Development of fluorescent biomarkers in *Listeria monocytogenes* to better predict the impact of food processing on its survival and virulence in dairy products

Description of the subject

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 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 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 available in the scientific literature, will be used to improve predictive models for microbiological risk in dairy products.

The thesis will focus on the pathogen *L. monocytogenes* and will be carried out in the academic laboratories of the UMR PAM (Dijon) and the UMR SECALIM (Nantes), according to a schedule 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 thesis will be structured around 4 axes

Axis 1: Selection of two out of six strains of *L. monocytogenes* and two relevant stress conditions

The main objective of this axis will be to evaluate the resistance of six strains of *L. monocytogenes* to two stress conditions encountered in unit operations chains in the dairy industry. Statistical analysis of these phenotypes of interest will allow us to select, from these six strains, two with different levels of resistance. These strains will be used throughout the thesis.

Axis 2: Identification of mRNAs specific to each phenotype of interest

The aim of this axis is to identify virulence or stress genes that are over-expressed when the two stress conditions are applied to the two strains selected in axis 1. The level of expression of the genes of interest, identified by a biobibliographical study, will be measured by RT-qPCR. In order to generate new fundamental knowledge, an unbiased transcriptomic analysis will be performed by RNAseq, combined with a bioinformatics analysis (ontology, identification of promoter sequences specifically involved in each phenotype, etc.).

Axis 3: Development of a fluorescent mutant bank by transcriptional (chromosomal) fusion

The genes of interest identified in Axis 2 will be considered as candidates for the construction of a mutant bank from the two previously selected strains (Axis 1). These mutants, which will be fluorescent due to the insertion of the gene encoding a fluorescent protein (GFP, RFP, CFP or YFP), will be used to generate new knowledge on the impact of stress conditions on the resistance and virulence of *L. monocytogenes*.

Axis 4: Quantification of mutant fluorescence under different stress conditions

In this area, we will evaluate the fluorescence emitted by mutants placed under previously established stress conditions. In particular, the experimental conditions will be optimized to avoid artifacts such as autofluorescence of the milk matrix.

Recent references on the topic:

den Besten, H. M. W., A. Amézquita, S. Bover-Cid, S. Dagnas, M. Ellouze, S. Guillou, G. Nychas, C. O'Mahony, F. Pérez-Rodriguez and J.-M. Membré 2018. Next generation of microbiological risk assessment: Potential of omics data for exposure assessment. *Int J Food Microbiol* **287**: 18-27.

Duqué, B., S. Rezé, A. Rossero, J.-M. Membré, S. Guillou and N. Haddad 2021. Quantification of *Campylobacter jejuni* gene expression after successive stresses mimicking poultry slaughtering steps. *Food Microbiol*: 103795.

Duqué, B., N. Haddad, A. Rossero, J.-M. Membré and S. Guillou 2019. Influence of cell history on the subsequent inactivation of *Campylobacter jejuni* during cold storage under modified atmosphere. *Food Microbiol* **84**: 103263.

Guillou, S. and J.-M. Membré 2019. Inactivation of *Listeria monocytogenes, Staphylococcus aureus*, and *Salmonella enterica* under high hydrostatic pressure: A quantitative analysis of existing literature data. *J Food Prot* **82**(10): 1802–1814.

Ignatova, M., B. Guével, E. Com, N. Haddad, A. Rossero, P. Bogard, H. Prévost and S. Guillou 2013. Two-dimensional fluorescence difference gel electrophoresis analysis of *Listeria monocytogenes* submitted to a redox shock. *J Proteomics* **79**(0): 13-27.

Ignatova, M., H. Prévost, I. Leguerinel and S. Guillou 2010. Growth and reducing capacity of *Listeria monocytogenes* under different initial redox potential. *J Appl Microbiol* **108**(1): 256-265.

