Institut "Jožef Stefan", Ljubljana, Slovenija

center za prenos tehnologij in inovacij
na Institutu "Jožef Stefan"

Licensing opportunity

Single plasmid systems for inducible dual protein expression and gene regulation in lactic acid bacterium *Lactococcus lactis* for microbial cell factories in industrial producing proteins

Field of use
Microbiology, molecular biology

Current state of technology
Stage of Development:
Available for demonstration

IPR status
Know-how

Publication
TBA

Developed by
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Reference
TBA

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Background

Tools for recombinant protein expression have been relatively well developed. *L. lactis* is therefore comparable to other well established bacterial expression systems, such as *Escherichia coli* and *Bacillus subtilis*. Advanced techniques for genetic engineering are required to develop *L. lactis* further as a microbial cell factory. Simultaneous expression of two or more proteins is beneficial for various applications, including the expression of multi-subunit proteins, the use of *L. lactis* as a mucosal delivery vehicle or as a multistep biocatalyst.

Description of the Invention

Here, plasmids for co-expression of two recombinant proteins in *L. lactis* have been developed and their effectiveness assessed by the expression of model proteins. Plasmids were further upgraded and a single plasmid CRISPR-Cas9 system has been developed. Duplication of the nisin promoter enabled the balanced, inducible expression of two model proteins in *L. lactis*, thus constituting a new tool for recombinant protein expression in this organism. A similar strategy resulted in a single plasmid CRISPR-Cas9 system that can be used, among other possible applications, for plasmid curing or CRISPRi-mediated gene regulation in *L. lactis*.
Plasmids will be applied in the future research in *L. lactis* for concomitant expression of therapeutic and reporter proteins, as well as for plasmid curing and gene silencing.

Main Advantages

- New tool for recombinant protein expression in *L. lactis* was developed.
- Duplication of the nisin promoter enabled the balanced, inducible expression of two model proteins in *L. lactis*.