctt gctcccaaag attagcggcg gacgggtgag taacacgtgg gcaacctgcc
tgtaagactg ggataactcc gggaaaccgg ggctaatacc ggataattca tttcctctca
tgaggaaatg ctgaaagacg gcttctcgct gtcacttaca gatgggcccg cggcgcatta
gctagttggt gaggtaacgg ctcaccaagg ccacgatgcg tagccgacct gagagggtga
tcggccacac tgggactgag acacggccca gactcctacg ggaggcagca gtagggaatc
ttccgcaatg gacgaaagtc tgacggagca acgccgcgtg agcgatgaag gccttcgggt
cgtaaagctc tgttgttagg gaagaacaag tatcggagta actgccggta ccttgacggt
acctaaccag aaagccacgg ctaactacgt gccagcagcc gcggtaatac gtaggtggca
agcgttgtcc ggaattattg ggcgtaaagc gcgcgcaggt ggttccttaa gtctgatgtg
aaagcccacg gctcaaccgt ggagggtcat tggaaactgg ggaacttgag tgcagaagag
gaaagtggaa ttccacgtgt agcggtgaaa tgcgtagaga tgtggaggaa caccagtggc
gaaggcgact ttctggtctg taactgacac tgaggcgcga aagcgtgggg agcgaacagg
attagatacc ctggtagtcc acgccgtaaa cgatgagtgc taagtgttag agggtttccg
ccctttagtg ctgcagcaaa cgcattaagc actccgcctg gggagt

Tiago *et al*. (2006) isolated *Bacillus foraminis* sp. nov, which is genetically related to species of the genus *Bacillus*, from alkaline non-saline groundwater and they described this species' members as rod-shaped cells, Gr+ve, aerobic, and heterotrophic bacteria. Furthermore, they found that this species could not form spores under varied conditions.

The colour changed from yellow to wine red after adding 0.0393g of HAucl4.3H_2O to 100 ml of N.B to obtain (1mM as mentioned in 2-1) cultured with ShAm 98 bacterial strain which initially indicated the synthesis of AuNPs as shown in Fig. (2).

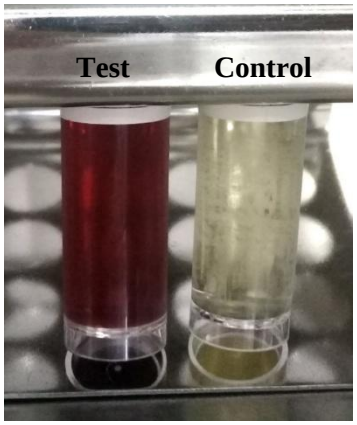


Fig. 2: Change in colour from yellow to wine red.

According to Zhang *et al*. (2016), the change in colour is observed when there is an increase in the size of the particles. In the case of GNPs, the change is from deep red to a range of colours which is due to the localised surface plasmon resonance (LSPR) that they exhibit.

Gold and silver nanoparticles can be extracellular, synthesized by thermophilic bacteria, which is considered to be an excellent tool for this purpose (Gomathy and Sabarinahtan, 2010). Because of their inexpensive medium, their ease of handling and safety, and their potential to adsorb and reduce metal ions into nanoparticles using enzymes produced by metabolic processes, the microorganisms have been used for the synthesis of nanoparticles (Kumar *et al*., 2014; Luo *et al*., 2014).

Many members of the family bacilliaceae were used for GNPs synthesis (e.g *Bacillus subtilis*, *B. cereus*, *B. niabensis* 45, and Geobacillus spp.) (Daniela *et al*., 2013; Sunkar *et al*., 2014; Li *et al*., 2016). However, in our research, we could not find any study about *Mesobacillus foraminis* where they were used for GNPs synthesis therefore, there is no more reference to explain the exact mechanisms of GNPs synthesis by this novel strain which opens a great field to study this bacterium in more detail.

