Research Article

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Actinomycetes-mediated Fabrication of ZnO–doped TiO_2 Nanoparticles: A Sustainable Approach for Antimicrobial and Photocatalytic Applications

S. Indumathi[1], R. Jagadeesan[2], S. Vijayakumar[3], A. Rathinavel[3], M. Arunthirumeni[1] and L. Ramkumar[1*]

[1]Department of Microbiology, K. S. Rangasamy College of Arts and Science (A), Tiruchengode, Namakkal, TN, India
[2]Department of Horticulture, ICAR- Krishi Vigyan Kendra, Virinjipuram, Vellore, TN, India
[3]Department of Chemistry, K. S. Rangasamy College of Arts and Science (A), Tiruchengode, Namakkal, TN, India
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*lakshmananram75@gmail.com

ABSTRACT

The present study focuses on the synthesis, characterization and determining multifunctional properties of ZnO-doped TiO_2 nanoparticles. UV–Vis spectroscopic analysis demonstrated a strong absorption peak confirming the role of ZnO doping in modulating the electronic configuration of TiO_2. The FTIR spectra exhibited characteristic vibrational features which imply the presence of capping agent in secondary metabolites of actinomycetes, while XRD analysis confirmed the coexistence of anatase TiO_2 and wurtzite ZnO phases. The SEM micrographs demonstrated predominantly spherical particles with slight agglomeration with an average size of 22 nm. Antimicrobial activity assays revealed remarkable zone of inhibition, with the most intense activity against *Staphylococcus aureus*, succeeded by *Bacillus subtilis* and *Escherichia coli*. Further, the photocatalytic efficiency of the nanoparticles was validated through methylene blue degradation studies. The ZnO-TiO_2 nanoparticles exhibited substantial dye removal, attributable to enhanced electron-hole separation and extended visible light absorption. Collectively, the findings suggest that ZnO-doped TiO_2 nanoparticles combine desirable optical, structural, antimicrobial, and photocatalytic properties, making them promising candidates for biomedical and environmental remediation applications.

Keywords: Actinomycetes; ZnO-doped TiO_2 nanoparticles; Antimicrobial activity; Photocatalytic activity; Methylene blue.

1. INTRODUCTION

Nanomaterials have drawn significant interest towards the health sector due to their unique physicochemical properties and various applications. Among these, zinc oxide (ZnO) and titanium oxide (TiO_2) have specifically emerged as high-potential candidates owing to their relatively lower toxicity compared to other metal oxide nanoparticles (Karthikeyan *et al.* 2022). Chemically fabricated nanoparticles have usually faced constraints for biomedical use due to the inherent toxicity of the chemical precursors and their by-products. In this context, eco-friendly or green synthesis has gained momentum as a viable option, especially through plant and microbial-mediated pathways. Among the microbial sources, actinomycetes in recent times have become notable for the biosynthesis of metallic nanoparticles due to their biocompatibility and lowered toxicity, hence posing enormous potential for varied biomedical applications (Yaradoddi *et al.* 2022).

Actinomycetes are Gram positive eubacteria which are different from other eubacteria in terms of elevated level of G+ C content in their genetic material. The most significant role in industrial use is played by the genus *Streptomyces* through the production of therapeutic compounds (Venkateswaran *et al.* 2022). Active biocompounds, organic acids, enzymes released by the actinomycetes are efficient reducing agents which indeed enable the conversion of the bulk metals into nano-form and quantum dots. This bioreduction process occurs both extracellularly and intracellularly, where metal ions are translocated into the cell and are subsequently reduced within the respective compartments (Kumari *et al.* 2020; Manimaran and Kannabiran, 2017).

The actinomycetes-based synthesis route exerts tremendous control over particle size and morphology and further it may provide operative bio-coatings impelled from microbial metabolites affording an augmented stability and biological efficacy. Actinomycetes are attractive bio-factories for the synthesis of nanoparticles, which have immense applications in drug delivery, agriculture, and environmental clean-up (Gupta *et al.* 2019). Rapid emergence of multi-drug resistance bacterial strains is a

critical global public health issue that warrants immediate attention and exploration for alternative therapies, such as those used in metallic nanoparticles. (Mba and Nweze, 2021). ZnO nanoparticles were found to possess significant antioxidant activity, where about 50% inhibition was achieved and protection against DNA damage was provided. The protective effect on DNA was also found to be directly proportional to the increase in concentration. Owing to their biocompatibility and biosafety, biosynthesized ZnO nanoparticles offer a promising platform for antibacterial use and exploration of novel therapeutics (Al-darwesh *et al.* 2024).

