PhD proposal - CEA fellowship starting October 2024
Michael Lafond (BBF-INRAE Marseille) - Victor Duarte (CBM-CEA Grenoble)

Biosynthesis and functional evaluation of novel antimicrobial peptides from mammalian gut microbiomes

Context of the project

Since the discovery of penicillin, humans have widely developed and used antibiotics to prevent microbial infections. However, the intensive and sometimes unjustified use of these compounds has led to the emergence of pathogens resistant or multi-resistant to all classes of antibiotics. In 2016, J. O'Neill reported that, if no action is taken, the number of deaths due to antimicrobial resistance could reach 10 million a year by 2050. This major public health threat is prompting scientists to find new molecules, ideally natural ones, with different structures and modes of action to counter resistance phenomena evolutionary developed. A promising alternative is antimicrobial peptides for which some of them are currently in clinical development or have been approved.

In this context, bacteria are a treasure trove of multiple classes of naturally occurring antimicrobial peptides, more commonly known as bacteriocins. One example is the ribosomally synthesized and post-translationally modified peptides (RiPPs). Of peptidic nature, their ribosomal synthesis differentiates them from conventional antibiotics. An advantage of these peptides is that they have an activity spectrum often directed against species in the same ecological niche as the producing bacteria. Numerous studies show that the intestinal microbiome, plays a very important role in the health of the host. For example, the barrier effect implemented by commensal bacteria is achieved through the production of antimicrobial molecules including bacteriocins.

PhD proposal

Our past and current work on the antimicrobial peptides focuses on the bacterium *Ruminococcus gnavus* present in the digestive tract of about 90% of the population. This strain produces five compounds, the Ruminococcins C (RumC1 to C5) belonging to the sactipeptide family. The results obtained with RumC isoforms are very encouraging since these peptides are active at micro-molar concentrations on Gram-positive pathogens, including resistant and multi-resistant strains. Moreover, we reported that the most active isoform (RumC1) is also effective in vivo to protect mice from *Clostridium perfringens* infection at a lower dose than the control antibiotic.

We recently explored the tremendous diversity in four mammalian gut microbiome environments to discover RumC-like natural orthologues by using metagenomic approaches (Collaboration Dr. Gabrielle Potocki-Veronese, Toulouse Biotechnology Institute). A BLAST search, of more than 68 million genes retrieved from the human, mouse, pig and bovine gut metagenomes, against the five RumC sequences, allowed us to select ten novel RumC-like sequences from different species. Importantly, these sequences were selected based on the presence of crucial motifs that we know to be essential for the antibacterial activity of RumC peptides.

Similarly, to the RumC bacteriocins, the biosynthesis of the new RumC-like peptides involves modifying enzymes that belong to the radical-SAM family. As reported for the RumC isoforms, the biologically active peptides will be produced heterologously in *E. coli* using an optimized expression protocol. The biochemical characterization of both the peptides and maturase enzymes will be conducted with various techniques such as site directed mutagenesis, mass spectrometry and UV-vis spectroscopy. The structural studies will be addressed by using NMR and X-ray crystallography. An important part of the project is also devoted to the biological activity of these compounds on resistant and multi-resistant strains. Finally, the mode of action as well as the safety will be investigated for the most efficient peptides.

In order to offer the student the broadest possible training, she/he will carry out all the experiments from the production to the biological studies. The CEA Grenoble and the CBM lab offer the entire scientific and technical environment to carry out this project. The biological evaluation and the mode of action of the peptides will be addressed in collaboration with the Fungal Biodiversity and Biotechnology Laboratory at the INRAE Marseille.

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Skills required

A background in biochemistry, with good knowledge of recombinant protein production and purification techniques, is an asset. Knowledge on microbiology and an interest in mass spectrometry and structural biology are desirable.

Funding

CEA fellowship already obtained, starting in October 2024.

Contact

Please send a CV and a cover letter to:
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Publications of the teams on the topic

1- Mechanistic and functional aspects of the Ruminococcin C sactipeptide isoforms. Shamseddine L, et al., iScience, 2023, 26, 107563. https://doi.org/10.1016/j.isci.2023.107563.

2- Peptide and Protein Engineering for Biotechnological and Therapeutic Applications. Chapter 3: Antimicrobial Ribosomally Synthesized and Post-Translationally Modified Peptides as a Source of Alternatives to Antibiotics: A Focus on the Sactipeptides and Ranthipeptides Subclasses. Roblin C, et al., World Scientific Publishing Co., 2023, 57 – 114. https://doi.org/10.1142/9789811261664_0003

3-The Multifunctional Sactipeptide Ruminococcin C1 Displays Potent Antibacterial Activity In Vivo as Well as Other Beneficial Properties for Human Health. Roblin C, et al., International Journal of Molecular Sciences. 2021; 22(6):3253. https://doi.org/10.3390/ijms22063253.

4- The unusual structure of Ruminococcin C1 antimicrobial peptide confers clinical properties. Roblin C, et al., Proc. Natl. Acad. Sci., 2020, doi/10.1073/pnas.2004045117.

5- Ruminococcin C, a promising antibiotic produced by a human gut symbiont. Chiumento S, et al., Science Advances, 2019 Sep 25;5(9):eaaw9969. doi: 10.1126/sciadv.aaw9969. eCollection 2019 Sep.