According to Hulkoti and Taranath (2014), the presence of amino acid and organic phosphate compound in the metabolic process, contributes to GNPs biosynthesis. Montero *et al.* (2018) found that no viable bacterial cells were detected after 96 hours of incubating the bacteria with $HAuCl_4.3H_2O$. This study proposed that the bacterial cell compound will lyse and release different charged organic compounds into the surrounding media which will actively contribute to the GNPs formation and self-assembly polymers development.

To prove presence of the AuNPs crystalline nature and to determine phase and mean size of the bioproduced GNPs in the supernatant of the ShAm 98 bacterial culture, XRD analysis was done, and two peaks of AuNPs were observed, representing the index as (111) and (200) respectively as shown in Fig. (3).

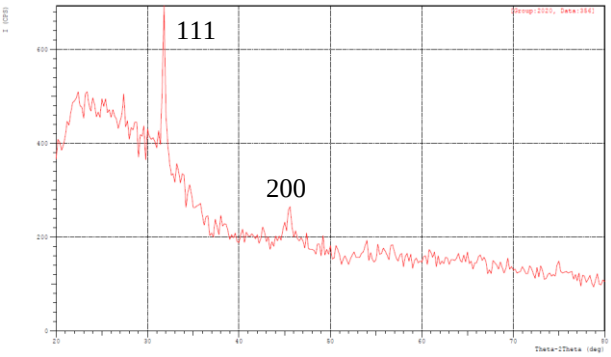


Fig. 3: XRD of biologically synthesized gold nanoparticles.

Parab *et al*. (2011) found that the best formation of crystalline GNPs was seen at (111) crystal plane in XRD pattern which explains the similarity to these to metallic gold, while the presence of the other peaks was due to the impurities present in the bacterial culture supernatant as they were directly examined after AuNPs production (Pourali *et al*., 2017).

The size of GNPs ranged from 30 to 130 nm according to the results of AFM analysis, while the average of GNPs diameters was 66.61 which was distributed in 30.0 nm (10%) and 105 nm (90%), and the roughness average was 3.31 nm as illustrated in Fig. (4).

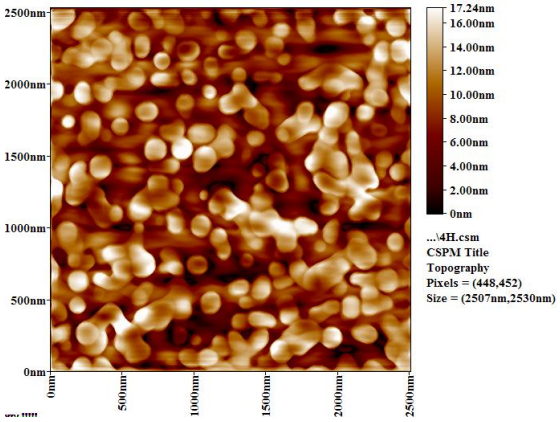


Fig. 4: Atomic force microscopy analysis of GNPs.

Characterizing of GNPs by using AFM has several advantages in comparison to TEM. The former technique offers visualization in 3 dimensions, so that it is possible to measure not only the height, length, and width, but also the morphology and surface texture.

The results of TEM and SEM show the polymerised and crystalline nature of GNPs as illustrated in Fig. (5a, 5b) respectively.

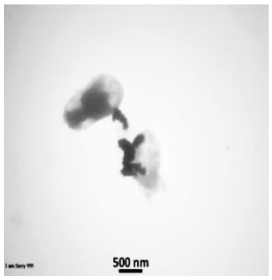
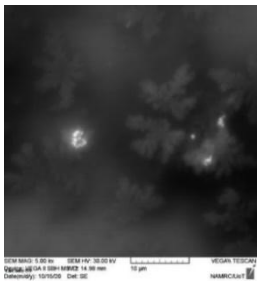


Fig. 5: (a) TEM image bacterial cells with crystalline GNPs.



(b) SEM image polymers of GNPs with a shiny crystalline appearance.