ZnO nanoparticles are also found to have applications in the treatment of cancer and diabetes. They are also used in drug delivery. In addition, they are used in the formulations of biofertilizers (Dangana *et al.* 2023). TiO_2 is also a well-established promising multifunctional material with great physical stability and non-toxicity that makes it a revolutionary cost-effective material for photocatalytic utilization (Ansari *et al.* 2022). Due to photochemical behavior of TiO_2, it is widely attractive in cosmetic, pharmaceuticals and diagnostic (Sagadevan *et al.* 2022). Methylene blue (MB) is a clinically important photosensitizer used in photodynamic therapy for cancer treatment as well as in antimicrobial applications.

Methylene blue degradation studies not only find environmental relevance but also yield important information on the biomedical potential of metallic nanophotocatalysts (Lim *et al.* 2021).

Within this context, an attempt to synthesize ZnO-doped TiO_2 nanoparticles mediated by *Streptomyces maritimus* IA17 was carried out. Physical and chemical nature of the synthesized doped material was analyzed using UV-vis Spectrophotometer, Fourier Transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM) with EDAX. ZnO-doped TiO_2 nanoparticles were explored to study the antimicrobial properties and to investigate the photocatalytic activity in the degradation of MB dye.

2. METHODOLOGY

2.1 Microbial Synthesis of ZnO-Doped TiO_2 Nanoparticles

The well–studied actinomycetes isolate *Streptomyces maritimus* IA17 was inoculated in International Streptomyces Project Medium 2 and maintained at room temperature for 12 days. After incubation period, the broth culture was subjected to centrifugation to extract the supernatant from the biomass. Centrifugation was carried out at 5000 rpm for 15 minutes. The supernatant was taken in a sterile flask and stored at room temperature for further analysis. A slightly modified protocol of Subhapriya and Gomathipriya (2018) was adopted for the synthesis of TiO_2 nanoparticles, where 0.5 M of titanium oxysulfate was taken and dissolved in 25 mL distilled water.

2.2 Doping with Zinc Acetate Dihydrate

Doping is a method that modifies the physical and chemical nature of nanoparticles. Doping of nanoparticles also enhances the biocompatibility of the metallic nanoparticles for increased invasion into cells. For the biosynthesis of ZnO-doped TiO_2 nanoparticles, 0.5 M zinc acetate dihydrate was used as a precursor and added to 0.5 M of titanium oxysulfate solution under stirring. The resulting mixture was then combined with 50 mL of supernatant from an actinomycetes culture. The culture contained bioactive compounds that act as capping agents during doping and nanoparticle formation. Once the precipitate was formed, the solution was subjected to centrifugation at 3000 rpm for 15 minutes to get the pellet. The pellet was dried at 400°C in muffle furnace for 8 hours. ZnO-doped TiO_2 nanoparticles were obtained in powder form and stored in a sterile container (Rajivgandhi *et al.* 2022).

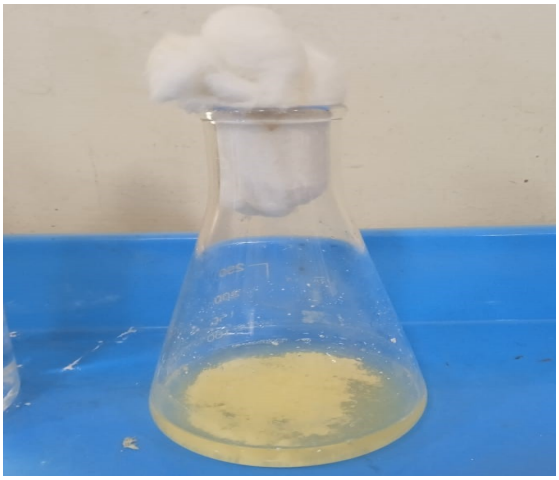


Fig. 1: Formation of white precipitate after adding culture extract with ZnO and TiO_2 solution

2.3 Physico-chemical Analysis of ZnO-Doped TiO_2 Nanoparticle

Optical characteristics of the ZnO-doped TiO_2 nanoparticles were determined using UV-Vis spectroscopy based on absorption maxima. The vibrational characteristics exhibited by doped nanoparticles were analyzed by FTIR. Different phases of the synthesized ZnO-doped TiO_2 nanoparticles were determined by X-Ray diffraction studies. The morphology and size of nanoparticles was determined using SEM. The purity of the doped nanoparticle without any significant impurity was confirmed by EDAX.

