

Short Communication: Characterization and biological synthesis of zinc oxide nanoparticles by new strain of *Bacillus foraminis*

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Abstract. *El-Ghwas DE. 2021. Short Communication: Characterization and biological synthesis of zinc oxide nanoparticles by new strain of Bacillus foraminis. Biodiversitas 23: 548-553.* The production of ZnO Nanoparticles (ZnONPs) has gained a lot of interest because of its wide antibacterial and eco-friendly activity. This work was performed for the biosynthesis of ZnONPs using isolated bacteria strains. A new bacterial strain isolated from farm soil in Jeddah, Saudi Arabia, was identified by molecular identification using 16S ribosomal RNA. The isolated sequence had the highest similarity (99 %) with *Bacillus foraminis*. On the other hand, the appearance of white precipitate at the end of incubation time confirms the production of ZnONPs. Also, UV, TEM, and FTIR were used to investigate the stability and structure of ZnONPs. The absorbance of ZnONPs was measured at 386 nm in a UV experiment. TEM result revealed that the particles were polydisperse and usually spherical, with maximum particles in the 5.40 - 6.79 nm range. In addition, FTIR confirms the reduction of zinc ions by bacteria. This is the first report on the biosynthesis of ZnONPs by *B. foraminis*.

Keywords: *Bacillus foraminis*, biosynthesis, FTIR, TEM, UV, ZnO nanoparticles

Abbreviation: FTIR: Fourier-transform infrared spectroscopy, TEM: Transmission Electron Microscope, UV: Ultra Violet, ZnONPs: ZnO Nanoparticles

INTRODUCTION

In the preceding decade, nanotechnology has emerged as a technology that has altered every field of applied science. Nanoparticles are tiny particles ranging from 1 to 100 nm. NPs have unusual features because of their large surface-to-volume ratio and extremely small size, resulting in considerable variances in attributes compared to their bulk counterparts (Piccinno et al. 2012). By providing creative solutions, nanoparticles have been successfully integrated into various sectors. ZnO is a type of metal nanoparticle made from metal oxides (Hedayati et al. 2017). ZnO is an inorganic substance with distinctive features such as broad radiation absorption, pyroelectricity, semiconductor, high catalytic activity, and piezoelectricity (Sharma et al. 2011).

Furthermore, the US Food and Drug Administration has classified ZnO as "Generally known as Safe" (FDA 2015) because it has non-toxic properties and is safe to both humans and animals (Sirelkhatim et al. 2015). Therefore, zinc oxide nanoparticles have attracted much attention in recent years. Several studies have been published on the efficacy of ZnONPs in preventing the growth of a wide range of pathogens (Swain et al. 2016) and suggesting that they may eventually replace antibiotics. Chemical and physical techniques have traditionally been used to make ZnONPs, which provide best control over the size of NPs and a higher production rate, but their high energy requirements, high capital

costs, hazardous chemicals, and poisonous effects make these technologies unfavorable. Also, these characteristics cause secondary contamination in the environment.

Furthermore, a prior study found that the chemical creation of NPs is hazardous and incompatible with living organisms, but they have limited biological and clinical applications. As a result, the development and research of biocompatible, cost-effective, environmentally friendly, and cleaner NP syntheses are required. The green production of NPs has developed as a viable alternative to traditional physical and chemical procedures in recent years. These processes are eco-friendly, non-toxic, and cheap. The microorganisms serve as a nano-factory, converting metal ions into metal NPs with the help of enzymes and other biomolecule components produced or manufactured by the bacteria. Despite this, only a few microorganisms are capable of producing ZnONPs. So, other microorganisms with the capacity to synthesize ZnONPs must be investigated.

Therefore, this research was performed to find a new bacterial strain that synthesis ZnONPs and also to study its properties.

MATERIALS AND METHODS

Collection of soil samples

A total of ten soil samples were obtained from various cow farms in the Makkah region of Saudi Arabia. The soil

surface was scraped to remove large pieces of debris, roughly 10 g of material was extracted at a depth of 2-5cm and brought to the laboratory at the University of Jeddah's, Faculty of Science and kept at 4°C for further processing.

