

Original Research Article

Green synthesis of zinc oxide nanoparticles using *Candida tropicalis*

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Sent for review: 23 September 2025

Revised accepted: 19 November 2025

Abstract

Purpose: To determine the properties of green-synthesized zinc oxide (ZnO) nanoparticles (NPs) prepared using *Candida tropicalis* and evaluate their bioactivity.

Methods: All the bacterial and yeast isolates obtained from soil samples were identified microscopically and genetically. *Candida tropicalis* was chosen as a yeast strain capable of zinc oxide (ZnO) nanoparticle formation. A biosynthesis method was developed utilizing *Candida tropicalis*, isolated and named H-406, for the production of spherical ZnO nanoparticles. The nanoparticles were characterized using scanning electron microscopy (SEM), zeta sizer, ultraviolet-visible (UV) spectroscopy, and Fourier transform infrared spectroscopy. Antimicrobial activities of ZnO NPs were also assessed using the agar well diffusion method.

Results: The soil samples yielded successful recoveries of eight bacterial isolates and two yeast isolates. These particles had a peak UV absorbance at 354 nm and exhibited a peak at 546.9 cm⁻¹, which indicates a bond in the composition and stability of the particles that was confirmed as ZnO nanoparticles. The nanoparticles tested demonstrated a positive antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Conclusions: These results demonstrate that a soil-derived strain of *Candida tropicalis*, which was isolated, has the potential for green ZnO nanoparticle synthesis.

Keywords: *Candida tropicalis*, Internal Transcribed Spacer (ITS) gene, Zinc oxide (ZnO) nanoparticles (NPs), Green biosynthesis, New *Bacillus* strains, 16S rDNA

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INTRODUCTION

The soil is a complex microhabitat that harbors a multitude of tiny organisms such as fungi, bacteria, archaea and algae. This variation occurs from the interactions of multiple environmental conditions, a wide variety of nutritional sources and complex interactions among different dietary components. The large numbers of soil microorganisms, as well as the

diversity of the metabolic pathways they are involved in, are very important for mediating most of the biogeochemical cycles, which leads to efficient cycling of nutrients, and also for healthy and vigorous plant growth [1]. The diversity of soil microorganisms has high potential for the synthesis of valuable products, including nanoparticles. The growing attention to nanotechnology is related to its tremendous

promise for applied science, especially for physics, chemistry and biology [2].

Nanoparticles (NPs), particles with diameters ranging from 1 to 1000 nm, have a high surface-to-volume ratio that enables the acquisition of certain physicochemical properties, including a high active surface area and abundant reactive sites, resulting in improved catalytic and physicochemical stability [3]. They are classified into organic and inorganic NPs, and inorganic NPs are obtained from different metal and metal oxide resources, such as zinc oxide (ZnO). Because of their safety and non-toxic properties, as well as effective antibacterial performance, many researchers have shown interest in the clinical applications of ZnO NPs. Their effect is mainly due to the generation of oxygen-free radicals at the surface. The antibacterial activity exerted by these NPs is not uniform, but instead varies according to nanoparticles' physicochemical features such as composition, crystallinity structure, surface, size and shape [4].

Early trials to synthesize NPs exploited expensive, complicated, physical and chemical approaches in the presence of poisonous and hazardous compounds. Much like chemical precipitation and physical grinding, traditional synthesis methods have high production yields, but they also have their disadvantages. Such techniques employ dangerous and harmful chemicals, which pose a threat to the environment and the surrounding individuals. Additionally, they require a lot of energy, and their unstable particles, unlike particles synthesized biologically, are prone to agglomeration. On the other hand, green synthesis methods mitigates the drawbacks through reduction and capping of agglomerated particles while utilizing green capping to increase the capping efficiency. Lowering the environmental burden through renewable and benign resource utilization through green synthesis is thus a more effective method [4]. Biological synthesis methods using microbes such as bacteria, fungi and yeast present fast, eco-friendly and low-cost alternatives. Yeast-based NPs biosynthesis, rather than bacteria, has the advantage of fast growth, simplicity and easily scalable culture media, high tolerance and uptake of metals and a metal-binding cell wall. Moreover, yeast culture supernatants are enriched with biomolecules, which are necessary for acting as reducing, stabilizing and capping agents [5].

Although green synthesis has been reported, there is still demand for the development of new, efficient and affordable biological agents,

especially from unexplored populations such as soil.

