

## Production and Optimization of Biosyntheses Gold nanoparticle(AuNPs) from *Bacillus subtilis*

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### Abstract :

Gold nanoparticle (AuNPs) have many advantages and a wide range of application in various fields, for that, there is a growing interest for the syntheses of AuNPs using an economical, safe and less complex. This study demonstrated that the extracellular biosynthesis of gold nanoparticle (AuNPs) using *Bacillus subtilis*, characterized using UV-Vis spectroscopy, and studied optimization condition of culture broth using the OVAT method. Strain of *Bacillus subtilis* was isolated from local soil sample to enhance the production of gold nanoparticle by the bacteria. The bacteria extracellular extract production in Brain Heart Broth. The whole optimum condition was conducted at 150 rpm stirring speed. The highest amount of gold nanoparticle synthesis was demonstrated by the isolated *Bacillus subtilis* at 37°C, 96 h of incubation, pH 7, Starch carbon source, and Beef extract nitrogen source. Optimized medium is given double results for AuNPs production.

**Key words:** *Bacillus subtilis*, Biosyntheses, gold nanoparticle, HAuCl<sub>4</sub>, AuNPs.

### Introduction

In previous studies, nanoparticles (NPs) have attracted great interest in various applications in medicine, agriculture, and biosensors. It is known that nanoparticles (size less than 100nm) have an effective use in most applications due to their similar size to biological molecules and their easy penetration (1). Gold nanoparticles (AuNPs) have optical and electrical properties that are different from traditional materials and have a future in the field of medicine (2). These characteristics include a high surface area-to-volume ratio, stability, surface plasmon resonance, surface chemistry, AuNPs may advantageously of which easily production in various forms and sizes (3). AuNPs have been developed by physical, chemical, and biological methods (4). Biological syntheses of AuNPs has been interesting attention because avoid hazardous and chemical substances. In addition, the synthesis of AuNPs is at appropriate conditions of pH, temperature, and no require the addition of stabilizing agent which less their toxicity and expands their medical application (5). The biological synthesis of NPs is a non-hazardous, and energy-efficient technique of manufacturing NPs. This technique employs in various sources (protein, fatty acids, sugars, enzymes, and phenolic substances) play an important role in both the bioreduction of metallic ion to NPs and their stability. AuNPs produced biologically are more stable than those produced by other techniques. AuNPs may be produced easily using chemical processes, the great danger is the creation of by-products (secondary products) that are detrimental to human health and the environment. Many biological systems, including plants, bacteria, yeast, and fungi, are thus new methods for the synthesis of safe nanoproducts, such as AuNPs (6). Microbial extract was excellent factories that worked to absorb metal ions and reduce them to NPs by producing enzymes through metabolism using the extracellular method to facilitate their extraction and do not require for other processes (7). *Bacillus* was used to biosynthesize AuNPs such as *B. niabensis*, *Lactobacillus*, *Lysinibacillus*, *B. cereus*, *B. subtilis*, *B. megaterium* and *B. marisfla*

(8). This research study aimed to investigate the ability of microorganisms *B.subtilis* collected from the local soil of southern Thi- Qar and to study the ideal conditions affecting a fermentation medium and to study its effect on the production of AuNPs.

## Materials and Methods

### *Samples Collection*

Soil samples were collected from the Al-alfuhud marshes in Thi-Qar, the samples were kept in sterile bags and stored at 4°C in laboratory for further experiments (9).

### *Microbial Cultures*

Serial dilutions ( $10^{-1}$  –  $10^{-6}$ ) were performed on all soil samples, where 1g of soil was taken and placed in test tubes containing 9 ml of distilled water, and the tubes were placed in a water bath device at a temperature of 80°C for 10min. 0.5 ml of each dilution was taken, then spread on nutrient agar, then incubated for 24 h, at a temperature of 37°C (10).

### *Strains and cultivation of bacteria*

*B. subtilis* was identified by Vitek-2, Its grown in Brain Heart Broth medium, Culture was incubated at 30°C degrees for 72h in a shaking incubator at 150 rpm (11).

### *Biosynthesis of Gold nanoparticle (AuNPs)*

*B. subtilis* fermentative media was centrifuged for 20 min at 10,000 rpm, The supernatant is sterilized with a Milipore filter unit (0.22 µm). The resulting extract is mixed with HAuCl<sub>4</sub> (1mM) (1/9) v/v on a magnetic stirrer. The mixture is incubated in a vibrating incubator at 37°C. The synthesis of AuNPs is observed through the color change and the reduction of the gold ion to a wine red color, as well as the UV-vis spectrum at Range (500-600) nm (12).

