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Antibacterial effects of ZnONPs produced by Bacillus clausii on growth of Pseudomonas aeruginosa

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***Abstract*---**Nanotechnology's most recent advancements Nanoparticles of metals and metal oxides have a wide range of uses in a variety of sectors, research institutes, and enterprises. The most frequent metal oxide nanoparticles are zinc oxide nanoparticles. (ZnO NPs) Because of its unique features and applications, zinc-tolerant probiotics of Bacillus spp are capable of tolerating high concentrations of Zinc+2 and creating Zinc Oxide Nanoparticles, These bacteria are gaining popularity as a natural microbial cell nano-factory for a more efficient and ecologically acceptable technique of nanoparticle production. X-ray diffraction was used to determine the morphological and structural properties of ZnO NPs , The produced nanoparticles were crystalline, relatively stable, roughly spherical, and pure, according to transmission electron microscopy (TEM). (ZnONPs) biosynthesized by Bacillus clausii have antibacterial efficacy against Pseudomonas aeruginosa.

***Keywords*---**Zinc oxide nanoparticles ZnONPs, Bacillus clausii.

Introduction

“Materials that are generated, exploitedand synthesized on a scale smaller than 1 μm are referred to “nanotechnology” [1]. “Nanoparticles are metal particles with a diameter of 1–100 nm with a range of geometries such as spherical, wedge-shaped and rod ”. The term "nano" derives from a Greek expression "dwarf" or "very thin.” [2] Bacillus have the obvious benefit over other prospective probiotics in that they can be generated easily and economically effectively by drying and last well through shelf life. They will also survive gastric acidity and make it into the intestine, the rumored location of action. [3] . Bacillus clausii is a Gram-positive, rod-shaped, non-pathogenic, spore-forming bacterium that can survive transit through the acidic environment of the stomach and colonize the intestine

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even in the presence of antibiotics[4]. Zinc oxide nanoparticles have sparked global attention as multifunctional nanoparticles due to their unique capabilities as adaptable semi-conductors with piezoelectric properties. [5]. Microbial metal nanoparticle production has lately become popular due to its low cost, biocompatibility, and environmental friendliness[6]. Many key properties of ZnO include chemical and physical stability, excellent catalysis, and effective antibacterial activity[7] Symbiotic microbes may use NPs as a safe source of carbon. The creation of microbial nanoparticles offers more advantages than conventional chemical and physical methods. Nanoparticles have several medical uses. Furthermore, the bacterial nanoparticles might be employed to regulate human bacterial pathogens[8].

Methodology

Isolate of Bacteria

“Bacillus clausii was chosen as a more effective biological model for ZnO NP production. This strain was isolated from dairy products housed at the Advanced Microbiology Lab at the University of Babylon. We grew the isolate for 24 hours on MRS agar at 38°C before diagnosing it using the Vitek2 method”.

Zinc oxide Nanoparticles’ “Biosynthesis by *Bacillus clausii* “

“The Bacillus clausii pure culture was inoculated in a flask containing brain heart broth that had been autoclave sterilized, at 37° incubated C for 24 hours at 100rpm. After the incubation time, we centrifuged the supernatant at 5000 rpm for 25 minutes. To delay the transformation process, the pH of the supernatant was controlled with 0.4 M NaOH to be neutral we added the NaOH to reach pH 7 to eliminate the influence of organic acids. 20 g. 0.4M Zinc actate (CH3.COO)2 Zn.2H2O) dissolved in 1000 ml distilled water, was added to 250 ml supernatant, and then reheated for 5-10 minutes in an 85 ° C water bath The development of a white precipitate at the bottom of the flask indicates the transformation process. The flask was subsequently incubated at 37° C for 12 hours, during which time all of the particles accumulated in the flask's bottom. The product was centrifuged at 10000 rpm for 20 minutes to remove the black precipitate before being rinsed with D.W. The technique was then repeated three times to get pure goods, following which the product was dried for four hours at 60 ° C in a hot air oven”. [9].