2.4 Antimicrobial Activity

The antimicrobial potential of ZnO-doped TiO_2 nanoparticles was evaluated against selected bacterial pathogens by the agar well diffusion method. Fresh cultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were prepared and adjusted to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL). Nutrient agar plates were swabbed uniformly with bacterial inoculum. Different concentrations of the nanoparticle suspension (25, 50 and 100 µg/mL) were loaded into the wells, while DMSO served as negative control and standard antibiotic streptomycin served as positive control. The plates were incubated at 37°C for 24 hours, and the antibacterial efficacy was determined by measuring the inhibition zone diameter around each well.

2.5 Photocatalytic Potential

The degradation of MB dye under light source was assessed to evaluate the photcatalytic efficiency of ZnO-doped TiO_2 nanoparticles. A standard solution of MB (10 mg/L) was prepared, and a 100 mL portion of dye solution was treated with a fixed concentration of nanoparticles (1 g/100 mL). Before irradiation, the suspension was stirred magnetically in the dark for 30 min to achieve adsorption–desorption equilibrium between the dye molecules and catalyst surface. Photocatalytic reactions were conducted under UV/visible light irradiation and aliquots of the reaction mixture were withdrawn at regular time intervals. The samples were centrifuged to remove nanoparticles, and the residual dye concentration was subjected to UV-visible spectroscopy study to measure the change in the absorption maxima at different time intervals (Loh *et al.* 2018).

3. RESULTS AND DISCUSSION

The biosynthesis of ZnO-doped TiO_2 was achieved using *Streptomyces maritimus* IA17 extracellularly. The synthesis was visually evidenced by the formation of white precipitate from milky white solution (Fig. 1).

3.1 UV-visible Spectroscopy

The optical properties of the ZnO-doped TiO_2 nanocomposites were characterized by UV–Vis spectroscopy. The absorption spectrum had a well-defined maximum at 341 nm, which indicates an electronic transition in the material (Fig. 2). From Planck's relation (E=hc/λ), the photon energy of the wavelength was computed. Putting h=6.626 × 10^{-34} J=3.0 × 108 ms^{-1}, and λ=341 × 10^{-9} m, the computed energy was 3.64 eV. This value is the calculated optical band gap derived directly from the absorption maximum (Gunasekaran *et al.* 2023).