Many researchers and most studies used TEM and/ or SEM analysis to demonstrate different shapes of AuNPs ranging from spherical, triangle, and cubic to snow-flak like self-assembly using different methods of physical, chemical, and biological synthesis of GNPs (Parab *et al*., 2011; Rajeshkumar, 2016). On comparing the results of the fluorescent microscope with SEM, it was found that there are no differences in their results when showing the morphology of GNPs. Moreover, the fluorescent images show amazing GNPs polymers with shiny spots with more details of dendroid structures in the polymers as is shown in Fig. (6).

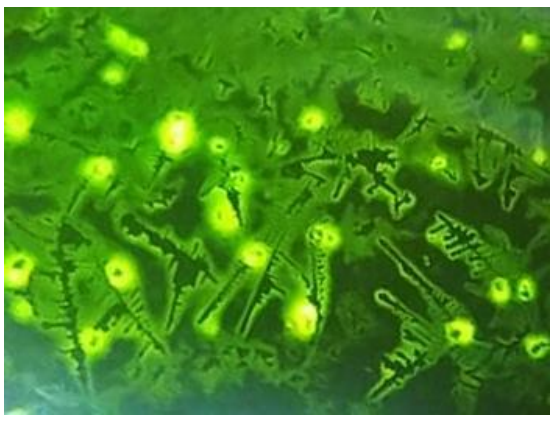


Fig. 6: GNPs polymers with shiny spots.

Since there was no previous study using a fluorescent microscope and acridine orange stain in the field of nanotechnology, this study is considered the first effort to characterize biosynthesized GNPs using this technique.

CONCLUSIONS

The new strain "*Mesobacillus foraminis* ShAm 98", which was isolated from hot spring water, was used for the first time as a micro-factory for GNPs, produced by a simple, cost-effective, and eco-friendly method that can have a variety of applications in many fields. Additionally, acridine orange was used for GNPs staining and examined under a fluorescent microscope as a new method of determining the characteristic morphology of GNPs.

REFERENCES

Abdullah, M.N. (2024). Theoretical investigation on transportance for single-walled nanotubes, and bi-layer graphene. *Raf. J. Sci.* **33**(1), 121-129. DOI: 10.33899/rjs.2024.182836

Aljobori, M.; Al-Rawi, A.M. (2024). Effect of chitosan nanoparticles on growth, swarming motility, and biofilm formation in proteus mirabilis isolated from urinary tract infections. *Raf. J. Sci.*, **33**(1), 32-41. DOI: 10.33899/rjs.2024.182824

Amjad, S.H.M. (2021). A review on gold nanoparticles (GNPs) and their advancement in cancer therapy. *Inter. J. Nanom. Nanotech. Nanomed.*, **7**(1), 19-25. DOI: 10.17352/2455-3492.000040

Anderson, M.L.; Morris, C.A.; Stroud, R.M.; Merzbacher, C.L.; Rolison, D.R. (1999). Colloidal gold aerogels: Preparation, prosperities, and characterization. *Langm.*, **15**(3), 674-81.

Boisselier, E.; Astruc, D. (2009). Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. *Chem. Soc. Rev.*, **38**(6), 1759-1782. DOI: 10.1039/b806051g

Daniela, N.; Sebastian, A.; Miguel, E.; Patricio, A.; Jenny, M. (2013). Gold nanoparticles synthesized by *Geobacillus* sp. Strain ID17 a thermophilic bacterium isolated from Deception Island, Antarctica. *Microb. Cell Fact. (BMC)*, **6**(n/a), 12-75. DOI: 10.1186/1475-2859-12-75

Du, L.; Jiang, H.; Liu, X.; Wang, E. (2007). Biosynthesis of gold nanoparticles assisted by *Escherichia coli* Dh5α and its application on direct electrochemistry of hemoglobin. *Electroch. Commun.*, **9**(n/a), 1165-70. DOI: 10.1016/J.ELECOM

Gomathy, M.; Sabarinathan, K.G. (2010). Microbial mechanisms of heavy metal tolerance. *Agricul. Rev.*, **31**(2), 133-138.

Hall, B.G. (2011). "Phylogenetic Trees Made Easy: How to Manual". 4th ed., Sinauer Associates.

