INSTITUT DES SCIENCES ANALYTIQUES

UNIVERSITÉ DE LYON

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Lyon 1

SUJET DE THESE :

Caractérisation des phospho-relais et des toxines chez les bactéries par couplage photo-dissociation laser et spectrométrie de masse

PHD PROPOSAL :

Characterization of bacteria phospho-relays and toxins by coupling mass spectrometry and laser photo-dissociation

Keywords: mass spectrometry, proteomic, optical spectroscopy, bacteria

Laboratory: Institute of Analytical Sciences (ISA), UMR 5280– Université de Lyon, UCBL, CNRS

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Scientific content: Today, approximately 6 million people die each year from bacterial infections[1]. Death incidences result both from a considerable downshift in the discovery of new antibiotics and by the appearance of strains expressing multiple resistance to antibiotics caused by the selection pressure during antibiotherapy. Indeed, the current diagnosis methods require 24 hours before bacterial strain and resistance identification. Thus, during this very long delay, broad-spectrum antibiotherapy are often necessary for critical patients. We recently reported a unique 45-minutes mass spectrometry (MS)-based analytical method, called Scout-MRM, allowing the quantification a large panel of proteins involved in antibiotic resistance mechanisms of bacteria[2]. In addition, the identification of toxin proteins secreted by infectious germs can be a very important indicator in the case of bacterial diagnosis. Moreover, characterization of post-translational modifications is important to understand the defense mechanisms of bacteria. However, these indications are difficult to obtain due to the sample complexity.

In order to improve the detection specificity for minor proteins, we make use of an experimental setup coupling MS and laser induced dissociation (LID) in the visible range (473 nm). This association adds a stringent optical specificity to the mass selectivity[3]. Since proteins do not naturally absorb in the visible range, this new methodological approach relies on the chemical derivatization of specific proteins with a chromophore. Thus, only the targeted ions that absorb at the laser wavelength are specifically fragmented, increasing detection sensitivity.

Research project: The first part of the PhD project will be devoted to the characterization of post-translational changes in two-component systems (TCS). Phosphorylation of aspartic acid residues are involved in regulatory mechanisms of bacteria. However, the characterization of these changes, especially by mass spectrometry, is difficult because phosphoaspartate (pD) groups are intrinsically labile. The proposed strategy is therefore to derive phosphorylated proteins from bacteria with a Dabcyl chromophore containing hydroxylamine function in order to stabilize phosphorylated aspartic acid residues[4] and induce a specificity for LID (Figure 1). After enzymatic digestion, the TCS protein-reporting peptides containing a pD residue will be identified and quantified by LID-MS/MS in targeted analysis. The characterization of these phosphorylations will lead to a better understanding of the signaling mechanisms of two-component systems in response to environmental changes. In addition, different levels of phosphorylations may be correlated with the resistance and/or virulence of different strains of bacteria.

1. Santajit, S.; Indrawattana, N., *BioMed Research International*, **2016**, 8
2. Rougemont B *et al., Anal Chem* **2017**, 89:1421–1426
3. M. Girod*, et al.*, *The Analyst,* **2014**, 139, 5523-5530
4. J.W. Chang *et al.*, *Angew Chem Int Ed*, **2018**, 57, 15712-15716

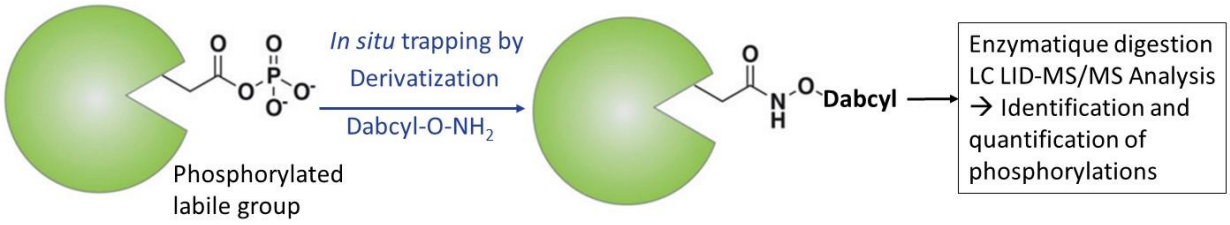

Figure 1: LID-MS/MS strategy for detection and quantification of phosphorylations after derivatization

The second aspect of the research project will consist in quantification of toxins in bacteria. Bacterial toxins are proteins secreted by infectious germs and have pathogenic effects. Analysis of these proteins within the bacteria remains difficult because of their very low concentration. We therefore plan to use the specificity of LID to improve the detection of these toxins, by reducing interfering signals. In this case, cysteine peptides of toxins derivatized with a Dabcyl maleimide chromophore will be specifically targeted by LID-MRM. The different levels of toxins detected may be correlated with the virulence of different strains of bacteria.

The PhD student will have to work during his thesis at the interface of analytical chemistry and biochemistry including:

- evaluation of photo-dissociation behavior of non-commercial chromophores (collaboration with chemistry laboratory - UMR 5182 - Ecole Normale Supérieure de Lyon)
- development of protocols for grafting of chromophores on peptides and preparation of bacteria samples
- implementation of laser dissociation in a new triple quadrupole mass spectrometer
- development of a proteomic global study on a cohort of clinical samples

Lab and environment: The project is funded by the ANR and the RHU Grants awarded to Jérôme Lemoine in 2020, in collaboration with the Hospices Civils de Lyon. The laboratory has all the necessary equipment to carry out the project. Different strains of bacteria from blood cultures are available in the laboratory.

Required Background: We are looking for a highly motivated person with a strong background in analytical chemistry or biochemistry (Master degree). Experience in mass spectrometry and/or proteomics will be appreciated. A good motivation to learn, autonomy, communication skills, curiosity, and agreeable team spirit are also among important qualities.

How to apply: Interested persons are requested to send a detailed CV with a covering letter and the marks and rank obtained in Master 2. Letter of recommendation or contact to a person who can provide it would be a welcome addition.

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