

MICROBIAL-ASSISTED GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES AND THEIR POTENTIAL APPLICATION

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Abstract. Nanomaterials are the rapidly growing field of nanotechnology. The development of reliable strategies for the synthesis of nanomaterials with different chemical compositions, sizes, and high monodispersity is one of the most challenging issues these days. Recent studies on the use of microorganisms in the synthesis of nanoparticles (NPs) are a relatively new and exciting area of research with considerable potential for development. This mini-review highlights the use of different microorganisms in the biosynthesis of zinc oxide nanoparticles (ZnO NPs) and their biological applications.

Keywords: ZnO NPs, microorganisms, biological applications.

Introduction

One of the fastest-growing fields of study is nanotechnology, which has a wide range of applications in the biomedical sciences, chemical catalysis, drug delivery enabled by nanotechnology, drug formulations using nanotechnology, contrast agents in biomedical imaging, biosensors for the detection of various disease biomarkers, antimicrobial agents, anticancer agents etc. NPs possess size range of 1 to 100 nm. Because of their large surface area and nanoscale size, NPs have special physical and chemical characteristics [1]. NPs can be synthesized using various physical, chemical methods, and biological methods [2]. The biological methods are safe, non-toxic, economical, environmentally friendly, and clean [3]. They generally comprise two main systems: i) microbial [4] and ii) plant systems [5]. However, the microbial synthesis of the NPs has its advantages over plant-mediated synthesis including rapid and simple manipulation and also reproduction of microbes [6] as well as the cultivation without seasonal and geographical area restrictions [7] compared to plant counterparts. Several microbes including fungi like *Fusarium* sp., *Aspergillus fumigatus*, *Aspergillus aeneus*, *Aspergillus terreus*, *Aspergillus niger*, [8] yeasts; *Pichia fermentas*, *Candida albicans*, *Pichia kudriavzevii* [9] bacteria like *Aeromonas hydrophila* [10] *Bacillus* sp., [11] *Lactobacillus* sp. [12], *Pseudomonas aeruginosa* [13], *Staphylococcus aureus* [14], *Streptomyces* sp. [15], have been exploited to synthesize ZnO NPs. Out of various types of microbes, bacteria are preferred due to their ease of handling, rapid growth, and genetic manipulative attributes compared to other eukaryotic microorganisms [16]. ZnO NPs are the most widely utilized type of metal oxide-based nanoparticles due to their versatility in a range of applications, including cosmetics, biosensors, antibacterial agents, and biomedical applications [17]. At ambient temperature, the ZnO NPs exhibit a high excitation binding energy of 60 meV and a direct wide bandgap of roughly 3.3 eV, which falls in the near UV wavelength range [18]. Apart from various biological applications, ZnO NPs can also be used for environmental remediation such as wastewater treatment, photocatalytic degradation of dyes etc. [19].

2. Methodology used for microbial-assisted synthesis of NPs

Compared to chemical and physical approaches, the production of NPs from biological sources, such as bacteria, actinobacteria, yeast, fungi, algae, and viruses, is safer and environment-friendly. Bacteria are the preferred microorganism to use in the production of NPs because they can be cultivated in artificial environments at an ambient growth rate [20]. There are mainly two pathways i.e. intracellular or extracellular for the microbial-assisted synthesis of NPs.

To fabricate NPs extracellularly, microorganisms are cultured in an appropriate medium. The microbial cell-containing broth is centrifuged, and the microbial enzyme-containing supernatant is utilized for producing NPs. In a different vessel, the reductase enzyme-containing supernatant is allowed to react with the metal ions. NPs are produced when metal ions undergo bioreduction in a supernatant free of cells. The characterisation of newly produced NPs in terms of their morphology, homogeneity etc. are then evaluated using FT-IR, XRD, SEM, and TEM spectroscopic techniques. The production of NPs occurs intracellularly through the cellular mechanism of microbial organisms. To produce the biomass pellet, the microbial biomass is cleaned with sterile distilled water and centrifuged. The microbial cultures are kept in the proper liquid media. A metal aqueous solution is added and the microbial biomass reacts and grows under the appropriate incubation conditions. NP formation is indicated by specific colour changes [21].