The use of soil-type microorganisms for biosynthesis of NPs has not been systematically explored in recent literature and this is the major focus of this study. The soil-dwelling yeast isolate, *Candida tropicalis*, belongs to the group of yeasts that are known for their ability to synthesize enzymes and biomolecules in their culture supernatant. Although it is mostly described as a pathogen, *C. tropicalis* has high resistance to strong metal-binding to heavy metals, which enables it to contribute to efficient nanoparticle production [6]. Though previous studies have shown that certain yeast strains, such as *Saccharomyces cerevisiae*, have been used to synthesize silver NPs [7], and *Rhodotorula* has been used to synthesize gold NPs [8]. The use of soil-derived *C. tropicalis* for synthesizing ZnO nanoparticles is still under investigation and its products function as reducing and capping agents for NPs. The main objectives of this study include the isolation and identification of a soil microorganism that biosynthesizes ZnO nanoparticles, development of a new green synthesis method with the selected isolate, characterization of the biosynthesized nanoparticles, and assessment of the antimicrobial activity of nanoparticles against different pathogens. This study represents a novel and sustainable strategy for synthesis of these high-value nanoparticles, in addition to expanding the role of this microorganism in the field of green nanotechnology.

METHODS

Sampling and initial preparation

Soil and compost were sampled at a uniform depth of 11 cm from gardens, riverbanks and oily areas in Basrah, Iraq. The samples were then transferred into plastic boxes and moved to the Advanced Biotechnology Laboratory in the Pharmacy College, Basrah University, Iraq. Once within the lab, the soil samples were air-dried and sifted before use.

Isolation of microorganisms

To isolate microorganisms, 1 g of hydrated sieved soil sample was suspended in 9 mL of distilled water and vigorously stirred for 5 minutes. Serial dilutions of this suspension were plated onto Sabouraud dextrose agar (SDA). To prevent fungal bloom, 1 mL of 0.1 mg/mL cycloheximide was delivered to SDA. For three days, the plates were incubated at 28 – 30 °C. The microbial colonies that appeared in the

plates were streaked onto new SDA plates and subcultured after incubation [9].

Microscopic analysis of isolates

The Gram-staining approach was used to evaluate the soil isolates microscopically. This procedure was used to identify their morphological characteristics, including Gram reaction, cellular shape and purity of the culture [9].

Genetic identification of the isolated microorganisms

Genomic DNA of cultured isolate was extracted using FavorPrep™ Microbial Genomic DNA Extraction Mini Kit (FAVROGEN Biotech Corp., Cat. No: FABGK100). The extracted DNA was then analyzed via electrophoresis on a 1 % agarose gel stained with 0.5 µL ethidium bromide, and run at a force of 60 volts for 90 min. Subsequently, 16S ribosomal DNA (rDNA) gene (for bacterial isolates) and Internal Transcribed Spacer (ITS) gene (for yeast isolates) were amplified using forward and reverse primer sets listed in Table 1 [10]. Each 25 µL polymerase chain reaction (PCR) mixture comprised of 12.5 µL of 2X GoTaq Master Mix (Promega, USA), 1 µL (10 pmol) of each forward and reverse primer, 2 µL of DNA template, and 8.8 µL of nuclease-free water. Amplification of the bacterial and yeast genes was conducted under the following thermal cycling conditions, shown in Table 2.

Gene amplicons were visualized after electrophoresis on a 2 % (w/v) gel of agarose. The gel was previously stained with 0.5 µL ethidium bromide during gel preparation. A 5 µL aliquot of each PCR product was loaded onto the gel and a 1 kb DNA ladder (BIONEER, Korea) was used as molecular weight reference. The expected fragment sizes were approximately

1500 base pairs for the 16S rRNA gene and 646 base pairs for the ITS region.

Sequencing and construction of phylogenetic tree

Following amplification of each gene, the amplicons were submitted to a DNA sequencing facility (MacroGen Company, Seoul, South Korea) for automated DNA sequencing. The obtained sequences were analyzed and taxonomically identified using the BLASTn (Basic Local Alignment Search Tool of nucleotides) algorithm available on the NCBI website [11]. Finally, a comparative analysis of all sequences was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 7.0.26 (7170509-X86_64). For each bacterial and yeast isolate, the sequenced 16S rDNA and ITS gene data were first aligned. The concatenated sequences, which ranged in length from 476 to 678 bp, were then used for phylogenetic tree construction using the same software.