Isolation and purification of bacterial strains

On nutrient agar plates, bacteria were isolated and purified using serial dilution method and incubated at 35-37°C, for 24 hours. After incubation, bacterial colonies were separated from the mixed culture, transferred to separate agar plates, and incubated for another 24 hours at 35-37°C. Then, pure colonies were kept at 4-5°C for further research (Atlas 2006).

Screening for synthesis of ZnO nanoparticles

The isolated bacteria strains were examined to synthesize ZnO nanoparticles as follows: - each bacterial strain was seeded on a 250 ml conical flask containing nutrient broth medium for 24 hours at 35-37°C under 200 rpm shaking condition. After that, supernatant was used separately to synthesize ZnO nanoparticles according to (Mishra et al. 2013; Selvarajan and Srinivasan 2013) with slight modification. 50 ml of 0.1 M zinc sulfate and 0.4 M sodium hydroxide were mixed with 50 ml of each culture filtrate of bacteria strains, followed by aggressive shaking and heating at 40°C for 15 minutes. The flasks were placed in the microwave for 1-2 minutes before chilling for 1 hour to allow the nanoparticles to settle. The presence of white color deposition at the bottom of the flask confirmed the formation of nanoparticles. After that, deionized water was added to the nanoparticles and centrifuged for 10 minutes at 3000 rpm. Then, pellet was carefully cleaned with deionized water after each centrifugation. Finally, pellet was collected on a tiny plate and dried in an oven at 40°C for 8 hours until completely dry, after which powdered ZnO nanoparticles were obtained and placed in a small vial in the refrigerator for further study. Identification of most potent strain

Morphological identification of bacterial strain

The identification of bacterial isolates was based on Bergey's Manual of Systematic Bacteriology, Vol. 4, and the International Journal of Systematic Bacteriology.

Automated identification systems (VITEK)

The VITEK system was developed in the 1970s as an automated system for microbial identification and antimicrobial susceptibility testing (AST). After the production and standardization of primary inoculum, it performed all the procedures required for identification and AST (Eigner et al. 2005). In a 12 × 75 mm clear plastic (polystyrene) test tube, suspended a sufficient number of pure colonies in 3.0 ml of sterile saline (aqueous 0.45 % to 0.50 % NaCl, pH 4.5 to 7.0). The DensiChek™ turbidity meter was used to measure the turbidity. 0.50-0.63 for Gram positive and 0.50-0.63 for Gram negative bacteria. The identity cards were inoculated with the bacterial suspensions using an integrated vacuum device. A test tube containing the bacterial suspension was placed into a special rack (cassette). The identification card was placed

in a neighboring slot while inserting the transfer tube into the corresponding suspension tube. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction was read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm was used to eliminate false readings due to small bubbles that may be present. Genetic identification of synthesized ZnONPs bacteria strain

Detection of genomic DNA was performed by amplification and PCR of 16S-rRNA gene was performed according to (Al-Tamimi 2015). PCR product analysis was done according to (Watanabe et al. 2001). The nucleotide sequences were compared to the nucleotide database by BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST/>) (Clark et al. 1974).

Properties of ZnO nanoparticles

UV-visible spectroscopy

T80+UV/VIS Spectrometer, PG Instrument Ltd. range: 190-1000 nm was used to analyze the UV spectrum.

Transmission electron microscopy (TEM)

Transmission electron microscopy was used to detect the size and shape of zinc oxide nanoparticles. The TEM image was made at the National Research Centre in Cairo, Egypt, using the JEOL - JXA 840A, Model Japan, electron probe micro-analyzer. Thin films of the sample were generated by depositing a small portion of the sample on a coated copper grid and nanoparticle images were taken after the film had dried on the TEM grid.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum was obtained by mixing potassium bromide at 1: 100 ratio with a 2 mm semi-transparent disc and compressing it for 2 minutes. The Nexus 670 FTIR spectrophotometer (Iclet Co, USA) was used to record spectra over a wide range of wavelengths (4000-400 cm⁻¹).

