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Research Article

ANTIMICROBIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES BIOSYNTHESIS BY BACILLUS SUBTILIS ON SOME PATHOGENIC BACTERIA

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Abstract

In the presented study, characterisation and synthesis of zinc oxide NPs (ZnONPs) through *Bacillus subtilis (B. subtilis)* are studied. To analyze the formation of ZnONPs, B. subtilis has been cultured in Nutrient broth as well as incubated at a temperature of 37 Celsius for a period of 24 hrs. The creation of ZnONPs was demonstrated when the supernatant following centrifugation was introduced to 1 mM ZnO and the color changed from yellow to light yellow. The structure, morphology and stability of the created ZnONPs have been examined with the use of morphological and structural properties technologies as FE-SEM analysis and XRD. Additionally, optical properties, like UV and FTIR spectra have been used. The results showed that the created NPs have been largely stable, hexagonal in phase, roughly spherical, with maximum particles with a diameter between 7 and 19 nm. The antibacterial effectiveness of ZnONPs against several dangerous microorganisms was also examined in this study. According to the research, ZnONPs have both a fatal and higher suppressive effect on pathogenic bacteria.

Keywords: ZnONPs, *Bacillus subtilis*, Biosynthesis, Characterization, and antibacterial activity.

INTRODUCTION

An environmentally friendly and dependable alternative to physical and chemical methods has been made possible by the employment of bacteria in the creation of biological nanoparticles [1]. Fungi and bacteria are naturally capable of reducing or oxidizing metal ions into oxidized or metallic NPs through functioning as miniature nano-factories [2]. The expansion of NPs' biological applications depends on the creation of trustworthy, safe, and ecologically acceptable production systems. Among metal oxide NPs, ZnONPs stand out for a number of reasons. Some advantages include effective antibacterial action, high catalysis, and physical and chemical stability [3]. ZnONPs are multifunctional inorganic NPs which can be employed in a range of applications as a result of their distinctive properties, which include powerful UV as well as infrared adsorption capacities [4]. Low toxicity and biodegradability are the two most significant features of ZnONPs [5].

METHODOLOGY

Bacteria used in Biosynthesis

Bacillus subtilis has been chosen as the biological model for creation of NPs from various strains after evaluating the ability of each strain to produce them. This strain was obtained spontaneously from nonpathogenic soil bacteria in a sophisticated microbiology facility. These bacteria were identified by microscopic analysis of the colony using Gram stain, colony morphology on various substrates, and the fundamental biochemical system. After that, there's a test, and then there's Vitek 2.

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Biosynthesis of ZnO NPs by *Bacillus subtilis*

Pure *B. subtilis* culture has been inoculated into a flask containing nutrient broth and cultured at a temperature of 37 Celsius for a period of 24 hrs at 100 rpm to create ZnONPs. Following incubation time, culture broth's pH has been raised to 0.40M NaOH in order to delay the transformation. The flask containing culture solution received 0.1% ZnSO4H2O solution after being heated on a water bath to a temperature of 80 Celsius for a period between 5 and 10 mins. The emergence of a white precipice at flask's bottom as a result of transformation caused the flask to be removed from water bath. To make sure that all particles gel in the flask's bottom, incubate at a temperature of 37 Celsius for a period of 12 hrs. The next step was to dry the product for 4 hrs at 40 degrees in a hot air oven after filtering and washing it with deionized water [6].

Characterization of Biosynthesized ZnO NPs

X-ray diffraction (XRD), FT-IR, UV-Vis spectroscopy, and Scanning Electron Microscopy (SEM) have been utilized for analyzing the bio-synthesized ZnO NPs [7].

The X-Ray Diffraction Measurements

Materials that had dried out were used for XRD. Utilizing CuKa1 radiations (l 14 1.540598A °), at 40kV and 40mA, with a 10mm divergence slit, an analytical X-ray diffractometer called X0Pert has been utilized in order to measure ZnO NPs' crystalline size. In order to identify the phases of the particles throughout a 2Y range of 30 to 80, JCPDS Cards have been utilized as standards. The size of crystallites was calculated using the Debye-Scherer equation [8].