The instruments that used in determining the properties of ZnO nanoparticles biosynthetic by *lactobacillus plantarum*

“X-ray diffraction analysis (XRD)”.,”Field Emission - Scanning Electron Microscopy (FESEM)” and “Transmission Electron Microscopy (TEM)”

Antibacterial efficacy of ZnONP : Disc Diffusion Method

This method was accomplish on Muller Hinton media as follows:

- Concentrations were obtained from each bacterial isolate and compared to McFarland solution to determine the appropriate concentration for each.
- The proper focus 0.1 ml of each bacterial isolate was put to plates containing Muller Hinton agar, which was distributed over the dish's surface using a spreader and left for an hour.
- ZnONPs were created in cork borers, each with a 6 mm diameter. There was an identical distance between the film and the well.
- To get final concentrations, the nanoparticles were dissolved in distal water.
- The ZnO nanoparticles inhibitory zones were measured using a ruler after 24 hours of incubation at 37°C.[9].

Results and Discussion

Biosynthesis of Zinc Oxide Nanoparticles by Bacillus clausii

“The biosynthesis of nanoparticles by *Bacillus clausii.* “The reduction of ZnO into ZnO NPs” after exposure to bacterial extract was validated by discovering a change in solution color during the creation of ZnONPs. The different color was white deposited at the bottom of the flask after mixing Bacillus clausii with Zinc actate (CH3.COO)2 Zn.2H2O) and biosynthesis of ZnO NPs by all of these bacteria. The color of the result varies during the reaction, from light brown to yellow after 24 hours. This finding was consistent with the study done by Bacillus clausii, which plays a vital role in the formation of ZnO NPs “[10]. the final stage of ZnONPsbiosynthesis by *Bacillus clausii* after the process of drying these nanoparticles in the oven .

Morphological & Structural properties of Zn- ONPs biosynthesized by *Bacillus clausii*

Moderately stable ZnONPs have been synthesized using *Bacillus clausii* .The effect of reaction time plays a vital role in the morphology of nanoparticles[11,12]

X-Ray diffraction analysis (hexagonal phase)

Research revealed that the produced nanoparticles were crystalline and pure in nature. Figure shows the respection lines of spherical ZNO NPs (1) The average particle size of ZnO NPs was calculated using the Scherrer equation maximum FWHM }$=0.9\lambda/ \Delta(2\theta).\cos(\theta)$ [13][14] ….. Thus, D is the size of the crystallite, k is the wavelength of the X-ray source, and cos 16= 0.968583. The FWHM is beta, and the diffraction angle is h”. The nanoparticles generated were crystalline and pure in nature, according to X-Ray diffraction study (hexagonal phase). Figure 1 shows the peaks at 2 = 46 ZnONPs. The average particle size of ZnONP was calculated using the “Scherrer equation”, and this result coincided with the values given in [15].

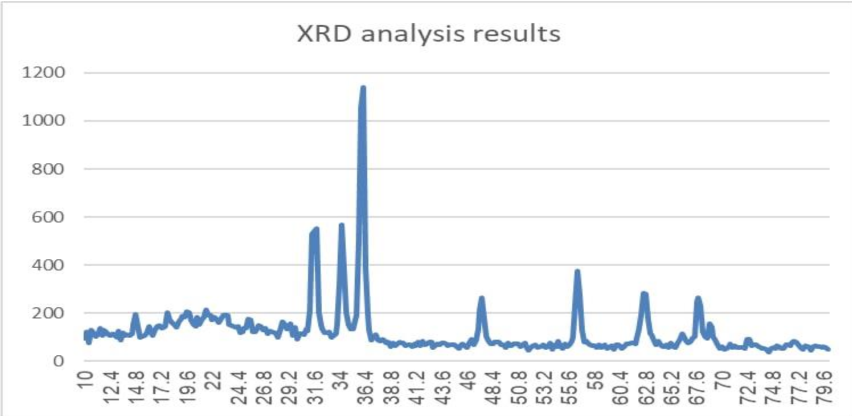

Figure 1. XRD analysis for . Zn NPs biosynthesized.

Fourier Transform Infrared (FTIR)

“The details of functional groups involved in Zn NPs production were determined using the FT IR spectra of Zn NPs nanoparticles. The FT IR spectra of ZnO NPs revealed notable peaks at 3448.84, 1660.77, 1020.38, and 549.73 cm–1. The broad vibrational band detected at 3448 cm–1 is caused by the symmetrical stretching mode of water molecules. The vibrational bending mode is ascribed to the band of water molecules found at 1660.77 and 1020.38 cm–1. The peak observed to the stretching vibrations of ZnO NPs at 549.73 cm–1 matches to Figure(2). A large, centered peak at 3448 cm–1 is ascribed to stretching hydrogen-bonded –OH groups on the surface of the nanoparticles in the ZnO spectrumFurthermore, a sharp peak at 453 cm–1 is detected, which corresponds to the stretching of the Zn – O bonds; these findings were consistent with the findings of [16]. The stretching vibration of the zinc-oxygen link was responsible for the well-resolved, wide, and strong transmission band below 549 cm–1 [17, 18]. A minor peak shift at can be attributed to a change in particle size caused by metal doping”.

