# ECO-FRIENDLY PRODUCTION OF ZINC OXIDE NANOPARTICLES BY *STENOTROPHOMONAS PAVANII* FOR ANTIMICROBIAL APPLICATION

Mohamed Hosny [1], Moaz AlSedawy [2] and Huda. S. El-Sheshtawy [1] *

[1] Processes Design and Develop Department, Egyptian Petroleum Research Institute, Nasr City, 11727 Cairo, Egypt

[2] Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

***Corresponding author:Email:** hudaelsheshtawy@yahoo.com

## ABSTRACT

Green nanoparticle synthesis is gaining popularity and has been proposed instead of using physical and chemical processes. In this work, the bacterial strain *Stenotrophomonas pavanii* was used to produce zinc oxide nanoparticles (ZnO-NPs) in a low-cost and eco-friendly manner. The biosynthesized nanoparticles were examined using dynamic light scattering (DLS), X-ray diffraction (XRD) and transmission electron microscopy (TEM). The ZnO nanoparticles synthesized were clean, principally round in form, and vary in size from 0.9 to 17.1 nm. In the present work, the biosynthesized ZnO nanoparticles have been used for antimicrobial applications against Gram-positive and Gram-negative bacteria, yeast, and fungi strains. ZnO NPs had better antibacterial efficacy against tested Gram-negative bacteria than they did against Gram-positive bacterial and fungal strains. The highest antimicrobial activity against *Escherichia coli* (28 mm) and *Pseudomonas putida* (29 mm). The extracellular production of zinc oxide nanoparticles from culture *Stenotrophomonas pavanii* showed good antibacterial activity.

**Keywords:** Eco Friendly production; nanoparticles; *Stenotrophomonas pavanii*; antimicrobial activity

## 1. INTRODUCTION

The term nano materials refer to materials with a diameter ranging between 1.0 and 100 nm. nano materials have attained prominence in technological advancement as a result of their unique flexible and adaptable optical, mechanical, electrical, biological catalytic, and magnetic characteristics that are superior to their bulk form **(Mutukwa *et al.*, 2022).**

Metal oxide nanoparticles (NPs) such as copper oxide, tin oxide, zinc oxide (ZnO), and titanium dioxide are among the most studied NMs due to their numerous diverse properties and applications. Zinc oxide is one such metal oxide that has received extensive research due to its chemical stability, non-toxic nature, cost-effectiveness, biocompatibility, and biodegradable nature **(Alsaiari *et al.*, 2023).** ZnO is utilized as a food stabilizer; hence, the FDA categorizes it as a chemical that is "generally recognized as safe." (GRAS). ZnO-NPs have a wider range of uses than other metal oxide nanoparticles, including medicine, gas sensors, biosensors, cosmetics, and storage **(Deka *et al.*, ٢٠٢٢)**. ZnO-NPs have shown substantial antimicrobial activity against common food pathogens such as *Campylobacter jejuni, E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* indicating its usefulness as a food preservative. They can block the growth of both Gram-positive and Gram-negative bacteria. Gram-positive bacteria, including *S. aureus, Streptococcus pyogenes* and *Enterococcus faecalis* have shown 95% growth inhibition in the presence of ZnO nanoparticles **(Mokammel *et al.*, 2022).**

Different studies demonstrate that ZnO-NPs can be synthesised using microbe-assisted synthesis **(Mutukwa *et al.*, 2022).** Suba et al. successfully synthesised ZnO-NPs using bacteria isolated from cow milk, and the synthesised ZnO-NPs exhibited good antibacterial and anticancer activity. The ZnO-NPs were hexagonal in structure and spherical in shape and the average particle size was reported to be 32 nm **(Suba *et al.*, 2021)**. Additionally, **Hefny et al.** successfully synthesised ZnO-NPs using five fungal cultures isolated from five fungal species (*Aspergillus niger, Aspergillus tubulin, Aspergillus fumigatus, Penicillium citrinum, and Fusarium oxysporum*) and the synthesised ZnO-NPs also exhibited good antibacterial activity. The synthesised ZnO-NPs were crystalline with a hexagonal wurtzite structure, which is the typical structure of ZnO, and the particle size was between 30 and 100 nm **(Hefny *et al.*, 2019)**. Bacteria are preferred synthesize ZnO-NPs, as they are simpler to handle and enable genetic manipulation more easily than other eukaryotic microbes do **(Velusamy *et al.*, 2016)**.

Biological synthesis is an approach that utilizes plants, fungi, bacteria, algae, etc., for the preparation of NPs. This green synthesis approach eliminates the need for reducing and capping agents during the synthesis of NPs. This is due to the ability of microbes and plants to reduce metal salt precursors while also stabilizing the synthesized NPs using naturally occurring substances such as enzymes and secondary metabolites. Thus, making biological synthesis a safer and low-cost alternative to conventional NPs synthesis methods **(Dhaka *et al.*, 2023).** The size of the NPs can be estimated using a variety of techniques, including dynamic light scattering (DLS),

scanning electron microscope (SEM), Transmission electron microscopy (TEM) and X-ray diffraction (XRD) **(Kestens *et al.*, 2016).**

This study aimed for the biosynthesis of ZnO-NPs by the action of cell-free broth of *Stenotrophomonas pavanii* isolated from soil, and testing their ability to inhibit the growth of a numerous microorganism, such as *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556, *Pseudomonas putida* ATCC 10145 and *Escherichia coli* ATCC 23282, *Aspergillus niger* IMI-31276, and *Candida albicans* IMRU-3669, using the agar diffusion technique.

