

Study the Viability of Some Bacterial Species Isolated from Contaminated Soils on the Manufacture of Nanoparticles

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Abstract: Researchers in nanoparticle manufacturing have recently turned to biomanufacturing to reduce silver ions into silver nanoparticles because these microorganisms work as ecofriendly nanofactories, which were controlled on the size and shape of manufactured nanoparticles, this technique can occur either inter the cellular structure or extracellular structure, but the extracellular synthesis is cheaper and require simple processing technique .Therefore, in the current study bacterial strains isolated from contaminated soil with motor oil were selected to synthesize silver nanoparticles by extracellular method at 37C° for (72-168)hours. The result of fabrication was observed by the shift in the color of reacted solution into yellowish brown and confirmed the fabrication of Ag NPs by UV-Visible spectroscopy that measured the absorption spectra of Ag NPs which was located between (404 -444) nm. Also, FTR analysis was used to determine the functional groups of bacteria that participated in the reduction of Ag⁺ into Ago through notice the presence of band located between (3344-3310) cm⁻¹ related to NH(Amide) and the presence of band between (1633-1636) cm⁻¹ assigned to C=O(carbonyl amide). Finally, AgNPs fabricated by supernatant of Bacillus strains when reacted with 3mM AgNO₃ solution showed more effective against *Staphylococcus aureus* than *E. coli*.

Keyword: Biosynthesis of Ag NPs, *Bacillus spp.*, UV-Visible Spectroscopy ,FTR ,Antibacterial Activity.

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1.Introduction:

One of the most important fields for researches in nanotechnology is biosynthesis of metal nanoparticles due to having numerous applications in various fields like therapeutics, bimolecular detection, catalysis [1,2]. Therefore, the studies in this field were turned on to use biological approach because these nanofactories method have significant advantages compared to other process because these nanofactories have the ability to synthesize nanoparticles in an ecofriendly method [3] bacteria is the most favorable between microorganisms because it is relatively easy to handle and can produce bio component like enzymes, proteins and bio-surfactant (lipopeptides) ,these biomolecules form bio-reducing agent by bacterial cell metabolic activities[4,5,6]. In many bacteria strains these biomolecules are used as capping agent which makes nanoparticles more stable. Bio reduction of silver ions by microorganism can occur by extracellular or intracellular[7]. Extracellular manufacturing of nanoparticles happens by limitation metal on the surface of bacteria while intracellular manufacturing occurs inside bacteria and needs extra steps to obtain nanoparticles like treatment by ultrasound to release the synthesized silver nanoparticles. Many studies concentrated on Extracellular synthesis because of it is cheap, simple and provides large scale production[8,1]. Recent researches reported the synthesis of AgNPs by *Bacillus subtilis* and *Enterococcus spp.*, *Morganella spp.* [9,10,4]. Bacterial strains isolated from soil contaminated with hydrocarbons materials were tested for their strength to reduce Ag ions to Ag NPs, which identified biochemical and molecular by 16SrRNA as Gram-positive Bacilli bacterium forming spores related to genus *Bacillus* [11]

2. Material and methods:

2.1. Isolation and identification of bacteria from contaminated soil :

10 gm. of each soil sample contaminated with generator oils in Mosul was collected and taken from a depth of 5cm using a sterile spatula and placed in sterile plastic bags, then transferred to the laboratory for making serial of ten dilution by adding it to 90ml of sterile D.W. to obtain the first dilution 10^{-1} [12]. Then added 0.1ml of 10^{-4} , 10^{-5} dilution separately to sterile Petridis containing Nutrient Agar and incubated at 37°C for 24 hours to obtain bacterial colonies that were subculture individually on N.A. for purification. Later bacterial isolates identified biochemically and molecularly based on 16srRNA gene sequence analysis described [13,14,15].

