LUE - PhD thesis: SynCom - Engineering of Synthetic Communities producing antibacterial biomolecules inhibiting the pathogenic bacterium *Listeria monocytogenes*

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1. Scientific program

The consumption of **food contaminated with pathogens is an important cause of morbidity and mortality worldwide**. Every year, approximately 600 million people – 1 in 10 people – get sick from foodborne pathogens, 420,000 of whom die. Human damage caused by foodborne pathogens results in colossal economic losses amounting to USD 110 billion due to lost productivity and health expenses (Borges et al., 2022; World Health Organization, 2015).

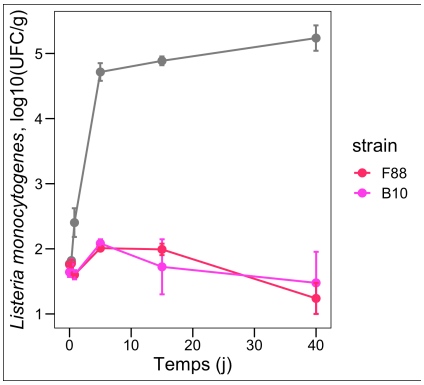

Figure 1: Inhibition of *L. monocytogenes* in camembert cheese by *C. maltaromaticum* F88 and B10. The pathogen and the protective strains were added to the milk prior cheese making at the pilot scale.

Bacteriocins are antimicrobial peptides of bacterial origin with a high potential for economic use in the agri-food sector. They are used in **biopreservation** applications to control the presence of undesirable microorganisms in the agri-food industry (Borges et al., 2022). **Lactic acid bacteria such as *Carnobacterium maltaromaticum*** are good candidates for such applications because the frequency of bacteriocin production is high in these bacteria and they benefit from a presumption of safety status making their use favourable (Afzal et al., 2010). In addition, *C. maltaromaticum* is a generalist species capable of colonizing a wide range of habitats (Iskandar et al., 2017), which makes it particularly suitable for biopreservation applications of diverse food products. LIBio has built up a collection of approximately 100 strains showing a high level of diversity, indicating that *C. maltaromaticum* is a species of choice for exploring functional diversity (Rahman et al., 2014; Ramia et al., 2018). Based on the use of a genetically engineered bioluminescent strain constructed by C. Riedel (Riedel et al., 2007), LIBio has developed a **high-throughput screening strategy of strains inhibiting *Listeria monocytogenes*** (El Kheir et al., 2018), **a food-borne pathogen of major concern** in the agri-food industry (Datta and Burall, 2018). This method was applied to the collection of *C. maltaromaticum* **to identify two strains (F88 and B10) with potent and robust anti-*L. monocytogenes* properties.** A mining strategy based on the combination of interference competition network and genome analyses allowed to delineate new Bacteriocin Gene Clusters (BGC) including one that encodes the synthesis of a **newly identified piscicolin 126.2** that could be **responsible for the potent anti-*L. monocytogenes* activity** of these two *C. maltaromaticum* strains (Gontijo et al., 2022). LIBio has shown that strains F88 and B10 are able to **efficiently inhibit the growth of *L. monocytogenes* in camembert and Saint-Nectaire cheese** (Figure 1). These remarkable properties have led to a **patent** (Borges and Revol-Junelles, 2021), which has very recently been **licensed to an industrial supplier** of food ingredients. However, as with most biopreservation technologies, the **effectiveness of these strains may vary depending on the cheese technology and the pathogen may persist at low levels in the food** (Figure 1).

The biopreservation technologies described in the literature are based on engineering approaches that **do not take into account the properties of the microbial communities** forming the microbiomes of fermented food products. Omics approaches have shown that depending on its structure, the **food microbiota can either improve or reduce the effectiveness of biopreservation** agents (Borges et al., 2022). However, **little is known about the ecological mechanisms responsible for these opposing effects**. Yet, understanding these mechanisms could allow the development of new biopreservation approaches based on the engineering of synthetic communities.

The objective of the project is to design a synthetic community engineering approach of microorganisms producing antimicrobial substances inhibiting the pathogenic bacterium *L. monocytogenes.*

2. Scientific environment

Laboratory of Biomolecules Engineering LIBio – University of Lorraine

The LIBio based at University of Lorraine has been studying the mode of action of bacteriocins in model systems as well as in the food matrix. LIBio has an expertise in the field of microbial competition in food systems. The research aims at understanding the role of the interactions in the structuration of microbial communities in food. Two main types of interactions are considered: (i) interactions between food microorganisms, with a special focus on competition mediated by bacteriocins, and (ii) interactions between bacteria and the food matrix. These issues are studied using complementary approaches of biophysics and microbiology including classical microbiology techniques, genomics, genetic engineering, metabarcoding and high throughput microbiological phenotyping. The final aim is to control food quality and safety. The coordinator of the French partner (Frédéric BORGES) has (co)authored 47 peer reviewed research publications and review articles with an H index of 20. He has (co)directed 10 PhD students and was involved in several national and international projects funded by ANR, ANRT, CARNOT-ICEEL, SATT-SAYENS, INTERREG. He coordinated 6 projects and is task leader and member of the management board in the ANR project "PATHOFOOD" where he is highly involved in the executive management of the project. He is co-leader of sub-Working Group Regulatory of the eCOST PIMENTO. As a co-inventor, he patented strains of *C. maltaromaticum* with anti-*L. monocytogenes* for biopreservation applications (national: FR19/11895 the 24/10/2019, international: PCT/EP2020/078966) for which an exclusive licence has been granted to the microbial culture industry. He is also an expert of the Specialized Expert Committee CES BIORISK at ANSES (2022-2026).

Institute of Microbiology and Biotechnology - University of Ulm (Germany)

The German co-applicant is affiliated to the Institute of Molecular Biology and Biotechnology of Prokaryotes at the University of Ulm. The group of Christian RIEDEL (coordinator of the German partners) has scientific expertise in all aspects of microbiology and genetic engineering of microorganisms relevant to the project. Research of the group is focused on pathogenic and commensal microorganisms of the human intestinal tract, their interaction with the host, and the application of the results of basic research into biotechnological production of health-related consumer goods. The group has expertise in identification and characterization of bacterial peptides including AMPs as well as the design, construction and implementation of sensor and reporter systems in bacteria. C. RIEDEL is a member of the editorial board of several scientific journals (e.g. *Applied and Environmental Microbiology*). Additionally, he has been a participating member of several national and international research projects funded by BMBF, H2020/BBI, ERA-IB, etc. and is coordinating two BMBF-funded research consortia (AMPLIFY, EXAMPLIFY). Until present, he has (co)authored 75 peer reviewed research publications and review articles, 3 book chapters and 4 patents with a h-index of 33 and total of +4200 citations. One of the patent applications filed recently is related to biotechnological production of bacteriocins (UK IPO Ref. No. 2105707.0). Also, an invention disclosure on technologies related to the sensor strain approach to identify, purify, and characterize novel bacteriocins to the technical transfer office of UULM (internal #: 40.041:0765).

3. Application file

The application file must include the following documents:

- Resume,
- Letter of motivation,
- Grades obtained for the degree conferring the Master's degree and copy of the diploma if available,
- Two letters of recommendation from the Head of the training course and the tutor of the end-of-studies internship,
- Tangible elements on the initiation to research (research paper, publication, ...).

Applications should be sent by e-mail to:
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Christian Riedel: christian.riedel@uni-ulm.de

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https://www.researchgate.net/profile/Christian-Riedel-7

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