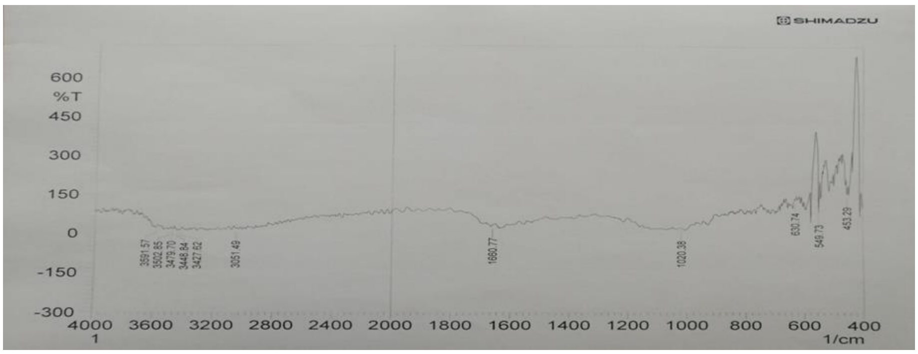

Figure 2. FT IR spectra of ZnO NPs biosynthesized by Bacillus clausii

X-Transmission- Electron- Microscopy (TEM)

Figure 3 shows TEM pictures of ZnO NPs nanoparticles . The particle size reduces as the quantity of metal doped rises. Transmission Electron Microscopy was utilized to characterize the size and crystallinity of the synthesized ZnO NPs, and "Dynamic Light Scattering was employed to assess the zeta potential of the biosynthesized ZnO NPs (DLS)". The average size of the nanoparticles was determined using DLS and was establish between 35 and 45 nanometers. The average size of biosynthesized ZnO NPs has been determined to be nm [19]. Transmission electron microscopy (TEM) is the most effective electron microscopy method for establishing the morphological identities of ZnO NPs and other metal nanoparticles [20].

