Jožef Stefan Institute
Center for Technology Transfer and Innovation

TECHNOLOGY OFFER

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

Fields of use

Cellular and Molecular Biology, Enzyme Technology, Synthetic Biology, Microbiology, Bioprocesses, Industrial genetic engineering applications, Medical genetic engineering applications, Cellular and Molecular Biology, Enzymology, Protein Engineering, Fermentation, Drug delivery and other equipment

Current state of technology

Available for demonstration

Type of cooperation

Research cooperation agreement, Technical cooperation agreement

Intellectual property

Secret Know-how

Developed by

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More information about the invention

Summary

A Slovenian research organization has developed a new series of plasmids for advanced genetic modification of lactic acid bacterium Lactococcus lactis. The method is useful in the dairy industry as a cell factory and as a host for recombinant protein expression. Partners are sought in industry and academia in biotechnology for technical and research cooperation agreements.

Description of the invention

A Slovenian research organization has developed a new tool for recombinant protein expression and gene regulation in Lactococcus lactis - a food-grade lactic acid bacterium that is used in the dairy industry and as a cell factory and as a host for recombinant protein expression. The nisin-controlled inducible expression (NICE) system is frequently applied in L. lactis. Its advantages are generally recognized as safe (GRAS) status and absence of endotoxins. L. lactis was recently recognized as a probiotic and has been genetically engineered as a vector for the delivery of antigens and therapeutic proteins to the mucosal surfaces.

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.

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.

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Slovenian research group expertise is related to probiotic and other lactic acid bacteria and best described by the following three focal points and references:

- Genetically engineered lactic acid bacteria as vehicles for the delivery of therapeutic molecules to different mucosal surfaces – treatment of inflammatory bowel disease, binding the Shiga toxin, targeting cancer cells and delivering viral antigen for vaccination.
- Methods that facilitate the study of lactic acid bacteria - assessment of novel anchors for nonGMO surface display, in vivo imaging of lactic acid bacteria in mice, and first CRISPR-based tools for genomic engineering of Lactococcus lactis.
- Advanced delivery systems for probiotics – lactobacilli incorporated in viable nanofibers enabling the formation of dosage form for probiotics.

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.

Partner Sought

Partners sought are companies and academia related to the field of biotechnology, molecular biology and medicine. Partners are sought for:

- Technical cooperation: due to broad range application field of this kind of plasmid systems the invited partners interested in genome editing, gene regulation, production of next-generation antimicrobials, imaging and other applications.
- Research cooperation: future research in L. lactis for concomitant expression of therapeutic and reporter proteins, as well as for plasmid curing and gene silencing.