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

Different soil samples, rice straw and corn stover samples were collected from Egyptian agricultural areas.

### 2.2. Isolation of bacterial isolates from soil samples

The bacterial isolates in the soil samples were isolated using mineral salts medium (MSM) (g/L) ($FeSO_47H_2O$, 0.1, $MgSO_47H_2O$, 0.5, $KH_2PO_4$, 0.5, KCl, 0.1, $NH_4NO_3$, 4.0, $K_2HPO_4$, 1.0, NaCl, 2.0, and $CaCl_2$, 0.1 and just 1.0 % glucose was employed), pH was set to 7.0. One gram of soil samples was placed in 250 mL of MSM and then incubated for 2.0 days in a shaking incubator at 30°C with 150 rpm of agitation. A loopful from each flask was streaked on nutrient agar for the separation of single pure bacterial isolates **(Larik *et al.*, 2019)**. The isolated organisms were selected for screening for the synthesis of nanoparticles.

### 2.3. Screening for synthesis of ZnO nanoparticles by bacterial isolates

The cell-free broth of isolated bacterial isolates (B1-B8) were screened for the synthesis of ZnO nanoparticles.

#### 2.3.1. Preparation of cell-free broth of the bacterial isolates

One mL inoculum of each bacterial isolate containing $10^6$ CFU/mL was added to 100 mL Muller-Hinton-Broth (MH) (g/L) together with 300 gm of dehydrated beef, casein hydrolysate 17.5 gm, starch 1.5 gm, and an adjusted pH of 7.3. then incubated in a shaking incubator at 37$^o$C for 18 to 24 h at a shaking rate of 150 rpm. The bacterial cultures were centrifuged for 30 minutes at room temperature and 5000 rpm to obtain cell-free broth. The remaining cells in the cell-free broth were removed by using a membrane filter (0.22µm) and the cell-free broth was collected for future investigation. The sterility of the media was examined using the controlled autoclaved without inoculation **(El-Sheshtawy *et al.*, 2021 and Khalil *et al.*, 2022).**

### 2.3.2. Synthesis of nanoparticles extracellularly by the cell-free broth of bacterial isolates

The cell-free broth of eight bacterial isolates (B1-B8) was tested for ZnO-NPs synthesis extracellularly by adding 1mM $ZnSO_4.7H_2O$ as a precursor for nanoparticles to the gathered cell-free broth at a volume ratio (5:50 v/v).

To counteract the effects of light, all combinations were incubated at room temperature and maintained in the dark for 24 hours. After incubation, the mixture was centrifuged at 5000 rpm for 30 minutes to precipitate the collected metals NPs, followed by a wash in deionized water and a 24h drying period in an oven at 80 $^oC$. To determine if bacteria were involved in the creation of nanoparticles, a control experiment using uninoculated media was carried out **(Sunkar and Nachiyar, 2017).**

### 2.3.3. Screening for the best-produced ZnO-NPs

The particle size of ZnO-NPs produced by each bacterial isolate was determined by the dynamic light scattering (DLS) technique using the Zeta Sizer Nano equipment (Malvern, UK). To ensure uniformity, the measurement was performed three times for each sample **(El-Sheshtawy *et al.,* 2021)**. ZnO-NPs showed the smallest particle size was selected for further characterization and testing of its antimicrobial activity.

## 2.4.Characterization of the selected biosynthesized ZnO-NPs

### 2.4.1. Dynamic light scattering (DLS)

The analytical technique of DLS was used to determine the particle sizes of the produced samples. Malvern Instruments Ltd (UK). provided the Zeta Sizer Nano equipment, which was used to measure the particle sizes. To ensure uniformity, the measurement was performed five times for each sample **(El-Sheshtawy *et al.,* 2021)**.

### 2.4.2. X-ray diffraction (XRD) analysis

A straightforward, non-destructive, and quick analytical method for identifying the phase of crystalline materials is X-ray powder diffraction (XRD). This is accomplished by comparing a sample's diffraction spectrum peak positions with a standard XRD spectrum from the database of the Joint Committee on Powder Diffraction Standards (JCPD) **(Hjiri *et al.,* 2019).**

### 2.4.3. High-Resolution Transmission Electron Microscopy (HRTEM)

HRTEM was used to analyze the shape and structure of the ZnO nanoparticles. The samples were examined using the JEM-200CX model (JEOL, Japan) and an accelerating voltage of 200 kV **(Mclaren *et al.,* 2009)**.

### 2.5. Antimicrobial efficacy of ZnO-NPs' against the selected strains

The effectiveness of the synthesized nanoparticles against standard strains of Gram-positive and Gram-negative bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556, *Pseudomonas putida* ATCC 10145 and *Escherichia coli* ATCC 23282), yeast-like fungi (*Candida albicans* IMRU-3669) and filamentous-like fungi (*Aspergillus niger* IMI-31276) was assessed using the agar well-diffusion approach **(Jayaseelan *et al.*, 2012)**. Streptomycin and fluconazole were the standard drugs for testing the bacterial and fungal resistance. All chemicals were soluble in distilled water at a concentration of 5.0 mg/mL. For bacterial strains, the plates were incubated for 24 h at 30 $^{o}$C. For fungal strains, it was 25 $^{o}$C for 3-5 days. The diameter of the inhibitory zone produced by the biosynthesized nanoparticles on the examined microorganisms was measured **(Magiatis *et al.*, 2002)**.

