PHD PROJECT DESCRIPTION

(4000 characters max., including the aims and work plan to de published online)

Project title: Deciphering the lipidomic landscapes of multidrug-resistant *Klebsiella* sp. via MALDI-TOF mass spectrometry.

1.1. Project goals

The central objective is to elucidate the molecular lipidomic architecture of multidrug-resistant (MDR) *Klebsiella* spp. and identify specific lipid biomarkers associated with antibiotic resistance. The project aims to establish a standardized MALDI-TOF MS framework for rapid molecular phenotyping, enabling the detection of adaptive membrane remodeling that occurs under antimicrobial selective pressure, which traditional proteomic methods often fail to capture.

1.2. Outline

The rapid dissemination of carbapenemase-producing *Klebsiella pneumoniae* (e.g., NDM, KPC) in Polish hospitals represents a critical epidemiological threat. Current diagnostic standards, including protein-based MALDI-TOF MS, frequently lack the resolution to distinguish specific resistance phenotypes or closely related clones. This project shifts the focus to lipidomics—specifically targeting lipid A and other membrane constituents—as a more sensitive indicator of bacterial survival strategies. By utilizing MALDI-TOF MS in both positive and negative ionization modes, the research will map the structural lipid alterations that underpin resistance. This approach will bridge the knowledge gap in microbial lipidomics and provide a foundation for a new generation of rapid, lipid-based diagnostic tools.

1.3. Work plan

A. **Standardization of Protocols**: Selection of reference MDR *Klebsiella* strains and optimization of culture conditions (media, growth kinetics) to ensure reproducible lipidomic signatures. Refinement of liquid-liquid extraction (LLE) methods (e.g., Folch) to maximize lipid recovery from encapsulated strains.
B. **Optimization of MALDI-TOF MS Parameters:** Comparative evaluation of various organic matrices (e.g., DHB, CHCA, 9-AA, THAP) and automated deposition techniques to enhance ionization efficiency and spectral quality across different lipid classes.
C. **Clinical Isolate Profiling:** Systematic generation of lipidomic profiles from a diverse cohort of clinical MDR isolates (KPC, NDM, and colistin-resistant strains). This involves correlating mass spectra with phenotypic resistance levels (MIC values) determined by gradient diffusion methods.
D. **Data Analysis and Biomarker Mapping:** Statistical validation of MS data using multivariate analysis (PCA, PLS-DA) to identify discriminatory m/z signals. Creation of a validated library of lipidomic fingerprints that serve as reliable biomarkers for specific resistance mechanisms in *Klebsiella* sp.

1.4. Literature (max. 7 listed as a suggestion for a PhD candidate preliminary study)

Maślak E, Arendowski A, Złoch M, et al. Silver Nanoparticle Targets Fabricated Using Chemical

Vapor Deposition Method for Differentiation of Bacteria Based on Lipidomic Profiles in Laser Desorption/Ionization Mass Spectrometry. Antibiotics. 2023;12(5):874. doi:10.3390/antibiotics12050874

Walczak-Skierska J, Monedeiro F, Maślak E, Złoch M. Lipidomics Characterization of the Microbiome in People with Diabetic Foot Infection Using MALDI-TOF MS. Anal Chem. 2023;95(44):16251-16262. doi:10.1021/ACS.ANALCHEM.3C03071

Fuchs B, Süß R, Schiller J. An update of MALDI-TOF mass spectrometry in lipid research. Prog Lipid Res. 2010;49(4):450-475. doi:10.1016/j.plipres.2010.07.001

Perry WJ, Patterson NH, Prentice BM, Neumann EK, Caprioli RM, Spraggins JM. Uncovering matrix effects on lipid analyses in MALDI imaging mass spectrometry experiments. J Mass Spectrom. 2020;55(4):e4491. doi:10.1002/jms.4491

Appala K, Bimpeh K, Freeman C, Hines KM. Recent applications of mass spectrometry in bacterial lipidomics. Anal Bioanal Chem. 2020;412(24):5935-5943. doi:10.1007/s00216-020-02541-8

Nishikawa M, Tang W, Kostrzewa M, et al. Discrimination of Klebsiella pneumoniae and Klebsiella quasipneumoniae by MALDI-TOF Mass Spectrometry Coupled With Machine Learning. Microbiologyopen. 2025;14(4):e70035. doi:10.1002/mbo3.70035

Leung LM, Fondrie WE, Doi Y, et al. Identification of the ESKAPE pathogens by mass spectrometric analysis of microbial membrane glycolipids. Sci Rep. 2017;7(1):1-10. doi:10.1038/s41598-017-04793-4

1.5. Required initial knowledge and skills of the PhD candidate

The ideal candidate should hold a Master's degree in Biology, Biotechnology, or a related field, with a strong focus on microbiology. Essential skills include proficiency in aseptic techniques, bacterial culturing, and phenotypic identification. A solid understanding of bacterial resistance mechanisms, particularly regarding *Klebsiella* species, is highly valued, alongside familiarity with standard antibiotic susceptibility testing like disc diffusion or MIC determination. While prior experience with MALDI-TOF mass spectrometry is beneficial, it is not mandatory; we prioritize a strong biological background and the manual precision necessary for lipid extraction protocols. Candidates should demonstrate an eagerness to learn advanced analytical methods and apply them to microbiological challenges. Additionally, the ability to review scientific literature in English and a basic grasp of statistical tools for biological data interpretation are expected. We seek a motivated individual eager to bridge classical microbiology with modern molecular diagnostics.

1.6. Expected development of the PhD candidate's knowledge and skills

Through this doctoral program, the candidate will acquire advanced expertise in microbial lipidomics and specialized mass spectrometry. They will master the technical operation of MALDI-TOF MS systems, transitioning from routine species identification to complex molecular profiling of multidrug-resistant pathogens. The researcher will develop high-level proficiency in

specialized lipid extraction protocols and sample preparation for encapsulated bacteria. Furthermore, the candidate will enhance their analytical skills in interpreting mass spectra and utilizing multivariate statistics to identify clinical biomarkers. Ultimately, the student will mature in experimental design and scientific communication, becoming an independent researcher at the intersection of microbiology and analytical chemistry.