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“Development of tools for precise genome engineering in *Lactococcus lactis*”

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Abstract

Strains of Lactic Acid Bacteria (LAB) have a broad range of applications in food industry, including manufacturing of cheese, sausages, and other fermented products. Due to their robustness and stress tolerance, LAB are also being explored as potential candidates for production of fine chemicals within a frame of several projects, including BacHBerry. The latter is focused on identification and production of novel high-value plant-borne polyphenolic compounds, such as flavonoids using bacterial cell factories. Many LAB strains are easily genetically accessible, as there exist efficient transformation protocols, as well as expression vectors, and classical tools for genome modification. Major drawbacks of currently available genome modification strategies are that they are time consuming, require several rounds of selection, and in most cases only a single locus can be targeted at a time. Recent studies were able to overcome these issues by using CRISPR/Cas (Clustered, Regularly Interspaced, Short Palindromic Repeats – CRISPR-associated proteins) and recombineering (recombination-mediated genetic engineering). The main goal of this project is to assemble a toolbox for rapid engineering of *Lactococcus lactis*, a well-studied species of LAB, which could be used for construction of efficient production strains. We plan to introduce and optimize a system for genome engineering of *L. lactis* in order to have a tool for rapid site-directed mutagenesis, as well as insertion and deletion of genetic elements. The most up-to-date results of the study would be discussed herein.