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

Project title: Development of new spectrometric and molecular biology methods used to microorganisms identification

1.1. Project goals of this project will be to create new microorganism identification (MI): environmental and clinical using:

- spectrometric laser desorption/ionization (LDI) techniques: MALDI TOF MS (matrix-assisted LDI with time-of-flight mass spectrometry,) based on low-molecular-weight (LMW) matrices and NALDI TOF MS (nanostructure-assisted LDI TOF MS) based on novel nanomaterials
- molecular biology techniques based on 16S rDNA for prokaryote and 18S rDNA for eukaryote gene sequencing as reference methods.

The use of hyphenated separation techniques such as capillary electrophoresis (CE)-MALDI/NALDI-TOF MS) will be examined.

1.2. Outline

The current MI methods of bacteria isolation is based on phenotypic characteristics. These techniques collectively allow high-level accuracy in identifying most bacterial isolates, but they are costly, non-specific and time-consuming. These problems can be overcome by using the LDI technologies: MALDI and NALDI techniques [1].

LDI approaches has revolutionized the method of MI in microbiology laboratories, as it is a rapid, high throughput, low-cost and efficient system. This technique identifies and quantifies molecules by analyzing the mass-to-charge ratios (*m/z*) of molecular ions. Matrix - a solution of a LMW organic compound that, after drying, forms a crystalline solid with low vapour pressure, plays a key role in MALDI-TOF MS analysis since enabled detection of large biomolecules such as proteins and lipids via the so-called soft ionization mechanism [2,3]. Classical matrix such as cinnamic acid derivatives implies the limitations: suppression of LMW compounds, generation of clusters and adducts [4]. Organic matrix molecules that are replaced to nanomaterials to assist LDI process in NALDI. NALDI field is fast-growing and perspective for applications for different analytical, environmental and medical purposes, but also has its challenges and limitations. One of the limitations of this field is preparation of target with deposited nanomaterials to get reproducible

and accurate results as nanomaterials can be labile in solid state after its drying on the target. Large number of nanomaterials are used nowadays for this purpose: metal and metal oxide nanoparticles, carbon-based nanomaterials, nanocomposites, metal organic frameworks (MOFs). Gold nanoparticles attracted much attention because of their intrinsic advantages as large surface area, strong absorption efficiency in UV-Vis region, high chemical stability, easy modification and preparation [5]. However, targets for NALDI analysis based on gold nanoparticles are mostly prepared by their *in-situ* analysis. Solvents and precursor salts consumption is quite large in this case and the reaction takes in general more than 3 days [6]. Therefore, the main goal of this project is to new methods for preparation of NALDI targets cheaper than existing analogues based on chemically synthesized metal and metal oxide nanoparticles for microbial identification.

LDI identification of microorganisms is primarily based on the detection of the proteins. As a result, this technique, apart from being used in the proteomic approach, can also be important part of the lipidomic, genomic or broadly defined metabolomic studies. Other big advantages of this technique are: possibility of an application to a wide range of microorganisms taxonomy, as is the case with DNA-based techniques; shorten time-to-results - final result can be obtained within a few minutes as well as high sensitivity, which means that for reliable analysis a relatively small amount of material ($<10^4$ microbial cells) is required [7]. Due to many benefits of LDI technique, we can find several examples of its successful application in the scope of microbiological studies within both clinical (e.g. blood, urine, saliva, wound swabs [8,9]) and environmental uses like honey [10] or dairy products [11].

Capillary electrophoresis characterized by short analysis time, low financial outlays and consumption of small amounts of samples and reagents, seems to be promising tool for purifying and/or concentrating bacterial cells prior to LDI analysis [12]. Despite several papers confirming usefulness of CE in microorganisms separation and identification [13], there is still a problem associated with the natural tendency of microorganisms to agglomerate and interact with column filling [14].

