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Abstract: Elucidating molecular mechanisms contributing to the prevalence of L. monocytogenes ST121 strains in food production environments

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Listeria (L.) monocytogenes is a gram-positive pathogen responsible for listeriosis, a rare but severe infection in humans and animals, which is acquired primarily through the consumption of contaminated food. A number of large recent listeriosis outbreaks worldwide e.g. in: Austria, Germany and the Czech Republic, the USA, Denmark, dramatically renewed the public interest in listeriosis including an amendment of the legal basis in Austria. Concomitantly, an increasing rate of listeriosis in several European countries, including Austria, has been reported in the recent years leading to an increased concern by the European Food Safety Authority. L. monocytogenes can survive and grow in multiple natural and man-made habitats; therefore prevention of the transmission of L. monocytogenes from the food processing environment to the final product is very challenging. It has been shown in many studies that some L. monocytogenes strains are able to persist for months or years in food processing plants. Studies on the molecular mechanisms of persistence are however still very rare.

Among a great diversity of L. monocytogenes strains isolated from food production, particularly strains belonging to sequence type (ST)121 are highly prevalent. The molecular reasons for the high abundance of ST121 strains are however currently unknown. Recently, we determined the genome sequences of two persistent L. monocytogenes ST121 strains and compared them with publicly available L. monocytogenes ST121 genomes. Our results show that the ST121 genomes are highly similar to each other and show a tremendously high degree of conservation in their prophages and particularly among their plasmids, suggesting that strong selective pressure is acting on these otherwise highly variable genetic elements. We hypothesize that plasmids and prophages are providing important adaptations for survival in food production environments. In addition, the ST121 genomes share common adaptations which might be related to their persistence such Tn6188, a transposon responsible for increased tolerance against quaternary ammonium compounds, a yet undescribed insertion harboring recombination hotspot (RHS) repeat proteins, which are most likely involved in competition against other bacteria.

In the proposed project we will analyze molecular mechanisms responsible for the high prevalence of L. monocytogenes ST121 strains on food production environments. The project consists of three main objectives: 1.) Sequencing additional ST121 genomes to verify the results from our recent genome analyses. 2.) Functional characterization of the RHS proteins of ST121 strains as they are possible mediators of competition against other bacteria, providing ST121 strains with important advantages. Here, it is planned to generate deletion mutants of the RHS genes, complementation as well as heterologous expression with the pBAD inducible vector system in E. coli. The mutant strains will be characterized phenotypically for survival and competition against other bacteria. 3.) Analyzing the influence of plasmids and prophages on the survival of ST121 strains in food production environments. For this, we first plan to perform

transcriptome sequencing of ST121 strains under stress conditions to analyze gene expression profiles with a particular focus on prophages and plasmids to analyze their contribution to survival. We will also cure ST121 strains from their plasmids and investigate phenotypic changes under various stress conditions using Biolog Phenotype MicroArrays.

The anticipated results of the proposed project will be of high relevance for food safety in general and for risk assessment in particular.