### 2.6. Molecular identification of the selected bacterial isolate

#### 2.6.1. Morphological and biochemical characterization of the selected isolate:

The selected bacterial isolate (B6) was characterized morphologically and biochemically according to the scheme of **Bergey's** manual of determinative bacteriology **(Holt *et al.*, 1994)** and **Procop *et al.* (2017)**. Definitive identification of this bacterial isolate at the species level was performed using molecular-based identification.

#### 2.6.2. Molecular identification of bacterial isolate

Identification of the selected bacterial isolate (B6) to the species level was carried out by sequence analysis of 16S rRNA gene and phylogenetic tree were performed in Gene Analysis Unit, Sigma Scientific Services Co., Egypt.

Genomic DNA of the selected isolate was extracted according to the method described by **Al-Ahmadi and Roodsari (2016),** and the 16S rRNA gene was amplified. Amplification of 16S rRNA gene was carried out with TransScript two steps RT-PCR SuperMix using forward primer 16S-f 5'-AGAGTTTGATCMTGGCTCAG-3', reverse primer 16S-r 5'-AGAAAGGAGGTGWTCCARCC-3', 2x TransTaqHiFi PCR SuperMix II and nuclease free water. PCR program for 16S rRNA gene amplification was pre-denaturation at 94℃ for 5.0 min followed by denaturation at 94℃ for 30 s, 30 s annealing at 57℃ and 90 s extension at 72℃ and a final extension step of 10 min at 72℃ withhold at 4 °C. The amplified 16S rRNA gene was separated on an 1.5% agarose gel using 100 bp DNA marker (Intron, Korea), placed in an electrophoresis chamber and covered with electrolyte solution in 1X TBE buffer ,allowing the PCR product to run in the gel at 95 V for 40 min then extracted from agarose gel using QIAquick Kit DNA purification kit (Intron, South Korea). The purified RT-PCR product was sequenced in the forward direction on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) using a ready reaction Big dye Terminator V31 cycle sequencing kit (Perkin-Elmer/Applied Bio-systems, Foster City, CA) with Cat. No. 4336817EMBL's Nucleotide Sequence Database. The forward sequence of 16S rRNA gene of the selected isolate were aligned and compared with the known

16S rRNA gene sequences in the Genbank database using the BLASTn search at NCBI. The taxonomic position and the phylogenetic tree of the isolates were constructed with MEGA-X.

### 2.7. Statistical analysis

The data were all expressed as the mean ± SD, and each experiment was carried out in triplicate (n ¼ 3). Values were expressed as three replicated means standard deviation. Using the one-way analysis of variance (ANOVA) function in the Minitab application, Tukey's HSD at p _ 0.05 found no statistically significant difference between the identical letters that appear in each column.

## 3. RESULTS

### 3.1. Isolation of bacterial isolates

In the current investigation, eight distinct bacterial isolates named (B1-B8) have been isolated from various soil sources depending on their colony morphology and microscopic examination. On MSM media, the bacterial strain (B6) from the soil sample is thought to be the predominant isolate. It was investigated whether the different bacterial isolates could create ZnO nanoparticles.

### 3.2. Screening the production of ZnO-NPs by cell-free broth of bacterial isolates

The data of the dynamic light scattering (DLS) technique in Figure (1) showed that the particle size of ZnO-NPs produced by the cell-free broth of bacterial isolates (B1-B8) ranged from 77.88 nm to 510.3nm. ZnO-NPs produced by the cell-free broth of B6 isolate has the smallest particle size (77.88 nm), followed by B8 (105.6nm), B2 (107.6 nm), B3 (116.9 nm), B5 (202.2 nm), B1(250.4 nm), B4 (320.3 nm) and B7 (510.3 nm). The B6 isolate was selected for identification by 16S rRNA, whereas ZnO NPs produced by this isolate were used to characterise and study its antimicrobial activity.

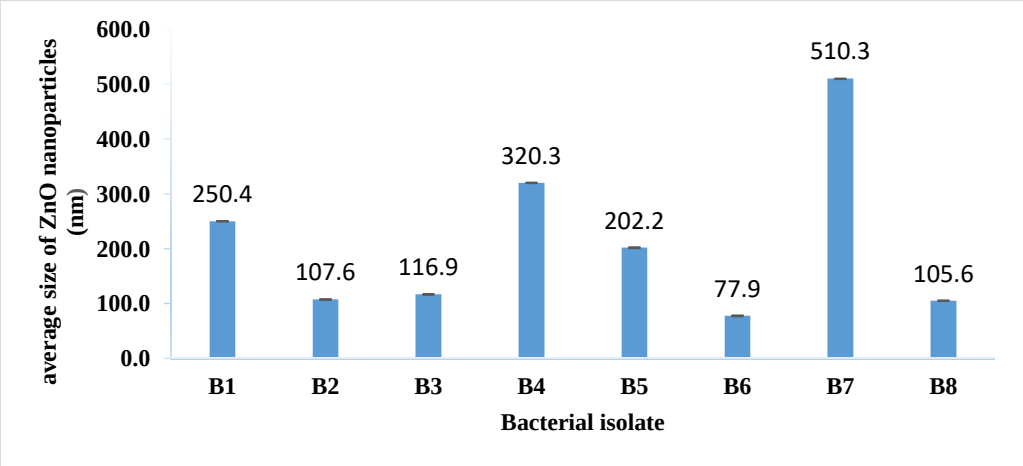


**Figure (1): Size of ZnO-NPs prepared by different bacterial isolates using DLS analysis. All values are expressed as mean ± SD and represent the average values from three experiments (n = 3).**

