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Original Article

Detection of Biosynthesis of CuNPs using *P. aeruginosa* Isolated from Soil

Sabah Raheem Hamady[1], Ismail Jumah Abbass[2], Kadhim Fadhil Kadhim[3]

[1]*Iraqi Ministry of Education, Basrah Education Directorate, Basrah, Iraq.*
[2,3]*Department of Biology, University of Basrah, College of Education/Quran, Basrah, Iraq.*

[3]*Corresponding Author : kadhim.fadhil@uobasrah.edu.iq*

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Abstract *- The production of nanoparticles, while controlling the size and shape of the particles, has recently occupied much research in the field of nanotechnology. The biosynthesis of metal nanoparticles is of great importance due to their suitability for the environment. The use of microorganisms to produce nanoparticles has progressed over chemical and physical methods due to their effectiveness, low cost, and environmentally friendly nature. The present study focused on the synthesis of copper nanoparticles (CuNPs) in a simple, efficient, and rapid manner by the bacterium Pseudomonas aeruginosa. Copper is important in this process due to its interesting physical and chemical properties. It has good therapeutic potential, and when copper-sulphate-solution is added to the bacterial suspension devoid of bacterial cells, it is observed that the color of the reaction mixture changes to green, and this is evidence of the formation of nanoparticles. Different techniques were relied upon to characterize CuNPs by ultraviolet spectroscopy, with the highest value at 314. An FT-IR examination was conducted to determine the active aggregates. A SEM examination was also conducted, confirming that the size of copper nanoparticles reached 56.40 nm. This study aimed to produce nanoparticles. CuNPs using a Pseudomonas aeruginosa.*

Keywords *- Bacteria, CuNPs, UV-Spectroscopy, SEM.*

1. Introduction

Nanotechnology is one of the most important research areas in modern materials science and technology. Nanotechnology deals with the manufacture of nanoparticles of different shapes and sizes. Nanoparticles have unique electrical, optical, and biological properties and are used to manufacture medicines, nanodevices, and medicine (Nair and Laurencin 2007). The green synthesis of nanoparticles is still in its infancy, and as a result of the great demand for the synthesis of various nanoparticles, new methods of synthesis must be invented and developed, and they must be less expensive and environmentally friendly. In addition, the method of making copper nanoparticles has not been discovered and known much by researchers, and there are many studies on the synthesis of gold and silver nanoparticles (Huang et al. 2007; Ahmad and Sharma 2012; Sharma et al. 2013). Therefore, copper nanoparticles are important due to their optical, mechanical, preparation, and electrical properties, as copper is the alternative material for important metals such as silver and gold (Pecharromán et al. 2006). Nanoparticles are also important in wound dressings (Borkow et al. 2009; Borkow et al. 2010). The biosynthesis of copper nanoparticles is from inorganic nanoparticles, including oxidant nanoparticles, metal nanoparticles, and sulfides (Singh et al. 2010). Copper has a major role in electronic circuits as a result of good electrical conductivity and is of great importance in industries, especially in the electricity sector, because of its abundance, low cost, and good conductive properties. Given the role of microorganisms in producing various products (Kadhim and Alrubayae 2019; Alrubayae and Kadhim 2020), our study sought to verify the production of copper nanoparticles by *P.aeruginosa.*

2. Materials and Methods

2.1. Sample Collection

Soil samples were collected (Qurna and Mudena city) to diagnose the *P. aeruginosa* bacteria after they were grown on nutrient-agar-medium and then purified and diagnosed.

2.2. Identify Bacteria

2.2.1. Phenotypic Diagnosis

The bacteria were grown on MacConkey-agar and Blood-agar to determine their cultural characteristics in terms of the color and shape of the colonies.

2.2.2. Microscopic Diagnosis

The bacterial isolates were examined using a microscopic examination by taking part of the bacterial colonies, transferring them to a glass slide, and then staining

them with Gram stain to know the shape of the bacterial cells and how they are grouped (Cowan and Steel 1965).

2.2.3. Biochemical Tests

The bacteria were grown on MacConkey agar and blood agar to conduct biochemical tests, and they were identical to what was stated in internationally approved diagnostic systems (Brooks 2010).

2.3. Preparation of P.aeruginosa Biomass and Synthesis of Copper Nanoparticles

2.3.1. Preparation of Bacterial Suspension

When the bacteria are activated and grow on the nutrient broth, they are placed in a shaking incubator at a temperature of 37°C for a period of 48 hours until the biomass is formed. Then, they are placed in a centrifuge at 5000 rpm to get rid of the bacteria and obtain the bacterial suspension, which is stored at a temperature of 4. until use.