### *Optimal Conditions of Production Gold nanoparticle (AuNPs) using one variable at a time (OVAT) method.*

***Effect of incubation period on Production Gold nanoparticle (AuNPs):*** to determine the effect of incubation period on biosynthesis Gold nanoparticle, *B.subtilis* isolate was grown on Brain Heart Broth and incubated at 30°C, for 24, 48, 72, 96, and 120h. under shaking at 150 rpm.

***Effect of temperature on Production Gold nanoparticle (AuNPs):*** to determine the effect of temperature on biosynthesis Gold nanoparticle, *B.subtilis* isolate was grown in Brain Heart Broth and incubated at 30, 35, 37, 40, and 45°C for 72h under shaking at 150 rpm (11).

***Effect of pH on Production Gold nanoparticle (AuNPs):*** Effect of culture media pH on biosynthesis Gold nanoparticle was performed by adjusting Brain Heart Broth to pH 5, 6, 7, 8, and 9 before bacterial inoculation. culture broth was incubated at 30°C, for 72 h under shaking 150 rpm. The pH was modified using the HCL(1M) and NaOH (1M) At different times (11).

***Effect of carbon sources on Production Gold nanoparticle (AuNPs):*** under optimum temperature, pH, and incubation period. Five different carbon sources (Starch, Arabinose, Maltose, Xylose, and Dextrose) with the same concentration of 0.2% were tested for biosynthesis Gold nanoparticle. the culture broth was inoculated with *B.subtilis* and incubated for 72 h at 30°C, under shaking 150 rpm, The difference was measured with the UV-vis Spectroscopy at Range (400-600) (13).

***Effect of nitrogen sources on Production Gold nanoparticle (AuNPs):*** The effect of different nitrogen sources were selected by adding 0.2% of the nitrogen sources (Yeast extract, Tryptone, Casein, Peptone, and Beef extract). All of these media were sterilized separately at Autoclave at 121°C for 15 min. The flasks were incubated at 30°C for 72 h under shaking 150 rpm, The difference was measured with the UV-vis Spectroscopy

at Rang ( 400-600) (13).

**Characterization of Gold nanoparticle (AuNPs)**

UV-Vis spectroscopy study using a spectrophotometer(EppendorfAG22331 Hamburg,Germany) at wavelength range of 200-700 nm confirmed the existence of produced nanostructures, For AuNPs. the deionized water used as a blank solution in these studies(14) .

**Results**

**Samples Collection and Identification of Bacteria**

Soil Sample were Collection from the Fuhud marshes in Thi-Qar, and the Sample were diagnosed using VITEK-2.

Table1:Characteristics the isolate using Vitek-2

Source: Soil

Collected: Apr 6, 2024

<b>Comments:</b>	

<b>Identification Information</b>	<b>Analysis Time:</b> 13.98 hours	<b>Status:</b> Final
<b>Selected Organism</b>	91% Probability <b>Bacillus subtilis</b>	
	<b>Bionumber:</b> 0125300574446261	
<b>ID Analysis Messages</b>		

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_R	+						

**Biosyntheses of Gold nanoparticle(AuNPs)**

In this study, isolated *B.subtili* strain, was used to investigate its ability to synthesis gold nanoparticle (AuNPs). The extracellular synthesis of gold nanoparticle was verified by incubation culture supernatants with HAuCl4 at concentration (1mM) and note gradual color change (optically) from Yellow to wine red after incubation, the negative control contain HAuCL4 solution didnot show character change in color (Fig. 1 (A) ).

**Ultraviolet-Visible Light (UV-Vis) Spectroscopy**

UV-visible spectroscopy is a useful technique to study the kinetics biosynthesis gold nanoparticle. The reaction mixture showed shift of peak maxima from 500 nm to 590 nm, Uv-spectra range surface plasmon resonance band with appearances can be seen in (Fig. 1(B)). The intensity of which increased gradually throught 24h, Uv-spectroscopy was used to determine purity of gold nanoparticle.

