Internship Offer

TEAM :
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PROJECT TITLE :
Biosynthesis of new pyrrolamide antibiotics by synthetic biology

PROJECT SUMMARY :

Natural products are widely used in clinics, especially as antibiotics and anti-tumor agents. Because of the increasing threats posed by multi or extremely drug resistant pathogens, an active search for new microbial natural products with high pharmaceutical potential is conducted in laboratories worldwide, based on newly developed methods (genome mining) and technologies (automated dereplication, HTS…). In parallel, new approaches are being developed to synthesize new "unnatural natural products". Two main strategies are explored: combinatorial biosynthesis and mutasynthesis. Combinatorial biosynthesis consists in the combination of genes involved in the biosynthesis of various natural products to design new biosynthetic pathways and thus produce new natural product analogues. Mutasynthesis is based on the feeding of microbial strains blocked in the biosynthesis of an intermediate with chemically synthesized intermediate analogues, resulting in “unnatural natural products”. Such approaches already resulted in the synthesis of aminocoumarine derivatives with antibacterial activities better than the ones of the antibiotic novobiocine, used in clinics.

The Molecular Microbiology of Actinomycetes team develops an approach based on combinatorial biosynthesis and mutasynthesis for the synthesis of new bioactive molecules of the pyrrolamide family. Pyrrolamides constitute a family of specialized metabolites produced by *Streptomyces* bacteria. These molecules bind non-covalently to the DNA minor groove with some sequence specificity (4 A/T bases). This confers on them numerous biological activities, such as antibacterial, antiviral or antitumor activities, but also renders them to cytotoxic for clinical usage. Our team has already elucidated the biosynthetic pathways of several pyrrolamides (congocidine, distamycin, anthelvencin) (Figure 1) and has undertaken the characterization of several others (pyrronamycins, amidinomycine). We have also constructed and verified the first gene cassettes for the production of pyrrolamide precursors and for the assembly of these precursors.

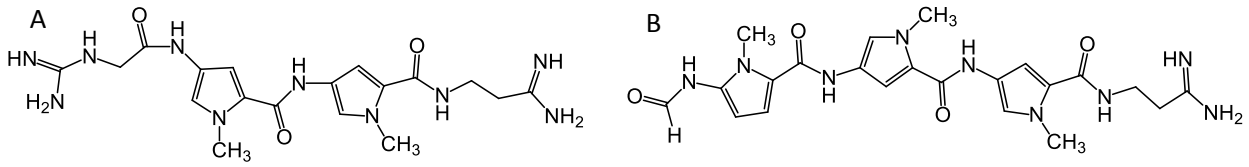

Figure 1: Structure de la congocidine (A) et de la distamycine (B)

The project will mainly aim at constructing new gene cassettes for the biosynthesis of pyrrolamide precursors, precursor assembly, pyrrolamide tailoring and resistance using a standardized approach. These cassettes will next be used to explore the factors (protein/protein interactions; substrate specificity…) that may affect the success of combinatorial biosynthesis approaches. This project will allow the intern to acquire skills in molecular biology (PCR, cloning using various techniques), microbiology (cultures, transformations, conjugation of bacterial strains) and analytical chemistry (flash chromatography, SPE, HPLC) for the purification and characterization of bacterial

metabolites. The structural characterization of new metabolites will be carry out in collaboration with chemists from the ICSN (Jamal Ouazzani team) or of the University Paris-Descartes. This project constitutes an opportunity to work on a multi-faceted project allying molecular microbiology, synthetic biology, and analytical chemistry.

KEYWORDS : Natural products, synthetic biology, combinatorial biosynthesis, antibiotics, *Streptomyces* bacteria

PUBLICATION ON THE SUBJECT

1 Vingadassalon A, Lorieux F et al. Natural combinatorial biosynthesis involving two clusters for the synthesis of three pyrrolamides in Streptomyces netropsis. **ACS Chem Biol**, 2015, **10**, 601-610.

2 Aigle B, Lautru S, et al. Genome mining of Streptomyces ambofaciens. **J Ind Microbiol Biotechnol**, 2014, **41**, 251-263

3 Lautru S., Song L., et al. A Sweet Origin for the Key Congocidine Precursor 4-Acetamidopyrrole-2-carboxylate **Angewandte Chemie Int. Ed.**, 2012, **51**, 7454-7458

4 Juguet M, Lautru S, et al. An iterative nonribosomal peptide synthetase assembles the pyrrole-amide antibiotic congocidine (netropsin) in *Streptomyces ambofaciens*. **Chemistry and Biology**, 2009, **16**, 421-431.