2.2. Synthesis of silver nanoparticles:

Some bacterial strains identified by molecular and biochemical methods were selected to synthesize silver nanoparticles by inoculating it individually in 100ml of sterile N.B. media and incubated in incubator shaker rotate at 200 rpm for 24 hours to obtain bacterial suspension with concentration 1.5×10^8 compared with McFarland tube (0.5). Then added 50ml of a bacterial strain to flask containing 50 ml of 1mM of AgNO_3 solution and incubated in 150 rpm for 72-168 hours in dark condition. After that supernatant of the reaction mixture was separated by centrifuged it at speed 10000 rpm for 10 min. Finally, the pellet of Ag NPs was collected and stored for further usage [13,16,17].

2.3. Characterization of silver nanoparticles :

The initial detection of Ag NPs was noted for color changing for solution resulting from the reaction to check the fabrication of nanoparticles by nanofactories through the extracellular method and analyzed optical characteristics of Ag NPs and scanned the absorption spectra between 200-900 nm. Also, FITR were analyzed for the reaction mixture to investigate functional groups that participated to reduce Ag^+ into silver atom Ag^0 [16,18].

2.4. Antimicrobial Activity of Fabricated AgNPs:

The antimicrobial activity of synthesized Ag NPs against some bacteria was investigated on Mueller-Hinton agar (M.H.A.) plates by using disk diffusion method. About 0.1ml of suspension for pathogenic bacteria like *E. coli*, and *staphylococcus aureus* at concentration 1.5×10^8 compared with McFarland tube after incubated it for 24 hours then was spread evenly on solid media of M.H.A. plate by sterile swabs. Later 20 μl for the pellet of Ag NPs and AgNO_3 at (3mM, 2mM) concentration and supernatant of *Bacillus* strains was added separately to the wells which was made by Cork borer then incubated it at 37°C for 24 hours. From the observation of inhibition zone appeared around the wells can determine antibacterial activity against pathogenic bacteria [19,20].

3. Results and Discussions

3.1. Identification of bacterial strains :

The isolates bacteria from soil were identified biochemically as gram positive bacilli forming spore and by 16srRNA gene sequences showed that the isolates M1, M2, M3, M4, S10 were identified as *Bacillus paramycoides*, *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus cereus*, *Bacillus wiedmannii* illustrated in the Table (1).

Table (2) Diagnosis of bacterial isolates according to 16srRNA

Number of strain	Name of strain	DNA Extraction	PCR Result 16srRNA 1250 bp
M1	<i>Bacillus paramycoides</i>	+	+
M2	<i>Bacillus subtilis</i>	+	+
M3	<i>Bacillus paramycoides</i>	+	+
M4	<i>Bacillus cereus</i>	+	+
S10	<i>Bacillus wiedmannii</i>	+	+

3.2.Characterization of Fabricated Ag NPs:

Formation of silver nanoparticles by supernatant of *Bacillus* strains when treated with a solution of AgNO_3 was observed visually by the color shift of reaction mixture from pale yellow to yellowish brown at the end of incubation. Brown properties may be due to excitation of the Plasmon Surface Resonance (SPR) of the nanoparticles and were provided appropriate substantiates of their fabrication. These color changes were shown in Fig. [1]