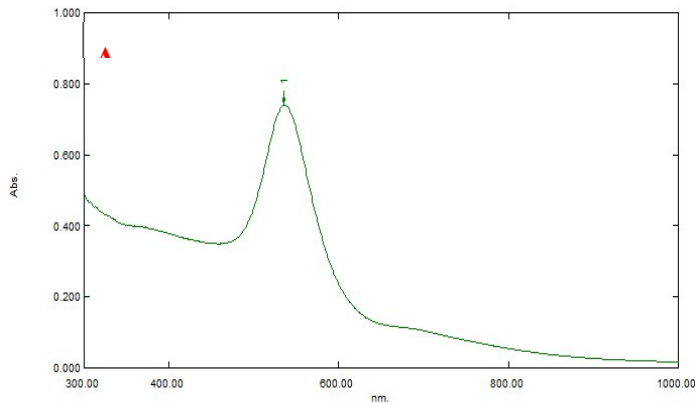


Fig. 1. (A) UV-vis spectra of AuNPs, (B) Biosynthesis of AuNPs using *B.subtilis* supernatant.

**Optimal Conditions of Production Gold nanoparticle ( AuNPs) using OVET method.**

**Effect of incubation period :** the production of the gold nanoparticle( AuNPs) from the *B.subtilis* was monitored through different incubation periods. It is shown in (Fig. 2 (A) and (B)). that biosynthesis of gold nanoparticles increased with increasing incubation period, reaching (0.412) after 24 h, reaching its maximum level of (0.722) after 96h. it decreases for reaching to (0.211) with incubation period to 120 h.

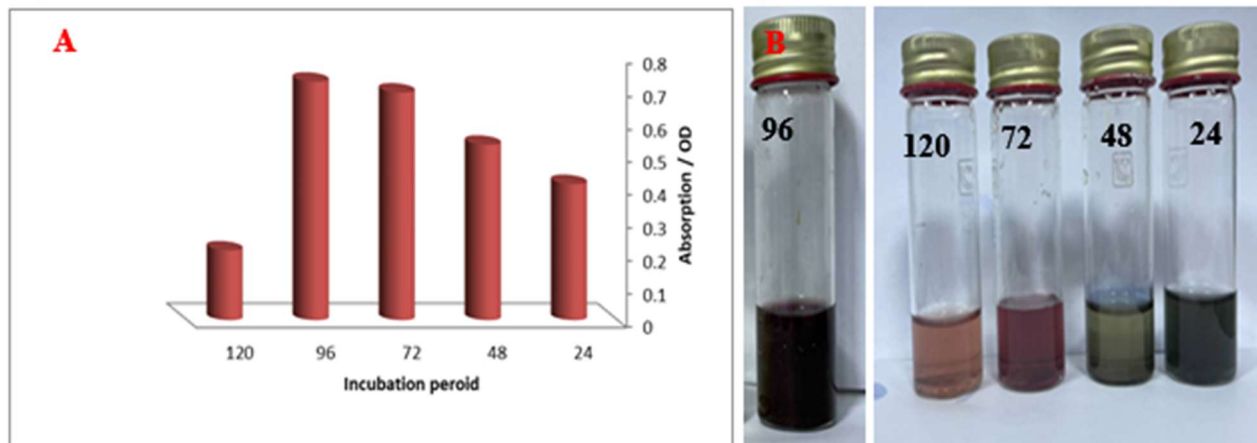


Fig. 2. (A) and (B) The effect of the Incubation period on the production of AuNPs.

**Effect of temperature:** Temperature is an important factor in determining the activity of microorganisms, growth rate, metabolism, physiological and functional characteristics of the microorganism. The gold nanoparticle was produced by the microorganism at a variety of temperatures, ranging from (30-45) °C, and it is shown in (Fig. 3 (A) and (B)). indicated that the best temperature was 37°C, where the AuNPs absorbed reached (0.632), noted that the AuNPs maintained good stability of AuNPs at temperatures 37°C.

