Summary

Experimental works on the bactericidal properties of nanoparticles (NPs) focused mainly on a partial analysis of their impact on selected metabolic processes, therefore, do not fully reflect their multi-level pressure on the functioning of microorganisms. Although current research on the induction of oxidative stress in bacterial cells by NPs is extensively conducted, it includes, in many cases, the production and identification of generated reactive oxygen species (ROS) after NPs photoactivation and rarely connect them with accompanying pathological changes such as lipid peroxidation and protein oxidation. Moreover, the available knowledge on the combined analysis of the expression level of antioxidant defense genes with the activity of their molecular counterparts is severely limited. Little is also known about the interaction of NPs with the outer layers of bacteria and the effect of NPs on fatty acid profiles. The above considerations justify the choice of the research topic and the consistent pursuit of elucidating the mechanisms underlying the biological activity and toxicity of NPs.

The aim of the doctoral dissertation was a multifaceted analysis of the mechanisms of oxidative stress in *Escherichia coli* (ATCC® 25922™), *Bacillus cereus* (ATCC® 11778™) and *Staphylococcus epidermidis* (ATCC® 12228™), exposed to inorganic nanoparticles: Ag-NPs, Cu-NPs, ZnO-NPs and TiO_2-NPs. As part of the conducted research, the following hypotheses were verified: (1) NPs affect the action of bacterial antioxidants; (2) NPs affect the expression level of oxidative stress genes; (3) NPs modify the respiratory activity of bacterial cells and (4) NPs interact with the outer layers of bacteria and cause cell morphological changes.

The primary issue in studying the antibacterial agents' toxicity is the precise explanation of their bactericidal and bacteriostatic effects on specific microorganisms. Herein, the analysis of the MIC, MBC and IC_{50} toxicological indices confirmed the antibacterial activity of all NPs against *E. coli*, *B. cereus* and *S. epidermidis*. Simultaneously, the tested strains exhibited varying sensitivity to particular types of NPs. Moreover, they were more susceptible to metal nanoparticles than metal oxides. The results of microbiological tests confirmed higher mortality of *E. coli* cells after treatment with Ag-NPs, ZnO-NPs and TiO_2-NPs compared to *B. cereus* and *S. epidermidis*.

A detailed analysis of oxidative stress induction and ROS generation in *E. coli*, *B. cereus* and *S. epidermidis* by NPs indicated that this is one of the basic mechanisms of their antimicrobial activity. Individual NPs generated different types of ROS and increased the overall level of ROS in bacterial cells. The most significant increase in the total concentration of ROS in *E. coli* and *B. cereus* cells occurred after exposure to Cu-NPs. In turn, ZnO-NPs and TiO_2-NPs had a considerable effect on the production of ROS in *S. epidermidis*. It was found that NPs induced ROS formation to a greater extent in *B. cereus* and *S. epidermidis* cells than in *E. coli*. Referring to the level of individual forms of ROS, the principal share in the total concentration of ROS in *E. coli* cells treated with Cu-NPs were $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$, in *S. epidermidis* cells treated with TiO_2-NPs were $O_2^{\bullet-}$ and $^{\bullet}OH$, and in *B. cereus* cells exposed to Cu-NPs and ZnO-NPs turned out to have $O_2^{\bullet-}$ and H_2O_2. The results presented in this dissertation also confirmed the fundamental importance of GSH in protecting *E. coli* and *S. epidermidis* cells against oxidative stress. This was evidenced by decreased GSH concentration in *E. coli* cells exposed to ZnO-NPs and Ag-NPs, and in *S. epidermidis* cells treated with ZnO-NPs. By comparison, the concentration of GSH in *B. cereus* cells did not change significantly, which indicates its small contribution to the protection against oxidative stress. It is worth emphasising that each

microorganism had a unique and specific defense mechanism, characterised by different content and participation of antioxidants in the protective mechanisms.

The production of ROS in bacterial cells is inextricably linked to the oxidation of various biomolecules, primarily lipids and proteins. The treatment of *E. coli*, *B. cereus* and *S. epidermidis* cells with Ag-NPs, Cu-NPs and TiO_2-NPs resulted in a significant increase in lipid peroxidation; however, ZnO-NPs did not cause this phenomenon. The most significant increase in lipid peroxidation in *B. cereus* and *S. epidermidis* cells compared to the control cells occurred after treatment with Cu-NPs. By comparison, this increase in *E. coli* cells was the highest in the presence of TiO_2-NPs. Statistical analysis confirmed the positive correlation between lipid peroxidation and ROS formation. For example, a significant increase in $^{\bullet}OH$ concentration in *B. cereus* and *S. epidermidis* cells was positively correlated with an increase in lipid peroxidation. However, the high level of $^1O_2$ in *E. coli* cells treated with TiO_2-NPs may explain the influence of this ROS on lipid peroxidation. The most commonly attributed modification of proteins induced by ROS is protein carbonylation and changes in the content of amino groups. The detailed analysis confirmed a significant increase in the content of carbonyl groups in the protein of *E. coli*, *B. cereus* and S. *epidermidis* exposed to all types of NPs. The highest increase in the content of protein carbonyl groups occurred in *E. coli* treated with Ag-NPs, ZnO-NPs and TiO_2-NPs. In turn, the greatest increase in the content of these groups in *B. cereus* and *S. epidermidis* appeared after contact of the cells with metal oxides. In the case of amino groups, the most significant increase in their content in all strains was found after exposure to ZnO-NPs.