### 3.3. Characterization of ZnO-NPs biosynthesized by B6 isolate

Figure (2a) displays the X-ray diffraction (XRD) pattern of the produced ZnO-NPs. Three powerful diffraction peaks at 2 of 31.7, 45.4, and 56.4° are responsible for the [100], [002], and [102] cell orientation planes, respectively, according to the XRD pattern. The p63mcspace group is strikingly similar to these diffraction peaks. The JCPDS data [crystal code: 36-1451] of **Saravanan *et al.* (2018)**, which uses the Scherer equation and the large diffraction peak at 31.7°, also supports this. These findings supported the successful synthesis of the ZnO crystal structure. Figure (2b) shows how morphological study using TEM images revealed that biosynthesized ZnO nanoparticles are spherical in shape and have a constrained size distribution. According to the TEM analysis, which supports the XRD results, the size of the as-prepared ZnO particles ranges from 0.9 to 17.1 nm (Figure 2b).

Additionally, the DLS investigation demonstrated that ZnO has a hydrodynamic size range of 34 to 122 nm. The creation of a hydrated shell around the surface or a potential particle aggregation in the aqueous environment during the analysis could both be contributing factors to the increased dynamic size of ZnO-NPs in the DLS spectra (Figure 2c). The characterization information presented above demonstrated that the production of ZnO-NPs from the bacterial strain *Stenotrophomonas pavanii* in Muller-Hinton broth medium was effectively validated.

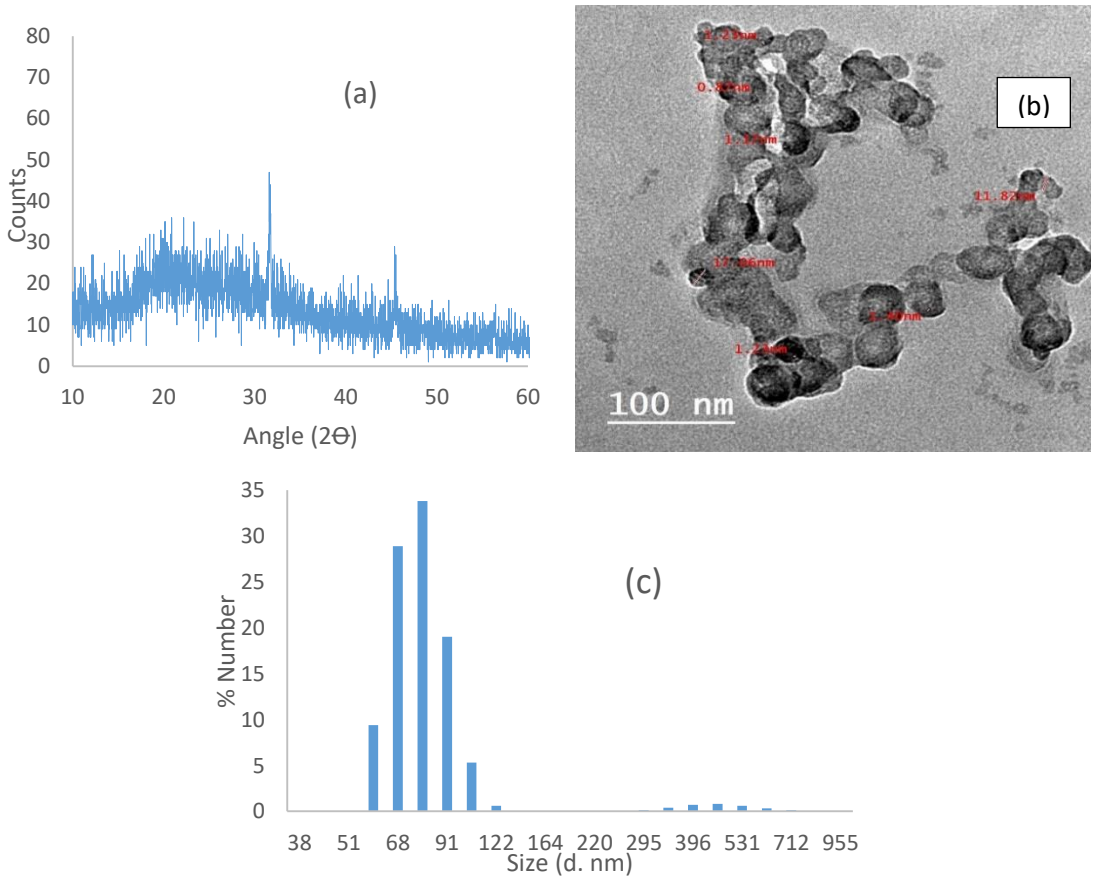


**Figure (2): Characterization data of the as-prepared ZnO nanoparticles: (a) XRD pattern, (b) TEM image, and (c) DLS spectrum.**

### 3.4. Application of the produced ZnO-nanoparticles for antimicrobial activity

ZnO-NPs produced by a cell-free broth of B6 isolate showed a variety of antimicrobial activity at a concentration 5.0 mg/ mL (Figure 3) against Gram-positive reference strains including *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 35556, Gram-negative reference strains including *Escherichia coli* ATCC 23282 and *Pseudomonas putida* ATCC 10145 and antifungal activity against yeast-like fungi (*Candida albicans* IMRU-3669) and filamentous-like fungi (*Aspergillus niger* IMI-31276).