Screening microorganisms for ZnO nanoparticle production

Initial screening for ZnO nanoparticle biosynthesis was performed on all isolated microorganisms, with the yeast isolate *C. tropicalis* strain H-406 being selected. The isolate was cultivated in conical flasks (250 mL) with nutrient broth medium and put in a shaking incubator (2.52 xg) overnight at 36 – 37 °C. After incubation, the suspension was centrifuged and the supernatant was used for ZnO nanoparticle biosynthesis using a modification of a previously reported method [12]. An equal volume (50 mL) of each of zinc sulfate pentahydrate (0.1 M) and sodium hydroxide (0.4 M) was added to *C. tropicalis* culture supernatant using a gentle stirring process as substrates.

Table 1: Primers for amplification of 16S rDNA and ITS genes

Primer	Orientation	Sequences
16S rDNA	27-Forward	5'-AGAGTTTGATCCTGGC-3'
	1492-Reverse	5'-GGTTACCTTGTTACGACTT-3'
ITS	Forward	5'-GGAAGKARAAGTCGTAACAAGG-3'
	Reverse	3'-GTTTCTTTTCCTCCGCTTA-5'

Table 2: PCR programs for amplification of the 16 rDNA and ITS genes

Step	16S rRNA gene program	Yeast ITS region program
Initial denaturation	92 °C for 2 minutes	94 °C for 3 minutes
Denaturation (per cycle)	94 °C for 30 seconds	94 °C for 30 seconds
Annealing (per cycle)	51.8 °C for 45 seconds	53 °C for 30 seconds
Extension (per cycle)	72 °C for 1.5 minutes	72 °C for 50 seconds
Final extension	72 °C for 5 minutes	72 °C for 7 minutes
Number of cycles	35	35

This was shaken vigorously and then incubated at 40 °C for 15 minutes. The reaction flask was subsequently heated in an oven at 100 °C for 5 – 7 min, followed by cooling in ambient air for 1 h until the nanoparticles formed sediments. The white sediment in the flask indicated successful synthesis of nanoparticles.

Characterization of ZnO nanoparticles

Ultraviolet-visible (UV-VIS) spectroscopy

The UV spectrum of ZnO NPs was analyzed using a double-beam UV/VIS 1800 spectrophotometer (Shimadzu, Japan) with a range of 200 - 400 nm.

Scanning Electron Microscopy (SEM) for ZnO NPs

To examine the shape and morphology of ZnO NPs, a NovaSEM 450 scanning electron microscope was used (SEM; FEI Co.).

Fourier-transform infrared (FTIR) spectroscopy for ZnO NPs

Fourier-transform infrared (FTIR) spectra were recorded using an FTIR ALPHA II BRUKER spectrophotometer over a wavelength range of 4000 - 400 cm⁻¹. The samples were prepared by mixing them with potassium bromide at a 1:100 ratio and compressing the mixture into a 2 mm translucent disk for two minutes.

Zeta sizer nano range

The size characteristics of ZnO NPs were elucidated using the Malvern ZETASIZER NANO apparatus [13].

Antimicrobial properties of ZnO NPs

The agar well diffusion method was adapted to determine the antimicrobial activity of the produced ZnO NPs against clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Clostridium albicans*. The clinical isolates were cultured until turbidity

equaled half McFarland standard. Each isolate was then evenly swabbed onto a nutrient agar plate. Using a sterilized cork borer, wells of 6 mm diameter were made and 75 - 100 µL of the biosynthesized ZnO nanoparticles (0.05 mg/mL) was added to each. After overnight incubation of plates at 36 - 37 °C, the inhibition zone diameters (mm) were measured [14].

RESULTS

Microbes isolated from soil

This study examined a total of ten isolates from various soil and compost samples in Basrah City. These included two yeast isolates (*C. tropicalis* isolate H-406 and *Rhodotorula diobovate* strain B2) and eight bacterial isolates, comprising both Gram-negative (-ve) and positive (+ve) strains, as shown in Table 3.

Identification of new global bacterial strains

After identification of all microbial isolates, two bacterial isolates were found to be new strains, which differed from the model strain in the number and position of some bases. Thus, one of the new bacterial strains, *Bacillus licheniformis* strain En.Ra.Sa.1.IRAQ, was published in GenBank as a new strain "PQ084580.1" that relates to *Bacillus licheniformis* FBL CN1 strain with 99.3 % identity but differs due to two transversion point mutations (C to G) at positions 177 and 194, as shown in Figure 1. The second new strain, *Bacillus cereus* strain En.Ra.Sa.3. IRAQ, also published in GenBank as a new strain "PQ323574.1", which is closely related to *Bacillus cereus* strain NPK1_1_45 in (99.2 %) identification percent and differs in transversion point mutation of A to C at position 92bp, transversion point mutation (G instead of C) in position 120bp, and transversion mutation of G to C at position 308bp. as shown in Figure 2. and the phylogenetic tree for all bacterial isolates, as well as the new strain (Figure 3), while the phylogenetic tree for isolated yeast is presented in Figure 4.

