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Evaluate the Ability of Environment isolates of Bacillus licheniformis to Synthesized Gold Nanoparticles

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Abstract

The current study aimed to investigate the ability of Bacillus licheniformis isolated from the environment to synthesize gold nanoparticles (AuNPs). The study included isolating and identifying of B. licheniformis from the soil and investigating its ability to synthesis AuNPs, then identifying AuNPs using XRD, UV -vis., FeSEM, EDS, FTIR, and AFM. The results showed the ability of B. licheniformis isolated from soil to synthesis AuNPs by changing the color of the solution from yellow to ruby pink and the optical absorption using ultraviolet spectrometry showed the appearance of a beak of AuNPs at a wavelength of 530 nm. FeSEM electron microscopy showed that synthesized AuNPs were spherical in shape and homogeneous with a diameter ranging from 20.47 - 76.50 nm. Also, the results of XRD, FTIR, EDS, and AFM revealed the nanoscale properties of AuNPs synthesized from B. licheniformis.

Key Words: AuNPs, B. licheniformis, XRD, FTIR, EDS, AFM.

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INTRODUCTION

Gold nanoparticles (AuNPs) is one of the most noble metals had been studied due to their unique properties and functionalities of surface that can be used in many aspects including biosensors, microbiology, pharmacy, biotechnology, catalysis, ect. (Rahi et al., 2015). Surfaces of AuNPs can be modified readily with ligands that contain the functional groups such as phosphines, thiols, and amines which exhibit affinity for gold surfaces (Mahato et al., 2019). Due to the wide range of AuNPs and biocidal properties, Au-based compound has been utilized as nontoxic inorganic agents, its compounds are non-toxic to cells of animal but toxic to microorganisms such as fungi and bacteria (Sayadi et al., 2021). AuNPs come in a variety of sizes and shapes such as spherical, decahedral, icosahedral multiple twined, sub-octahedral, nanoprisms, octahedral, nanotriangles, irregular shape, tetrahedral, hexagonal platelets and nanorods (A. K. Khan et al., 2014). Physical, chemical, and biological approaches were used to synthesize metal nanoparticles. Chemical and physical processes give higher yields, but they require specialized and expensive equipment, high energy consumption, difficult circumstances, in addition, these nanoparticles (NPs) cannot be used in the medicine because of health-related issue (Alzubaidi et al., 2023; Huq et al., 2022). Some Nanoparticles had been synthesized by such as silver nanoparticles which produced by B. licheniformis showed antimicrobial activity against human pathogens including gram negative and gram positive bacteria, fungi and viruses (Elbeshehy et al., 2015). Silver and Gold Nanoparticles were produced by using B. licheniformis (R. Singh et al., 2014; Sriram et al., 2012) and cadmium nanoparticles (Bakhshi & Hosseini, 2016). selenium nanoparticles derived biogenic that produced by B. licheniformis had been reported as new prevention strategy reducing prostate cancer (Sonkusre, 2020). B. licheniformis have ability to produce several including proteases, α-amylase, β-mannanase, pentosanase, cycloglucosyl transferase, penicillinase,and several pectinolytic enzyme (Rey et al., 2004).

METHODS

Isolation and Identification of B. licheniformis

Fifteen soil samples were collected from different sites including: garden, barren and river banks during November 2021. The soil samples were collected from 10 cm depth in a sterile screw caps and suspended in 9 ml of distilled water, mixed well, diluted and the second tube (10-2) was placed in water path at 80 °C for 10 min in order to kill all vegetative cells remaining only spores forming bacteria (Organji et al., 2015). One ml of the bacterial suspension was transferred to tubes containing 9 ml of BHI broth and incubated at 37°C for 24-48 hours. After incubation a swab from each positive tubes (tubes with turbidity) were transferred to nutrient agar and blood agar plates for the isolation and primary identification. In addition to morphological character of bacterial colonies and microscopic examination, a confirmed identification of B. licheniformis was carried out using vitek 2 compact system.