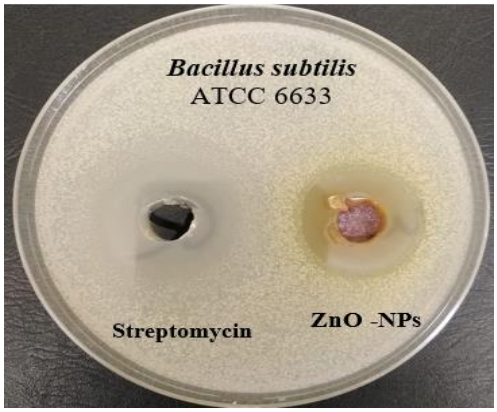


**Figure (3): Antimicrobial activities of biosynthesized ZnO-NPs (5.0 mg/mL) observed as inhibition clear zones against *Bacillus subtilis* ATCC 6633 compared to streptomycin as a reference antibiotic.**

The data in table (1) showed that the diameter of the clear zone produced by ZnO-NPs against Gram-negative reference strains is greater than that of Gram-positive and fungal reference strains when compared to streptomycin or fluconazole as a control. This indicates ZnO-NPs produced by B6 isolate exhibited more antimicrobial activity against Gram-negative bacteria than against Gram-positive bacteria, yeast-like or filamentous-like fungi.

**Table (1). Inhibition zone diameter (mm) produced by ZnO-NPs produced by B6 isolate.**

| Microorganisms | Inhibition zone diameter (mm) | |
|---|---|---|
| | ZnO-NPs | References of antimicrobial agent * |
| *Bacillus subtilis* ATCC 6633 | 25 | 28 |
| *Staphylococcus aureus* ATCC 35556 | 22 | 18 |
| *E. Coli* ATCC 23282 | 28 | 22 |
| *Pseudomonas putida* ATCC 10145 | 29 | 28 |
| *Candida albicans* IMRU-3669 | 20 | 0 |
| *Aspergillus niger* IMI-31276 | 22 | 20 |

**The data are presented as mean ± SD and represent the average values from three experiments (n = 3).**

**References*: Streptomycin: against Gram-positive and Gram-negative species; Fluconazole antifungal against fungal species**

### 3.5.Identification of the selected bacterial isolate and phylogenetic analysis

The results of the biochemical activities of the isolate (B6) in Table (2), indicate that it belongs to the genus Stenotrophomonas. By amplifying and sequencing the 16S rRNA gene, the sequence BLASTn of B6 isolate showed high similarity (100%) to *Stenotrophomonas pavanii* LMG 25348. Phylogenetic analysis of the 16S rRNA gene sequence of the B6 isolate showed that, it was closely related to *Stenotrophomonas pavanii* LMG 25348 (Figure 4). The bacterial isolate (B6) was definitely identified as *Stenotrophomonas pavanii* and took the symbol HM01. *Stenotrophomonas pavanii* HM01 is submitted in Genbank with accession number PP325747.1.

**Table (2): Results of biochemical tests of B6 isolate**

| **Test** | **Result** |
|---|---|
| Oxidase | +ve |
| Growth on MacConkey | Non-lactose fermenter |
| Motility | Motile |
| Indole | -ve |
| H2S production | -ve |
| Catalase | +ve |
| Citrate | -ve |
| Urease | -ve |
| Methyl red | -ve |
| Voges–Proskauer | -ve |
| Arginine dehydrogenase | -ve |
| Lysine decarboxylase | +ve |
| Ornithine decarboxylase | -ve |
| Esculin hydrolysis | +ve |
| DNase | +ve |
| Nitrate reduction | -ve |

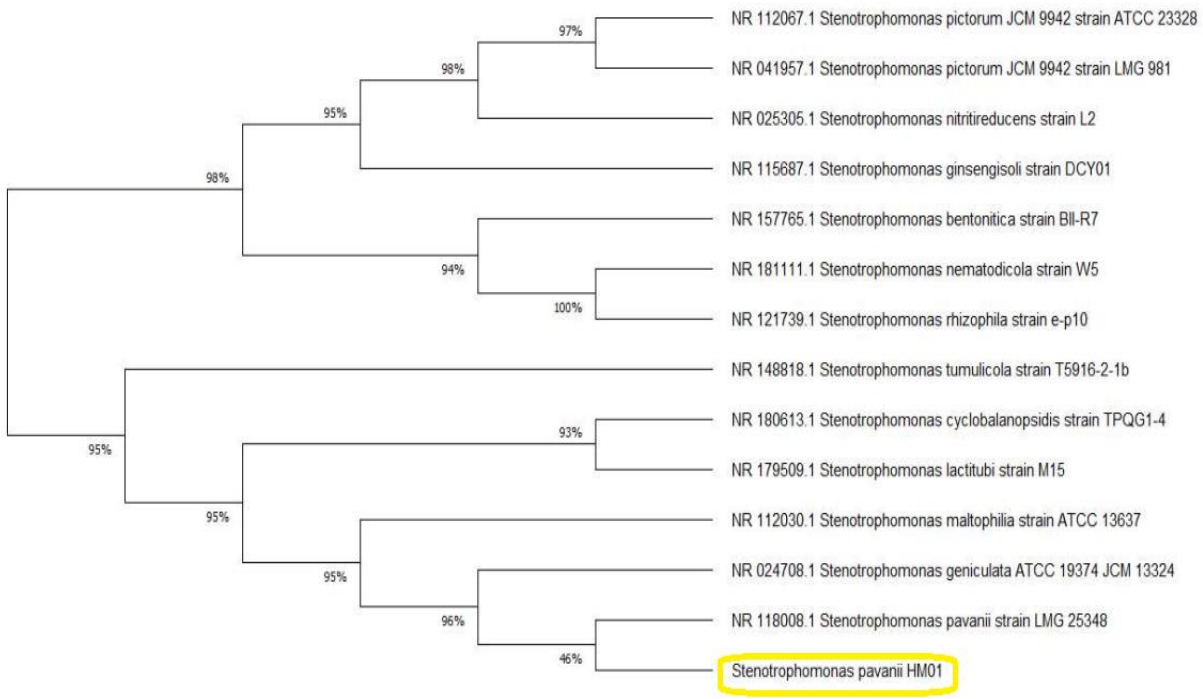


**Figure (4): A phylogenetic tree of *Stenotrophomonas pavanii* HM01 isolate evolutionary relationships to other strains, taken from the NCBI database and the phylogenetic tree done by the Mega X program**

