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Bio-Synthesis of Gold Nanoparticle Using B. subtilis and Their Antibacterial Activity Against Pathogenic Bacteria

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Abstract:

Background: In recent decades, research has focused on finding an alternative antibacterial agent due to the spread of resistance isolates and the side effects of antimicrobial agents. In the present study, the antibacterial activity of gold nanoparticles (AuNPs) against clinical bacterial isolates was studied.

Method: In this study, the supernatant of Bacillus subtilis was used to synthesize (AuNPs), and the resulting (AuNPs) were characterized by spectroscopic techniques. also, the antibacterial activity of (AuNPs) against Enterobacter cloacae, Pseudomonas aeruginosa, Proteus mirabilis, and Staphylococcus haemolyticus isolates were studied by disk diffusion method.

Results: The synthesis of (AuNPs) was confirmed by a color change. which was check were by UV-spectroscopy at a maximum absorption peak of 535 nm. Field Emission Scanning Electron Microscope (FE- SEM) confirmed the presence of small spherical nanoparticles with an average diameter of 21.86 - 31.94 nm. X-ray diffraction (XRD) shows the crystalline nature of (AuNPs) was verified with an average particle diameter of 41. 14 nm. AuNPs showed antibacterial activity with inhibition diameters of P.aeruginosa 13 mm, P. mirabilis 10 mm, S. haemolyticus 25 mm, E. clocae 25 mm.

Conclusion: It was found that the antibacterial activity of (AuNPs) was shown against both Gram-negative and Gram-positive isolates. based on the result, it was concluded that AuNPs are effective against pathogenic bacteria.

Key words: Antibacterial activity, AuNPs, Bacillus subtilis, Biosynthesis, Gold nanoparticle.

1-Introduction:

The genus Bacillus has taken an important role in technological applications through the production of antimicrobial compounds [1]. The high of antibiotic resistance is one of the greatest threats to public health, especially given the scarcity of new and effective antimicrobial drugs [2]. The World Health Organization (WHO) recently included ESKAPE pathogens in the list of bacteria that urgently need new antibiotics and approaches [3]. As a new context, nanotechnology has been exploited to produce new antimicrobial drugs, so the microbe is unable to develop resistance to them making a wide range of advantages, including effective targeting, low toxicity, and enhanced pharmacokinetics [4]. Several strategies such as the biosynthesis of AuNPs have been used as an alternative to antimicrobial commercial antibiotics [5]. Many fields of technology have used metallic nanoparticles as antibacterial agents, Studies have shown that these nanoparticles can be a susbstitute for functions commonly used commercial antibiotics [6]. Microorganisms such as bacteria, fungi, yeasts and plant extracts have been used to synthesize metal nanoparticles such as AuNPs. The biological extracts of these organisms contain a group of enzymes that play a role in reducing dissolved metal ions into stable nanoparticle [7]. Metal ions were reduced to nanoparticles by enzymes secreted during metabolic processes using extracellular extraction methods[8]. B. subtitlis genus was used to synthesize AuNPs [9]. The present study aims to develop an alternative strategy to overcome pathogens using AuNPs synthesized from B. subtitlis isolate.

2- Research Gap:

Many studies have shown the importance of nanoparticles on the medical side to fight disease or neutralize their spread, as a result of the emergence of disease-resistant, antibiotic-resistances.The investigation, synthesis of gold nanoparticles in safe biological methods, can pave the way for an effective and safe treatment to be an alternative to the use of chemical antibiotics.

3- Material and Methods:

3.1 Bacterial Isolates

B. subtilis is isolated from local soil samples and diagnosis using Vitek-2 system and used to synthesize AuNPs. The pathogenic bacteria P. mirabilis, P. aurginosa, E. clocae, S. haemolytic were isolated from different clinical samples and diagnosis using the morphology and biochemical test, this isolate was used in the current study [10].