Table 3: Bacterial isolates collected from soil samples

No.	Gram stain	Isolates name
1.	+ve	<i>Bacillus cereus</i> strain En.Ra.Sa.3. IRAQ
2.	-ve	<i>Lelliottia amnigena</i> strain MSR-M49
3.	+ve	<i>Bacillus subtilis</i> strain S12
4.	+ve	<i>Bacillus spizizenii</i> strain NBRC 101239
5.	+ve	<i>Bacillus amyloliquefaciens</i> strain D15
6.	+ve	<i>Bacillus licheniformis</i> strain En.Ra.Sa.1.IRAQ

Query	1	TTTTAGCTAGCACTTACAGATGGACCGGCGCGCATTAGCTAGTTGGTGAGGTAACGGCT	60
Sbjct	168	 C C	227
		177 194	
Query	61	CACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGAC	120
Sbjct	228		287
Query	121	ACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTG	180
Sbjct	288		347
Query	181	ACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTTCGGATCGTAAAACTCTGTTGTTAGGGA	240
Sbjct	348		407
Query	241	AGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGC	300
Sbjct	408		467
Query	301	TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGG	360

Figure 1: Transversion point mutations in the new strain *Bacillus licheniformis* strain En.Ra.Sa.1.IRAQ

Query	1	GGCGGACGGGTGAGTACCACGTGGGTAACCTGCCCATAGACTGCGATAACTCCGGGAAA	60
Sbjct	76	 A G	135
		92 120	
Query	61	CCGGGGCTAATACCGGATAACATTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCG	120
Sbjct	136		195
Query	121	GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA	180
Sbjct	196		255
Query	181	AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGACACACGGC	240
Sbjct	256	 G	315
		308	
Query	241	CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA	300
Sbjct	316		375
Query	301	GCAACGCCGCGTGAGTGATGAAGGCTTTTCGGGTGCGTAAAACTCTGTTGTTAGGGAAGAAC	360
Sbjct	376		435

Figure 2: Transversion point mutation in the new strain *Bacillus cereus* strain En.Ra.Sa.1.IRAQ

