

Unveiling the Role of Postbiotics in the Dissemination of Antibiotic Resistance Genes: Implications for Commensal and Pathogenic Bacteria

This project investigates whether **postbiotics**—substances derived from inactivated lactic acid bacteria (LAB) or their metabolic products—can unintentionally promote the spread of antibiotic resistance genes (ARGs) among both pathogenic and naturally occurring bacteria in food-related environments.

Postbiotics are increasingly being introduced into the food industry as natural preservatives that support the “clean label” trend. While they are valued for their antimicrobial properties and ability to inhibit spoilage organisms, their **long-term safety remains poorly understood**, especially in terms of their potential to serve as **silent vectors** for the transfer of antibiotic resistance.

The central goal of the project is to determine whether postbiotics produced from LAB strains carrying resistance genes can contribute to horizontal gene transfer (HGT), including to pathogenic species such as *Listeria monocytogenes* and *Staphylococcus aureus*, as well as to commensal bacteria. This issue is particularly urgent given the **absence of clear regulatory frameworks** regarding the genetic safety of LAB used for postbiotic production, both in Poland and across the EU.

To address this, postbiotics will be produced using several commonly applied inactivation techniques, including thermal, enzymatic, sonication, and high-pressure methods. The antibacterial activity of each preparation will be tested against a panel of bacteria relevant to the food industry, with a special focus on dairy-related pathogens.

In a stepwise experimental approach, the project will assess whether postbiotics can promote ARG transfer under different biological conditions: first in liquid cultures (planktonic state), then in biofilms—structured microbial communities that are known to support gene exchange and are especially resistant to cleaning processes. Finally, selected experiments will be conducted using real food matrices, such as artisanal and mozzarella cheeses, to simulate real-world storage and contamination scenarios.

Gene transfer events will be monitored using culture-based methods and PCR analysis, allowing precise detection of ARG acquisition by recipient bacteria. In addition, the project will examine changes in biofilm structure and membrane permeability to understand how postbiotics may influence bacterial physiology in ways that could facilitate gene uptake.

Although gene transfer from inactivated cells has been suggested in isolated studies, **no comprehensive research has yet explored whether postbiotics—despite being non-viable—can contribute to the dissemination of antibiotic resistance in food environments**. This project directly addresses this knowledge gap.

By combining microbiology, food technology, and molecular analysis, the project aims to provide evidence that can guide **future regulatory decisions** and risk assessment procedures. The findings may contribute to updated guidelines for postbiotic use in the food industry, helping ensure both product quality and consumer safety in the context of the growing global challenge of antimicrobial resistance.