Synthesized of AuNPs by B. licheniformis

The approach described by Singh & Kundu, 2014 , has been followed to assess extracellular production of AuNPs . Purified bacterial isolates were grown in 100 mL of nutrient broth for 24 hours at 37° C for biomass generation. The culture was centrifuged for 30 minutes at 8000 rpm/min to separate the bacterial cells. In a new sterile conical flask, the supernatant was collected. One mM of $HAuCl_4.3H_2O$ (chlolrouric acid trihadrates) has been added to 5 ml of the all isolates supernatant and incubated in a shaker at 37°C for 24 hours, the ability of bacterial supernatant to create AuNPs was assessed.

Characterization of AuNPs

Only isolates that showed high ability to synthesis AuNPs were characterized by UV visible, SEM, XRD, EDS, and FTIR. All these technique were carried out in the Biological Center of Tahran University / Iran, except UV visible characterized which carried out in Alameen Center For Advanced Research and Biotechnology (ACARB) in Najaf / Iraq.

RESULTS AND DISCUSSION

The results of primary isolation of B. licheniformis was shown in figure (1). Morphologically, on blood agar base its appear as opaque white colonies with a coarse and matte surface with irregular edge with no hemolytic activity while it appearance in nutrient agar was large opaque, adherent and irregular edges this was proven by researchers (Ghani et al., 2013). Microscopically, it is appear as a rod-shaped Gram-positive cells, arranged in diploid form or long chains. Vitek 2 Compact System confirmed the identification of B. licheniformis as mention in table 1.

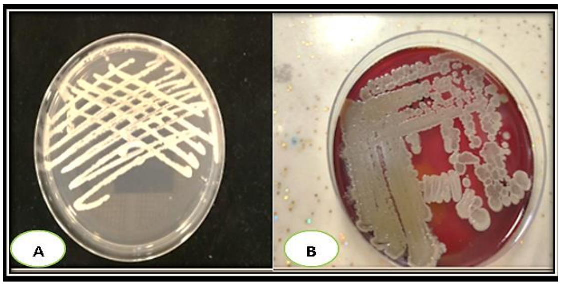
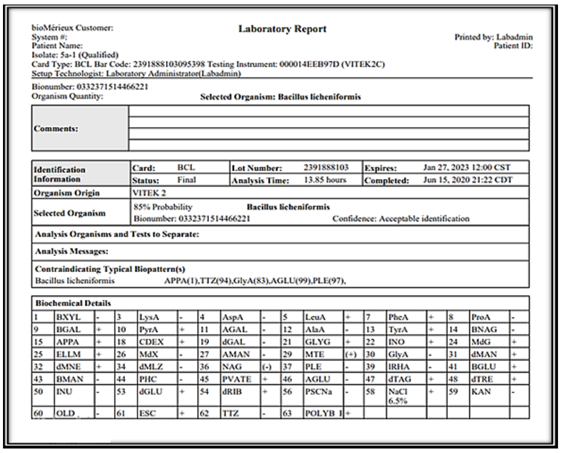


Fig.1. Bacillus licheniformis isolate on A: nutrient agar, B: blood agar base .

Table.1. The result of the vitek 2 compact system for identification of Bacillus licheniformis.



Laboratory Report

bioMérieux Customer:
System #:
Patient Name:
Isolate: 5a-1 (Qualified)
Card Type: BCL Bar Code: 2391888103095398 Testing Instrument: 000014EEB97D (VITEK2C)
Setup Technologist: Laboratory Administrator(Labadmin)

Printed by: Labadmin
Patient ID:

Bionumber: 0332371514466221
Organism Quantity:

Selected Organism: Bacillus licheniformis

Comments:

Identification Information	**Card:**	BCL	**Lot Number:**	2391888103	**Expires:**	Jan 27, 2023 12:00 CST
	Status:	Final	**Analysis Time:**	13.85 hours	**Completed:**	Jun 15, 2020 21:22 CDT
Organism Origin	VITEK 2					
Selected Organism	85% Probability **Bacillus licheniformis** Bionumber: 0332371514466221 Confidence: Acceptable identification					

Analysis Organisms and Tests to Separate:

Analysis Messages:

Contraindicating Typical Biopattern(s)
Bacillus licheniformis APPA(1),TTZ(94),GlyA(83),AGLU(99),PLE(97),