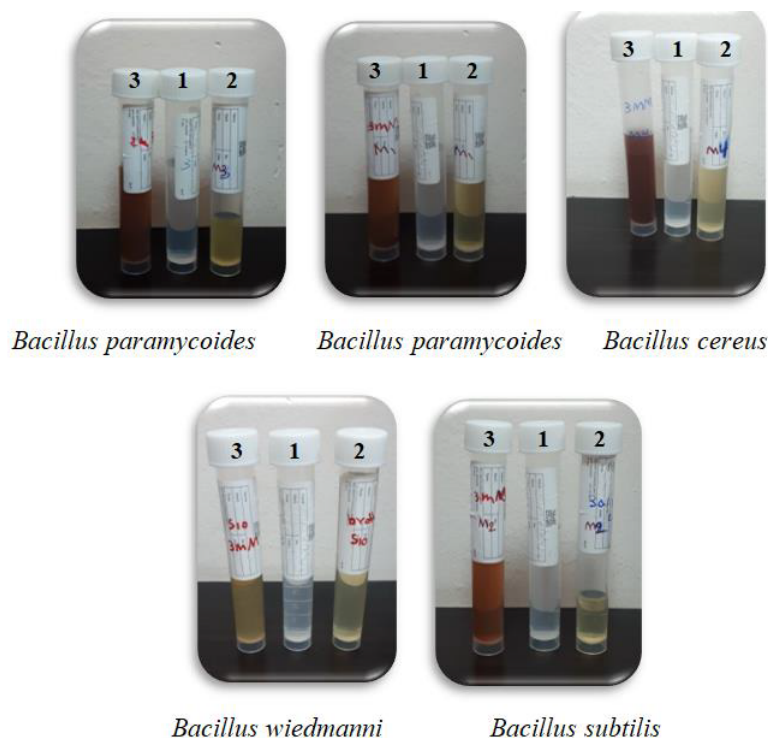


Figure (1) Visual observation of synthesized silver nanoparticles by supernatant of bacterial strains (1)(Control) AgNO_3 solution (2) supernatant of bacteria (3) Ag NPs solution

There was evidence that an electron shuttle or other reducing agents were released into the culture of *Bacillus spp.*, and participated in bioreduction of silver ions Ag^+ into silver atom [21,22]. By measurement of UV-Visible spectroscopy can be observed a absorption peak located between (404-444)nm suggest the presence of Surface Plasmon Resonance which is properties of silver nanoparticles that prepared using culture supernatant of selected strains used in this study

like *Bacillus wiedmannii*, *Bacillus paramycoids*, *Bacillus subtilis*, *Bacillus paramycoids* was concentrated at (404,424,438,444)nm respectively and illustrated in Fig.(2) similar observation was previously reported by [9,19,23].