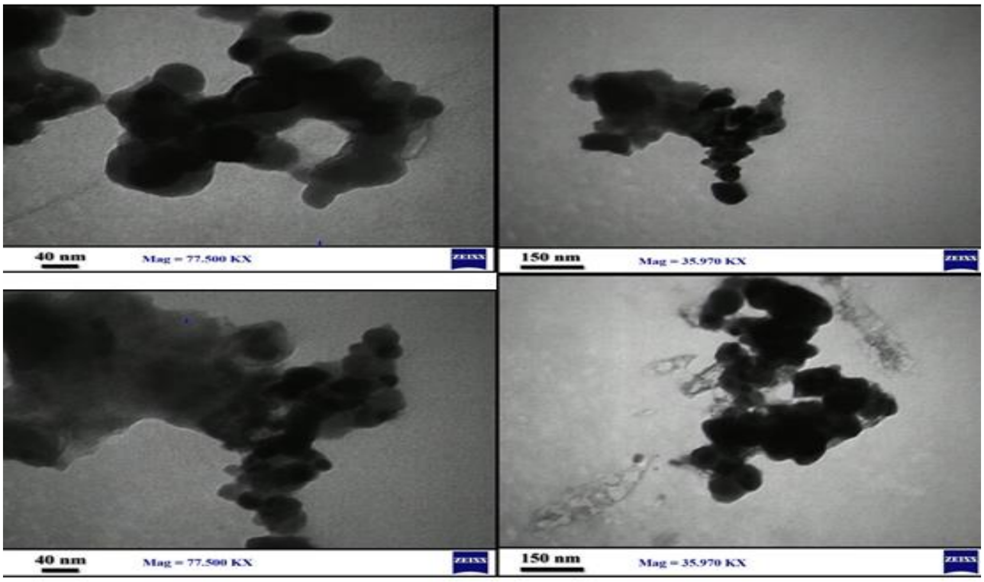

Figure 3. TEM micrographs of Zn NPs bio-synthesized by Bacillus clausii

Anti-Bacterial Efficacy of ZnO NPs against *Pseudomonas aeruginosa*

Antibacterial activity of zinc nanoparticles biosynthesized by Bacillus clausii against MDR *P.aerugenosa* bacterium isolate was investigated. At 200 g/ml MIC *P.aerugenosa* (26,22,18,16,7 mm), five different concentrations of Zn Nanoparticles (200, 100, 50, 25, 12.5 g/ml) were employed by disc diffusion agar technique. On MDR pathogenic bacterial isolates. The antibacterial effectiveness was caused by cell membrane disruption, which resulted in cell contents release and cell death. While the precise mechanism of action is unknown, the production of H2O2 (a powerful oxidizing agent detrimental to the cells of living creatures) from the surface of Zinc Oxide has been identified as the key factor of Zn-O, indicating that ZnONPs had an antibacterial impact. It was also discovered in this study that raising the concentration of ZnONPs improved growth inhibition. Depending on the pathogenic bacteria and ZnONP concentrations. Four concentrations of ZnONPs were used in this investigation to boost the antibacterial activity of nanocomposites [22].

Extremely low amounts of Zn NPs or ZnONPs cannot induce toxicity in human systems. The antibacterial effectiveness of Zn-ONPs was evaluated using the disc diffusion agar technique. The presence of an inhibition zone clearly indicates that rising ZnONP concentrations limit harmful bacterial development [24]. Zinc oxide nanoparticles are widely recognized to be antibacterial and to limit the development of germs by penetrating the cell membrane. Oxidative stress causes damage to lipids, carbohydrates, proteins, and DNA[25], and zincoxide nanoparticles have been shown to be harmful to both bacterial and eukaryotic cells. The MICs of zinc oxide nanoparticles against E. coli, *Pseudomonas aeruginosa*, and S. aureus were 500 and 125 g/ml, respectively.

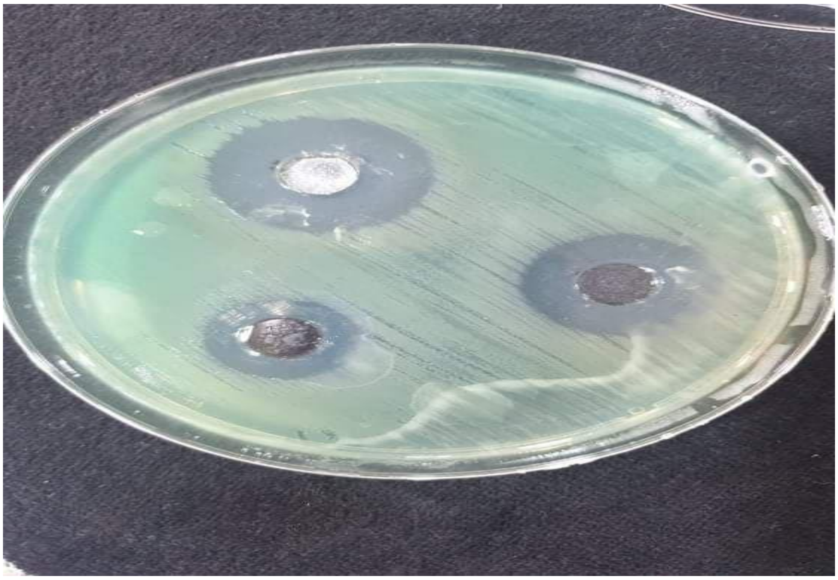

Figure 4. Anti-Bacterial Efficacy of ZnO NPs against Pseudomonas aeruginosa.

Conclusion

“The technique utilized in the “biological and catalytic” uses of Bacillus clausii in the synthesis of ZnO NPs, which offers a excessive chance to medicinal institutes biological activity and mode of synthesis from *Bacillus clausii*, which offers a great opportunity to medicinal institutions Our current condition investigation into the biosynthesis of ZnO NPs. More study should be done to improve “ZnO NPs' biological” uses, which are being studied by concentrating on ways to inhibit *Pseudomonas aeruginosa* development and employing them as “antibiotics” provide us fantastic outcomes, with minimal complications, cheap cost, and little resistance. This suggests that ZnONPS have a strong antibacterial effectiveness in inhibiting the development of Pseudomonas aeruginosa bacteria”.

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