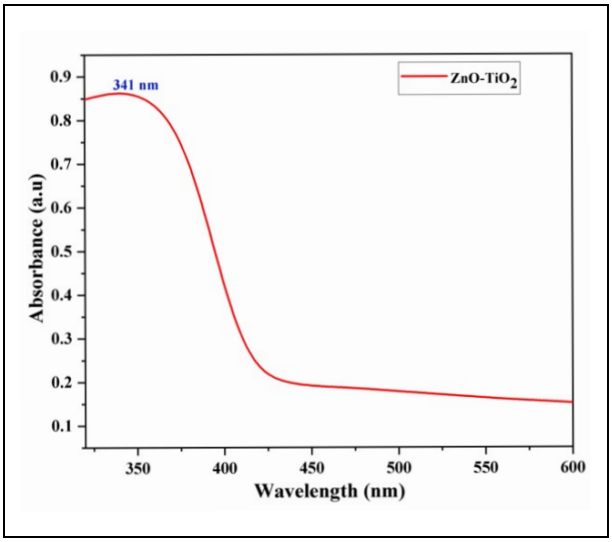


Fig. 2: UV-vis spectrum of ZnO-doped TiO_2 nanoparticle

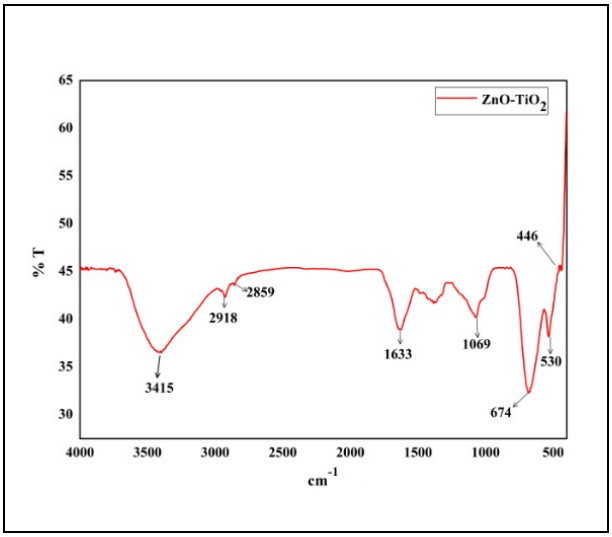


Fig. 3: FT-IR spectrum of ZnO-doped TiO_2 nanoparticles

3.2 Fourier Transmission Infrared Spectroscopy

Fourier Transmission Infrared Spectroscopy analysis of the ZnO-doped TiO_2 nanoparticles showed some key vibrational characteristics (Fig. 3). The band at 530 cm^{-1} represents metal–oxygen lattice vibrations, which confirm the presence of Zn–O and Ti–O bonds in the composite material. A peak at 674 cm^{-1} also confirms the occurrence of mixed-metal lattice modes. The 1069 cm^{-1} band is assigned to the Ti–O–Ti stretching, characteristic of the anatase TiO_2 structure (Siwinska *et al.* 2018). Water molecules and hydroxyl groups adsorbed over the surface are evidenced by the 1633 cm^{-1} and 3415 cm^{-1} bands (bending and O–H stretching), respectively. These are recognized to promote photocatalytic and antibacterial activities (Morsi and Elsalamony, 2016). The peaks at 2918 cm^{-1} and 2859 cm^{-1} are attributed to symmetric and asymmetric C–H stretching, possibly due to leftover organic moieties

during synthesis, which can serve as stabilizers on the surface (Nguyen *et al.* 2017)

3.3 XRD Analysis

The XRD pattern of the doped ZnO-TiO_2 nanoparticles revealed distinctive peaks at 2θ values of 26.28°, 34.90°, 38.50°, 43.30°, 53.30°, 55.81°, 62.10°, 63.10°, 6 8.30°, and 69.60 ° (Fig. 4). In comparison with the International Centre for Diffraction Data (ICDD–PDF, previously JCPDS), the 26.28° and 38.50° diffraction peaks belong to the (101) and (112) planes of the anatase phase of TiO_2 (JCPDS No. 21-1272) and the peak at 43.30° was attributed to the rutile phase of TiO_2 (210) (JCPDS No. 21-1276). Furthermore, the prominent peaks at 34.90°, 53.30°, 55.81°, 62.10°, 63.10°, 68.30°, and 69.60° were indexed to the (002), (102), (110), (103), (200), (112), and (201) planes of wurtzite ZnO structure (JCPDS No. 36-1451), thus proving successful incorporation of ZnO in the TiO_2 lattice. The lack of impurity peaks also proved that no secondary phases like $ZnTiO_2$ or other mixed oxides were developed during synthesis. The presence of anatase TiO_2 and ZnO diffraction peaks together indicate the formation of a TiO_2–ZnO heterojunction, which has been found to increase the separation of charges and hence the efficiency of photocatalysis and antimicrobial activity (Karthikeyan *et al.* 2022; Wang *et al.* 2023).