3.2 Preparation B. subtilis supernatant

A flask containing 100 ml of Brian Heart Broth was inoculated with B. subtilis isolate under sterile conditions. The isolates were incubated at 37°C, PH-7 for 72h in shaker incubation. The supernatant was then centrifuged and used as a reducing agent in AuNPs synthesis process [11].

3.3 Bio-synthesis Gold nanoparticles

AuNPs were prepared by mixed B. subtilis supernatant with 1mM of $HAuCl_4$ solution (2/9,v/v) and incubated at 37°C, in 150 rpm rotary shaker. The formation of AuNPs was determined by color change from yellow to wine red and the reduction of gold ion was confirmed by Uv-spectroscopy [12].

3.4 Characterization of Gold nanoparticles

After getting a stable color change in 24h, The formation AuNPs was scaned by a UV-Spectroscopy (Germany-ALS lab) from 200 to 700 nm [13]. AuNPs were analyzed by XRD using KU-KA radiation source by X-pert high

instrument at wavelength (λ=1.54060). The crystalline size of AuNPs was measured using Debye Scherrer equation [14]. (FE- SEM) was used to investigate the surface morphology and size of biosynthesized AuNPs [15].

3.5 Antibacterial activity of Gold nanoparticles

Four microorganisms P. mirabilis, S. haemolyticus, P. aeruginosa and E. cloacae were collection from different clinical sample for sensitivity testing of isolates to AuNPs. MacConkey agar and Blood agar wear used to purify pathogenic isolates. The antibacterial activity of AuNPs was determined by disk diffusion method, sterile filter discs were impregnated with AuNPs for 24h and placed on Muller Hinton agar plates swabbed with bacterial suspension. The plates were incubated in an incubator at 37°C for 18h - 24h, after which the diameter of inhibition zone were measured [16].

4- Results:

4.1 Bacterial Isolates

B. subtilis isolate was obtained from local soil samples, Vitek-2 system were performed, as in Table1. while pathogenic isolated P. mirbilis, S. haemolyticus, P. aeruginosa and E. cloacae were obtained from different clinical sources, after repeated isolation process of bacteria on MacConkey agar and Blood agar, pure isolates were obtained, morphological and biochemical tests were performed, as in Table 2.

Table1. Characteristic B. subtilis isolate using Vitek-2

Source:Soil **Collection:Apr6,2024**

Comments:	

Identification Information	Analysis Time 13.98 h	Status: Final
Selected Organism	91% Probability Bionumber:	
ID Analysis Messages		

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	NdG	-
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	NaCl6.5 %	+	59	KAN	-
60	OLD	-	61	ESC	+	62	TTZ	+	63	POLYB R	+						

Table 2. Morphological and Biochemical test of isolated B. subtilis, P. aeruginosa, E. clocae, S. haemolyticus

Morphological and Biochemical	B. subtilis	P. aeruginosa	P. mirbilis	E. cloacae	S. haemolyticus
Gram stain	+	-	-	-	+
Shape	Rod	Rod	Rod	Rod	Coccus
Methyl Red test	-	-	+	-	+
Indole test	-	-	-	-	-
Voges proskar test	+	-	-	+	+
Klikler Iron test	A/A	A/A	K/A	A/A	K/A
Catalase test	+	+	+	+	+
Oxidase	-	-	-	-	-
Motility	+	+	+	+	-

4.2 Preparation B. subtilis supernatant

After incubating B. subtilis at 37^0C for 72h and pH-7, a centrifuge was made and the supernatant was used as a reducing agent in the AuNPs synthesis process.

4.3 Bio-synthesis Gold nanoparticles

In this study, Extracellular biosynthesis of AuNPs by supernatants of B. subtilis with $HAuCl_4$ (1mM) was investigated. The results showed that color change after 24h of incubation at room temperature, the change in color of solution to wine red indicated the synthesis metallic AuNPs optically, as in the figure 1.