Field Emission-Scanning Electron microscopes (FE- SEM) Measurement

SEM takes pictures of a sample by scanning it with a high-energy electron beam [9]. Field Emission Scanning Electron Microscopes (SEM) model S-1640q from Hitachi Corporation (Japan) with various magnification powers and detectors were used to examine the topography of the produced powder. The size of the particles was also determined by it. Prior to the examination, it is necessary to wash and dry the samples. In a period between 3 and 4 mins, FE–SEM can be pumped down to 10–5mbar. The extra high tension (EHT) (beam voltage) of the gas panel has been set to necessary value, which typically begins at 5KV. The sample's surface was covered in gold so as to help surface charge discharge.

Fourier Transform Infrared (FTIR) Measurements

A FTIR spectrometer provided through Shimadzu was used on KBr pellets regarding the samples to produce mid-IR spectra ranging from 4000 to 400 cm1 for certain pure samples and all doped samples.

UV-Visible Spectroscopy

In order to find the wavelength with maximum absorbance, spectra scans have been performed in wavelength range of 200nm-800nm with the use of HACH DR5000 spectrophotometer using a ZnONPs concentration (5 mg/20 ml) produced for UV-Visible spectroscopy.

Antibacterial activity of ZnONP

1. For determining the appropriate concentration for each bacterial isolate, the concentrations regarding each have been measured and compared to McFarland solution.
2. On Muller Hinton agar-coated plates, 0.1ml of every bacterial isolate has been added and the plates were left for an hour. The spreader was used to cover the dish's surface.
3. Three cork borers with a diameter of 6 mm were used to create wells with the same spacing.
4. Zno Nanoparticles were dissolved in deionized water, yielding concentrations ranging from (2..5-0.313) g/ml.
5. 40 microliters of each substance under study were poured into each well, which was then incubated for a period of 24 hrs at a temperature of 37 Celsius.
6. A ruler was used to quantify the inhibition zones of nanoparticles [10].

RESULTS AND DISCUSSION

Biosynthesis of ZnO NPs

B. subtilis confirmed the creation of NPs through observing the white color deposit at the bottom of flasks (1). In a case when *Lactobacillus sporogens* have been employed, this discovery was consistent with [11], and the results were consistent with [12]. [13] *B. subtilis* confirmed the creation of NPs through observing the white colour deposit at the bottom of flasks. In the case when *Lactobacillus sporogens* have been employed, this discovery was consistent with [14], and the results were consistent with [15] [16]. The extracellular reduction regarding aqueous zinc nitrate by *Sphingobacterium thalpophilum* culture supernatant served as the basis for the development of a straightforward bioprocess for the manufacture of ZnONPs. It is clear that *B. subtilis* is essential for producing ZnONPs. The cause of the issue might be this bacterium's negative electrokinetic potential that causes cations to rapidly magnetize and begin the production of NPs. With oxygen present, *B. subtilis* could grow constantly, which increases its metabolic efficiency. It is obvious that *B. subtilis* is crucial to the creation of ZnONPs. This bacterium's negative electrokinetic potential, and that induces cations to readily magnetize and start the creation of NPs, could be the root of the problem. *B. subtilis* can grow continuously with the existence of oxygen, which makes it more metabolically efficient.

Figure 1. Bio-synthesis of ZnONPs from *Bacillus subtilis*

Morphological and Structural characteristics of Zn-ONPs bio-synthesized biosynthesis by *Bacillus subtilis*

X -ray diffraction analysis

Fig. 1 illustrates the XRD patterns regarding biogenic ZnONPs produced by Bacillus subtilis. The nanoparticles produced were natural and crystalline in nature, according to XRD analysis. The peaks at 2 = 31.7694, 34.4211, 36.2521, 47.5376, 56c.6016, 62.8624, 66z.3782, 67.9610, 69.0982, 72x.5631, and 76.9671 corresponded to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) resection traces. Equation (1), which uses the FWHM to represent the peak (101) reflected picture at 2, uses the Scherer equation [16] for predicting the average particle size of ZnO [17, 18].

$$Dx=\text{zero.Nine}(\theta)\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3-1$$
$$\Delta(2\theta).\text{Cos}$$

It was discovered that the average particle diameter is 7nm. The broadening of lines at the diffraction peaks suggests that generated materials are in the nanoscale range. The resultant NPs' syntheses is just too little due to the organic synthesis method utilized to produce them.