## 4. DISCUSSION

Biologically active microorganisms such as bacteria, yeast and fungi, are used to synthesise ZnO NPs. Due to its effectiveness, eco-friendly operations, usability, accessibility, and high production, this technology seems promising. Microbes are crucial in the biological synthesis of metal and metal oxide NPs. The present work reports the low-cost, green synthesis of zinc oxide nanoparticles (ZnO-NPs) using a cell-free broth of *Stenotrophomonas pavanii* HM01 isolate.

In the current study, *Stenotrophomonas pavanii* HM01 isolate was used for biosynthesis of ZnO-NPs with potential antimicrobial activity. The genus Stenotrophomonas is widely used by many authors for the biosynthesis of nanoparticles with different biological actions. Zaki et al. reported that *Stenotrophomonas rhizophila* strain 39M isolated from lake water to reduce silver to silver nanoparticles in the range of 5-50 nm which could be used successfully as an environmentally friendly disinfector of water and wastewater against pathogenic microorganisms **(Zaki *et al*. 2014).** Mishra et al. synthesized silver nanoparticles by *Stenotrophomonas* spp. BHU-S7 (MTCC 5978) with an average mean size of ~12 nm for the management of soil-borne and foliar phytopathogens **(Mishra *et al.*, 2017).** Nayaka et al. used *Stenotrophomonas maltophilia* Strain NS-24 supernatant for the biosynthesis of silver nanoparticles (AgNPs) with an average of 18.06 nm in size. AgNPs have antimicrobial activity against different Gram-negative and Gram-positive bacterial strains like *Escherichia coli* (MTCC 40), *Enterococcus faecalis* (MTCC 6845), *Streptococcus pneumoniae* (MTCC 8874) and *Staphylococcus aureus* (MTCC 2825) **(Nayaka *et al.*, 2020).** Kashyap et al. (2023) used *Stenotrophomonas maltophilia* Strain SCS1.1 for the

biosynthesis of copper nanoparticles with particle size 80 nm which exhibited strong antibacterial, as well as antifungal activity **(Kashyap *et al.* 2023).** This could conclude that green synthesis of nanoparticles is gaining importance and has been suggested as possible alternative to chemical and physical methods.

In this study, ZnO-NPs produced by a cell-free broth of *Stenotrophomonas pavanii* HM01 isolate showed a variety of antimicrobial activity at a concentration 5.0 mg/ mL against Gram-positive reference strains including *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 35556, Gram-negative reference strains including *Escherichia coli* ATCC 23282 and *Pseudomonas putida* ATCC 10145 and antifungal activity against yeast-like fungi (*Candida albicans* IMRU-3669) and filamentous-like fungi (*Aspergillus niger* IMI-31276). The produced ZnO-NPs have potential activity against Gram-negative reference strains greater than Gram-positive and fungal reference strains. This agrees with **Chunchegowda *et al.* (2021)** who reported that the ZnO-NPs synthesized by Leaf Extract of *Passiflora subpeltata* offered significant inhibition against the isolated Gram-negative *E. coli* with MIC at 62.5 µg/ mL **(Chunchegowda *et al.*, 2021).** In addition, the biosynthesized ZnO-NPs by *Lactobacillus plantarum* TA4 exhibited antibacterial activity against Gram-negative (*E. coli* and *Salmonella* sp.) and Gram-positive (*S. aureus* and *S. epidermidis*) bacteria in a concentration-dependent manner (**Mohd Yusof *et al.*, 2020**). In contrast, ZnO-NPs synthesized by *Azadirachta indica* (Neem) leaf extract exhibited greater activity towards Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) than other Gram-negative (*Escherichia coli*) bacteria due to Gram-negative bacteria have a thick outer cell membrane layer that makes them resistant to ZnO NPs compared to Gram-positive bacteria. Moreover, it was also suggested that the presence of lipopolysaccharide on the cell wall of Gram-negative bacteria exerted a strong aversion towards the NPs, making them resistant to NPs **(Bhuyan *et al.*, 2015).** This could conclude that the biosynthesized ZnO-NPs had a broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria acts by different mechanisms such as inhibiting cell wall synthesis.

## 5. CONCLUSION

Eight different bacterial isolates (B1-B8) were gathered for this investigation from a variety of samples that were collected, including a soil sample, rice straw, and corn. We considered selecting the best bacterial strains for the synthesis of nanoparticles. The DLS results demonstrate that B6 produced ZnO-NPs with the smallest particle size (77.88 nm) using $ZnSO_4.7H_2O$ as a precursor. The selected strain (B6) was recognized as *Stenotrophomonas pavanii* strain LMG 25348 using the 16S rRNA gene. ZnO NPs produced extracellularly by *Stenotrophomonas pavanii* are used to create an antibiotic. The antimicrobial activities of the ZnO-NPs were likewise ineffective against various dangerous pathogens, with *E. coli* and *Pseudomonas putida* exhibiting the most potent antimicrobial action. The culture filtrate was used to get our conclusion. We concluded that using the culture filtrate of *Stenotrophomonas pavanii*, extracellular production of zinc oxide nanoparticles showed good antibacterial activity.