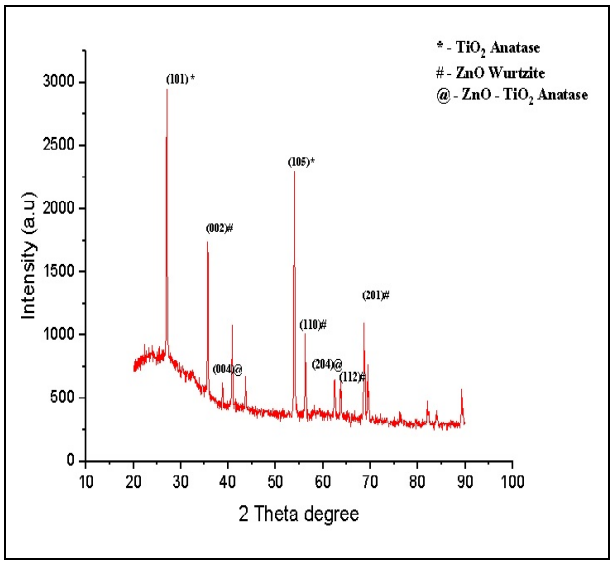


Fig. 4: XRD profile of ZnO-doped TiO_2 nanoparticles

3.4 Structural Analysis by SEM

The SEM image analysis revealed that the ZnO-doped TiO_2 nanoparticles exhibited a mean particle size of about 22 nm, which is consistent with nanoscale materials (Fig. 5). The relatively broad size distribution is attributed to the presence of agglomerates, a common feature of metal oxide nanoparticles due to high surface energy and inter-particle interactions (Gupta *et al.* 2019). The primary nanoparticles remain in the nanometer range, confirming the results obtained from XRD crystallite size calculations. Fig. 6 shows the purity of the compound determined by EDAX with the composition of 70.5% Ti, 3.7% Zn and 25.8% O.

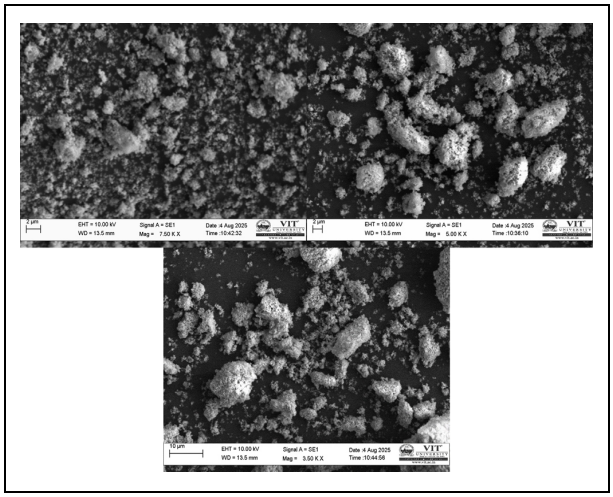


Fig. 5: SEM of ZnO-doped TiO_2

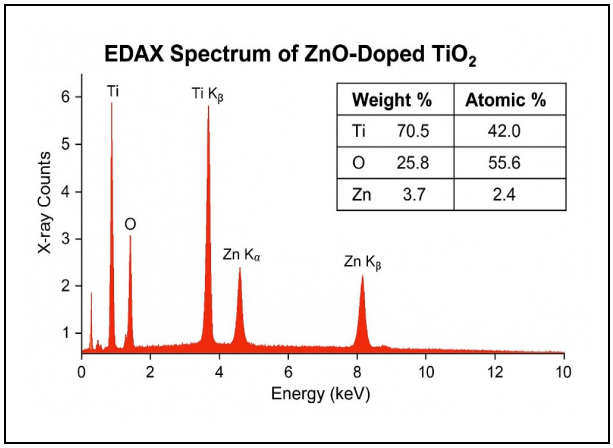


Fig. 6: ZnO-doped TiO_2 purity analysis with EDAX

3.5 Antimicrobial Activity

The antimicrobial efficacy of ZnO-doped TiO_2 nanoparticles was tested against a selection of bacterial pathogens. Streptomycin (positive control) showed anticipated inhibition, while DMSO (negative control) displayed no activity, confirming the validity of the assay (Fig. 7). Of the pathogens tested, maximum zone of inhibition was against *S. aureus* (22 mm), followed by *B. subtilis* (21 mm), *E. coli* (19 mm), *K. pneumoniae* (18 mm), and *P. aeruginosa* (16 mm). These results indicate that ZnO-doped TiO_2 has superior antibacterial activity, especially on Gram-positive bacteria, consistent with explanations offered in recent literature. ZnO doping specifically enhanced the surface area and ROS production which act as key factors in disruption of cell membrane and elevate the antimicrobial efficacy

(Pinzari, 2024). Similarly, Gunasekaran *et al.* (2023) reported strong antibacterial effectiveness of ZnO-TiO_2 nanocomposites, which supports our findings.

