

GREEN BIOSYNTHESIS OF ZINC NANOPARTICLES

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Currently there is a growing need for the development of an environmentally friendly process of synthesis of nanoparticles, during which no toxic chemicals are used. That is why an important area of research in nanotechnology sphere is the synthesis of metal nanoparticles by microorganisms such as bacteria and yeast (detoxification often occurs by reduction of metal ions/formation of metal sulfides). Bacteria are the organism of choice due to their fast growth, high efficiency and low cost. Metal nanoparticles exhibit antimicrobial properties, but the properties of nanoparticles depend on their size and shape, making them specific for different applications. Nevertheless, the desired size and shape of nanoparticles can be obtained by optimizing the synthesis process through manipulating their reaction conditions. Microbial synthesis of nanoparticles is an alternative to chemical and physical methods, as it is non-toxic and biocompatible. Despite the relevance of the application of the “green synthesis” method in the field of nanotechnology, biosynthesis by bacterial organisms has certain disadvantages, such as a high probability of pathogenicity, labour-intensive cultivation, and pollution problems. Ultimately, there is a need to explore more potential microorganisms for the synthesis of metal nanoparticles. The paper provides a review of literature data on the biosynthesis of zinc nanoparticles using lactic acid microorganisms. It was shown that bacteria are capable of synthesizing both extracellular and intracellular nanoparticles in the wavelength range of 315-392 nm. Data on the manifestation of antimicrobial properties by zinc nanoparticles against various gram-positive and gram-negative bacterial microorganisms and micromycetes.

Keywords: nanoparticles, green biosynthesis, *Lactobacillus*

INTRODUCTION

In recent years, green synthesis has been a new method not featuring some of the disadvantages of physical and chemical methods. In this approach, bacteria, archaeobacteria, fungi and plants can be used without the use of toxic and expensive materials to produce metal nanoparticles (MNPs) (Baraton, 2002; Shkotova *et al.*, 2019).

Since such particles are close to molecules in size, nanomaterials are able to exhibit unique physical and chemical properties close to the properties of individual molecules. The nanometre measurement range of sizes 1÷100 nm opens up new properties and fields of application of substances and materials (Baraton, 2002).

A unique property of nanoparticles is a developed specific surface of ~1000 m²/g, open to the action of environmental molecules. The smaller is the size of the nanoparticle, the greater is the number of atoms in relation to the volume present on the surface and the higher is its reactivity (Baraton, 2002; Pomastowski *et al.*, 2020).

Optical properties of nanoparticles are radically different from the properties of bulk material. Shall the shape and size of the particles change, the spectral characteristics change significantly for almost all nanoparticles. By varying the geometric parameters of nanoparticles, it is possible to achieve the required optical properties. When proceeding to the consideration of ensembles of nanoparticles, it is necessary to take into account the interaction between individual particles. The spectral properties of hybrid nanoparticles differ from the properties of the components they are composed of (Rodrigo, 2012; Shkotova *et al.*, 2019).

Nanoparticles have such chemical properties as catalytic and adsorption. The physical properties of nanoparticles arise due to surface or quantum-dimensional effects. Magnetic characteristics are of great importance for nanoparticles: here the difference between compact magnetic materials and the corresponding nanoparticles is most clearly revealed (Baraton, 2002).

One of the main reasons for the change in the physical and chemical properties of small particles as their size decreases is the increase in the relative share of “surface” atoms that are in different conditions (coordination number, symmetry of the local environment, etc.) than atoms inside the bulk phase. From the energy point of view, the decrease in particle size leads to an increase in the role of surface energy (Baraton, 2002; Voloshyna *et al.*, 2019).

BIOSYNTHESIS OF ZINC NANOPARTICLES

Nanoparticles of zinc oxide (ZnO) have attracted attention due to their unique properties, in particular, the properties of combating a wide range of pathogenic microorganisms. Bacterial, fungal and yeast cultures are used for intracellular or extracellular synthesis of ZnO nanoparticles by microbial cells or enzymes, proteins and other biomolecular compounds. Numerous studies indicate that nanoparticles of ZnO have great potential in biological applications, in particular as antimicrobial agents (El-Sayed *et al.*, 2021).

Lactobacillus gasseri

Research was conducted on the biosynthesis of zinc oxide nanoparticles (ZnO-NP) using lactobacillus strains. Biosynthesized zinc nanoparticles thanks to *Lactobacillus gasseri* were investigated using the UV-visible absorption spectrum in the wavelength range from 345 to 350 nm. This range is typical for the wavelengths of zinc nanoparticles. Biosynthesized nanoparticles of ZnO exhibits a surface plasmon resonance (SPR) band at 377 nm. In addition, the presence of glucose in the MRS medium used for the biosynthesis of zinc nanoparticles tends to decrease the value of the oxidation-reduction potential. A white precipitate at the bottom of the flask indicates the presence of zinc nanoparticles. ZnO molecules evolve slowly and form spherical and cubic shaped structures with a size of 22 nm. (El-Sayed *et al.*, 2021). In addition, the integrated biosynthesis of ZnO-NPs in yogurt had a positive effect on the shelf life of yogurt for four weeks without changes in sensory evaluation (El-Sayed *et al.*, 2021).