3. Some Biological applications of microbial-assisted synthesised zinc oxide nanoparticles:

ZnONPs have been widely studied and green synthesized due to their vast applications. ZnO NPs have been synthesized recently using the supernatant and cell biomass of the zinc-tolerant bacteria *Lactobacillus plantarum* TA4. According to TEM analysis, bacterial cell biomass was used to biosynthesize irregularly shaped ZnO NPs (size 191.8 nm) and flower-like ZnO NPs (size 291.1 nm) using a cell-free supernatant. When biosynthesized ZnO NPs were tested against Gram-positive (*S. epidermidis* and *S. aureus*) and Gram-negative (*Salmonella sp.* and *E. coli*) bacteria, it was shown that the former were more susceptible to the antibacterial action of ZnO NPs [22]. Barani et al. used two novel aquatic bacteria, *Marinobacter sp.* 2C8 and *Vibrio sp.* VLA, to investigate a microbial cell-free extract for the synthesis of ZnO NPs. The hydroxyl, amine, and carboxyl groups of the bacterial proteins appeared to be primarily in charge of stabilizing the biosynthesized ZnO NPs, according to an FTIR study. The XRD pattern verified that ZnO NPs had formed a hexagonal wurtzite structure. The average particle size for ZnO-2C8 NPs and ZnO-VLA NPs was approximately 10.23 ± 2.48 nm and 20.26 ± 4.44 nm, respectively, indicating a spherical shape of the nanoparticles. The high stability of the biosynthesized ZnO NP was indicated by the values of the zeta potential. The ZnO NPs showed remarkable antibiofilm activity, with a maximum inhibition of approximately 96.55% at 250 µg/mL, and demonstrated antibacterial activity against both Gram-positive and Gram-negative pathogens [23]. In another study by Gad El-Rab et al. in which they used a supernatant of an *Escherichia hermannii* strain that was isolated from raw milk was used to create ZnO NPs. This study selected the *E. hermannii* strain because it produces a higher concentration of ZnO NPs than other strains. The minimum inhibitory concentration (MIC) of these ZnO NPs against *K. pneumoniae* and *E. coli* was found to be 40 µg/mL and 10 µg/mL respectively [24].

Sharma et al synthesized ZnO NPs were extracellularly bio-fabricated using *Aspergillus terreus* AF1 in an intriguing study, and their biological properties were evaluated. FTIR analysis shows that the proteins released by the bacteria served as a capping agent for the ZnO NPs. These green-synthesized, spherical ZnO NPs, which ranged in size from 10 to 45 nm, showed good cytotoxic action against Vero and Caco cell lines and strong antibacterial activity against *P.*

aeruginosa, *S. aureus*, *B. subtilis*, and *E. coli*. ZnO NPs were mixed with cotton fabric, and SEM was used to verify that ZnO NPs had been deposited on the cotton fabric. This mixture was found to have modest antibacterial action against *P. aeruginosa* and *E. coli*, as well as to inhibit UV penetration [25]. Mohamed et al. investigated the impact of fungal strains on the shape and bioactivity of zinc oxide nanoparticles in another significant study. Two fungi that were isolated from soil—*A. niger* strain (G3-1) and *F. keratoplasticum* strain (A1-3) were used in this investigation to produce ZnO NPs. It was reported that various fungal strains were used to produce ZnO NPs with varying morphologies. *A. niger* strain (G3-1) was used to synthesize nanorods (size 8–32 nm) while *F. keratoplasticum* strain (A1-3) was used to create hexagonal ZnO NPs (size 10–42 nm). Furthermore, it was discovered that hexagonal ZnO NPs were less efficient than nanorod ZnO NPs against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative *E. coli* and *P. aeruginosa* [26]. Sumanth et al. produced ZnO NPs by utilizing *Xylaria acuta* that was isolated from *Millingtonia hortensis*. The hexagonal ZnO NPs, which ranged in size from 34 to 55 nm, had good anticancer effects against human MDA-MB 134 mammary gland carcinoma cells as well as strong antibacterial characteristics against the bacteria *P. aeruginosa*, *E. coli*, *S. aureus*, and *B. cereus* as well as the fungus *Cladosporium cladosporioides* [27].

Conclusion and future prospects

Microbial cells can be utilized to produce NPs with the correct nature and structure quickly and safely. ZnO NPs produced by using different microbial strain have numerous biological applications as well as environmental remediation like waste water treatment, dye degradation etc. However The production of NPs through microbial synthesis has numerous disadvantages also, one of which could be the inability to produce NPs with a uniform size, composition, shape, and symmetry because these factors depend on the medium's pH and temperature. Despite the many benefits of fabricating nanoparticles (NPs) with genetically modified organisms, including cost-effectiveness, environmental safety, and industrial feasibility, the employment of recombinant organisms is still relatively uncommon. Improved NP synthesis by genetically modified organisms still has to address issues with NP repeatability, purity, and separation as well as recombinant strain longevity. When employing genetically modified organisms to fabricate nanoparticles, researchers must also consider issue of safety and security before NPs are commercialized, issues including toxicity, dosages, and the host immune response during therapy still need to be resolved.

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