RESULTS AND DISCUSSION

Isolation and identification of bacteria

The results showed that a total of fifty pure isolate strains were obtained. The frequency and percentage of specific bacterial isolates are presented in Figure (1). The highest frequency was reported in *Bacillus* spp.16 (32%) followed by *Streptococcus* spp.9 (18%), *Agrobacterium* spp.5 and *Pseudomonas* spp.5 (10% each), *Corynebacterium* spp.4, *Arthrobacter* spp. 4, *Micrococcus* spp.4 (8% each), and *E. coli* 3 (6%).

Screening for the biosynthesis of ZnONPs by some isolated bacterial strains

It was observed that due to the change in color of filtrate and the production of a white precipitate after 24 hours of incubation, only one isolate i.e., *Bacillus* spp. was able to synthesize ZnONPs using zinc sulfate powder.

Whereas the color of the nanoparticles was not changed in the control and other isolates.

Genetic identification of isolate *Bacillus* spp.

Molecular investigation of 16S RNA was used to identify *Bacillus* spp. that can synthesize ZnONPs from zinc sulfate powder. PCR was used to amplify the 16S rDNA, which was done with universal forward and reverse primers. After purification of DNA, it was sequenced using an automated sequencer. The sequencing data of the isolates were then compared to known bacterium genes in the GenBank database. The results showed that the sequence of the isolate had highest similarity (99 %) with *Bacillus foraminis*, as shown in Figure (2).

Properties of ZnO nanoparticles (ZnONPs)

Transmission electron microscopy (TEM)

The morphology and size of nanoparticles were studied using TEM. It can detect mono and polydisperse nanoparticles purity and particle size during their production. Results showed that ZnONPs in the reaction solution has a higher grain size, homogeneous shape, and polycrystalline. Under a magnification of 50 X, it appeared approximately spherical and was polydisperse with various diameters ranging from 16 to 25 nm (Figure 3).

Ultraviolet spectrum (UV)

Bacillus foraminis was used to show the manufacture of zinc oxide nanoparticles using ultraviolet spectrum (UV) approach. The ZnO nanoparticles had a well-defined plasmon band with an absorbance value of 2.89 at a wavelength of 380 nm (Figure 4).

Fourier transform infrared spectroscopy (FTIR)

The probable biomolecules responsible for the efficient stabilization and capping of the metal nanoparticles were identified using FTIR studies. Furthermore, the metal ions reduction by *B. foraminis* was confirmed by FTIR measurement. The detected peaks were 3434, 1640, 1412, 1039, 1039 and 519 cm^{-1} , as shown in Figure 5. The vibration stretch of ZnO nanoparticles was detected at 519 cm^{-1} . C-O stretching esters, alcohols, ether, phenol, and carboxylic acids all peak at about 1000-1320 cm^{-1} . The peak for C-C stretch (in-ring) aromatics was 1412 cm^{-1} . N-H bend primary amines have a peak about 1580-1650 cm^{-1} . The peak shown was 1640 cm^{-1} , which leads to N-H bend primary amines. For amides, primary and secondary amines, the peak about 3250-3400 cm^{-1} leads to N-H stretching. For amides, primary and secondary amines, the shown peak of 3434 cm^{-1} corresponds to N-H stretching.

