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

Project title: Advancing Microbial Profiling Through Spectroscopic and Spectrometric Integration on Nanostructured Platforms

1.1. Project goals.

The development of prompt and precise methods for identifying microorganisms is crucial in mitigating the impacts of infections, ensuring the microbiological safety of food and production processes, and discovering novel strains for industrial use. Existing microbial detection methodologies in standard laboratory settings are plagued by several drawbacks, including significant labor and time requirements, high expenses, and limited specificity. Furthermore, the continual emergence of new microbial species necessitates ongoing adjustments to current laboratory protocols. Consequently, there is a growing focus on developing contemporary, accurate, and expedient microorganism detection methods. These methods should be adaptable for various sample types and analysis objectives, such as rapid detection, comprehensive identification, drug resistance evaluation, and detailed enzymatic property analysis. Our preliminary findings suggest that these demands can be satisfied by profiling the microbial proteome and lipidome using laser desorption/ionization (LDI) techniques, specifically nanoparticle-assisted laser desorption/ionization (NALDI) alongside matrix-assisted laser desorption/ionization (MALDI) as a reference approach. We intend to conduct extensive research employing these methods and expand them to include advanced spectroscopic techniques like surface-enhanced Raman scattering (SERS). These techniques will utilize a universal platform, whose surface will be nanostructurally modified with metallic nanoparticles. This platform - nanostructurally modified substrates - aims to analyze closely related species and environmental strains, addressing challenges faced by conventional methods such as 16SrDNA and MALDI. The project's objective is to develop nanostructurally modified substrates through chemical and physical vapor deposition (C/PVD) and atomic layer deposition (ALD) processes. This development aims to establish new, universal platforms for microbial identification using spectrometric and spectroscopic methods. A comprehensive examination of the relationship between the structure, size, morphology of metallic nanoparticles (Ag, Au), and their optical properties will be conducted. The focus will be on employing these developed platforms in three specific techniques involving optical excitation of the nanoparticles: NALDI, SERS.

1.2. Outline

The "gold standard" 16S rRNA sequencing method for microorganism identification has limitations including long turnaround times and high costs. MALDI-TOF mass spectrometry has emerged as a faster tool, with recent attention on direct detection of antimicrobial resistance biomarkers. However, challenges in sample preparation and variability hinder its application. Nano-assisted laser desorption ionization (NALDI) offers a solution, but target preparation is complex. Expanding targets for other methods like surface-enhanced Raman scattering (SERS) presents further opportunities. In this project, we aim to evaluate metallic nanoparticles (e.g., silver, gold, rhodium) to enhance optical excitation methods like NALDI and SERS. [1-8].

1.3. Work plan will be realized according to main research task:

(1) C/PVD and ALD synthesis of silver, gold nanoparticles will be performed with the selection of precursor type, amount of precursor, time of deposition and kind of substrate. Within the present project, metallic nanoparticles of different shapes and varying size and polydispersity, obtained by C/PVD and ALD on different kind of substrates, such as stainless steel (e.g 316L), brass and nanostructurally modified titanium alloy, are planned to be obtained. Their physicochemical characterization, as well as structure and morphology analysis will be carried out with the use of a wide range of instrumental analyzes, such as: XRD, Raman, , SEM, SEM/EDX, SEM-FIB, HRTEM, cry-TEM, AFM, UV/VIS-DRS, contact angle studies and nanoindentation analyses. Their optical excitation will be checked in the simultaneous identification of microorganism isolated from different biological matrices.

(2). The methodology section is aimed at refining the conditions for culturing diverse microorganisms that hold clinical and industrial significance. Optimal culture environments will be determined for isolating (a) clinically relevant bacterial species from swabs of chronic wounds (including surgical, diabetic, and pressure ulcers), saliva, urine, and/or blood collected from medical and palliative care institutions; and (b) bacterial species pertinent to the food sector—specifically, lactic acid bacteria (LAB), and pathogens recognized by the International Commission on Microbiological Specifications for Foods, such as Listeria monocytogenes, Salmonella spp., Escherichia coli, and Staphylococcus aureus—sourced from raw materials and end products supplied by dairy processors and/or other food production sectors through project collaboration. The selection process will involve the alteration of media compositions (both liquid and solid, including universal and selective media), adjustment of cultural conditions (atmospheric composition, CO2 enrichment, and temperature variations), and the enhancement of media with porous materials (zeolites or bio-silica) patent application no. P.445952, 2023. The assessment will also include measuring growth durations to determine the initial bacterial culture phase, as well as the minimum and optimal culture times. The collection of various microbial strains is planned as follows: (i) clinical samples, such as urine, catheters, and swabs from non-healing wounds, with a minimum of 750 samples; (ii) environmental samples, such as water, soil, manure, plant materials, and honey, with at least 300 samples; and (iii) food samples, such as whey, cheese, raw milk, and processed milk, with no less than 250 samples. The project anticipates achieving a minimum spectrum count of 2500 for clinical, 900 for environmental, and 750 for food samples. The estimated number of strains for analysis includes 30 reference strains, a minimum of 60 clinical strains, and at least 110 environmental (including food-related) strains. For reference identification, analyses will be conducted using 16S rDNA sequencing, MALDI Biotyper, ESX Zbybio system (conforming to IVD standards), and IR Biotyper methodologies.

(3). The extraction of the bacterial proteome using formic acid and acetonitrile will be performed using the addition of silica and biosilica as the adsorbent increasing the extraction of hydrophilic proteins to increase the extraction of hydrophobic proteins. Another step will be extraction of lipids and in case of them 3 extraction protocols will be stated: Folch bacterial lipid isolation protocol, Bligh & Dyer (B&D) and Matyash method.