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Sorbonne Université/ China Scholarship Council program 2020

Thesis proposal

Title of the research project: **Chemical diversity and biology of non-ribosomal peptide synthetase (NRPS)-pathway derived alkaloids in bacteria**

Keywords: Natural product chemistry, chemical biology, NRPS, biosynthesis, bacteria

Joint supervision: yes (Dr. Sébastien Prévost, Ecole Polytechnique)

Joint PhD (cotutelle): no

Thesis supervisor: Yanyan Li

Email address of the thesis supervisor: yanyanli@mnhn.fr

Institution: National Museum of Natural History (MNHN), CNRS

Doctoral school (N°+name): ED227 MNHN-SU « Science of Nature and Man: Evolution and Ecology »

Research laboratory: Molecules of Communication and Adaptation of Microorganisms (MCAM), UMR 7245 CNRS-MNHN

Address of the laboratory: CP54, 57 rue Cuvier, 75005 Paris

Name of the laboratory director: Prof. Philippe Grellier

Email address of the laboratory director: grellier@mnhn.fr

Subject description (2 pages max):

1) Study context

Secondary or specialized metabolism in bacteria typically follow a diversity-oriented biosynthetic logic [1], in order to fulfil different functions according under specific environmental conditions. Remarkable examples of diversity-oriented biosynthesis are found in non-ribosomal peptide

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synthetase (NRPS) pathways [2]. These modular mega-enzymes machineries are responsible for the biosynthesis of many bioactive compounds in bacteria. Recently, the pyrrolizidine alkaloid (PA) pathways in bacteria have been discovered, which involve typically an NRPS and a monoxygenase together with other accessory enzymes. The PA pathways can lead to diverse azabicycles as well as cyclocarbamate structures. We recently identified unusual PAs that were found to attenuate the virulence of the producing bacteria while interacting with the host [3]. However the molecular mechanisms underlining structural diversification in the PA pathways and their biological activity remain unknown. The objectives of this four-year thesis proposal is 1) to explore the natural diversity of PA pathways in bacteria, both pathogenic and environmental; 2) to understand their biosynthetic mechanism in view to combinatorial biosynthesis; and 3) to understand their biological functions in a chosen human pathogen.

2) Details of the proposal

Programme and methodology

2.1 Understanding the chemical diversity of PA pathways in bacteria (0-24 months)

Guided by genome mining approaches, bacterial strains with PA biosynthetic gene clusters (BGCs) with unusual features will be selected (Actinobacteria or Proteobacteria). The targeted BGCs will be inactivated and comparison of metabolic profiles between the mutant and the wildtype strain will be performed, in order to identify the PA compound. The new molecule will subsequently subject to purification and structural elucidation by standard natural product chemistry methods.

2.2 Understanding PA biosynthesis towards combinatorial biosynthesis (0-42 months)

Using the PA pathway currently studied in the laboratory [3] as a model, key biosynthetic enzymes including the NRPS and the monooxygenase will be produced recombinantly in *Escherichia coli* for biochemical characterization *in vitro*. Their proposed substrates will be synthesized under the supervision of Dr. S. Prévost (Ecole Polytechnique). Mechanistic aspects of these enzymes will be probed by site-directed mutagenesis and kinetic determination. Interesting candidates of the accessory enzymes will also be characterized biochemically. Based on the obtaining results, genetic engineering in vivo will be performed in order to generate hybrid PA pathways that would lead to new compounds.

2.3 Understanding the PA biological function in a human pathogen (30-45 months)

Natural PAs identified in task 2.1 and PA analogs generated in task 2.2 will be tested as anti-virulence agents in the human pathogen *Pseudomonas aeruginosa*. For those displaying such effect, whole-genome transcriptomes will be analyzed and compared under conditions with or without the

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compound. This is to identify potential target of the molecule. Moreover, their antibacterial, antifungal and cytotoxicity will be assayed, in order to identify new activities.

2.4 Thesis writing and defense (44-48 months)

3) References (publication from the host lab is highlighted)

1. Fischbach MA, Clardy J. (**2007**) *Nat. Chem. Biol.* 3, 353-355.

2. Payne JA, Schoppet M, Hansen MH, Cryle MJ (**2016**) *Mol. Biosyst.* 13, 9-22.

3. Hong Z, Bolard A, Giraud C, Prévost S, Genta-Jouve G, Deregnaucourt C, Häussler S, Jeannot K, Li Y (**2019**) *Angew. Chem. Int. Ed.* 58, 3178-3182.

4°) Profile of the Applicant (skills/diploma...)

We are looking for a highly motivated, dynamic candidate holding a master degree in any of the following disciplines: natural product chemistry, chemical biology, biotechnology, enzymology, microbiology and/or analytical chemistry, to work on this multidisciplinary project at the interface of chemistry and biology. Candidates with a background/experience in chemical synthesis or natural product biosynthesis are considered advantageous, however these skills are not mandatory to apply.

Contacts:

Thesis supervisor

Dr. Yanyan Li

Email address of the thesis supervisor: yanyanli@mnhn.fr