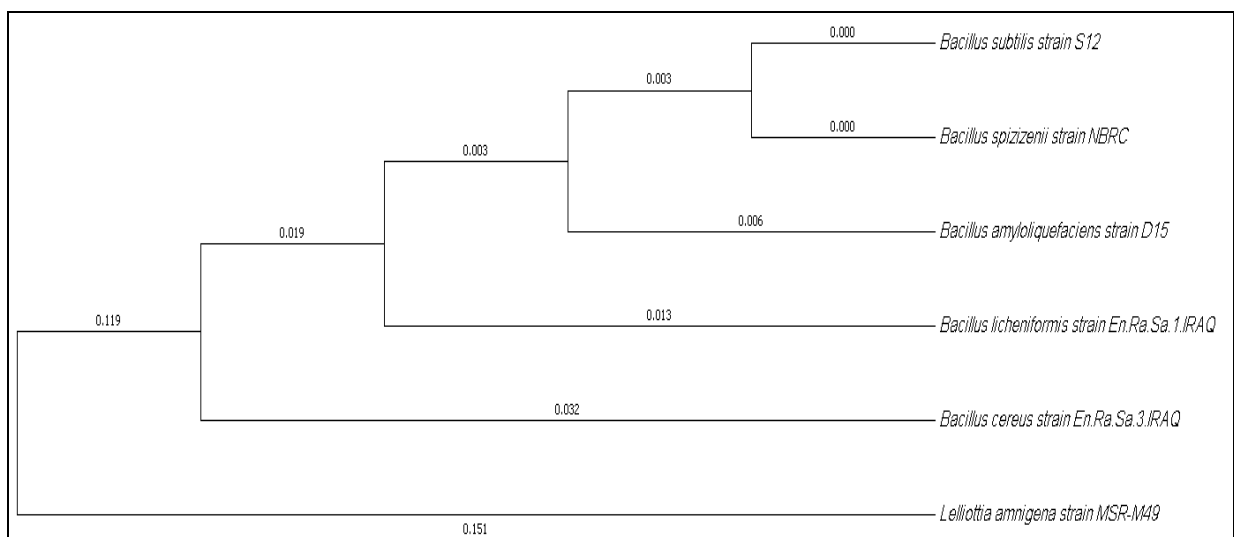


Figure 3: Phylogenetic tree of bacterial isolates from soil isolates

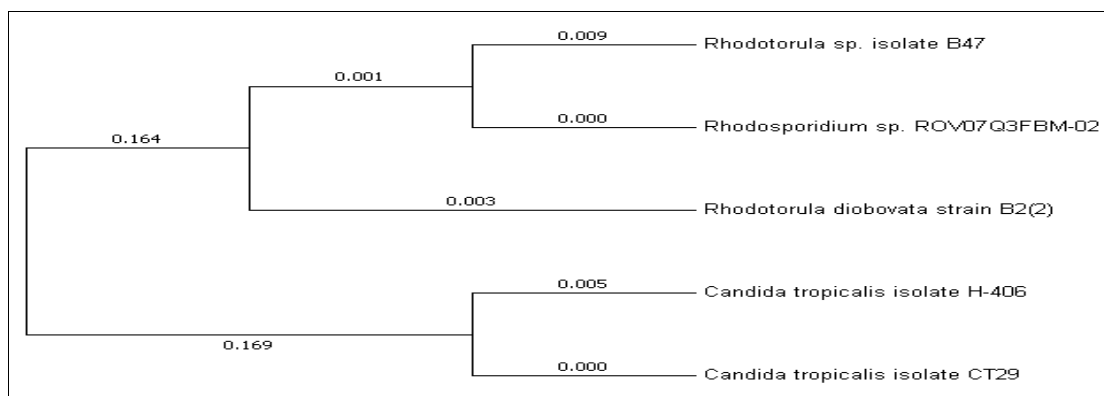


Figure 4: Phylogenetic tree of yeast isolates from soil samples

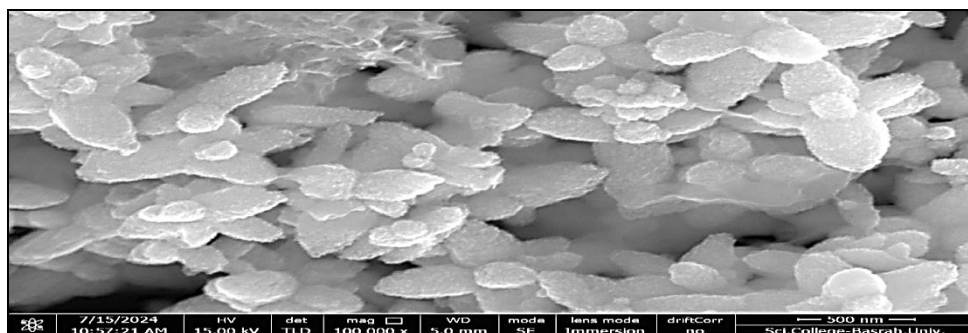


Figure 5: The SEM of ZnO nanoparticle

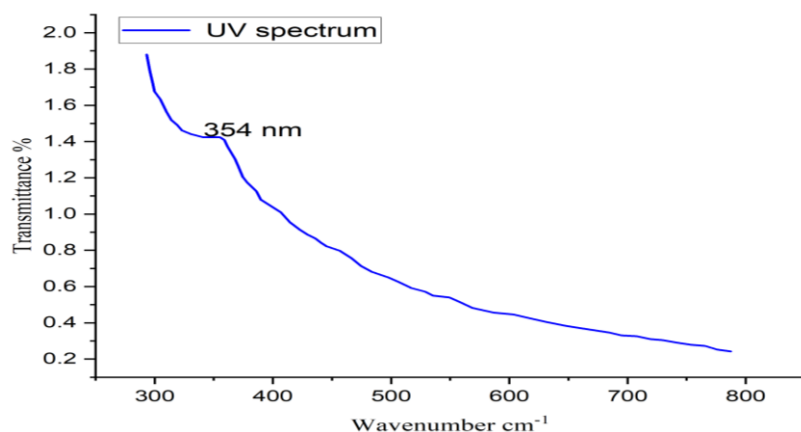


Figure 6: UV-visible absorption spectroscopy of ZnO NP

Synthesis of ZnO nanoparticles

All identified isolates were able to synthesize ZnO nanoparticles, although to varying degrees, with *C. tropicalis* isolate showing the strongest ability to produce ZnO nanoparticles. The white precipitate formation, a key indicator of successful ZnO nanoparticle production, marked the reaction between the *Candida* culture and the $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$ precursor.