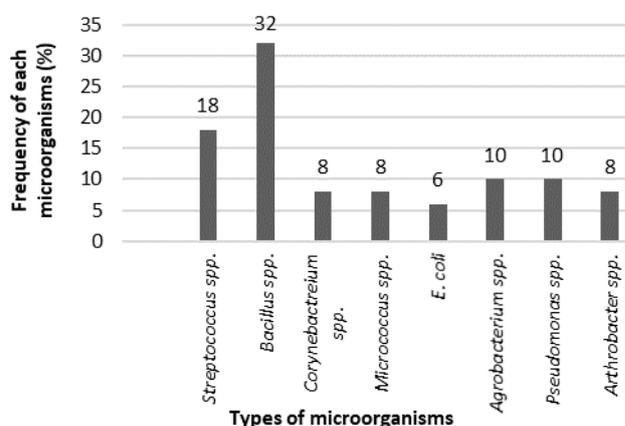


Figure 1. Frequency of identified bacterial isolates

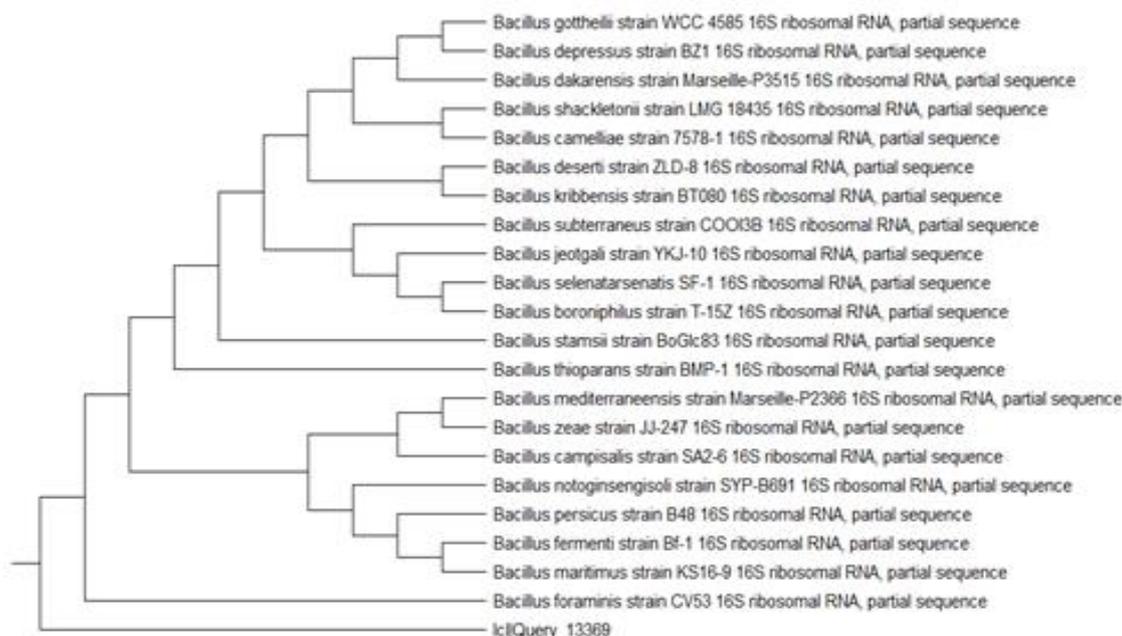


Figure 2. The phylogenetic tree of *Bacillus foraminis* and other *Bacillus* spp. constructed using the neighbor-joining method