Biochemical Details

1	BXYL	-	3	LysA	-	4	AspA	-	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	+	10	PyrA	+	11	AGAL	-	12	AlaA	-	13	TyrA	+	14	BNAG	-
15	APPA	+	18	CDEX	+	19	dGAL	-	21	GLYG	+	22	INO	+	24	MdG	+
25	ELLM	+	26	MdX	-	27	AMAN	-	29	MTE	(+)	30	GlyA	-	31	dMAN	+
32	dMNE	+	34	dMLZ	-	36	NAG	(-)	37	PLE	-	39	IRHA	-	41	BGLU	+
43	BMAN	-	44	PHC	-	45	PVATE	+	46	AGLU	-	47	dTAG	+	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	-
60	OLD	-	61	ESC	+	62	TTZ	-	63	POLYB	+						

Biosynthesis of AuNPs

A large amount of AuNPs have been synthesized by B. licheniformis, the results showed the efficiency of isolate to synthesis AuNPs by changing the color of bacterial supernatant from yellow to pink- ruby (Figure 2).

Fig.2. Biosynthesis of Gold nanoparticles by Bacillus licheniformis showed the color variation from yellow to pink-ruby.

CHARACTERIZATION OF SYNTHESIZED AUNPS

UV-visible spectroscopic examination

The results of UV-visible spectroscopic showed that the absorption peak of AuNPs synthesized by B. licheniformis was at a wavelength of approximately 520 nm. Change the color of bacterial suspension from yellow to red and measurement of the absorbance bands using UV-visible spectroscopy can confirm the biogenesis of AuNPs by B. licheniformis (Figure 3). The UV-visible spectrophotometric method is a true and tried method for identifying nanoparticles. The color of reaction mixture changed after 24 hours of incubation which indicating the synthesis of nanoparticles in the mixture. The AuNPs synthesized using bacteria produced SPR values in the range of 400–700 nm (Menon et al., 2017; Mussa, 2019) or (500-600) (Diego et al., 2018). As time progresses, there is a possibility of aggregation of NPs which results in a shift of longer wavelength region (Soliman et al., 2022). AuNPs synthesized by plant extract showed an absorption band at 225 nm (Rotimi et al., 2019). While that synthesized by Bacillus subtilis bacteria was 545nm (Srinath et al., 2018).

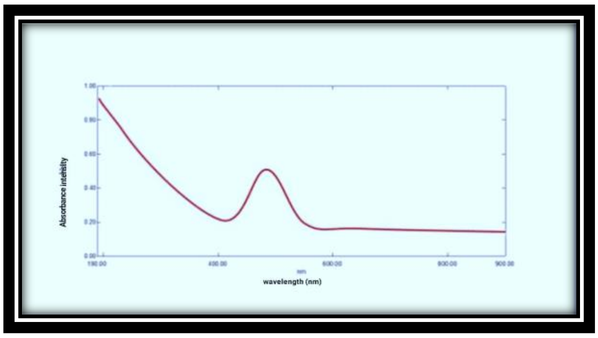


Fig.3. UV-VIS spectrum of gold nanoparticles synthesis by Bacillus licheniformis.

Field Emission-Scanning Electron Microscopy (FE-SEM) Analysis

The results of FE-SEM of AuNPs synthesized by B. licheniformis showed a homogenous in diameter and spherical shapes nanoparticles were the diameter of AuNPs ranges between 20.47-76.50 with average 33.6 nm as showed in figure (4). Many of studies showed the spherical shape of AuNPs (Yuan et al., 2023; Zhao et al., 2022; Depciuch et al., 2020). It has been confirmed that the reduction of biomolecules by mineral salts results in spherical nanoparticles (Sun et al., 2019)The variation in the shape and size of synthesized AuNPs may due to differences preparation method which lead to different shape and size of AuNPs (Gu et al., 2021).