Characterization of ZnO nanoparticles

The size and morphology of the NPs were characterized using SEM, capable of detecting

both monodisperse and polydisperse nanoparticles. At a magnification of 100,000X, ZnO nanoparticles were observed to be nearly spherical in shape. The nanoparticles also grew and accumulated to form flower-shaped bundles with asymmetrical rods of various diameters ranging from 66 to 125 nm (Figure 5).

In addition, UV-visible absorption spectroscopy of ZnO NP is shown in Figure 6. The UV-Vis spectroscopic study revealed a Plasmon resonance peak at 354 nm, confirming metal ions' reduction and the NPs' formation.

In addition, Zeta size analysis of ZnO nanoparticle, performed to determine the average particle size, revealed an NP size of 50.21 nm (Figure 7) and a polydispersity index (PDI) of 0.413.

Finally, FTIR spectral analysis of ZnO NPs confirmed the reduction of the metal ions by the reducing factors present in *C. tropicalis*. A detailed list of the detected peaks is provided in Table 4 and Figure 8.

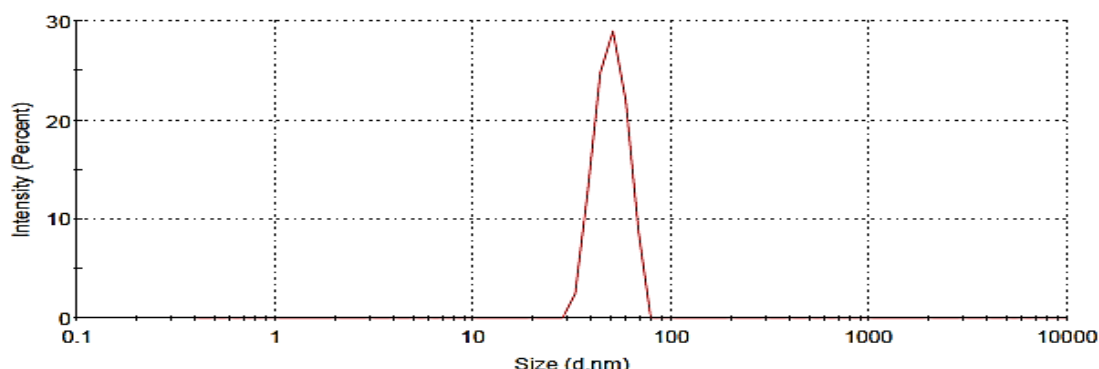


Figure 7: The size distribution intensity determined by Zeta potential analysis of ZnO NP

Table 4: Chemical groups seen in ZnO NPs

Wavenumber (cm ⁻¹)	Peak intensity	Functional group/Bond assignment
1600	Strong	Amide N–H bend (primary amines), ring C=C stretch (aromatics)
1482	Strong	Ring C=C stretch (aromatics)
1401	Not specified	Possibly C–H bending or carboxylate ion
1297 – 1234	Moderate	C–O stretching (esters, alcohols, ethers, phenol, carboxylic acids)
1040	Moderate	C–O stretching (various oxygen-containing groups)
903	Very strong	C–H bending
623	Strong	≡C–H bending (alkynes), possibly also out-of-plane bending
546	Sharp, strong	Zn–O bond (ZnO nanoparticle vibration stretch)
2333	Strong	C≡N or C≡C triple bond stretching

