Extracellular vesicles and lipidomes of *Cutibacterium* spp. with therapeutic and diagnostic potential

The genus *Cutibacterium* comprises facultative anaerobic microorganisms that are part of the human skin microbiota. Concurrently, they are also opportunistic pathogens, and may therefore pose a risk to individuals with immunodeficiency. The most significant and most thoroughly researched species within this genus is *Cutibacterium acnes* (formerly *Propionibacterium acnes*), which has been documented to play a role in the pathogenesis of acne vulgaris. Given the rapid increase in antibiotic resistance of these microorganisms and their widespread distribution, it is imperative to implement effective prevention strategies and accurate diagnostic techniques to combat *C. acnes* infections in humans.

Extracellular vesicles are spherical nanostructures secreted by prokaryotic cells that transport a variety of substances, including proteins, nucleic acids, cellular metabolites, bacterial virulence factors, and other molecules. These vesicles serve as mediators of intercellular communication. Due to these characteristics, they can selectively stimulate the host immune system, rendering them suitable for use as vaccine components.

The objective of this study was to conduct a comprehensive analysis of the lipidome of bacteria belonging to the genus *Cutibacterium* and to identify specific lipid markers with diagnostic potential for distinguishing between individual species and phylotypes. A further objective was to conduct a detailed analysis of the extracellular vesicles secreted by *C. acnes* cells, including their morphological characterisation and a comparative analysis of protein and lipid profiles. This enabled the preparation of a research platform for the potential use of these structures in vaccines.

The dissertation is comprised of a series of three publications, each of which is thematically related to the preceding one: a review and two research articles.

The review (Postepy Hig. Med. Dosw., 2020, 74, 572-588) provides a comprehensive overview of the current state of knowledge regarding extracellular vesicles secreted by bacterial cells as mediators of intercellular communication.

In the initial phase of the doctoral project outlined in the research article (Int. J. Mol. Sci., 2022, 23, 5797), extracellular vesicles were isolated from three phylotypes of *C. acnes* (IA1, IB and II) which were subjected to morphological analysis and their protein and lipid

profiles were compared. Significant differences were observed in the protein and lipid profiles of the extracellular vesicles under investigation, suggesting the presence of antigenic structures that may elicit a selective immune response. This is a valuable feature that indicates the potential of the vesicles as adjuvants or vaccine components.

The dynamic development of lipidomics has led to a shift in the perception of lipid compounds, which are now understood to be not just structural components of cell membranes but also antigens and signalling molecules. In the second phase of the PhD project described in the article (mSphere, 2024, 10.1128/msphere.00054-24), a comparative lipidomic analysis of eight *Cutibacterium* strains was conducted: four phylotypes of *C. acnes*, two strains of *C. granulosum*, and *C. avidum* and *C. namnetense*. The results obtained enabled the identification of marker lipids specific to the strains under investigation. The identification of cardiolipins and fatty acid amides in *Cutibacterium* spp. represents a novel finding.

This doctoral project has led to a comprehensive characterisation of the extracellular vesicles of *C. acnes*. The genotypic and phenotypic distinctiveness of *C. acnes* phylotypes was reflected in the differences in the proteins and lipids that constitute extracellular vesicles. A comparative characterisation of the lipidome of the genus *Cutibacterium*, together with the identification of specific molecular markers, has revealed their diagnostic potential in clinical microbiology.