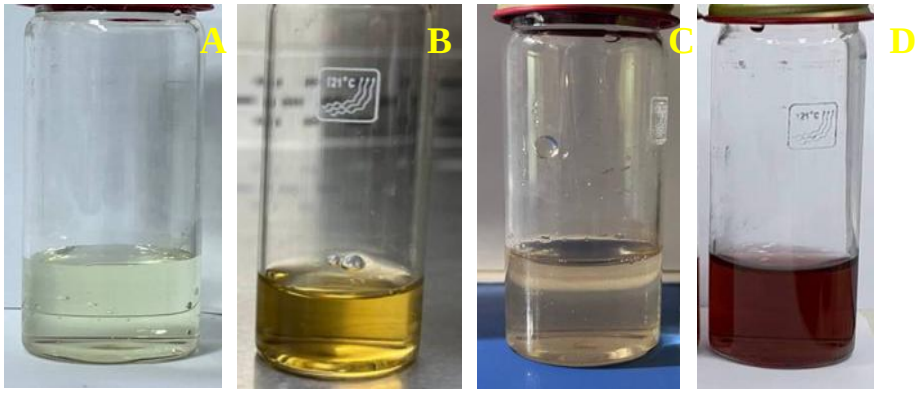

Figure1. (A) $HAuCl_4$ solution (1mM), (B) B. subtilis supernatant, (C) (AuNPs) after distillation, (D) AuNPs after 24h.

4.4 Characterization of Gold nanoparticles

4.4.1 Ultraviolet-Visible Light (UV-Vis) Spectroscopy

The optical properties of the biosynthesized AuNPs were examined employing a UV-Vis spectrometer. Illustrates the absorbance wavelength range of the AuNPs was 535 nm, inside the nano-range at room temperature. The accomplished UV-Vis crest demonstrates a coordinate electron recombination between the valence and conduction groups, as in the figure 2.

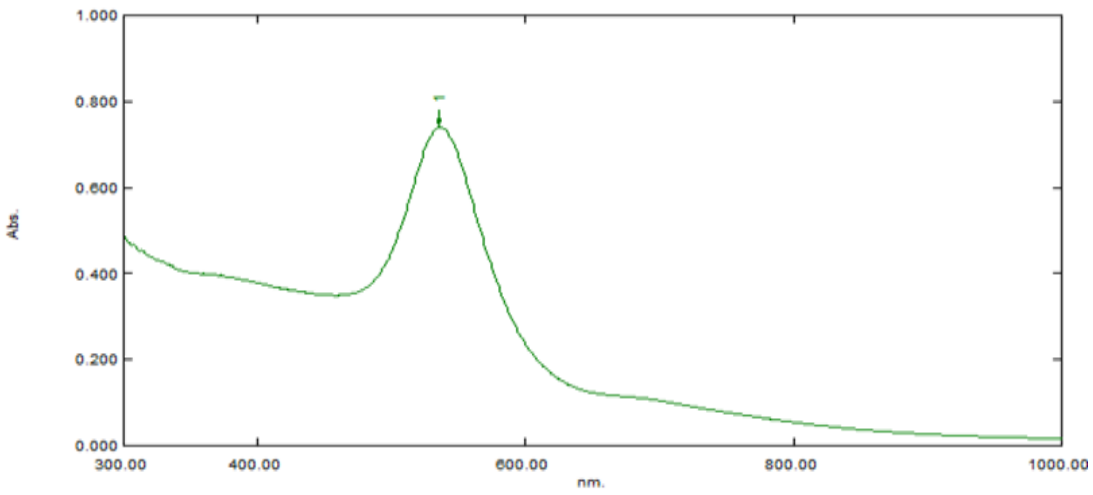

Figure 2. UV- vis absorbance spectrum of AuNPs by B. subtilis supernatant.

4.4.2 FE-SEM of Au nanoparticles

The surface morphology obtained using the FE-SEM technique was investigated and the topographical analyses were presented based on the surface investigation. Moreover, the prepared AuNPs sample exhibited small spherical particles. It is worth mentioning that the average nanoparticle diameter was found to be around 21.86 - 31.94 nm using Image J software as in the figure 3.

