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- Introduction

The ability to digest lactose, that characterizes almost everyone during childhood, is often lost during adulthood. In fact, approximately 75% of the world's adult population is lactose intolerant, due to a reduction of the intestinal lactase level. Upon lactose ingestion, people who suffer from lactose intolerance experience gastrointestinal symptoms. To date, the only effective strategy to avoid these symptoms is to reduce the consumption of lactose-containing food and drink. Since milk and other dairy products contain calcium, proteins and vitamins, and since lactose can promote the absorption of minerals such as magnesium and zinc, the removal of dairy products from the diet can cause several kinds of health problems.

With the aim to transiently restore the ability to digest lactose, we decided to use the lactic acid bacterium *Lactococcus lactis*, as a vector for the delivery of functional galactosidases.

- Material and methods

Galactosidases used in this work were selected based on their activity at fixed pH and temperature, PCR-amplified from the genome of the respective organism and cloned into p-GEM vector in order to create a "stock" for future experiments.

Expression vectors based on the NICE system (NIsin Controlled gene Expression) were constructed using a ligase-based cloning method, the sequence of the galactosidases PCR-amplified from the p-GEM vectors mentioned above, and the sequences for the expression, secretion and membrane anchoring, PCR-amplified using different bacterial genomic sources as template.

Further experiments will include the electroporation of these vectors within *Lactococcus lactis* and the analysis of the galactosidase's secretion/anchorage and activity.

The MIVO system will be used to simulate the intestinal digestion and absorption of lactose and compare the results obtained with or without the expression of the exogenous galactosidases.

- Results

We selected four galactosidases, two from bacteria (*Bacillus licheniformis* and *Lactobacillus bulgaricus*) one from yeast (*Kluyveromyces lactis*) and the human *LCT* gene. Based on their optimal pH and temperature, these galactosidases are predicted to show a good activity within the human digestive tract, especially at small and large intestine level.

We constructed eight different expression vectors. Four of these vectors harbor a nisin-inducible promoter followed by the bacterial secretion signal EXP4 and the galactosidase sequence to allow the expression and secretion of the selected galactosidases. The other four, harbor the same promoter followed by the spUSP45 signal, the galactosidase sequence, and the CWA (cell wall anchoring) sequence, for the expression and anchoring of the galactosidases.

Seminari seguiti

"Le cardiomiopatie eredo-familiari: da meccanismi molecolari e cellulari alla terapia personalizzata" Relatore: Marina Carmosino (23/11/2018)

"Pathogenesis of juvenile idiopathic arthritis" Relatore: Rolando Cimaz (19/04/2019; videoconferenza GenoMec)

"European HBOC management for asymptomatic patient carrier of a BRCA mutation" Relatore: Christophe Cvordier (07/05/2019; videoconferenza GenoMec)

"From encoded combinatorial libraries to targeted therapeutics" Relatore: Dario Neri (17/05/2019; videoconferenza GenoMec)

"Droplet Digital PCR Scientific Conference2019" Relatore: conferenza Biorad (21/5/2019).

Pubblicazioni

DNA polymerases in the risk and prognosis of colorectal and pancreatic cancers.
Mutagenesis, 13 September 2019; DOI: 10.1093/mutage/gez031
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T-type calcium channels drive the proliferation of androgen-receptor negative prostate cancer cells. *Prostate,* 23 July 2019; doi: 10.1002/pros.23879
Roberto Silvestri, Perla Pucci, Erik Venalainen, Chrysanthi Matheou, Rebecca Mather, Stephen Chandler, Romina Aceto, Sushilaben Rigas, Yuzhuo Wang, Katja Rietdorf, Martin Bootman, Francesco Crea.