Honary, S.; Fathobad, E.; Paji, Z.; Eslamifar, M. (2012). A novel biological synthesis of gold nanoparticle by Enterobacteriaceae family. *Trop. J. Pharmac. Res.*, **11**(6), 887-91. DOI: 10.4314/tjpr.v11i6.3

Hulkoti, N.I.; Taranath, T.C. (2014). Biosynthesis of nanoparticles using microbes-A review. *Coll. Surf. B: Bioint.*, **121**(n/a), 474-83. DOI: 10.1016/j.colsurfb.2014.05.027

Jazayeri, M.H.; Amani, H.; Pourfatollah, A.A.; Pazoki-Toroudi, H.; Sedighimoghdelam, B. (2016). Various methods of gold nanoparticles (GNPs) conjugation to antibodies. *Sens Biosen. Res*., **9**(n/a), 17-22. DOI: 10.1016/j.sbsr.2016.04.002

Kumar, K.S.; Kumar, G.; Prokhorov, E.; Sanchez. I.C. (2014). Exploitation of anaerobic enriched mixed bacteria (AEMR) for the silver and gold nanoparticles synthesis. *Coll. Surf. A. Physicoch. Eng. Asp.*, **462**(n/a), 264-70.

Li, Yu.; Li, Ya.; Li, Q.; Fan, X.; Geo, J.; Luo, Y. (2016). Rapid biosynthesis of gold nanoparticles by the extracellular Secretion of Bacillus niabensis 45: Characterization and antibiofilm activity. *J. Chem.*, **2016**(n/a), 1-7. DOI: 10.1155/2016/2781347

Luo, P.; Liu, Y.; Xia, Y.; Xu, H.; Xie, G. (2014). Aptamer biosensor for sensitive detection of toxin A of *Clostridium difficile* using gold nanoparticles synthesized by *Bacillus stearothermophilus*. *Biosen. Bioelec.*, **54**(n/a), 217-21.

MacCarthy, L.R.; Senne, J.E. (1980). Evalution of acridine orange for detection of microorganisms in blood culture. *Clin. Microb.*, **11**(3), 281-285.

Menon, S.; Rajeshkumar, S.; Venkat, K.S. (2017). A review on biogenic synthesis of gold nanoparticles, characterization, and its applications. *Res.-Effic. Tech.*, **3**(4), 516-527. DOI: 10.1016/j.reffit.2017.08.002

Montero-Silva, F.; Durán, N.; Seeger, M. (2018). Synthesis of extracellular gold nanoparticles using *Cupriavidus metallidurans* CH34 cells. *IET Nanobio.*, **12**(n/a), 40-6.

Narayananm, K.B.; Sakthivel, N. (2010). Phytosynthesis of gold nanoparticles using leaf extract of *Coleus amboinicus* Lour. *Material Character.*, **61**(11), 1232-8. DOI: 10.1016/j.matchar.2010.08.003

Parab, H.; Jung, Ch.; Woo, M.A.; Park, H.G. (2011). Anisotropic snow flake-like structural assembly of polymer-capped gold nanoparticles. *Nanopart. Res.*, **13**(n/a), 2173-2180. DOI: 10.1007/s11051-010-9975-5

Patra, J.K.; Baek, K.H. (2015). Novel green synthesis of gold nanoparticles using Citrullus innatus rind and investigation of proteasome inhibitory activity, antibacterial, and antioxidant potential. *Inter. J. Nanomed.*, **10**(n/a), 7253–64.

Pourali, P.; Badiee S.H.; Manafi, S.; Noorani, T.; Rezaei, A.; Yahyaei, B. (2017). Biosynthesis of gold nanoparticles by two bacterial and fungal strains, *Bacillus* cereus and *Fusarium oxysporm*, and assessment and comparison of their nanotoxicity *in vitro* by direct and indirect assays. *Elect. J. Biotech.*, **29**(n/a), 86-93. DOI: 10.1016/j.ejbt.2017.07.005

Rajeshkumar, S. (2016). Anticancer activity of eco-friendly gold naonparticles against lung and liver cancer cells. *Genetic Eng. Biotech.*, **14**(1), 195-202. DOI: 10.1016/j.jgeb.2016.05.007

Reith, F.; Lengke, M.; Falconer, D.; Graw, D.; Southam, G. (2007). The geomicrobiology of gold. *Inter. Soc. Microb. Ecology (ISME)*, **1**(n/a), 567-84. DOI: 10.1038/ismej.2007.75

Sun, A.; Hing, Z.; Yang, F.; Li, X. (2015). Characteristic of newly isolated *Geobacillus* sp. ZY-10 degrading hydrocarbons in crude oil. *Polish J. Microb. (PJM)*, **64**(3), 253–63.