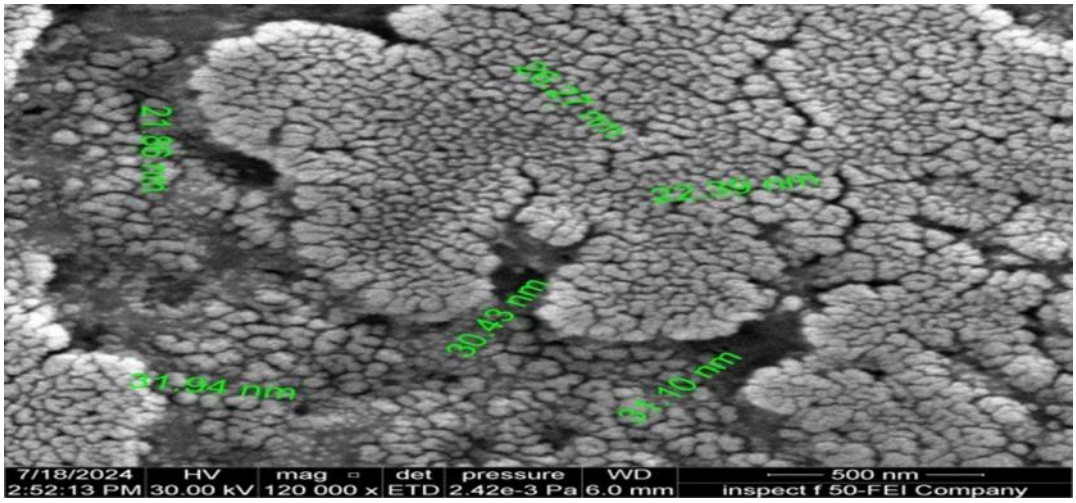

Figure 3. The FE-SEM micrograph of AuNPs biosynthesis by B. subtilis supernatant.

4.4.3 XRD analyasis

XRD was used to study the structural and crystalline properties of AuNPs synthesized from B. subtilis as a reducing agent. Where values obtained with Bragg reflections at 2 Θ = 28.7653, 47.6749 and 69.4391 degrees are consistent with the crystal structure of the AuNPs obtained where peak (778) 28 is 33 nm, peak (496) 47 is 10 nm and peak (338) 69 is 79 nm, as in the Table 3. where the mentioned peaks are consistent with different standard data standard (JCPDS) In addition, these peaks are consistent with the basal levels (111, 200, 311), as shown in the figure 4. If the AuNPs were subjected to XRD measurement to prove the crystal structure and find the average size of 41.14 nm AuNPs using the Debye-Scherer equation. These manufactured AuNPs are considered pure because they all belong to the AuNPs peaks and this means that these particles have been manufactured using specific and advanced techniques to obtain a pure and impurity-free product.

Table 3. Data of XRD anaylsis of size AuNPs in use Debye Scherrer.

No.	Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	Area [cts*°2θ]	Crystallite Size only [Å]	Debye Scherrer equation (nm)	Average diameter (nm)
1	28.7653	778.3	0.2559	321.56	270	33.49	41.14
2	47.6749	496.43	0.8622	269.93	84	10.53	
3	69.4391	33824.93	0.1272	5195.51	1218	79.40	

