ABSTRACT

Development of CRISPR-Cas9 based technology for genetic modification of *Lactococcus lactis subsp. cremoris*

Lactococcus lactis, a bacterium from the group of Lactic Acid Bacteria (LAB), is a widely used bacterium and has a property of lactic acid production from lactose. It is an important microorganism used in fermentation of cheese products, but it also became the first genetically modified microorganism used alive for therapeutic reasons. LAB are also common probiotics taken as a supplement in mild diarrhea. The aim of this study is to develop technology that allows to modify *Lactococcus lactis* using Clustered Regularly Interspaced Palindromic Repeats- Cas9 system, that will become faster, easier and relatively cheap tool for genetic engineering of this bacterium.

First part of the project is designed to test cells containing two plasmids, and how efficiently Cas9 expressed from one plasmid is cutting a targeted gene on another plasmid (also called plasmid curing). For this I implemented the erythromycin resistance gene and designed CRISPR-Cas9 system aimed to disable this gene and measured activity of Cas9 protein by growing cells with designed plasmids in medium with or without erythromycin and comparing their optical density.

The second part of the project was based on genetic modification of cell's chromosome using homologous recombination and applying CRISPR-Cas9 as a tool for eliminating cells which remained unchanged.

For both experiments I was using NIsin Controlled gene Expression system (Mo Bi Tech, 2008). Plasmid genes expression was induced by introduction of nisin into the growth medium.

Experiments showed some promising results although the genetic design of plasmids and the protocols of cell growth still require some changes and adjustments. The project was developed by the team of scientists in the laboratory of biotechnology in Jožef Stefan's Institute, Ljubljana, Slovenia (Berlec et al., 2017).