

## **Development of fluorescent biomarkers in *Bacillus cereus* to better predict the impact of perturbations on its resistance and virulence in dairy products.**

### Context

This work is part of the FluoPath project funded by the French National Research Agency (ANR) for the period April 2024-March 2028. This project brings together 7 partners, including 4 academic research laboratories (UMR PAM, UMR SECALIM, UMR SQPOV and LUBEM), 2 agro-industrial technical institutes (AERIAL and ADRIA) and the dairy interprofession (CNIEL).

The thesis will be carried out in the academic laboratories of the UMR SQPOV (Avignon) and LUBEM (UBO, Quimper), according to a timetable that does not require the student to travel regularly between the 2 cities due to their geographical distance (travel expenses will be covered by the host laboratories). The 3-year thesis will start in October 2024 and end in September 2027.

The main objective of the FluoPath project is to identify new biomarkers (promoters that induce the expression of genes of interest) coupled to a fluorescent biosensor in order to gain new insights into the physiological state of two pathogens (*Listeria monocytogenes* and *Bacillus cereus*) in dairy environments (milk, diluted model cheese and, if possible, solid model cheese) in relation to the impact of technological perturbations. Ultimately, this knowledge, combined with that already available in the scientific literature, will be used to improve predictive models for microbiological risk in dairy products.

This thesis focuses on the bacteria of the *B. cereus* group, ubiquitous spore-forming Gram+ bacteria in the environment distributed in different phylogenetic groups<sup>1</sup>. They are likely to contaminate all raw materials and foods within the supply chain and production processing, and can alter their organoleptic qualities, their shelf life, or even represent a health risk. Indeed, certain strains are responsible for foodborne toxic infections resulting in abdominal pain and diarrhea (enteropathogenic strains), symptoms to which are added vomiting and nausea (emetic strains) due to the emetic toxin, the cereulide, synthesized by *B. cereus* group III in food and widely contaminating meat, eggs, milk and starchy foods.

The objective of this thesis will be: 1) To evaluate, by RT-qPCR, the expression of potential known biomarkers linked to toxin production, virulence or sporulation in emetic strains. 2) To identify by RNA seq new biomarkers for less studied phenomena (for example, the latency phase<sup>2</sup>). 3) To construct a collection of fluorescent mutants (transcriptional fusions between the biomarkers of interest and the gene encoding the Green Fluorescent Protein). These mutants will be exposed to various stresses in a milk or even cheese environment and their performance will be characterized by flow cytometry and fluorescence microscopy.

### **Approaches implemented:**

Microbial genetics and physiology for the identification of candidate genes and the construction of mutant strains. Fluorescence microscopy and flow cytometry for studying the behavior of mutants at the single-cell level.

### **Teams:**

**SporAlim** within the SQPOV (Safety and Quality of Plant-Based Products) unit. SQPOV is a UMR made up of 70 agents on average (permanent and non-permanent), with 2 supervisions (Avignon University and INRAE), hosted by INRAE and classified as a restrictive regime zone (ZRR). (<https://www6.paca.inrae.fr/sqpv/Equipes-Personnes/SporAlim>). The doctoral student will be required to handle class 2 pathogenic bacteria.

**LUBEM** within the University of Western Brittany (University Laboratory of Biodiversity and Microbial Ecology). LUBEM is a research unit made up of 50 agents on average (permanent and non-permanent), from the University of Western Brittany. LUBEM is also a unit under INRAe contract (<https://www.univ-brest.fr/lubem/>).

### **Obligation of neutrality and principle of secularism**

Public service employees, both tenured and contractual, as well as interns, whether or not they are in contact with users, must respect the obligation of neutrality. While carrying out their duties, they must not express their opinions, by how they behave or by what they wear, whether religious or political, both with respect to the public and to their colleagues, nor affirm their preference for a religion.

**Supervision and contact:** Sandrine PONCET (CR INRAE) sandrine.poncet-mouturat@inrae.fr. Noémie DESRIAC (Mdc UBO) [noemie.desriac@univ-brest.fr](mailto:noemie.desriac@univ-brest.fr). Weekly and fixed monitoring will be put in place from the start of the thesis. The training of the doctoral student will respect the rules set by ED 536 of Avignon University.

The ANR FluoPath project provides funding for the doctoral student. The scientific, material and financial resources for carrying out the research project are put in place in the host units and teams. The SporAlim team has very well-equipped laboratories for aerobic and anaerobic microbiology (including reactors), a laboratory for handling class 2 pathogenic germs, a laboratory for molecular biology and biochemistry, as well as an epifluorescence microscope coupled to an analysis system of images. Flow cytometry tests will be carried out at LUBEM. The doctoral student will have access to a training by qualified personnel and the IT and bibliographic resources of the UMR SQPOV and LUBEM.

### **Training and skills sought**

The candidate will have a Master 2 in microbiology or an equivalent diploma (engineering school).

He/she must have solid training in molecular biology, biochemistry and microbial genetics. Fluency in French will be appreciated and a level in advanced scientific English is expected.

**Skills sought:** curious, diligent, ability to set up and carry out well-designed experiments, good presentation skills, ability to work in a team.

Application deadline (at 11:59 p.m.): July 15, 2024

<sup>1</sup> Guinebretière, M.-H. *et al.* Ecological diversification in the *Bacillus cereus* Group. *Environ Microbiol* **10**, 851–865 (2008) doi: 10.1111/j.1462-2920.2007.01495.x .

<sup>2</sup> François M, Carlin F, Broussolle V, Nguyen-Thé C. *Bacillus cereus cshA* Is Expressed during the Lag Phase of Growth and Serves as a Potential Marker of Early Adaptation to Low Temperature and pH. *Appl Environ Microbiol.* 2019 Jul 1;85(14):e00486-19. doi: 10.1128/AEM.00486-19. PMID: 31076436; PMCID: PMC6606889.