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Spray Drying of Starter Cultures. Diverse Solutions within Lactococcus lactis to Improve Robustness

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Summary

Lactic acid bacteria (LAB) are widely employed in food fermentations because of their spoilage-preventing, texture-enhancing and flavor-forming characteristics. *Lactococcus lactis* is one of the most extensively applied LAB, specifically in dairy fermentations. Industrial food fermentation processes are typically initiated with the addition of starter cultures containing high concentrations of one or multiple LAB. To preserve starter cultures prior to application in food fermentation processes, drying or freezing techniques are used. Freezing and freeze drying are currently the most applied preservation methods. Spray drying is a more cost-effective and energy-efficient processes to preserve starter cultures, but generally results in a greater loss of viability due to exposure to heat and oxidative stress as compared with other preservation methods. The viability of starter cultures is essential for an adequate contribution to the fermentation process and end-product. Therefore, robustness, the ability to remain viable during harsh conditions, is an important characteristic of starter cultures.

In this thesis a high diversity of heat and oxidative stress survival in a collection of 39 *L. lactis* strains was established. This demonstrates the importance of selection of starter culture strains for their robustness characteristics besides their flavor-forming and acidifying properties. Moreover, the heat and oxidative stress survival was shown to predict survival during spray drying. Comparison of the genetic content of the robust and sensitive strains associated several genes with robustness towards heat and/or oxidative stress. Furthermore, it was demonstrated that fermentation conditions prior to exposure to heat or oxidative stress had a large impact on survival of *L. lactis* strain MG1363. Comparison of alterations in robustness phenotypes and genome-wide gene expression levels induced by various fermentation conditions revealed a transcriptome signature associated with robustness towards heat or oxidative stress survival. This included the *metC-cysK* operon, involved in sulfur amino acid metabolism. By culturing strain MG1363 in absence of cysteine, which induced the operon, a concomitant increase in oxidative stress survival was achieved, confirming the role of sulfur amino acid metabolism in robustness.

The transcriptome signature associated with robustness of MG1363 was compared with those of the three *L. lactis* strains IL1403, KF147 and SK11, revealing highly strain-specific strategies to enhance robustness. Also the effect of specific modifications in the fermentation conditions did not always result in similar alterations of robustness in all strains. This complicates the development of a general fermentation strategy for improved robustness of *L. lactis*. Nevertheless, a generic transcriptome signature for robustness of *L. lactis* was identified as well. This transcriptome signature could function as an indicator for robustness for the complete *L. lactis* species and might aid the selection of optimal fermentation conditions resulting in increased robustness during spray drying.

Repeated exposure to heat stress enabled isolation of robust derivatives of the spray drying-sensitive strain SK11. These derivatives displayed an increased robustness towards heat stress and spray drying, which remained stable after prolonged culturing. Transcriptome analysis of independently obtained robust derivatives revealed that distinct mechanisms for survival exist in SK11. Nevertheless, when comparing all derivatives with the original strain, differential regulation of genes involved in zinc transport was consistently observed.

Overall, it was demonstrated that through selection of an appropriate strain, selection of optimal fermentation conditions and/or isolation of robust derivatives robustness during spray drying can be increased dramatically, potentially unleashing this technology as an economically feasible alternative for the currently applied preservation technologies for *L. lactis* starter cultures.