2.3.2. Preparation of Copper Nanoparticles

0.250g of copper-sulphate was dissolved in 100 ml to obtain a concentration of 1 mM. It was stored and ready for use. Where 100 ml of copper sulphate is mixed with 100 ml of bacterial suspension, we will notice the occurrence of color variation.

2.3.3. Nanoparticle Purification

The reaction mixture was placed in a centrifuge at 8000 rpm, where the supernatants were removed. The precipitate resulting from the centrifugation was then washed three times with distilled water (DDW) for 30 minutes, after which the precipitate was collected and dried in a hot air oven at 100 degrees Celsius for 3 hours, where it was ready to be used for further examination to determine the size, shape, and chemical composition of the nanoparticles.

2.4. Description of Copper Nanoparticles

2.4.1. Heterochromia

It was observed through the identification of copper nanoparticles that a color change occurred from yellow to green, which indicates the formation of nanoparticles.

2.4.2. Ultraviolet Absorbance Spectrum Examination

The sample under study was prepared to examine the absorbance spectrum, where 2 ml of the prepared copper-sulphate solution was taken and treated with the bacterial suspension and shaken well for the solution to be homogeneous. The device was then filtered with sterile distilled water and then examined with a spectrophotometer at wavelengths (300–800) nanometers. It was compared with bacterial suspension not treated with copper sulphate as a control agent (Joseph et al. 2016). The examination was carried out in the laboratory of the College of Education – Qurna / University of Basrah.

2.4.3. FT – Infra Red Spectrum (FT IR)

The infrared spectrum was recorded to determine the effective groups of biomolecules, and the samples were examined in the laboratories of the Qurna College of Education/University of Basrah.

2.4.4. Scanning Electron Microscope (SEM)

The samples were examined in the electron microscopy unit in Iran/University of Tehran using a Scanning Electron Microscope (SEM) to characterize the morphological shapes and size of the copper nanoparticles (Caroling et al. 2013).

3. Results and Discussion

The results of diagnosing the bacteria showed the form of smooth, circular colonies, as their colonies were of the beta-hemolytic type when grown on blood agar. In contrast, they were grown on MacConkey-agar-medium, as they appeared not to be fermenting the sugar lactose, as they were pale and had a green color due to their production of the pyocyanin formula. It had the smell of fermenting grapes. As for the microscopic diagnosis, it was gram-stain-negative and had a rod-like shape that did not form spores, and this agrees with (Brooks et al. 2010).

3.1. Biosynthesis of CuNPs

3.1.1. Heterochromia

The results of the current study showed the ability of bacteria to biosynthesize copper nanoparticles by extracting the *P. aeruginosa* bacterial suspension and treating it with CuSO4. Through observation, bioreduction occurs, which is responsible for the color change in the *P. aeruginosa* bacterial suspension after observation of the treatment. Where the bacterial suspension *P. aeruginosa* showed a color change from light yellow to green, and this result matches what was found (Shantkriti and Rani 2014), as in Figure (1).

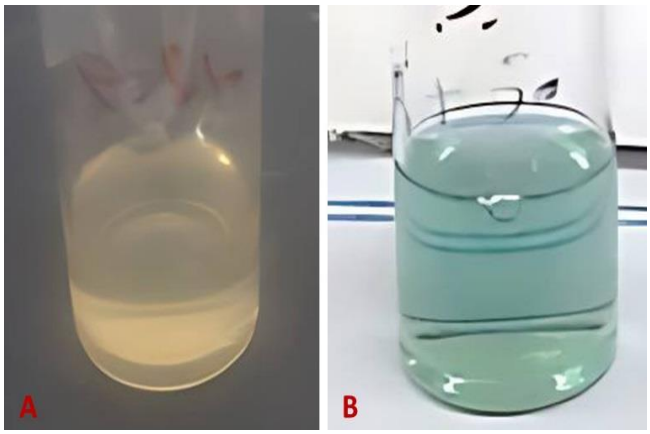

Fig. 1 A. Bacterial suspension without treatment with copper sulphate, B. The color changes from yellow to green when the bacterial suspension is treated with copper sulphate

Table 1. The results of biochemical tests for Bacteria *P.aeruginosa*

Bacteria	Oxidase	MR	VP	Urease	H_2S production	citrate	Gas production	Triple sugar Iron	Indole	Coagulase
P. aeruginosa	+	+	-	-	-	+	-	A/K	-	-