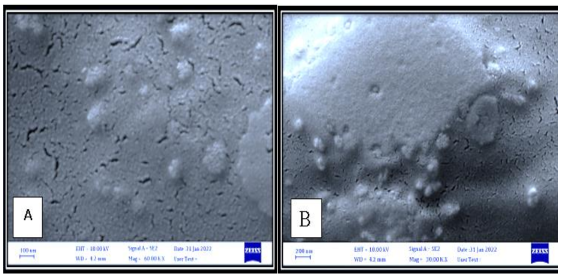


Fig.4. FE-SEM examination of gold nanoparticles synthesized by Bacillus licheniformis at: A: 100 nm, B: 200 nm.

X-Ray Diffraction (XRD) Analysis of AuNPs

As mention in figure 5, the results showed that the extracellular AuNPs synthesized by B. licheniformis are greatly crystalline in nature where four distinct peaks at 2θ values of 64.5, 44.1, 37,8 and 77,5 corresponding to 111, 200, 220, and 311 planes of AuNPs synthesis by B. licheniformis. XRD spectra of pure crystalline Au structures was published by the Joint Committee on Powder Diffraction Standards (file nos. 04-0784). The size of these AuNPs were 34.5 nm according XRD analysis. These results are compatible reported findings of characterization of AuNPs (Fereig et al., 2022; Xie, et al., 2019; Rajeshkumar, 2016).

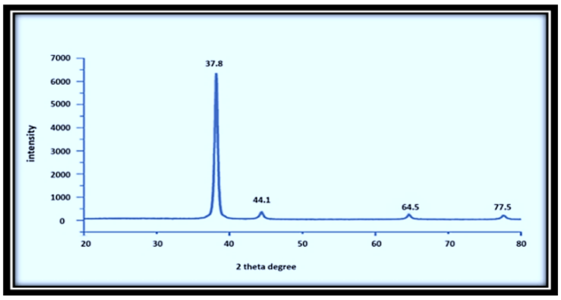


Fig.5. XRD of gold nanoparticles synthesis by Bacillus licheniformis.

Fourier Transform Infrared (FTIR) Analysis

The results of FTIR chart (Figure 6) showed the appearance of different peaks at range between 500-4000 cm^{-1}. The occurrence of

different peaks of absorption referred to the presence of other chemical groups such as O-H stretching group, C-H stretching group of alkane and N-H group. As mention in table (2) the peak of absorption of carboxylic group occur at 3911.5-3375.90 cm-1 and 3448.80 cm-1 which referred to the present of carboxylic group in carbohydrate, proteins amino acid residue that accumulated during the process of reduction of gold ions for synthesis of AuNPs (Diego et al., 2018). The presence of different functional groups in the AuNPs synthesized by bacteria indicate the role of these proteins in the synthesis and stabilization (preventing agglomeration) of the nanoparticles where the original proteins and the mechanisms of synthesis are not clear(San Diego et al., 2021).
(Rabeea et al., 2020) suggest that the FTIR explain the attachments of polysaccharides, phenol and amino acids as reducer agents on AuNPs .The materials used in the manufacture of nanoparticles often contain a wide range of biologically active substances, which makes it difficult to determine the exact component responsible for the synthesis (F. Khan et al., 2021).

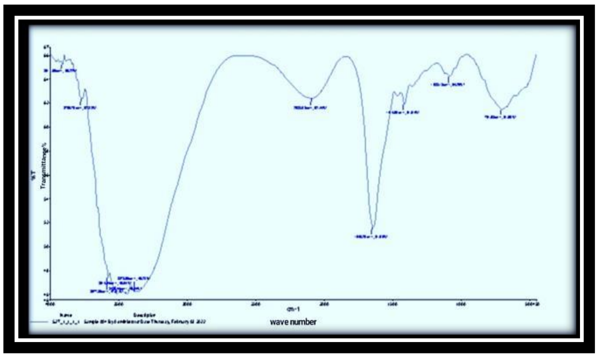


Fig.6. Fourier transform infrared spectroscopy (FTIR) of Nano biosynthesized Gold Nanoparticles by Bacillus licheniformis.

