Development of tools for precise genome engineering and biosynthetic pathway construction in lactic acid bacteria

Dudnik, Alexey

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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 *L. lactis*, a well-studied species of LAB, and construction of efficient production strains. First 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. Moreover, we also aim at establishing a set of compatible vectors that would allow simultaneous and tuneable co-expression of complex biosynthetic pathways. Obtained materials would be further used for construction and optimization of platform strains for production of polyphenolic compounds.