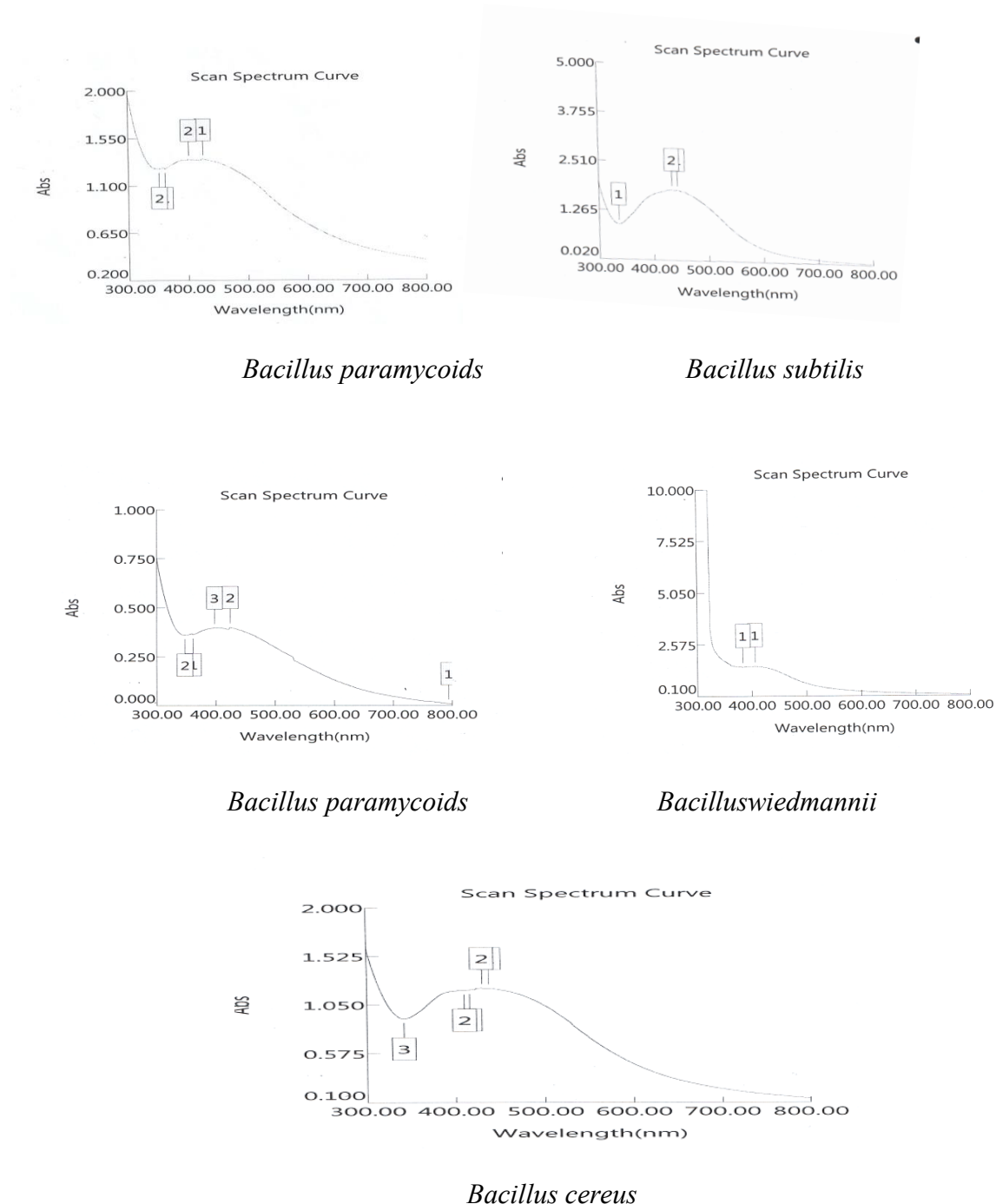
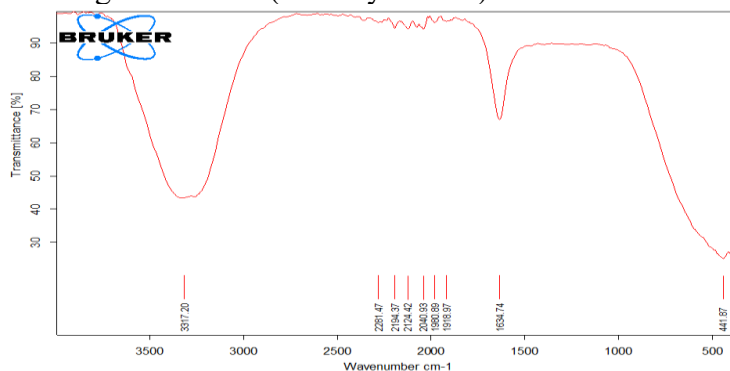


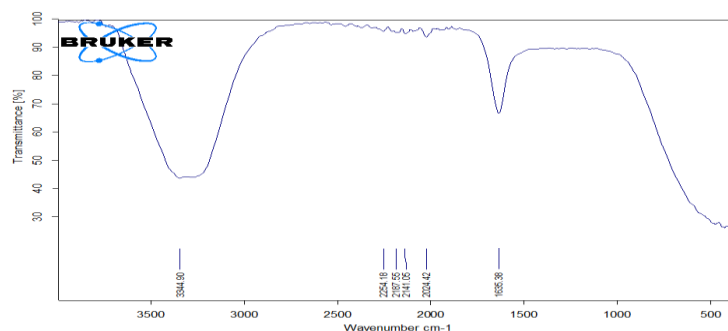
Figure (2) Show UV-Visible absorption beak of Ag NPS synthesized by supernatant of *Bacillus SPP*.