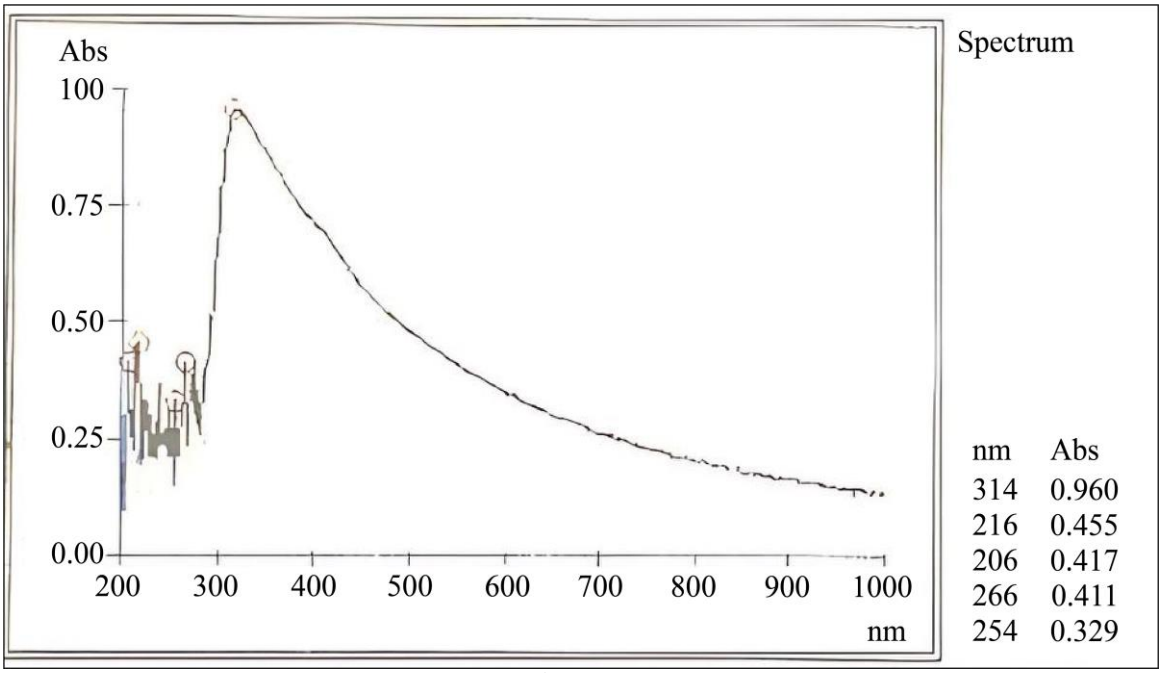

Fig. 2 UV-visible spectra of produced CuNPs by *P. aeruginosa*

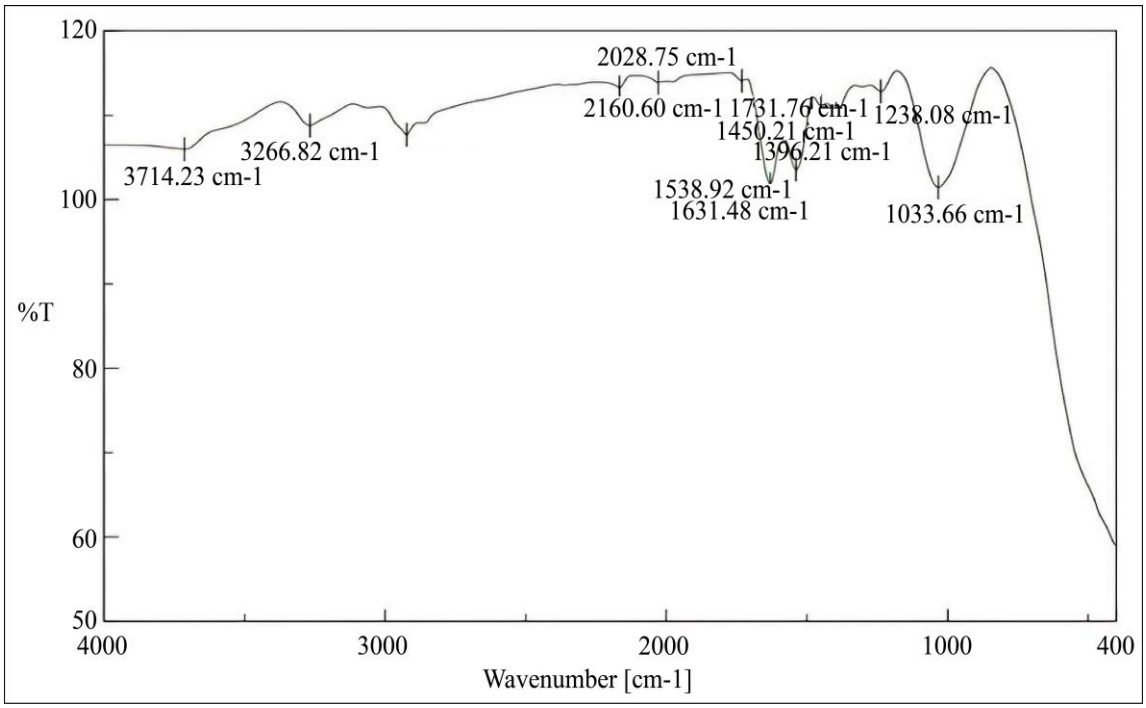

Fig. 3 FTIR spectrum of CuNPs biosynthesized by *P. aeruginosa* with distinct peaks

3.1.2. Ultraviolet Spectrum Analysis

The results of the ultraviolet and visible spectroscopy of the current study of the nanoparticle solution showed surface plasmon resonance spectra, where the result of examining the absorbance was at the wavelength (300–800) nm, and the highest value was at (314) nm, and it was identical to (Tiwari et al. 2014; Tiwari et al. 2016), the CuNPs showed a green color as a result of the excitation of the surface plasmon vibration of the CuNPs, Figure (2).

3.1.3. FTIR Analysis

An examination was conducted of a nano-copper solution that was bio-synthesized by bacteria, where it occurred between the copper sulphate solution and the bacterial biomass produced by the bacteria (enzymes and proteins), which are responsible for the reduction of copper ions and the formation of copper nanoparticles.

The results of the IR spectrum study showed, as in figure (), where it gave absorption bands at (3714.23) cm-1, which belong to the amplitude-frequency of the (H-O) group, and as at the location (3266.82) cm-1, which belongs to the amplitude-frequency of the (H) group. – O), while at the location (2923.56) cm-1, where it returns to the amplitude-frequency of the bond (H-N), and the absorption beam at (2167.6) cm-1, as it returns to the amplitude-frequency of the group (C ≡ C), as well as at the location (2038.75). Where it returns to the amplitude-frequency (S=C=N), while at the location (1631.48) cm-1, it returns to the amplitude-frequency of the joint (H-N), and also at the location (1238.08) cm-1, where it returns to the amplitude-frequency of the joint (O-C).