## REFERENCES

**Alsaiari, N. S., Alzahrani, F. M., Amari, A., Osman, H., Harharah, H. N., Elboughdiri, N., and Tahoon, M. A. (2023).** Plant and microbial approaches as green methods for the synthesis of nanomaterials: Synthesis, applications, and future perspectives. *Molecules*, *28*(1), 463.

**Bhuyan, T., Mishra, K., Khanuja, M., Prasad, R., Ajit Varma, A., (2015).** Biosynthesis of zinc oxide nanoparticles from *Azadirachta indica* for antibacterial and photocatalytic applications. *Materials Science in Semiconductor Processing*, Volume 32.

**Chunchegowda, U. A., Shivaram, A. B., Mahadevamurthy, M., Ramachndrappa, L. T., Lalitha, S. G., Krishnappa, H. K. N., and Ramachandrappa, N. S. (2021).** Biosynthesis of zinc oxide nanoparticles using leaf extract of Passiflora subpeltata: characterization and antibacterial activity against *Escherichia coli* isolated from poultry faeces. *Journal of Cluster Science*, *32*, 1663-1672.

**Deka, B., Baruah, C., Babu, A., and Kalita, P. (2022).** Biological and non-conventional synthesis of zinc oxide nanoparticles (ZnO-NPs): their potential applications. *Journal of Nanotechnology and Nanomaterials*, *3*(2), 79-89.

**Dhaka, A., Mali, S. C., Sharma, S., and Trivedi, R. (2023).** A review on biological synthesis of silver nanoparticles and their potential applications. *Results in Chemistry*, 101108.

**El-Sheshtawy, H. S., Hefni, H. H. H., Aboutaleb, W. A., Elaasser, M. M., Mady, M. F., and El-Shiekh, H. H. (2021).** Green Synthesis of Chitosan Bio-Nanocomposites and Investigation of their Antimicrobial and Antitumor Effects. *Iranian Journal of Science and Technology, Transactions A: Science*, *45*, 1247-1261

**Hefny, M. E., El-Zamek, F. I., El-Fattah, A., and Mahgoub, S. A. M. (2019).** Biosynthesis of zinc nanoparticles using culture filtrates of Aspergillus, Fusarium and Penicillium fungal species and their antibacterial properties against gram-positive and gram-negative bacteria. *Zagazig Journal of Agricultural Research*, *46*(6), 2009-2021.

**Hjiri, M., Aida, M. S., and Neri, G. (2019).** $NO_2$ selective sensor based on α-Fe2O3 nanoparticles synthesized via hydrothermal technique. *Sensors*, *19*(1), 167.

**Holt, J. G.; Krieg, N. R.; Sneath, P. H.; Staley, J. T. and Williams, S. T. (1994).** Bergey's manual of determinative bacteriology. 9th edition. *Baltimor: William and Wilkins Publishers*, Maryland, USA.

**Jayaseelan, C., Rahuman, A. A., Kirthi, A. V., Marimuthu, S., Santhoshkumar, T., Bagavan, A., and Rao, K. B. (2012).** Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *90*, 78-84.

**Kashyap, P., Shirkot, P., Das, R., Pandey, H., and Singh, D. (2023):** Biosynthesis and characterization of copper nanoparticles from Stenotrophomonas maltophilia and its effect on plant pathogens and pesticide degradation. *Journal of Agriculture and Food Research*, 100654.

**Kestens, V., Roebben, G., Herrmann, J., Jämting, Å., Coleman, V., Minelli, C., ... and Emons, H. (2016).** Challenges in the size analysis of a silica nanoparticle mixture as candidate certified reference material. *Journal of Nanoparticle Research*, *18*, 1-22.

**Khalil, H. A., El-Sheshtawy, H. S., Hosny, M., EL-Awady, A. T. and Hashem, A. I. (2022):** Biosynthesis of iron nanoparticles by petroleum degrading bacteria and evaluation of their potential for removal of petroleum contaminants. *J Environ Sci.*; *1(5)*: 33-70.

**Larik, I. A., Qazi, M. A., Phulpoto, A. H., Haleem, A., Ahmed, S., and Kanhar, N. A. (2019).** *Stenotrophomonas maltophilia* strain 5DMD: an efficient biosurfactant-producing bacterium for biodegradation of diesel oil and used engine oil. *International Journal of Environmental Science and Technology*, *16*, 259-268.

**Magiatis, P., Skaltsounis, A. L., Chinou, I., and Haroutounian, S. A. (2002).** Chemical composition and in-vitro antimicrobial activity of the essential oils of three Greek Achillea species. *Zeitschrift für Naturforschung C*, *57*(3-4), 287-290.

**Mclaren, A., Valdes-Solis, T., Li, G., and Tsang, S. C. (2009).** Shape and size effects of ZnO nanocrystals on photocatalytic activity. *Journal of the American Chemical Society*, *131*(35), 12540-12541.

**Mishra, S., Singh, B. R., Naqvi, A. H., and Singh, H. B. (2017**): Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. *Scientific reports*, 7(1), 45154.