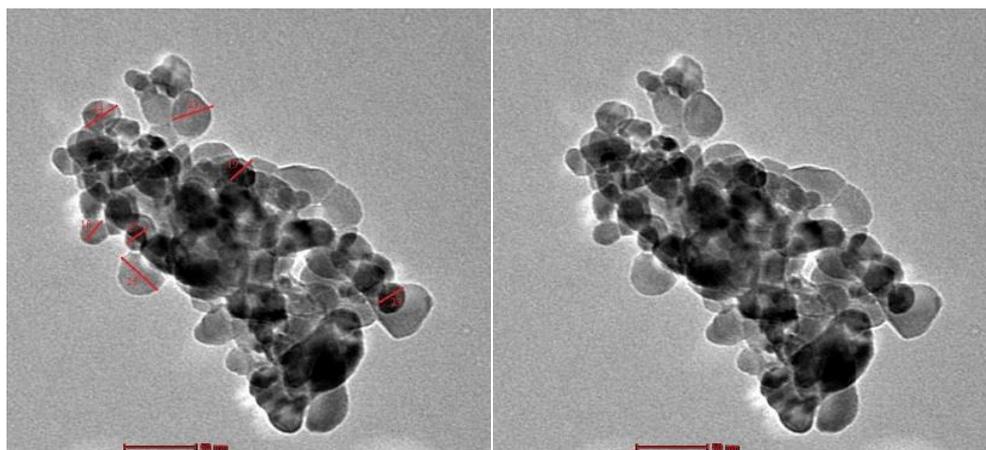


Figure 3. TEM analysis of ZnONPs synthesis by *Bacillus foraminis*

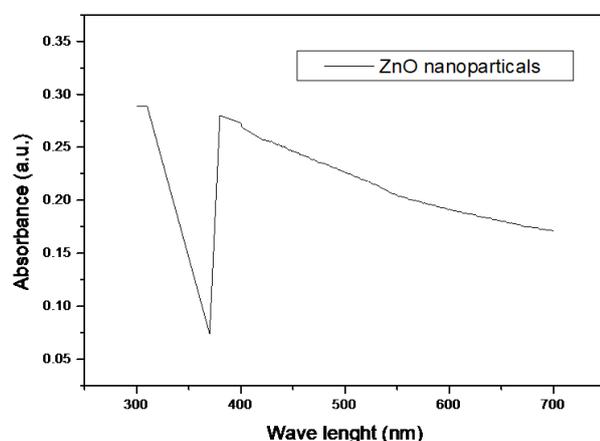


Figure 4. UV-spectrum of ZnONPs synthesis by *Bacillus foraminis*

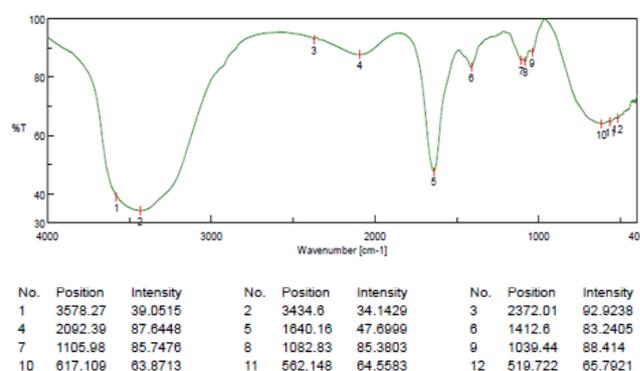


Figure 5. FTIR-spectrum of ZnONPs synthesized using *Bacillus foraminis*

Discussion

Biological nanoparticle synthesis is clean, cost-effective, non-toxic, and ecologically friendly method (Li 2011). The present investigation aimed to create zinc oxide nanoparticles (ZnONPs) using *B. foraminis* and study its properties. Bacteria were cultivated on nutrient agar plates and incubated for 24 hours at 35-37°C. According to Brown (2007), bacteria were identified based on microscopic examination and colony characteristics of stained smears that show microbial structure, shape, agreement, and Gram stain reactivity. The identification was then validated using the Biomerieux-recommended VITEK 2 compact system (McFadden 2000).

The results showed that *B. foraminis* was able to synthesis ZnONPs and after 24 hours of incubation with zinc sulfate powder, a white colored precipitate was formed due to the surface plasmon resonance phenomenon. This finding was in accordance with Hussein et al. (2009), who demonstrated the use of *Bacillus cereus* as a bio templating agent in creating zinc oxide nanoparticles. Shamsuzzaman et al. (2014) made a low-cost, and straightforward method for biosynthesis of ZnONPs by employing *Bacillus subtilis*.

Also, Jayaseelan et al. (2012) reported the synthesis of ZnONPs from ZnO solution using the reproducible bacteria *Aeromonas hydrophila* at room temperature over 24 h. In addition, Singh et al. (2014) described a biological method for producing zinc oxide nanoparticles by utilizing *Pseudomonas aeruginosa* rhamnolipids (RLs). Salman et al. (2018) reported the production of zinc oxide (ZnO) nanoparticles utilizing *Lactobacillus* spp.

Moreover, Selvarajan and Mohanasrinivasan (2013) used *Lactobacillus plantarum* VITES07 to demonstrate intracellular ZnONPs production. Also, Mishra et al. (2013) demonstrated that employing *Lactobacillus sporogens* to synthesize ZnONPs may form a hexagonal shape with varying diameters. On the other hand, several scientists have described the mechanism of synthesis of zinc nanoparticles by bacteria. Yusof et al. (2019) reported that NADH dependent enzymes, protein, NADH and other compounds synthesis by microbes are important factors in the biosynthesis of metal nanoparticles process. However, information on the chemical components involved in producing NPs is not available. Microbes that have an inherent ability to generate inorganic NPs that can be

routed either intracellularly or extracellularly (Markus et al. 2016; Yusof et al. 2019).