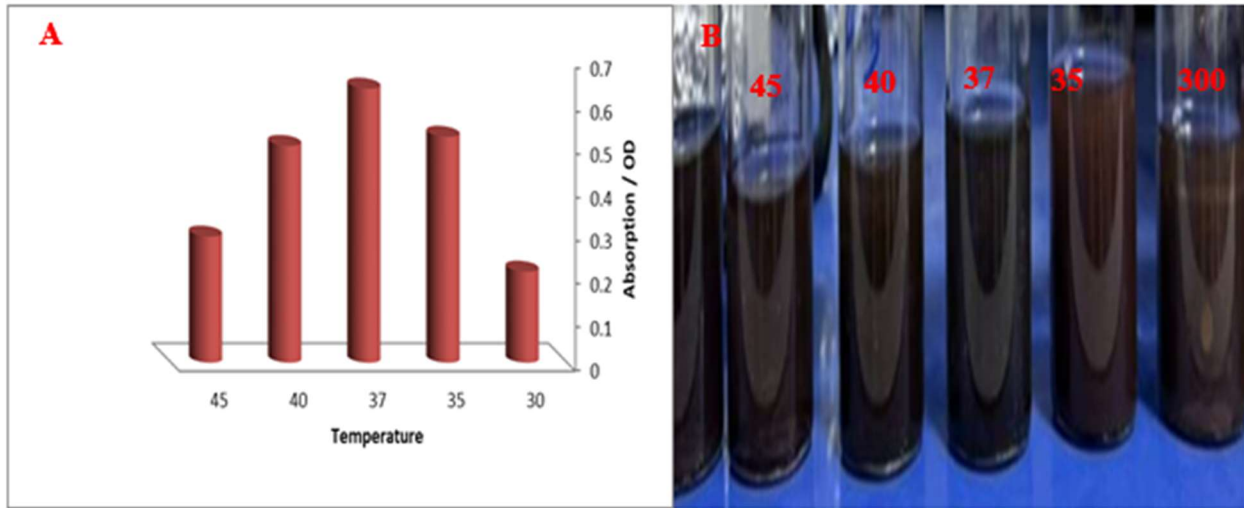


Fig. 3. (A) and (B) The effect of the Temperature on the production of AuNPs.

**Effect of PH:** It has been studied ability of the *B.subtilis* isolate to produce the gold nanoparticle was selected using the production medium with different pH range from (5-9), as shown in (Fig. 4 (A) and (B)) that the highest productive of the AuNPs was at a pH of 7, as its value was (0.528), Its show high stability after 24h.

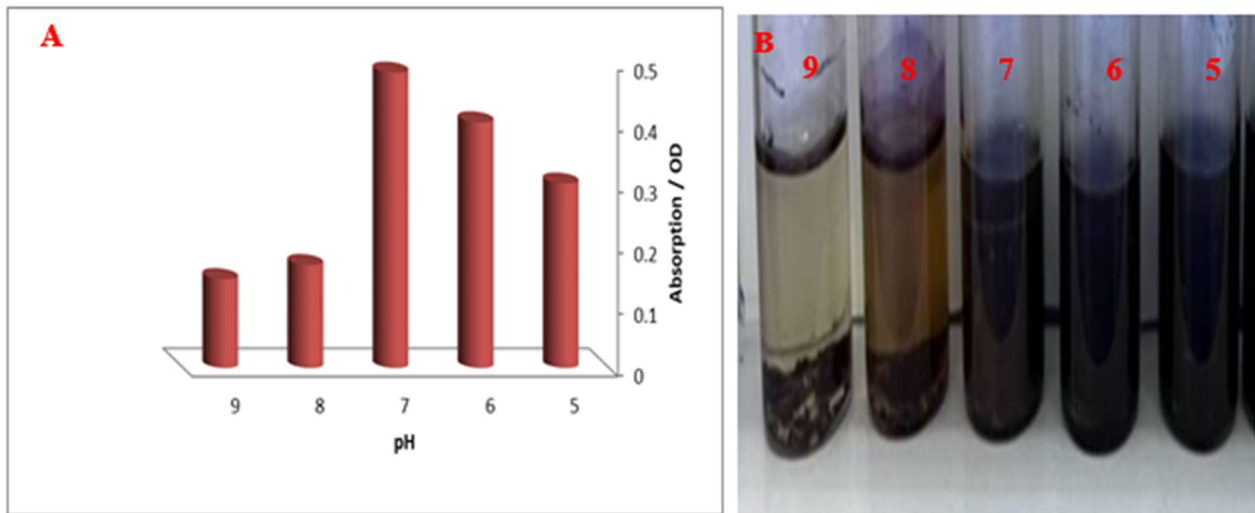


Fig. 4. (A) and (B) The effect of the PH on the production of AuNPs.

**Effect of carbon sources:** The result are shown in (Fig. 5 (A) and (B)). the carbon source, Starch, gave the highest biosynthesis gold nanoparticle, which amounted to (0.993), then the carbon source of Arbinose, which gave (0.542) of biosynthesis gold nanoparticle, then carbon source of Maltose, which gave(0.494), then the carbon source is Xylose, which obtained of absorbation of AuNPs was (0.191), and then carbon source of

Dextrose, which provided (0.185).