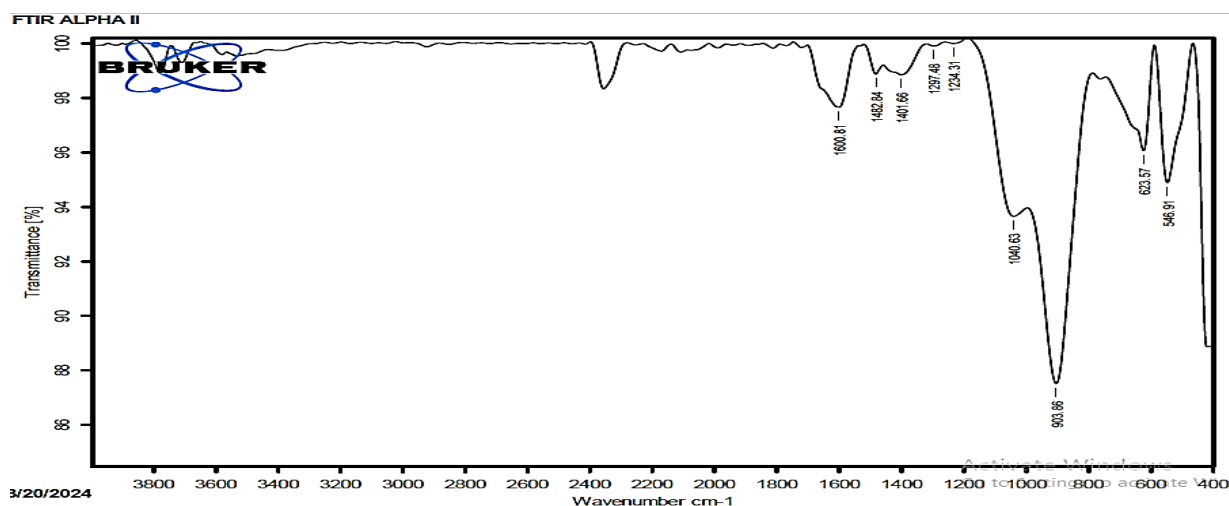


Figure 8: FT-IR spectral analysis of ZnO nanoparticles showing selected peaks of the FT-IR for ZnO nanoparticles

Antimicrobial activity of ZnO nanoparticles

The antimicrobial characteristics of ZnO NPs were assessed via a qualitative agar well diffusion test against 3 pathogenic isolates: *S. aureus*, *E. coli* and *C. albicans*. Clear zones of inhibition on the Mueller-Hinton agar plates

confirmed the nanoparticles' growth-inhibitory effects (Figure 9). Notably, ZnO nanoparticles were effective against *S. aureus*, *C. albicans*, and *E. coli*, with inhibition zone diameters of 18, 16, and 20 mm for the pathogenic isolates, respectively.

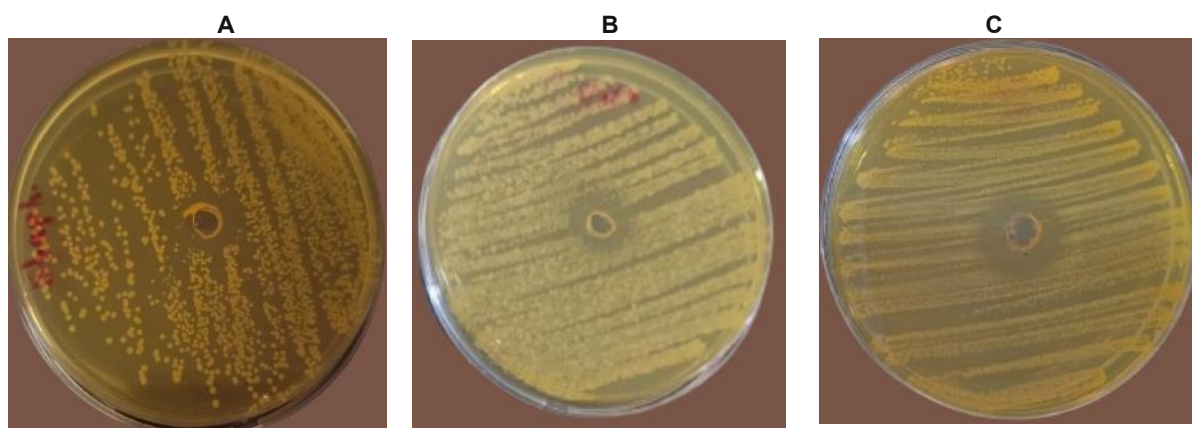


Figure 9: Antibacterial activity of ZnO NP against (A) *S. aureus*, (B) *C. albicans* and (C) *E. coli*

DISCUSSION

Numerous environmental microorganisms, particularly those in soil, have been used to synthesize metal nanoparticles. The efficiency of nanoparticle production varies among microbial species and is influenced by their unique enzyme systems. Soil microbial communities are vital for healthy and productive ecosystems. These communities comprise bacteria, fungi and other microorganisms. From soil samples collected across Basrah City, eight bacterial isolates were obtained. Genetic identification revealed that most of these isolates were various species of Gram-positive Bacilli, with only one Gram-negative isolate. The dominance of *Bacillus* species may be attributed to their extensive genetic and metabolic versatility, which enables them to fulfill a wide range of ecological roles within soil environments, including nutrient cycling and enhancing stress resilience in soil-dwelling organisms [15]. As the most abundant microorganisms, bacteria dominate soil communities; hence, millions are found in just one gram of soil, with the vast majority (more than 99 %) resisting cultivation in the laboratory, which explains the continuous appearance of new bacterial strains [16].