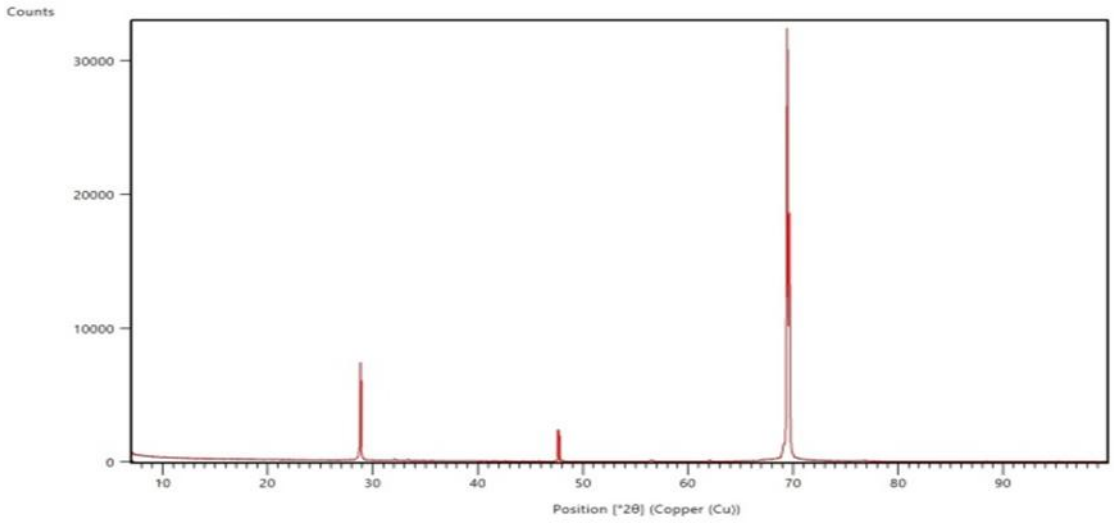

Figure 4. XRD Pattern of AuNPs synthesis by B. subtilis supernatant.

4.4.4 Antibacterial activity of Gold nanoparticles

The present study, E. cloacae, P. mirabilis, P. aeruginosa, S. haemolyticus isolate show antibacterial activity of AuNPs synthesized from B. subtilis supertanent, as in the figure 5, where the P. mirabilis isolate gave an inhibition zone around the disc (10) mm, while the E. cloacae isolate gave an inhibition zone around the disc (25) mm, and the P.aeruginosa isolate gave an inhibition zone (13) mm, while the S. haemolyticus isolates gave the inhibition zone reaching (25) mm. That exposure to these particles led to a high antibacterial effect, as in the Table 4.

Table 4. Antibacterial activity of AuNPs against pathogenic bacteria.

Pathogenic bacteria	Inhibition zone
E. clocae	25
P. aeruginosa	13
P. mirabilis	10
S. haemolyticus	25

