Development of tools for precise genome engineering in lactic acid bacteria

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Relevant activities

Our research group is a member of an EU-funded consortium called "BacHBerry" (Bacterial hosts for production of bioactive phenolics from berry fruits to products). The overall aim of this project is to develop a set of methodologies to investigate and explore the potential assortment of the bioactive phenolic compounds found in variety of berry species. Our part of the project aims at the engineering of lactic acid bacteria, such as *Lactococcus lactis*, for optimized production of the identified high-value compounds. My work, in particular, focuses on designing a set of tools for rapid genome engineering and protein expression.

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. Many LAB strains are easily genetically accessible, as there exist efficient transformation protocols, as well as expression vectors, and tools for genome modification. Major drawbacks of currently available genome modification strategies are 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). The system utilizes a set of short RNAs for directing Cas proteins to cleave foreign DNA. By modifying these RNAs, almost any DNA sequence within a cell's genome could potentially be targeted. DNA breaks activate repair mechanisms which in bacteria involve homologous recombination. Thus, co-transformation of the engineered CRISPR/Cas and synthetic oligonucleotides carrying a modified sequenced would result in replacement of the target genomic region with the desired modification. The aim of this project is to implement the CRISPR/Cas system in LAB in order to shorten the time and effort required for introduction of heterologous biosynthetic pathways into these organisms.