Soil is a typical habitat for yeasts. In the current study, two yeast isolates, *Candida tropicalis* and *Rhodotorula diobovata*, were identified. The number of yeast cells per gram of soil varies from a few to several thousand cells. Generally, yeasts are abundant in the top 10 cm of soil and are present in relatively low numbers compared to bacteria [17]. For these reasons, only two yeast isolates could be identified in the current study. *Candida tropicalis* is a milky white colony that grows rapidly and matures within 3 days. The majority of pathogenic fungi live in soil, water and organic debris [6] and this explains the

appearance of pathogenic isolate *C. tropicalis* in soil.

Candida tropicalis isolate had the strongest ability to produce ZnO NPs, which may be attributed to the cytoplasmic volume of yeasts compared to bacteria. This suggests that more complex metabolic systems and higher enzymatic activities can significantly support the production of ZnO NPs [17].

Candida isolates possess the ability to act as reducing, stabilizing and capping agents, thereby facilitating the biosynthesis of ZnO NPs [18]. Studies of nanoparticle morphology and size using SEM, allowing detection of monodisperse and polydisperse nanoparticles at 100000x magnification, showed ZnO NP as approximately spherical shapes. Also, the nanoparticles grew and accumulated to form flower-shaped bundles with asymmetrical rods and having various diameters ranging from 66 to 125 nm. The average particle size of the NP, as determined by a zeta sizer, was 50.21 nm and was found to possess a PDI of 0.413. A PDI less than 0.5 indicates a uniformity in the distribution of particle size across the sample. This homogeneity is an important observation since the size, as well as particle distribution, are critical in determining the properties of a nanoparticle, specifically its antimicrobial stability. Smaller and more uniform particles give a higher surface area-to-volume ratio and this is connected with an increased biological effect. This size-uniformity of nanoparticles is crucial for their biomedical use (e.g., drug delivery and antimicrobial) because it allows predictable functionality and dosages, which is a strong benefit compared to their chemically synthesized counterparts, which often display greater size polydispersity [19].

The UV-Vis absorption peak of the synthesized nanoparticles was observed at 354 nm, aligning

with earlier findings, which reported that ZnO nanoparticles exhibit strong absorption within the ultraviolet range (315 – 400 nm) [20]. The FTIR determination of ZnO nanoparticles showed a sharp, strong peak at 546.9 cm^{-1} due to the typical stretching vibration of Zn-O bond. The peaks of other functional groups (such as the peaks of different oxygen-containing compounds and amines) revealed that there were some bioorganic molecules from the *Candida tropicalis* culture supernatant that played roles as reducing, capping and stabilizing agents. This further verifies that the bio-moieties of the yeasts mediated the stable bio-fabrication of ZnO particles and inhibited the immediate agglomeration of particles, which is an added advantage of the green methodology [5].

High inhibitory activity of the nanoparticles against Gram-positive and negative isolates, as well as *Candida*, a fungal species, was recorded and agrees with previous reports [21]. Generally, the inhibitory activity of the nanoparticles against clinical isolates can be attributed to various forms of destruction of microbial cells. These mechanisms include adhesion to cell surfaces, which results in membrane damage and alterations in transport processes; penetration into cells, where they interfere with essential biological components such as DNA and ribosomes; increased production of reactive oxygen species; and ion transport between cells [22].

CONCLUSION

This study uses *Candida tropicalis* strain H-406, a new pathogenic yeast isolated from soil samples collected in Basrah City, for the biosynthesis of ZnO nanoparticles. These nanoparticles show significant inhibitory effects against clinical bacterial isolates and *Candida albicans*. This work introduces a process for making efficient antimicrobials and takes advantage of already known properties of biological synthesis without the need for potentially toxic chemicals. This has far-reaching implications in the generation of green syntheses using environmentally isolated microorganisms.

DECLARATIONS

Acknowledgement/Funding

The authors acknowledge the Molecular Biology Laboratory (College of Pharmacy) and the Nanolaboratory (Department of Physics, College of Science) at Basrah University, Iraq, for their support.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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