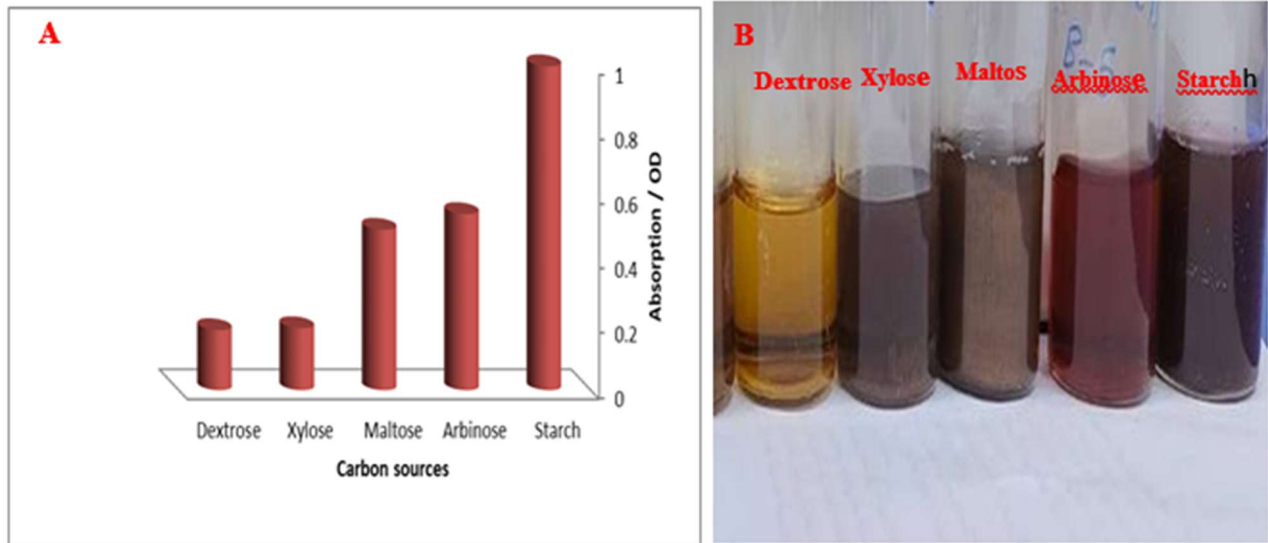


Fig. 5. (A) and (B) The effect of the carbon source on the production of AuNPs.

**Effect of nitrogen sources:** The result are shown in (Fig. 6(A) and (B)). the nitrogen source, beef extract, gave the biosynthesis gold nanoparticle, which amounted to (0.323), then the nitrogen source of pepton, which gave (0.253) of biosynthesis gold nanoparticle, then nitrogen source of casin, which gave (0.219), then the nitrogen source is yeast extract, which gave (0.210) of AuNPs, and then nitrogen source of trypton, which provided (0.215).

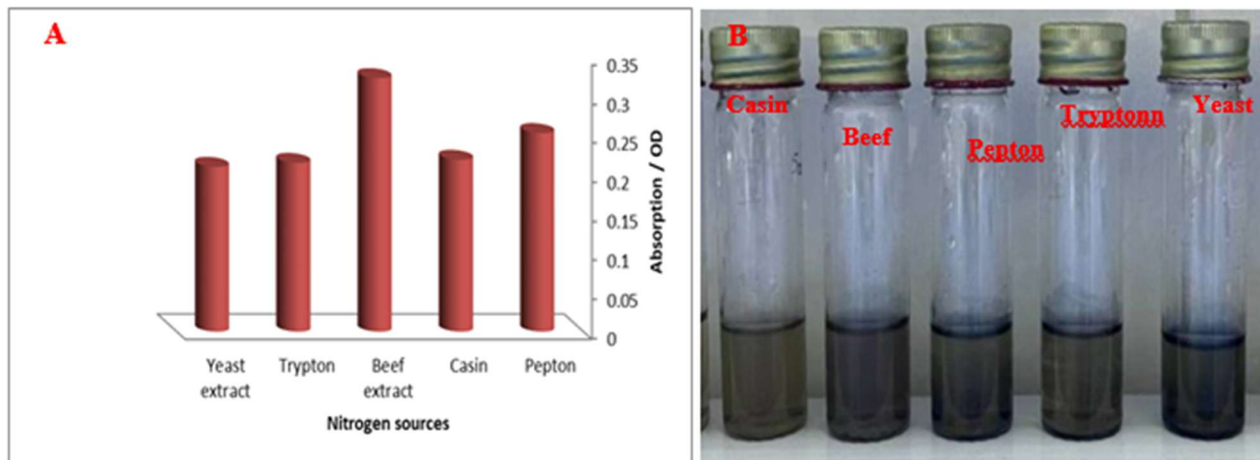


Fig. 6. (A) and (B) The effect of the nitrogen source on the production of AuNPs.

