1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: A new approach to the analysis of low molecular weight compounds in roughage using electrochemical, separation and spectral techniques

1.1. **Project goals:** The project aims to isolate and identify low molecular weight biological compounds from ensiled plant material (corn silage, grass mixtures with legumes) used to feed polygastric animals. Polyphenolic compounds and microorganisms (bacteria, yeasts) will be obtained from plant biomass to test the synergistic effect of fortified microflora on the process of mycotoxins neutralization in silages. By employing advanced "-omics", electrochemical, separation and spectral techniques, the project significantly enhances the level of detection and specificity in the analysis of various biological compounds.

1.2. **Outline:** Nowadays, feed and food contaminated by mycotoxins can significantly harm animal health and productivity and may also cause health problems in humans. Feed contaminated with these toxins can cause mycotoxicosis in animals, characterized by a variety of clinical signs depending on the toxin, which can cause significant losses to the ruminant industry. Mycotoxins, produced by molds, contaminate plant-based food and cause significant economic losses worldwide. Particularly hazardous are zearalenone and deoxynivalenol. Even low doses of these compounds can lead to health and reproductive problems in animals. In response to the problems outlined, we have planned a study to explore the potential for neutralizing mycotoxins and mold fungi using selected bacterial compositions. This initial stage involves the isolation of bacteria and yeasts from a variety of plant biomass suitable for ensiling and silage after fermentation. The second stage will employ modern identification techniques (16S rDNA and ITS sequencing, MALDI-TOF MS) to identify the microflora components. During the third stage polyphenolic compounds (e.g. cyclitols and gallic acid), will be obtained using ethanol extraction and accelerated solvent extraction (ASE). The extraction of these target compounds will be further enhanced through supercritical fluid extraction (SFE). Subsequently, the impact of the isolated polyphenolic-enriched yeast-bacterial combinations on antifungal and mycotoxins-neutralizing properties will be investigated. The final stage involves testing the survival of the yeast-bacterial compositions under varying lyophilization conditions and assessing the impact of the enriched microbial mixture on neutralization in the ensiled biomass.

1.3. **Work plan:** 1) Isolation of epiphytic microflora: the method of culturing on selective media will be applied, 2) Bacteria and yest identification (16S rDNA and ITS sequencing, MALDI-TOF MS), 3) Extraction of polyphenolic compounds: the methods will include accelerated solvent extraction (ASE) to maximize the efficiency and effectiveness of the process. Other potentially active compounds will be subjected to supercritical fluid extraction (SFE), facilitating the isolation of compounds with minimal losses and protecting them from thermal degradation, 4) Analysis of the isolated compounds: employing advanced chromatographic techniques (LC-QqQ-ESI-MS/MS) to enable precise quantitative and qualitative analysis of the isolated compounds, 5) Optimalisation of the lyophilization step: testing the survival of the yeast-bacterial compositions under varying lyophilization conditions, 6) Cytotoxicity testing: potential cytotoxic effects will be assessed using cell line and tests (Caco-2 cell line and MTT assay), allowing for the evaluation of the safety of using microbial composition in application-oriented research, 7) The impact of the enriched microbial mixture on mycotoxins neutralization in the ensiled biomass.