The protection of bacterial cells against oxidative stress requires the proper functioning of the catalytic antioxidant system. The activity of antioxidant enzymes, i.e. CAT, PER and SOD, is commonly used as a biomarker of oxidative stress. All NPs stimulated CAT, PER and SOD activity in *E. coli* and *B. cereus* cells, with the greatest increase in CAT and PER activity. The most significant influence on the activity of antioxidant enzymes in *E. coli* and *B. cereus* was established after treatment with Cu-NPs and ZnO-NPs. Changes in the CAT, PER and SOD activities in *S. epidermidis* exposed to NPs were difficult to interpret because each type of NPs affected the catalytic profile of these enzymes in a different and often opposite way. Disturbances in the functioning of antioxidant enzymes were associated with ROS levels and protein oxidation. The obtained results also indicated that all NPs changed the expression level of selected genes, which was correlated with impaired activity of antioxidant enzymes. The most significant differences between transcriptional and antioxidant profiles were found for proteins with CAT and PER-like activity. The analysis of the oxidative stress induction and the genotoxic properties of NPs also confirmed the relationship between ROS generation and the expression level of selected oxidative stress-related genes. For example, a positive correlation was found between the expression level of *sod*A2 and *sod*A genes in *B. cereus* and *S. epidermidis* cells, respectively, and increased levels of the $O_2^{\bullet-}$ radical.

A severe consequence of NPs interaction with the surface of bacterial cells was altered respiratory metabolism. Based on the obtained results, different DEH and ATPase activities and changes in total ATP concentration in *E. coli*, *B. cereus* and *S. epidermidis* cells treated with NPs were evidenced. The decrease in ATPase activity was positively correlated with a reduction in the total ATP concentration in all bacterial strains and changes in carbonyl group content. Interestingly, the analysis of the respiratory activity of bacterial cells also confirmed the relationship between DEH activity and general ATP levels. It is worth emphasising that the alternations mainly concerned intracellular ATP

concentration. It dynamically changes under stress conditions due to ATP utilisation in various cellular processes, including the synthesis of redox proteins.

Direct and indirect interaction of NPs with bacterial cell membranes can lead to irreversible changes in their structure and functioning. The most significant changes in cell membrane permeability and cytoplasm leakage were found in *E. coli* treated with ZnO-NPs and TiO_2-NPs. By comparison, statistical analysis showed no significant differences in membrane permeability between treated and untreated *B. cereus* and *S. epidermidis* cells. Substantial changes in the percentages of saturated and unsaturated fatty acids in the FAME profiles of all bacteria treated with NPs were also identified. The detailed analysis of the FAME profiles showed a differential and concentration-dependent effect of NPs on the percentages of *E. coli*, *B. cereus* and *S. epidermidis* fatty acids. In all bacteria, the most susceptible to NPs action were cyclopropane and/or hydroxyl fatty acids. Gram-positive bacteria turned out to be more sensitive to NPs than *E. coli*.

SEM analysis was performed to study the distribution and interaction of individual NPs with the bacterial surface and to identify potential damage to their outer layers. It was evidenced that individual NPs had a differential affinity to the surface of *E. coli*, *B. cereus* and *S. epidermidis* and were distributed on the bacteria's surface depending on their type. For example, ZnO-NPs were evenly dispersed over the entire surface of *E. coli* cells. Contrarily, they formed layered clusters in the form of agglomerates and aggregates of various shapes on the surface of *B. cereus* cells. It is worth emphasising that Ag-NPs slightly adhered to the surface of *E. coli*, *B. cereus* and *S. epidermidis* compared to the remaining NPs.

In conclusion, the conducted research provided new and solid evidence of the negative impact of NPs on microorganisms. Changes in the metabolism and structure of bacterial cells depended on the type of NPs and were species-specific. *E. coli* strain was characterised by an increased functioning of the antioxidant defense system and noteworthy changes in the permeability of cell membranes. On the other hand, the exposure of *B. cereus* and *S. epidermidis* to NPs resulted mainly in impaired respiratory metabolism. The biomarkers of oxidative stress and the proposed and optimised methodology may help standardise empirical and analytical methods in future studies of nanomaterial toxicity.