#### Study all optime condition in one experience

under optimum incubation period, temperature, pH, carbon source, and nitrogen source, The gold nanoparticle was synthesized using *B.subtilis* as a reductant agent, as shown in the figure (Fig. 7(A) and (B)), the culture medium of *B.subtilis* is treated with the optimum conditions studied in one experiment, which gave high stability, purity, biological activity, gold nanoparticle (AuNPs) and as it reached absorption to (1.161).

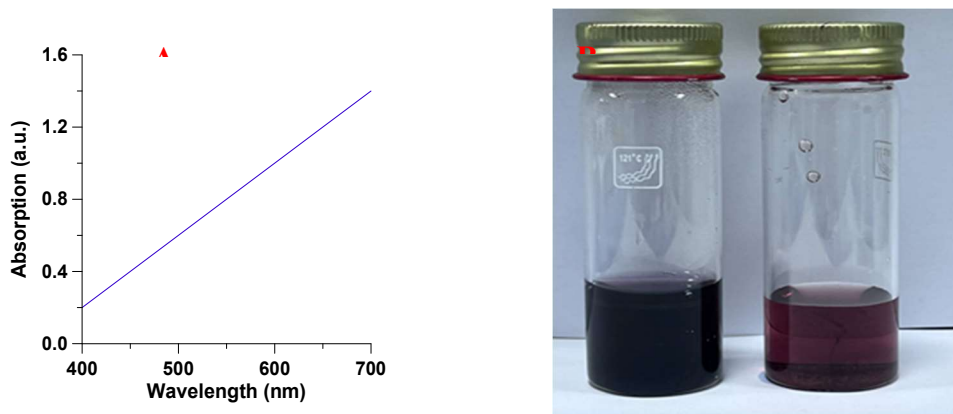


Fig. 7. Product AuNPs with all condition(A) UV-vis spectroscopy , (B) AuNPs before centrifugation, AuNPs after centrifugation (pure).

## 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. Using soil samples that were taken from the Al-fuhod Marshes in the South Thi-Qar. This study showed a gradual color change from yellow to wine red for a mixture reaction of  $\text{HAuCl}_4$  with the bacterial supernatant, and the color of the solution increases during a 24 h incubation period. UV-Spectrometer of AuNPs show maximum intensity at range (530 to 590). These results are consistent by (15). The change in the peak and color may be possible due to variable in size and shape of gold nanoparticle. isolate growing on nutrient agar medium for 24 h at  $37^\circ\text{C}$  and stained with Gram stain, the bacterial isolates appeared violet in the shape of groups, according to the results of light microscopy examination. Gram positive status is indicated by its rod-shaped. and indicated that the bacterial isolate was *B.subtilis* by vitek-2. The 96 h induction duration proved to be optimal for production (AuNPs), according to the results of the incubation period. After that, 120 h incubation showed a decrease in the production of AuNPs. the pH effect, which showed *B.subtilis* isolate exhibited a peak in absorption at pH 7, This is consistent with the results (16). The results of the isolate also demonstrated that the temperature at which biosynthesis AuNPs was highest,  $37^\circ\text{C}$ , This is due to the fact that it is an ideal temperature for the growth of isolated. The carbon source effect on AuNPs by adding an one carbon source to the culture medium, starch was significantly increased. Starch at a concentration of 0.2% was the most effective inducer of AuNPs by *B.subtilis*. The best carbon source of starch for the production AuNPs because ability this isolated on production amylase. There was less (AuNPs) production from other carbon sources, such as Dextrose and Xylose. Regarding the nitrogen source's influence, AuNPs production by *B.subtilis* isolates is best catalyzed by beef extract among all the nitrogen sources that were chosen; nevertheless, production was reduced when nitrogen sources such as and Casin and yeast extract were used. This result contradicted by (16), when said that the best source of carbon and Nitrogen to produce AuNPs is Dextrose and Casin.

## Conclusions

In brief, we report a biological synthesis for AuNPs using the cell-free extract of *B.subtilis* without introducing any chemical reducing agent. AuNPs were produced by an extracellular method, which gained importance because it does not require additional treatment and steps. Under optimum of incubation period, carbon sources, nitrogen, temperature, and pH, more stable Au nanoparticles were produced. They show significant antibacterial activity and delivery of medicines and medical fields.

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