1.3. Work plan will be realize according schema presented on Fig. 1.

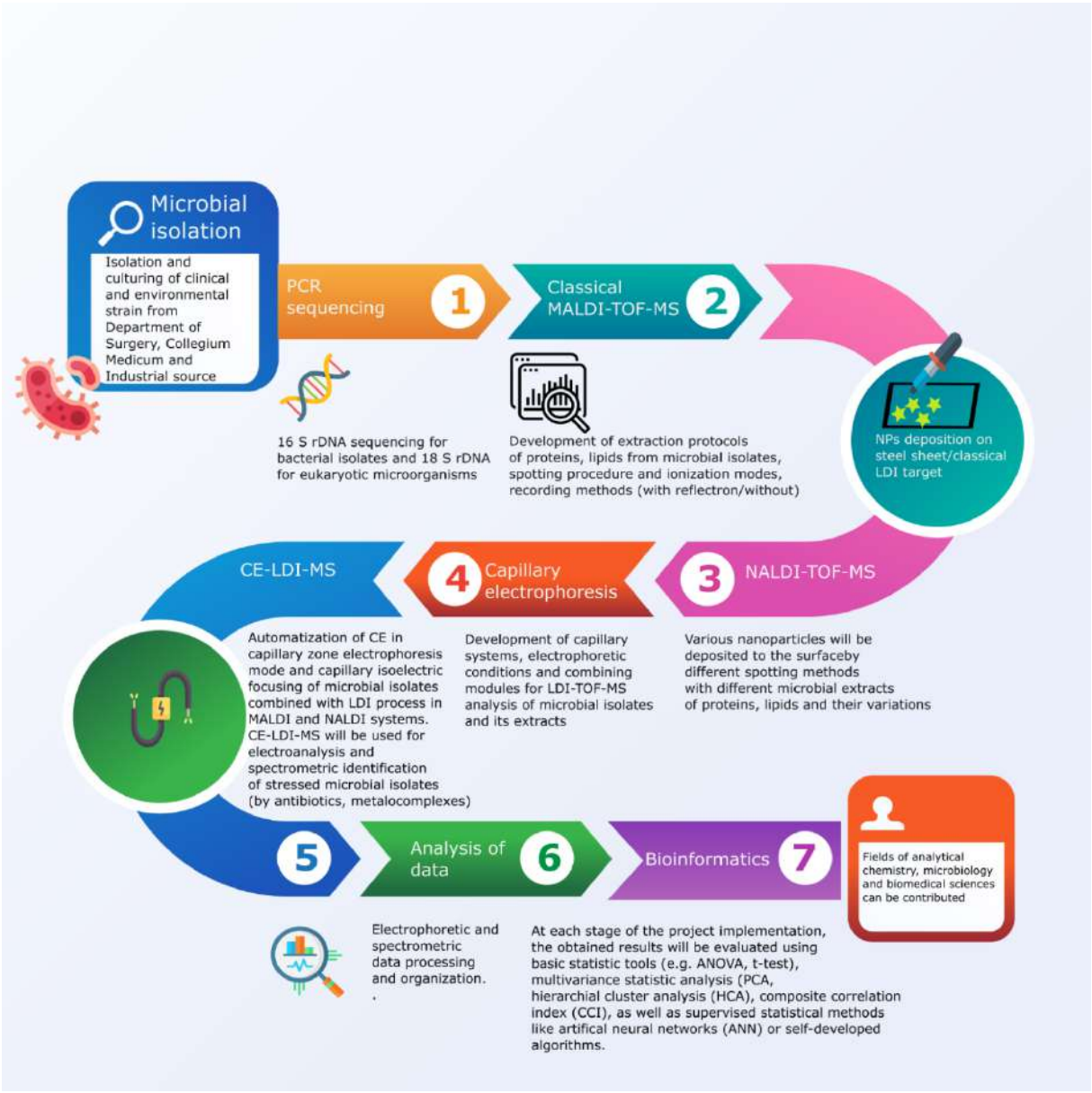

Fig. 1 Scheme of work plan

1.4. Literature

[1] Pomastowski P, Buszewski B 2019 Nanomaterials 9:260

[2] Albrethsen J 2007 Clin Chem 53:852

[3] Santos, IC et al 2016 Analyst 141:2827

[4] Arendowski A et al 2018 Bioanalysis 10:83–94.

[5] Lu M et al 2017 Nanomaterials 7:87

[6] Sekuła J et al 2015 Anal Chim Acta 895:45

[7] Angeletii, S., et al. 2017. J. Microbiol. Methods. 138:20-29.

[8] Lagace-Wiens, P.R., et al. 2012. J. Clin. Microbiol. 50:3324-3328.

[9] Ferreira, L., et al. 2010. J. Clin. Microbiol. 48:2110-2115.

[10] Pomastowski, P., et al. 2019. PLOS One 14(5):e0217078.

[11] Raelan-Plugaru, V., et al. 2017. Appl. Microbiol. Biotechnol. 101:1-13

[12] Dziubakiewicz, E., Buszewski, B. 2012. Malamut Publishing (Warsaw). 330-339.

[13] Kłodzińska, E., et al. 2007. Biomed. Chromatogr. 27(2):116.

[14] Szeliga, J. et al. 2011. Med. Sci. Monit. 17(10): MT91-MT96.

1.5. Required initial knowledge and skills of the PhD candidate

PhD candidate should be skillful and intellectually manipulative, familiar with the microorganisms isolation, culturing and identification as well as preparing different culture media. Experienced with the sample preparation and their further analysis using LDI TOF MS as well as skills in the mass spectra recoding. Knowledge in field of nanomaterial synthesis will be highly honoured. Knowledge about work in programs used to identify mass profiles of microorganisms like FlexAnalysis, FlexControl, and MALDI Biotyper will be favoured.

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

PhD candidate will gain knowledge and skills in field of microbiology, analytical chemistry and material science. Candidate will get specialized knowledge in microbial identification, nanoparticle synthesis and their physicochemical characterization especially for preparation of LDI targets.

Moreover, the interpretation of MS spectra and genetic data will be developed during PhD study. During the study student will be able to present obtained data in form of high-impact factor publication and posters and oral presentations at domestic and international conferences.