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Arginine deiminase pathway activity and nucleoside catabolism
in the fermented-meat starter culture *Lactobacillus sakei*

The industrial production of fermented sausages is mostly performed by the use of starter cultures. In this way the fermentation process can be controlled and shortened, and final fermented products with improved quality and safety can be obtained. *Lactobacillus sakei* is the most dominant lactic acid bacterium present in most artisan sausage fermentations and is, therefore, often applied as a starter culture for fermented sausage production.

This study dealt with the identification of several metabolic factors, encoded in the genome sequence of *L. sakei*, which could, at least partially, explain the superior dominance of *L. sakei* in meat fermentations. Although glucose is the preferential energy source for *L. sakei*, its concentration is rapidly depleted during meat fermentation. For this reason, a versatile use of energy sources other than glucose present in meat can improve the competitiveness and survival of *L. sakei* in the meat matrix. Therefore, this study focused on the capacity of *L. sakei* to grow on and convert arginine, nucleosides (inosine and adenosine), and glycerol, energy sources that are constantly released during fermentation and ripening of sausages.

All energy sources, except for glycerol, were consumed by several *L. sakei* strains tested in this study. Using a modelling approach, the conversion of arginine and nucleosides was described in detail for *L. sakei* CTC 494, a natural and highly competitive isolate from a Spanish spontaneously fermented sausage. The catabolism of both energy sources operated maximally under slightly acidic conditions, which are typical for sausage fermentations. The catabolism of nucleosides resulted in the production of a mixture of organic acids (lactic acid, acetic acid, and formic acid) and ethanol, while arginine conversion through the arginine deiminase (ADI) pathway resulted in the production of both citrulline and mainly ornithine.

The final ratio of citrulline to ornithine was modulated by environmental pH, with the highest production of ornithine under acidic conditions, thereby providing the strain with an energetic advantage. The genes coding for the ADI pathway in *L. sakei* CTC 494 were clustered in a single *arc* operon (*arcABCTDR*), showing the exact gene order as in *L. sakei* 23K. Moreover, differential expression of these genes was growth phase- and strain-dependent, suggesting a different role of the ADI pathway in different strains.

This study also unraveled the functional role of a previously uncharacterized gene, which is located downstream of the other genes of the *arc* operon in *L. sakei* CTC 494. This gene was shown to encode a citrulline/ornithine antiporter that is involved in the uptake of extracellular citrulline and thus contributes to the further conversion of citrulline into ornithine. Relative expression of this gene showed a different modulation as a function of environmental pH compared to the other genes of the *arc* operon. This differential modulation might result in an increased flexibility of the operation of the ADI pathway under stress conditions. Finally, this study identified the presence of an operational agmatine deiminase pathway for only a few strains of *L. sakei*, formerly annotated as a second *arc* operon. These *L. sakei* strains were thus able to convert extracellular agmatine into putrescine, both biogenic amines that are undesirable in meat.

The results obtained in this work contribute to the understanding of the adaptation mechanisms of *L. sakei* to the meat matrix, aiding its competitiveness and dominance in meat ecosystems. In particular, the contribution of the ADI pathway and the catabolism of nucleosides to the general competitiveness and adaptation of *L. sakei* were studied. The modelling approach allowed a quantitative description of these metabolic traits, which, when applied consistently, should allow for an objective comparison of various starter cultures and, hence, a rational choice for their commercial implementation in fermented sausage production.