Lactobacillus johnsonii

Extracellular biosynthesis of ZnO nanoparticles was carried out (average size between 4 and 9 nm) using *Lactobacillus johnsonii* culture fluid. The result of UV spectroscopy shows a broad peak of the absorption band at 406 nm. The time factor plays an important role in biosynthesis: maximum synthesis can be observed 24 hours after inoculation, but after 48 hours of incubation production decreases. A similar result was observed in the biosynthesis of titanium nanoparticles. UV spectroscopy showed that the optical properties of Zn nanoparticles indicate the presence of an absorption peak of Zn nanoparticles at 392 nm. In addition, the FTIR peak diagram of titanium confirmed the stronger ability of proteins to bind metal and increase the possibility of coating metal nanoparticles with proteins to prevent particle agglomeration. TEM image of Zn and Ti nanoparticles recorded a spherical shape and had average particle diameters of 18–105 nm (Al-Zahrani *et al.*, 2018; Pomastowski *et al.*, 2020).

Lactobacillus paracasei

The potential of *Lactobacillus paracasei* LC20 isolated from sweet whey was investigated as a new, effective and affordable source for the synthesis of ZnO nanocomposites after cultivation. It was established that *L. paracasei* LC20 is capable of synthesizing Zn nanoparticles, the presence of which, in turn, was confirmed by a white precipitate using the MRS medium (Krol *et al.*, 2018). *L. plantarum* can also be targeted for Zn nanoparticle synthesis in the range of 7–19 nm (Krol *et al.*, 2018).

Lactobacillus acidophilus

Currently, the antimicrobial effect of Zn and Ag nanoparticles on *Lactobacillus acidophilus* bacteria is sufficiently well studied due to the potential impact of these nanoparticles on human microflora. Cell morphological changes were observed during the experiment, but many cells remained normal in shape. Only a small amount of intracellular contents leaked through the nanoparticle treatment, and more live than dead cells were observed after exposure to metal nanoparticles. According to the results obtained in the study, it can be concluded that Zn and Ag nanoparticles have a slight inhibitory effect on *Lactobacillus acidophilus* (Selvarajan *et al.*, 2013).

Lactobacillus plantarum

In the course of zinc nanoparticles synthesis thanks to *Lactobacillus plantarum* the Fourier-IR spectroscopy analysis revealed the presence of proteins, carboxyl and hydroxyl groups on the surface of both biosynthesized Zn nanoparticles, which act as reducing agents and stabilizers. Surface plasmon resonance for biosynthesized nanoparticles was 349 nm and 351 nm. Biosynthesized Zn nanoparticles exhibit antibacterial and inhibitory activity against pathogenic bacteria depending on the concentration (Krol *et al.*, 2018; Mohd Yusof *et al.*, 2020a).

Lactic acid bacteria have mechanisms of tolerance to zinc ions. The main mechanism of resistance of *Lactobacillus* strains to zinc ions largely depends on the ability of microorganisms to interact with zinc ions through the processes of biosorption or bioaccumulation (Mohd Yusof *et al.*, 2020b).

Lactobacillus sporogens

Lactobacillus sporogens is also used for the synthesis of zinc nanoparticles. Absorption of radiation using UV-visible spectroscopy occurs in the range of 315 nm. X-ray diffraction analysis showed that zinc nanoparticles have a hexagonal cell structure with an average size of 145.7 nm (Mishra *et al.*, 2013).

Antimicrobial Activity of Zn Nanoparticles

Effective antimicrobial activity of Zn nanoparticles against various gram-positive and gram-negative bacterial pathogens and micromycetes, such as *Clostridium difficile*, *Clostridium perfringens*, *E. coli*, *Salmonella typhi*, *Candida albicans* and *Aspergillus flavus* (Mohd Yusof *et al.*, 2019), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia* and *Staphylococcus aureus* (Mishra *et al.*, 2013; Salman *et al.*, 2018) was established by the agar well diffusion method.

Among gram-positive bacteria, the diameter of the inhibition zone formed by zinc oxide nanoparticles against *S. aureus* showed a significant increase as compared to *B. subtilis*. Among gram-negative bacteria, the diameter of the zone of inhibition formed

by zinc oxide nanoparticles against *K. pneumonia* showed a significant increase as compared to *E. coli* and *P. aeruginosa*. Effect of zinc oxide nanoparticles on *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. Aureus* and *B. subtilis* showed strong antibacterial activity against *S. aureus* and *K. pneumoniae* (Rajan *et al.*, 2016).

CONCLUSION

Therefore, due to their properties, lactic acid microorganisms and micromycetes are capable of synthesizing zinc nanoparticles, which are capable of exhibiting antibacterial properties.

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