Lang, E., Guyot, S., Alvarez-Martin, P., Perrier-Cornet, J. M. & Gervais, P. Caco-2 Invasion by *Cronobacter sakazakii* and *Salmonella enterica* Exposed to Drying and Heat Treatments in Dried State in Milk Powder. *Front Microbiol* **8**, 1893 (2017).

Lerasle, M., S. Guillou, H. Simonin, V. Anthoine, R. Chéret, M. Federighi and J. M. Membré 2014. Assessment of *Salmonella* and L*isteria monocytogenes* level in ready-to-cook poultry meat: Effect of various high pressure treatments and potassium lactate concentrations. International Journal of *Food Microbiol* **186**(0): 74-83.

Ragon, M. *et al.* The Yin-Yang of the Green Fluorescent Protein: Impact on *Saccharomyces cerevisiae* stress resistance. *J Photochem Photobiol B, Biol* 112603 (2023) doi:10.1016/j.jphotobiol.2022.112603.

Zoz, F. *et al. Listeria monocytogenes* ability to survive desiccation: Influence of serotype, origin, virulence, and genotype. *Int J Food Microbiol* **248**, 82–89 (2017).

Zoz, F. *et al.* Control of relative air humidity as a potential means to improve hygiene on surfaces: a preliminary approach with *Listeria monocytogenes*. *PLOS ONE* **11**, e0148418 (2016).

Zoz, F. *et al.* Management of *Listeria monocytogenes* on surfaces via relative air humidity: Key Role of cell envelope. *Foods* **10**, 2002 (2021).

Type of financing

Presentation of the host institutions and laboratories

UMR Food and Microbiological Processes (PAM)

The proposed work will be carried out alternately within the Microbiological and Biotechnological Processes (PMB) team of the UMR PAM and the UMR SecAlim. The UMR PAM is supervised by the Institut Agro Dijon and the Université de Bourgogne. The PMB team focuses its research on understanding the mechanisms of cellular responses to different types of environmental and technological perturbations. The knowledge gained from this research has given the PMB team a strong expertise in the decontamination of food matrices and technological surfaces, as well as in the fields of fluorescence microscopy and spectroscopy via the DimaCell platform.

Website: http://www.umr-pam.fr

UMR SecAlim

UMR SecAlim is under the supervision of Oniris VetAgroBIo and INRAE. The unit's mission is to generate and disseminate scientific knowledge and methods in the field of microbiological safety of food, in response to industrial challenges and societal demands in terms of public health and control of food losses.

Its research activities are aimed at the characterization and control of microbial risks (health and spoilage) in food. Molecular, classical and predictive microbiology methods are used to understand, quantify and model the behavior of microorganisms during food processing, at the level of the microbial ecosystem or at the level of model bacterial species. Improved microbiological risk assessment enables us to provide expert, innovative solutions to ensure the microbiological safety of food.

Website: https://secalim.angers-nantes.hub.inrae.fr/

Doctoral School

Doctoral School n° 554 "Environnements-Santé, Dijon-Besançon

Title of the doctorate

Agro-Food Biotechnologies

Country of doctorate

France

Institution awarding the doctorate

University of Burgundy Franche-Comté

Doctoral School

Doctoral School Environment-Health

Candidate Profile

Requirements: Motivated candidates will have a Master 2 Research degree or equivalent in Food Microbiology and must be enrolled in the Doctoral School no. 554 "Environnements-Santé" (Burgundy-Franche-Comté).

Knowledge and skills required: rapid adaptability and autonomy, ability to work in a group, ability to listen and share tasks, standard analyses in microbiology, molecular biology, environmental genomics, food microbiology, bioinformatics concepts, fluency in English, writing and organizational skills. The candidate will be required to work in a Biosafety Level 2 laboratory.

Deadline for applications

30/06/2024

Application documents

All applications must include

- A complete and detailed curriculum vitae, with particular reference to the level of experience and qualifications required

- Letter of motivation

- References or contact details of referees

- Master's degree certificate or results of the current Master's degree (if the Master's degree will be completed for the 2023-2024 academic year).

Contact information

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