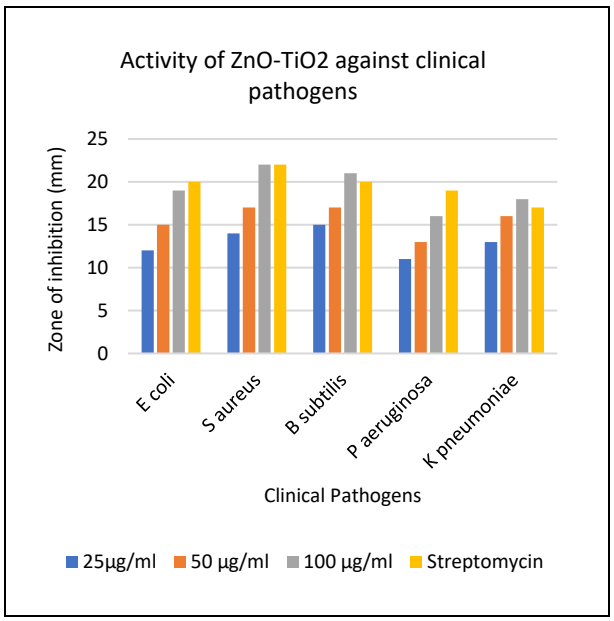


Fig. 7: Inhibitory activity of ZnO-doped TiO_2 against bacterial pathogens

3.6 Photocatalytic Potential

Methylene blue degradation experiment was performed under UV-visible irradiation to determine the photocatalytic performance of ZnO-doped TiO_2 nanoparticles. A progressive decline in the usual absorption band of MB at 664 nm with increasing irradiation time was observed, suggesting the gradual degradation of the dye (Fig. 8). ZnO-TiO_2 nanocomposite had greater degradation efficiency, which directly corresponds to its smaller band gap energy (3.64 eV) and the improved separation of the photogenerated electron–hole pair. Incorporation of ZnO not only shifted the light absorption to the near visible region but also inhibited the recombination of charges, both of which accelerated the speed of the degradation process. This enhanced performance is due to the structure and morphological characteristics established by FTIR, XRD, and SEM analyses, where the crystalline anatase phase and nanosized particles allowed photocatalysis. These findings are consistent with earlier observations which demonstrate that metal ion doping of TiO_2 structures effectively alters the band structure and enhances the surface reactivity, thereby improving dye degradation efficiency (Khan *et al.* 2022; Chamaraja *et al.* 2024). Thus, these findings suggest that ZnO–doped TiO_2 nanocomposites are very promising as low-cost material for wastewater treatment and other remediation strategies.

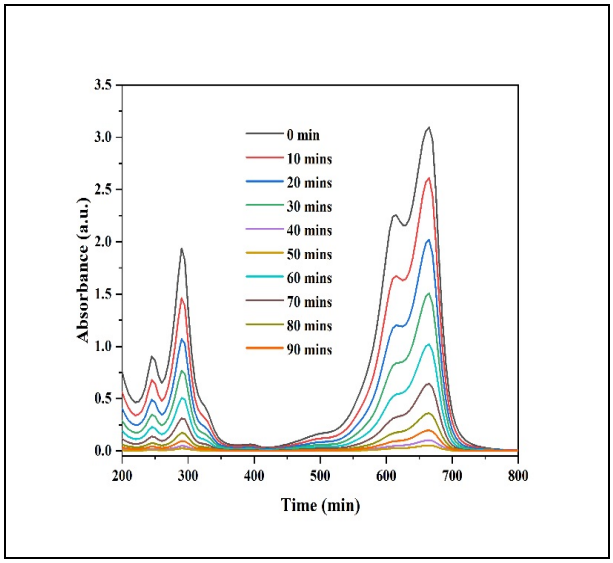


Fig. 8: Absorption spectra of MB at different time intervals after UV light irradiation in the presence of ZnO-doped TiO_2 nanoparticles

4. CONCLUSION

The current research achieved the biogenic synthesis of ZnO-doped TiO_2 nanoparticles *via Streptomyces maritimus* IA17 in a green approach. Structural and optical characterization revealed the co-occurrence of wurtzite ZnO and anatase TiO_2 phases and nanoscale crystallinity with narrowed band gap of 3.64 eV. The nanoparticles had significant activity against bacterial pathogens, with maximum inhibition against *Staphylococcus aureus* followed by *Bacillus subtilis* and *Escherichia coli*, indicating their therapeutic alternative against drug-resistant microorganisms. In addition, the ZnO-TiO_2 nanocomposites showed notable photocatalytic degradation of methylene blue under UV–visible light, due to increased band gap and broader light absorption range. In general, the actinomycetes–mediated incorporation of ZnO into TiO_2 not only enhanced the antimicrobial and photocatalytic activity but also supported biocompatible and multifunctional formation of nanomaterials. Future research can be aimed at understanding the mechanisms of antibacterial and photocatalytic activities of the nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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