Other peaks have shown in this study at 1645.26- 1083.52 cm-1 referred to carbonyl stretch and N-H bands vibration. N-H band vibration represent indicators of amid I and amid II groups of polypeptides as indicate by Faghihzadeh et al., 2016

Energy dispersive spectroscopy (EDS)

EDS analysis was used to define the crystal element of the presence of AuNPs by observed the optical absorption peaks. the element mapping visibly show the eminent amount of gold elements in the samples (Table 3). The results of EDS showed that the percentage of nanoparticles synthesized by B. licheniformis were higher (40.71%) than other elements present in solution where the lower percentage of oxygen (9.3%) was observed. The percentages of other elements were as follow: C = 19.2%, Cl = 16.1% and Na = 14.7% (Figure 7 and Table 2). high amount of AuNPs with low and moderate amount of other elements that accompany the presence of the gold element may be produced from the components of a chemical generator used to adjust the value of the broth (Gupta & Padmanabhan, 2018) or may originated from bacterial biomolecules bound to the surface of AuNPs (Murugan et al., 2014)

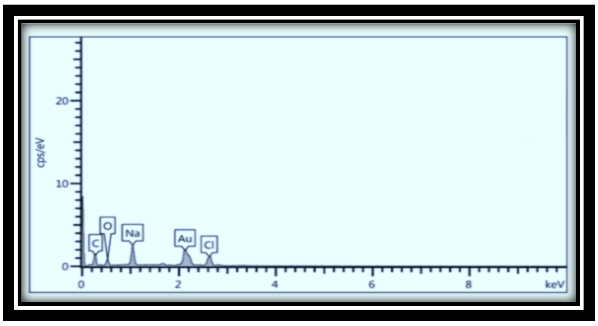


Fig.7. EDS analysis of gold nanoparticles synthesis by Bacillus licheniformis.

Table.2. The Percentage of Elements of gold nanoparticles synthesis by Bacillus licheniformis.

Elements	%
Au	40.7
C	19.2
Cl	16.1
Na	14.7
O	9.3
Total	100

ATOMIC FORCE MICROSCOPE (AFM)

The results of AFM analysis of AuNPs that synthesized by B. licheniformis showed (Figure 8) that AuNPs synthesized by B. licheniformis have physical size (6.397 × 6.397 µm) with average roughness about 4.294 nm. The rough surface depicts the synthesis of AuNPs. The hump depicts the big sized particles or the agglomerated AuNPs which fuse to give various exotic shapes in the later phase of reaction whereas the small individual peaks exposed in the images are of single spherical AuNPs. SEM measurements supported the AFM results about the structural features and surface morphology of AuNPs synthesized well-dispersed spherical shaped. homogeneous population nanoparticles had arranged with regular surface shape with the peaks towards the top. The results showed homogenous in diameter and spherical shapes nanoparticles. The different preparation methods lead to different nanoparticle sizes and size distributions (Gu et al., 2021) These results are almost identical to (R. Singh et al., 2014) who got a size of AuNPs with average 33.6 nm and match with the results of the researchers(Gupta & Padmanabhan, 2018; Khalid et al., 2020). In the other study the AuNPs synthesized by B. licheniformis were nanocubes with range of size 10-100 (Kalishwaralal et al., 2009).

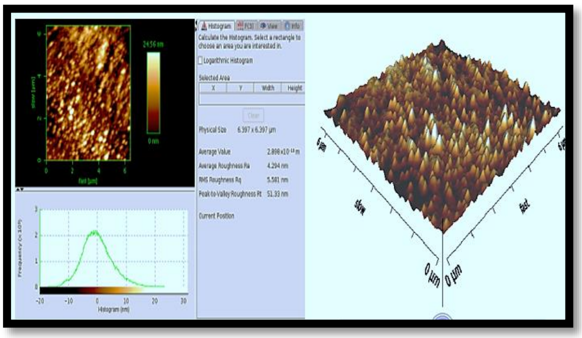


Fig.8. Atomic Force Microscopy (AFM) images of synthesized gold nanoparticles by Bacillus licheniformis.

CONCLUSION

The results showed the ability of B. licheniformis isolated from soil to synthesis AuNPs by changing the color of the solution from yellow to ruby pink and the optical absorption using ultraviolet spectrometry showed the appearance of a beak of AuNPs at a wavelength of 530 nm.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING SOURCE

None

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