The absorption beam gave (1538.66) cm-1 as it returned to the amplitude-frequency (O-N). In contrast, at the location (1033.66) cm-1 as it returns to the amplitude-frequency (O=S), and at The location (1390.21) cm-1, where it returns to the nine-band frequency (H-C), while at the location (1450), where it returns to the nine-band frequency (H-C). It has been shown that the reductive activity of copper ions may be due to the multiple active groups that appeared in this solution, as many studies have shown the ability of these groups to reduce in the process of preparing copper nanoparticles, as in Figure (3).

3.1.4. Scanning Electron Microscope (SEM)

Scanning electron microscope images showed the shapes and sizes of silver nanoparticles produced from bacterial suspension, using a magnification power of 200 kx. The microscopic images showed that the CuSO4 produced by *P. aeruginosa* was in the form of spherical aggregates with dimensions ranging between 56.40 nanometers and Figure (4).

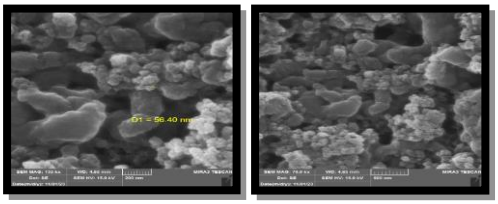

Fig. 4 The biosynthesized CuNPs in *P. aeruginosa* were depicted in an SEM-micrograph as spherical shapes aggregated with size ranges from 56.40 nm (magnification 200 K X).

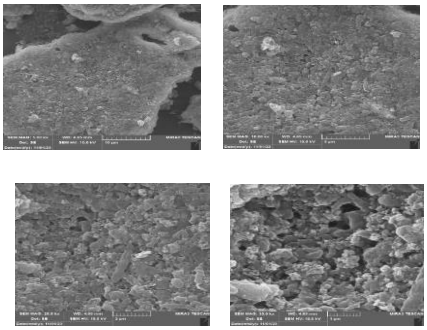

Fig. 5 The biosynthesized CuNPs in *P. aeruginosa* were depicted in an SEM-micrograph as spherical shapes aggregated (magnification 1µm). (magnification 2 µm). (magnification 5 µm) (magnification 10 µm).

4. Conclusion

Using several indicators such as: Chromogenicity, Spectroscopy, and Scan-Microscopically, the current study showed that *P. aeruginosa* isolated from soil could biosynthesize copper nanoparticles.

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