3.3.FITR analysis :

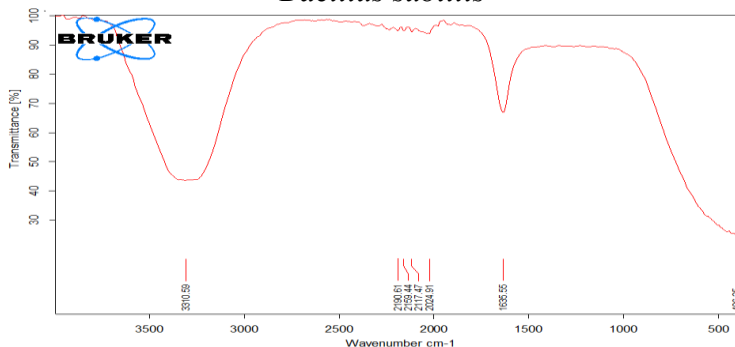
measurement of FTIR for solution Ag NPs showed the presence of bands located at region between (33310-3344) cm^{-1} were possibly related to NH (amide) that confirm presence of protein in the sample and corresponding with previously studies [9,19,23]. Also presence of band located between (2190-2281) cm^{-1} assigned to C=N (Nitrile) and the appearance of band at (1633-1634-1635-1636) cm^{-1} assigned to C=O (carbonyl amide). all bands illustrated in Fig (3)



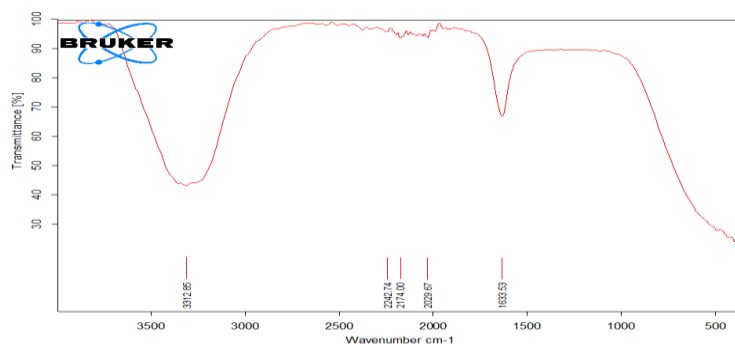
Bacillus paramycoids



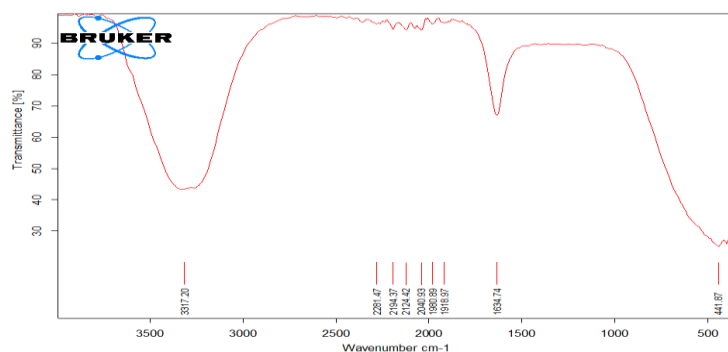
Bacillus subtilis



Bacillus paramycoids



Bacillus cereus



Bacilluswiedmannii

Figure (3) FITR analysis of Ag NPs fabricated by supernatant of *Bacillus* strains

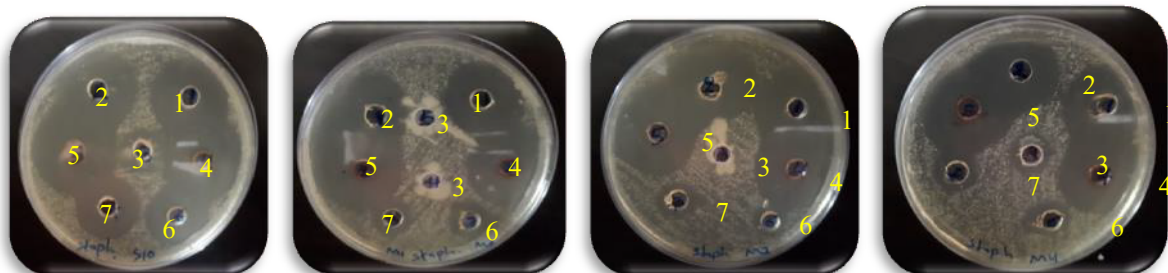
3.4. Antibacterial Effectiveness of AgNPs:

The synthesized Ag NPs by supernatant of *Bacillus subtilis* *Bacillus cereus* *Bacillus wiedmannii*, which incubated with 3mM AgNO₃ solution showed the highest antimicrobial efficiency against *staphylococcus aureus* comparison to *E. coli* the reason may be due the plasmolysis of the cell wall and separate cytoplasm from it, the mechanism of antibacterial activity of bio silver was differ from species to another and depending on the size of nanoparticles .this result was in agreement with previously studies by [19,24].The activity of Ag NPs was illustrated in Table(2)

Table (2) Antibacterial activity of Ag NPs synthesized by supernatant of *Bcillus* strains

Tested Bacterial strain	Type of bacillus	AgNO ₃ 3mM	AgNO ₃ 2mM	suspension of bacteria	sediment 3mM	Sediment 2mM	Filtrate 3mM	Filtrate 2mM
<i>Staph. aureus</i>	<i>Bacillus paramycoid</i>	15	12	6	21	15	14	13
	<i>Bacillus subtilis</i>	26	26	6	29	32	14	12

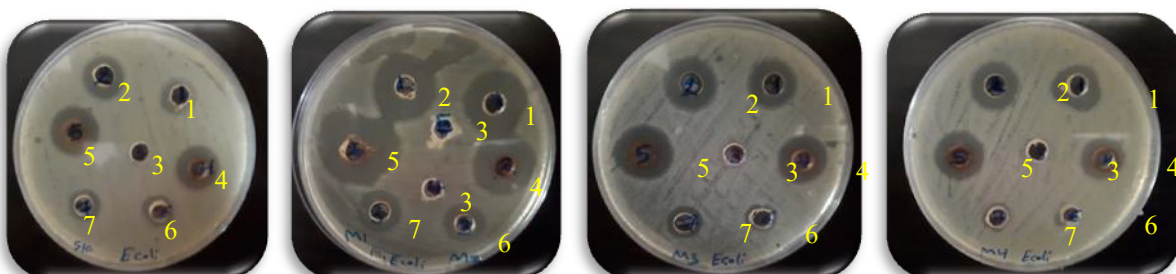
	<i>Bacillus cereus</i>	27	26	6	30	29	14	15
	<i>Bacillus</i>	30	29	6	35	32	11	11
<i>E. coli</i>	<i>Bacillus subtilis</i>	16	13	6	28	15	15	11
	<i>Bacillus</i>	16	15	6	20	19	12	11
	<i>Bacillus cereus</i>	14	15	6	22	20	6	6
	<i>Bacillus</i>	14	11	6	20	10	16	6



Bacillus wiedmannii *Bacillus subtilis*

Bacillus paramycoid *Bacillus cereus*

Antimicrobial activity of Ag NPssynthesized by supernatant of *Bacillus*SPP.against *staphylococcus aureus*



Bacillus wiedmannii *Bacillus subtilis*

Bacillus paramycoid *Bacillus cereus*

Figure (3) Illustrating antimicrobial activity of Ag NPssynthesized by supernatant of *Bacillus*SPP.against *E.coli*

- (1) 2mM AgNO₃ solution (2) 3mM AgNO₃ solution (3) supernatant of bacteria (4) sediment of 2mM AgNPs (5) sediment of 3mM AgNPs (6) filtrate of 2mM AgNPs (7) filtrate of 3mM of AgNPs

4.Conclusion:

We conclude from the present study the possibility of bacteria isolated from soil contaminated with motor oils to reduce silver ions to silver atoms by producing them for some effective compounds such as proteins and enzymes to form nanoparticles that is showed more effective against *staphylococcus aureus* than *E. coli* when the bacterial supernatant of some *Bacillus* strains reacted with 3mM AgNO₃.

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