

# Factors supporting persistence of *Listeria monocytogenes* in food processing environments

**FoodSafeR** is a 4-year Horizon Europe funded research project that started in October 2022.

The project aims to design, develop, and test the building blocks of an innovative proactive and holistic food safety warning and management system, which focuses on emerging of food safety hazards and associated risks.

**FoodSafeR** embodies integrated approaches to hazard characterisation and risk management in a comprehensive suite of future-oriented case studies, tools, methods, strategies, models, guidance, and training materials.

These resources are being made available in the **FoodSafeR Open Digital Hub**, a one-stop-shop platform uniting a community of professionals from the European and international food safety system.



This Project has Received funding from the European Union's Horizon Europe Research and Innovation Programme Under Grant Agreement No. **101060698** 



### Introduction

Listeria monocytogenes is a bacterium capable of surviving in food processing environments for long periods of time, posing the threat of product contamination and a risk to public health. This so-called persistence of food-borne pathogens presents an immense challenge to food producers, as persistent strains remain difficult to eradicate even using rigorous cleaning and disinfection strategies. Despite decades of research on phenotypic and genotypic traits associated with persistence, no clear patterns have arisen to adequately explain this phenomenon.

In the Horizon Europe Project FoodSafeR WP2 (Task 2.3), we aim to not only identify key patterns in the genome of L. monocytogenes that make it well-adapted to food processing facilities, but also draw upon concepts from the ecology and evolution of this organism toward improvement of future monitoring and management strategies. To do so, we combination of microbiological, apply а culture-independent, and bioinformatic approaches. The results of this work form the foundation of the "Persisters Database", which will be hosted as a resource on the FoodSafeR DigitalHub for use by professionals in the food safety sector.





## **The Team**

**The FoodSafeR** consortium of 19 leading academic, research and industry organisations from across Europe, is led by FFoQSI.

FFoQSI is the Austrian Competence Centre for Food and Food Quality, Safety and Innovation. It is a multidisciplinary joint research hub that encompasses scientific, national and international business partners food safety authorities and stakeholders, technology SMEs and startups from the food system.

**The FoodSafeR** Advisory Board of 25 leading stakeholders from the food safety field gives us a global reach.



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### **Materials and Methods**

We take a three-tiered approach toward understanding genetic and environmental factors that may underlie persistence, including:

(1) Meta-analysis of published studies to identify strain-type and gene-level patterns of persistence.

(2) Analysis of gene-level patterns across a database of whole genome sequences. Persistent strains are defined as identical isolates repeatedly sampled over at least two years with ≤20 whole genome SNP differences.

(3) Sampling food processing facilities across partner countries for L. monocytogenes isolates, co-occurring microbiota, and environmental data. Swab sampling and metadata collection (e.g., temperature, humidity) at three timepoints is ongoing in Austria (FFoQSI), Greece (AUA), and Spain (UBU). Isolates and the microbiome from swab samples will be sequenced and analyzed.

### **Summary**

(1) Meta-analysis of 45 published studies revealed only weak associations between specific genes and persistence. Specifically, disinfectant resistance genes and mobile genetic elements (MGEs) exhibit stronger links to persistence. This work highlights the need for a more robust definition for persistence across studies, as well as a deeper understanding of the context in which L. monocytogenes survives.

(2) Analysis of ~3000 genome sequences from Austrian food producers indicates that a high diversity of sequence types are capable of persistence, including ST4, ST8, ST9, and ST37, among others. Gene-specific patterns are being investigated, with emphasis on disinfectant resistance, MGEs, and persistence.

(3) Ongoing facility sampling demonstrates high variability in the prevalence of L. monocytogenes across facility types. Analysis of isolates from Austria in the context of historical data supports their classification as persistent strains, with less than 7 whole genome SNP differences identified across all genomes.