In comparison to the intracellular pathway, extracellular production is more favorable and has been frequently used. This is primarily because it may be used to manufacture huge quantities and requires minimal downstream processing this avoids a number of simple separation processes, synthesis, and industrialization. While the retrieval of NPs in intracellular production needs an extra step, like centrifuging the cell biomass and subjecting it to many cycles of ultrasonication for cell breakdown to obtain purified NPs (Markus et al. 2016; Yusof et al. 2019). The image of ZnONPs taken with a transmission electron microscope (TEM) revealed that it had a tiny size, homogeneous shape, and polycrystalline structure. ZnONPs in the samples were larger grain, almost spherical in shape, and polydisperse, with sizes ranging from 16 to 25 nm under 50 X magnification. This finding is consistent with Khajeh and Golzary (2014) who produced hexagonal ZnONPs with sizes ranging from 11 to 25 nm. Motshekga et al. (2018) investigated the morphology of ZnONPs and reported that they are spherical in shape. According to Al-Zahrani et al. (2018), ZnONPs are generally spherical in shape and polydisperse, with maximum particles in the 5-9 nm range. The size range of ZnONPs is 40-75 nm (Jayaseelan et al. 2012; Bhumi and Savithamma 2014). Also, using *Pseudomonas aeruginosa*, Singh et al. (2014), discovered spherical shaped ZnONPs with 35-80 nm in size. Selvarajan and Mohanasrinivasan (2013) also demonstrated the production of ZnONPs from *Lactobacillus plantarum* VITES07, which are crystalline and spherical in shape and range from 7 to 19 nm in size.

Furthermore, Sabir et al. (2020) reported that ZnONPs produced by *Bacillus subtilis* is spherical in shape and do not combine, with diameter ranging from 16 to 20 nm. The UV-vis spectrophotometer methodology is used to measure the structural characterization of nanoparticles by determining the absorbance to ensure the ZnONPs characterization (Haiss et al. 2007). The finding of UV-vis spectrophotometer revealed that ZnONP nanoparticles had an absorbance value of 2.89 at a wavelength of 380 nm. This UV absorption peak seems to be similar to the absorption band found in the results of Khana et al. (2019) who measured absorbance at 380 nm. Hudlikar et al. (2012) found that the absorption peaks of ZnO nanoparticles are between 340 and 385 nm. Mishra et al. (2013) also observed an absorption peak at 381 nm (3.26 eV). However, Sabir et al. (2020) discovered that tested ZnONPs had a well-defined plasmon band, with an absorbance value of 1.89 at 331 nm. Singh et al. (2011) demonstrated that the excitonic property of ZnO is observed at a wavelength of 368 nm at ambient temperature.

The FTIR data, on the other hand, was used to identify the probable biomolecules involved for the effective stabilization and capping of the zinc oxide nanoparticles produced by *B. foraminis*. Results revealed that the presence of carbonyl groups from amino acid residues was confirmed by FTIR analysis, and protein has a strong ability to bind metal designating proteins that can come

from metal nanoparticles, i.e., capping of zinc oxide nanoparticles, implying that biological molecules can perform dual functions of metal nanoparticle formation and removal (Vijayalakshmi et al. 2016; Jamdagni et al. 2018). The vibration stretch of ZnO nanoparticles has been determined at 519 cm^{-1} (Sangeetha et al. 2011; Raj and Lawrence 2018). In conclusion, the white precipitate of ZnO nanoparticles (ZnONPs) was obtained from a new bacterial strain of *B. foraminis* isolated from the soil of cow farms in the Jeddah region. The absorbance at 380 nm of ZnONPs was measured using a UV-vis spectrophotometer. Furthermore, TEM analysis revealed that the particles were generally spherical in form and polydisperse, with maximal particle sizes ranging from 16 to 25 nm.

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