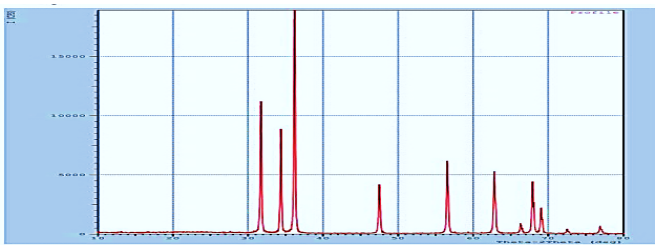

Figure 2. XRD analysis of ZnONPs synthesis by *Bacillus subtilis*

Feld Emission- scanning electron microscopy (FE-SEM) evaluation

ZnONP FE-SEM images were arranged using a biological technique and computed at 500 co for one hour, as demonstrated in parent.

With the use of a SEM, the size, surface area, and shape of ZnONPs have been determined. These images showed that the shape of the ZnONPs was irregular [19].

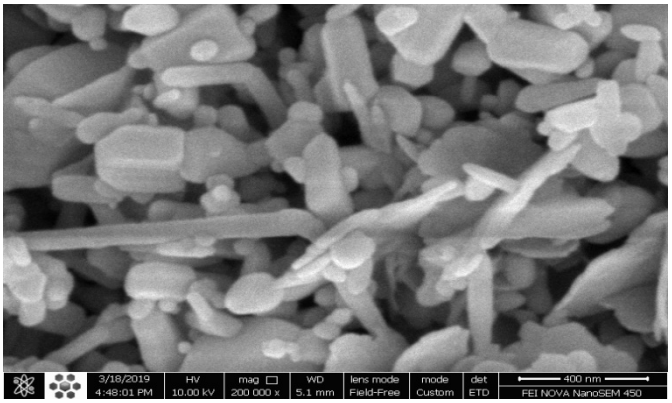

Figure 3. FE-SEM images of biological synthesis ZnO Nanoparticles *Bacillus subtilis*

Fourier Transform Infrared (FTIR)

FTIR spectra are utilized to identify the precise relevant agents involved in the production of ZnONPs. ZnONPs's first spectra that were received showed peaks at 3585, 1699, 1558, 1047, and 526cm. The wide vibrational band that had been observed at 3585 cm results from the symmetric stretching mode of water molecules. The bands found at 1699, 1558, and 1047cm are attributed to water molecules' bending vibrational mode. Figure (4) illustrates how a height of 590 cm corresponds to the stretching vibrations of ZnONPs. Zinc-oxygen bond stretching vibration was attributed to the intense, broad, and well-resolved transmission band beneath 526 cmq1v (17, 20). The change in the size of the particles as feature of metallic doping could be due to a minor height shift found throughout steel doping in this work (between 526 and 472 cm-1)...:.>q.

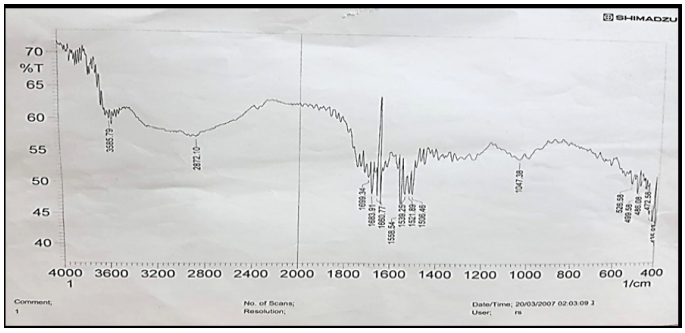

Figure 4. FTIR Spectra of Biosynthesis of ZnONPs through *B. subtilis*

UV -ViS diffuse reflectance

The samples have been isolated from biomass for analyzing the colloidal AnONPs' UV/ViS absorbance (425 nm). A sample acquired through centrifugation was believed to be useful. Yet, the color (yellow to light yellow) and absorbance (425 nm) of colloidal NP suspensions both decreased to practically zero. It could be caused by biomass capturing NPs or by their agglomeration. Centrifuging samples had thus shown detrimental effects on the NPs and ought to be evaded.