Sunkar, S.; Nachiyar, C.V. (2012). Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium *Bacillus cereus* isolated from *Gorcinia xanthochymus*. *Asian pacific J. Trop. Biomed.*, **2**(12), 953-9. DOI: 10.1016/S2221-1691(13)60006-4

Sunkar, S.; Nachiyar, C.V.; Renugadevi. K. (2014). Endophtytic *Bacillus cereus* mediated synthesis of gold nanoparticles and their stabilization using biopolymer chitosan. *Chem. Pharm. Res.*, **6**(11), 434–43.

Tiago, I.; Pires, C.; Mendes, V.; Morais, P.V.; Costa, M.S.; Verissimeo, A. (2006). *Bacillus foraminis* sp. nov., isolated from non- saline alkaline groundwater. *Inter. J. Sys. Evol. Microb.*, **56**(11), 2571-4. DOI: 10.1099/ijs.0.64281-0

Zhang, X.; Qu, Y.; Shen, W.; Zhou, J. (2016). Biogenic synthesis of gold nanoparticles by yeast *Megnusio mycesingens* Lh- FI for catalytic reduction of nitrophenols. *Coll. Surfaces A: Physicoch. Eng. Aspects.* **497**(5), 280-5. DOI: 10.1016/j.colsurfa.2016.02.033

تقنية تشخيص جديدة لجسيمات الذهب النانوية والمصنعة حيويا بواسطة بكتريا *Mesobacillus foraminis* ShAm 98 Iraq المعزولة من مياه الينابيع الحارة لحمام العليل في الموصل/ العراق

شفق طارق برهان **اميرة محمود الراوي**

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل

الملخص

تم عزل سلالة محلية جديدة من بكتريا *Mesobacillus foraminis* (*M. foraminis*) من مياه الينابيع الحارة لحمام العليل جنوب مدينة الموصل/ العراق وشخصت جزئيا باستخدام (phylogenetic analysis) لوحظ التغير اللوني كخطوة أولى لقابليتها على انتاج جسيمات الذهب النانوية (GNP) ولأثبات وجود جسيمات النانو العنقودية ، أستخدم تحليل حيود الاشعة السينية (XRD) وقد لوحظ ان افضل تكوين عناقيد الذهب النانوية كان عند المستوى (111) وقد استخدمت تقنية مجهر القوة الذرية لتحليل الشكل والحجم ونعومة سطح الجسيمات ألنانوية بينما أستخدم ألمجهر ألإلكتروني ألماسح وألنافذ (SEM+TEM) لتشخيص شكل وحجم جسيمات الذهب النانوية بالإضافة لذلك تم استخدام صبغة الاكريدين البرتقالية لصبغ GNPs وفحصها تحت المجهر الفلوريسيني كتقنية مبتكرة وجديدة لتحديد الصفات الشكلية لبوليمرات الذهب النانوية. وقد أظهرت النتائج صورا مذهلة لبوليمرات الذهب النانوية مع بقع مشعة وتفاصيل لتراكيب شجيرية متفرعة للبوليمر ونظرا لعدم وجود دراسات سابقة لاستخدام صبغة الاكريدين البرتقالية والمجهر الفلوريسيني في مجال تقنيات النانو فان هذه الدراسة تعد ألاولى في تشخيص جسيمات الذهب النانوية المصنعة حيويا باستخدام هذه التقنية.

الكلمات الدالة: *Mesobacillus foraminis*، Gold NP، المجهر الالكتروني، AFM، صبغة الاكريدين البرتقالية.