1.4. **Literature** *(max. 10 listed, as a suggestion for a PhD candidate)*

1. Janiszewska, D., Złoch, M., Pomastowski, P., & Szultka-Młyńska, M. (2023). Implications of Sample Preparation Methods on the MALDI-TOF MS Identification of Spore-Forming Bacillus Species from Food Samples: A Closer Look at Bacillus licheniformis, Peribacillus simplex, Lysinibacillus fusiformis, Bacillus flexus, and Bacillus marisflavi. ACS Omega, 8(38). **2.** Rogowska, A., Szultka-Młyńska, M., Kanawati, B., Pomastowski, P., Arendowski, A., Gołębiowski, A., Schmitt-Kopplin, P., Fordymacka, M., Sukiennik, J., Krzywik, J., & Buszewski, B. (2023). Advanced Mass Spectrometric Techniques for the Comprehensive Study of Synthesized Silicon-Based Silyl Organic Compounds: Identifying Fragmentation Pathways and Characterization. Materials, 16(9). **3.** Tuzimski, T., Petruczynik, A., Kaproń, B., Plech, T., Makuch-Kocka, A., Janiszewska, D., Sugajski, M., Buszewski, B., & Szultka-Młyńska, M. (2024). In Vitro and In Silico of Cholinesterases Inhibition and In Vitro and In Vivo Anti-Melanoma Activity Investigations of Extracts Obtained from Selected Berberis Species. Molecules, 29(5). **4.** Walczak-Skierska, J., Krakowska-Sieprawska, A., Monedeiro, F., Złoch, M., Pomastowski, P., Cichorek, M., Olszewski, J., Głowacka, K., Gużewska, G., & Szultka-Młyńska, M. (2023). Silicon's Influence on Polyphenol and Flavonoid Profiles in Pea (Pisum sativum L.) under Cadmium Exposure in Hydroponics: A Study of Metabolomics, Extraction Efficacy, and Antimicrobial Properties of Extracts. ACS Omega. **5.** Ran Xu, Elijah G. Kiarie, Alexandros Yiannikouris, Lvhui Sun and Niel A. Karrow (2022). Nutritional impact of mycotoxins in food animal production and strategies for mitigation. Journal of Animal Science and Biotechnology. **6.** Xin Mao, Wanzhao Chen, Huimin Wu, Ying Shao, Ya'ning Zhu, Qingyong Guo, Yanshen Li, Lining Xia (2023). Alternaria Mycotoxins Analysis and Exposure Investigation in Ruminant Feeds. Toxins.

1.5. Required initial knowledge and skills of the PhD candidate

Degree in Chemistry, Biotechnology, Biochemistry, or a related field: The candidate should have completed a master's degree or equivalent in areas such as chemistry, biotechnology, biochemistry, molecular biology, or pharmacology. **2. Technical Skills:** Chromatographic techniques (LC-QqQ-ESI-MS/MS), Extraction techniques: Experience with ethanol extraction, ASE, and SFE techniques will be highly beneficial. Knowledge of Polish and English, analytical thinking, eager to learn and work hard, knowledge of analytical chemistry and microbiology, methods of determination and identification of biologically active compounds, knowledge about advanced instrumental techniques. **3. Laboratory Skills**: Competence in standard laboratory practices with a strong emphasis on safety and precision in handling biological materials and chemicals. **4. Communication and Interpersonal Skills:** Ability to work effectively in a collaborative research environment, sharing tasks and responsibilities with other team members, **5. Desirable Additional Qualifications:** Previous experience in a research project related to natural products, plant biology, and microbiology. Knowledge of molecular biology techniques such as PCR, DNA isolation, and primer design.

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

1. Technical Skills: Mastery of chromatographic and spectroscopic techniques: The candidate will achieve proficiency in advanced analytical techniques such as LC-QqQ-ESI-MS/MS. Moreover, PhD candidate knows and can applied of two diagnostic approach (MALDI TOF-MS and 16S rRNA gene sequencing) for determination and identification of clinical isolates in the microbiology laboratory setting. PhD candidate is able to indicate bacterial strains characteristic of specific real matrices. On the other hand, PhD candidate is able to develop a new, previously unknown procedures and analytical methods for the determination and identification of different biologically active compounds and their metabolites with the use of (U)HPLC in combination with a different mass spectrometers. **2. Refinement of Extraction Skills:** Further refinement in various extraction methods, including ASE and SFE. **3. Experimental Design and Implementation:** The candidate will develop sophisticated skills in designing and implementing research experiments. **4. Data Analysis and Interpretation**: Advanced capabilities in statistical analysis and interpretation

of large data sets. **5. Scientific Writing and Communication**: Profound development in scientific writing and presentation skills by publication of research findings in high-impact journals and presentations the results at international conferences.