**Mohd Yusof, H., Abdul Rahman, N. A., Mohamad, R., Zaidan, U. H., and Samsudin, A. A. (2020):** Biosynthesis of zinc oxide nanoparticles by cell-biomass and supernatant of Lactobacillus plantarum TA4 and its antibacterial and biocompatibility properties. *Scientific reports*, 10(1), 19996.

**Mokammel, M. A., Islam, M. J., Hasanuzzaman, M., and Hashmi, M. S. J. (2022).** Nanoscale materials for self-cleaning and antibacterial applications. *Encyclopedia of Smart Materials*, 315-324.

**Mutukwa, D., Taziwa, R., and Khotseng, L. E. (2022).** A review of the green synthesis of ZnO nanoparticles utilising Southern African indigenous medicinal plants. *Nanomaterials*, *12*(19), 3456.

**Nayaka, S., Chakraborty, B., Airodgi, D., Bhat, M., Nagaraja, S., Swamy, P., and Rego, M. (2020):** In-vitro antimicrobial activity of biological synthesized silver

nanoparticles using *Stenotrophomonas maltophilia* strain NS-24 from non-rhizosphere soil. *Int. J. Pharm. Pharm. Sci*, 12, 73-79.

**Procop, G. W.; Church, D. L.; Hall, G. S.; Janda, W. M.; Koneman, E. W.; Schreckenberger, P. C. and Woods, G. L. (2017):** Koneman's color atlas and textbook of diagnostic microbiology. 7th edition. *Wolters Kluwer Health*, Philadelphia.

**Saravanan, M., Gopinath, V., Chaurasia, M. K., Syed, A., Ameen, F., and Purushothaman, N. (2018)**. Green synthesis of anisotropic zinc oxide nanoparticles with antibacterial and cytofriendly properties. *Microbial pathogenesis*, *115*, 57-63.

**Suba, S., Vijayakumar, S., Vidhya, E., Punitha, V. N., and Nilavukkarasi, M. (2021).** Microbial mediated synthesis of ZnO nanoparticles derived from Lactobacillus spp: Characterizations, antimicrobial and biocompatibility efficiencies. *Sensors International*, *2*, 100104.

**Sunkar, S., and Nachiyar, C. V. (2012):** Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium Bacillus cereus isolated from Garcinia xanthochymus. *Asian Pacific Journal of Tropical Biomedicine*, *2*(12), 953-959.

**Velusamy, P., Kumar, G. V., Jeyanthi, V., Das, J., and Pachaiappan, R. (2016).** Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application. *Toxicological research*, *32*, 95-102.

**Zaki, S., Kamal, A., Elkady, M., Abu-Elreesh, G., and Abd-El-Haleem, D. (2014):** Biosynthesis of silver nanoparticles using Stenotrophomonas rhizophila and its application as a disinfectant agent of water. *Eur. J. Exp*, 4, 662-669.

**Zare, E., Pourseyedi, S., Khatami, M., and Darezereshki, E. (2017).** Simple biosynthesis of zinc oxide nanoparticles using nature's source, and it's in vitro bio-activity. *Journal of Molecular Structure*, *1146*, 96-103.

# إنتاج صديق للبيئة لجسيمات أكسيد الزنك النانوية بواسطة *Stenotrophomonas pavanii* لتطبيق مضادات الميكروبات

محمد حسني[١]، معاذ السعداوي[٢] , هدى الششتاوي[١]*

[١] قسم تصميم وتطوير العمليات، المعهد المصري لبحوث البترول، مدينة نصر، ١١٧٢٧ القاهرة، مصر

[٢] قسم الميكروبيولوجيا والمناعة، كلية الصيدلة، جامعة الازهر، القاهرة- مصر

*البريد الالكتروني للباحث الرئيسي: hudaelsheshtawy@yahoo.com

**الملخص العربي**

يكتسب التخليق الاخضر للجسيمات النانوية شعبية وقد تم اقتراحه بدلاً من استخدام العمليات الفيزيائية والكيميائية. في هذا العمل، تم استخدام السلالة البكتيرية *Stenotrophomonas pavanii* لإنتاج جسيمات أكسيد الزنك النانوية (ZnONPs) بتكلفة منخفضة وبطريقة صديقة للبيئة. وقد تم فحص الجسيمات النانوية المصنعة حيويًا باستخدام تشتت الضوء الديناميكي (DLS)، حيود الأشعة السينية (XRD) والمجهر الإلكتروني النافذ (TEM). كانت جسيمات أكسيد الزنك النانوية المُصنَّعة نظيفة، ومستديرة الشكل بشكل أساسي، وتختلف في الحجم من ٠.٩ إلى ١٧.١ نانومتر. تم استخدام جسيمات أكسيد الزنك النانوية المصنعة حيويًا في تطبيقات مضادة للميكروبات ضد سلالات البكتيريا إيجابية الجرام وسالبة الجرام والخميرة والفطريات. كان لدى ZnO NPs فعالية مضادة للبكتيريا أفضل ضد البكتيريا سالبة الجرام التي تم اختبارها مقارنة بما كانت عليه ضد السلالات البكتيرية والفطرية إيجابية الجرام. أعلى نشاط مضاد للميكروبات ضد الإشريكية القولونية (٢٨ ملم) والزائفة بوتيدا (٢٩ ملم). أظهر إنتاج جسيمات أكسيد الزنك النانوية من مستنبت S*tenotrophomonas pavanii* نشاطًا جيدًا مضادًا للبكتيريا.

كلمات مفتاحية: إنتاج صديق للبيئة؛ الجسيمات النانوية. ستينوتروفوموناس بافاني. مضادات الميكروبات