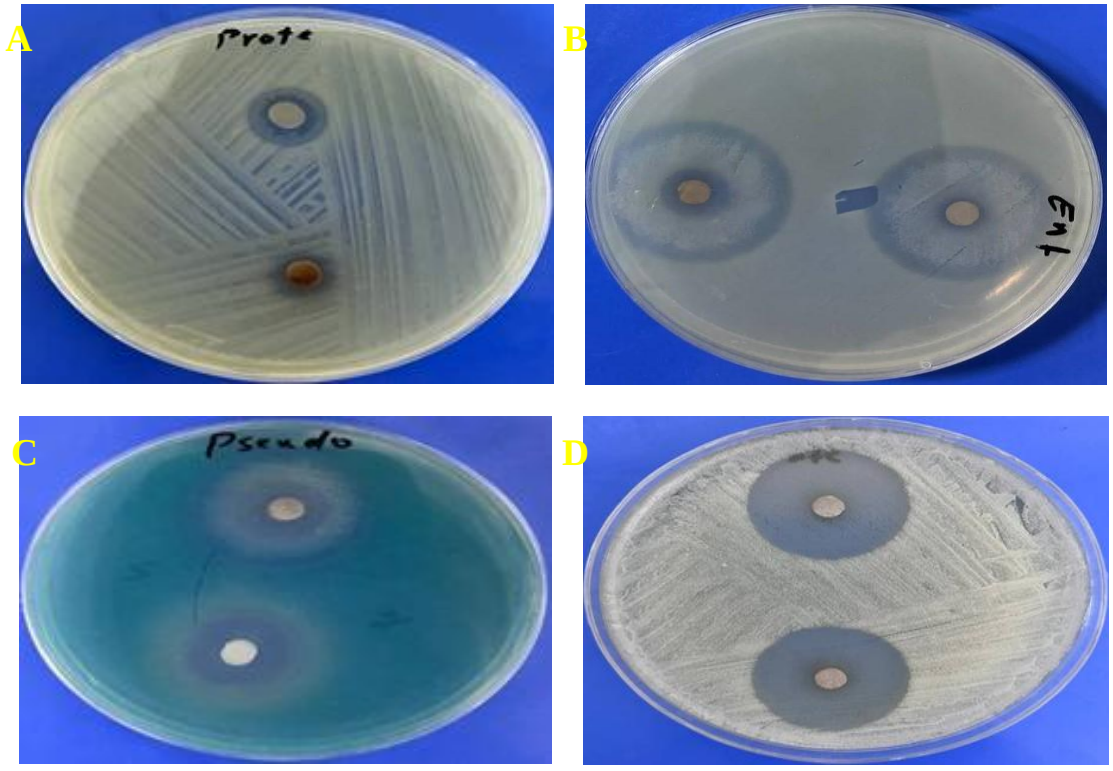

Figure 5. Antibacterial activity of (AuNPs) againt gram negative and positive organisms (A), P. mirabilis (B), E. cloacae (C), P.aeruginosa (D), S. haemolyticus.

5- Discussion:

The study was carried out in the Microbiology Laboratory at the College of Education for Pure Sciences/Department of Biology, Thi-Qar University, Iraq. The Pathogenic bacterial isolates were identified using morphological and biochemical test, also B. subtilis identified using Vitek-2. Blending the B. subtilis supertantent with HAuCl4 Solution (1mM) led to a color change from yellow to wine red. The color change is due to the surface plasmon resonance phenomenon in AuNPs that occurs as a result of the transformation from metallic to nanoscale size, this result is consistent with [17]. AuNPs appear pink, red, purple, ruby colors when the conditions for bacterial growth change, this study consistent with [18].

The AuNPs absorption peak at 353 nm wavelength indicates the presence of gold atoms that suffer from the quantum size effect, which affects their optical behavior and makes them absorb light in this region. UV-spectroscopy technique was used to confirm the presence of AuNPs manufactured using B. subtilis supertantent, where UV-spectroscopy spectra showed the surface plasmon resonance band 535 nm, this study are consistent with [19]. SEM analysis of AuNPs from B. subtilis is consistent with [20]. The average particle size of AuNPs was measured at incidence angles and peak widths, at peak (778) 28 nm, 33 nm, peak (496) 47 nm, 10 nm, and peak (338) 79 nm. This result is consistent with the result of (SEM) and studies [21], [22], [23]. The X-ray diffraction as shows result close to the JCPDS card as a standard reference for the composition of AuNPs. From the diffraction angles, it was proven that the AuNPs have a spherical shape, and the results of XRD are consistent with [17]. SEM was used during the study and the examination results gave diameters up to 21.86 - 31.94 nm. This nano-diameter shows an interpretation consistent with the results of UV-spectroscopy [24]. Antibacterial activeity of AuNPs showed inhibition zone close to commercial antibiotics against isolates P. mirabilis, P. aerginosa, S. haemolyticus, E. cloaca.

AuNPs have the ability to inhibit both gram-positive and gram-negative bacteria, these result are consistent with [25]. study indicated to different mechanisms that cause the inhibition, namely targeting of AuNPs to the bacterial cell wall, DNA, protein and their ability to penetrate due to their small size, where AuNPs target cell membranes and cause holes and lead to cell death, as AuNPs were work to bind the surface of the peptide to the glycan ports in the cell wall and break dow N-actylglucosamine and N-actylmuramice bound in the glycan [26].

6-Conclusions:

In conclusion, AuNPs can be synthesized using B. subtilis as a reducing agent. The UV-spectroscopy absorption spectra revealed the maximum absorption peak at 535 nm. SEM data revealed the particle sizes up to 21.86 - 31.94 mm. The crystalline nature was also shown to be spherical using XRD technique. The biologically prepared AuNPs showed antibacterial activity against gram-negative and gram-positive pathogenic isolates.

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