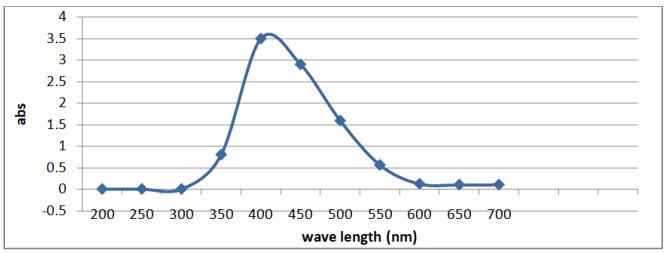

Figure 5. UV- Vis's spectrum, of ZnO NPs synthesizede by *Bacillus subtilius*

Antibacterial activityof ZnONPs against Pathogenic Bacteria

The synthesized ZnONPs have been tested for their anti-bacterial efficacy against four harmful bacteria, as indicated in table 1.

Table 1. Diameter of inhibition zone of the ZnONPs for some pathogenic bacteria

Bacteria Isolated	Gram stain	Type of sample	Source Obtained
E. coli	Negative	Urine	Kufa univ.– college of science
K. Pneumoniae	Negative	Swap (sputum)	Al-Hilla teaching hospital
S. aureus	Positive	Wab (sputum)	Babylon Univ.– College of Medicine
P. aeruginosa	Negative	Swap (sputum)	Hillah public health Lab

Disc diffusion agar methods utilized to evaluate the antibacterial activity of ZnONPs have been shown in Table 2.

Table 2. diameters of inhibition zones of ZnONPs for some of the pathogenic bacteria

No.	ZnONPs Con. (mg/ml)	ZnONPs (mm) *E.coli*	ZnONPs (mm) *Staph.aureus*	ZnONPs(mm) *Klebsiella Pneumonia*	ZnONPs(mm) *Pseudomonas aeruginos*
1	2.5	19	18	17	20
2	1.25	17	16	14	17
3	0.625	14	12	11	15
4	0.312	12	11	11	14

Those results demonstrate that, despite varying bactericidal dosages, ZnONPs had antibacterial activity against 4 pathogenic bacteria: *E. coli, S. aureus, K. pneumoniae, and P. aeruginosa*. The development of all bacteria was demonstrated to be mildly inhibited by ZnONPs at 0.312 mg/ml, but completely prevented by 2.5 mg/ml dosages. There is a chance that different microorganisms and ZnO or its NPs were used, leading to different concentrations. These findings also show that *K. pneumonia* has a lesser inhibition zone at the same dosage whereas *P. aeruginosa* has a higher inhibition zone at 2.5 mg compared to other bacteria. The results acquired are compatible with such findings because the ZnO NPs, at the same time, shown antibacterial activity against MRSA [21]. ZnONPs are thought to have an antibacterial effect on bacteria primarily by the production of more ROS, specifically hydroxyl radicals and singlet oxygen that break down bacterial cell wall. Second, the deposition of nano-particles on the bacteria surface or the accumulation of NPs in cytoplasm or periplasm impair cellular function and damage membranes. The outcomes also supported the findings of [22], which suggested that ZnONPs had an antibacterial effect on bacteria.

Conclusion

Bacillus subtilis may be utilized to make nanoparticles in a safe manner. Other chemical and physical techniques for nanoparticle production are less favorable than microbial synthesis. Nanoparticles are expected to be used in medicine and as sensors. Bacillus subtilis synthesizes ZnONPS, which are used as antibiotics and have excellent outcomes, minimal complications, a low cost, and little resistance when compared to conventional antibiotics.

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