Introducing a CRISPR-Cas9 based prime editing system for precision mutagenesis in lactobacilli

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Introduction: The Lactobacillaceae are key players in different ecological habitats, like food fermentations or important in the healthy state of diverse human, plant, and animal niches. Contrary to the industrial and medical importance of lactobacilli, there is surprisingly little known about the genetic factors behind these beneficial effects. One reason for this can be found in the limited amount of tools available for genetic modification, especially precision mutagenesis.

Methods: Here, we employ a recent advancement on the classical CRISPR-Cas method, called 'prime editing'. Prime editing uses an nCas9-reverse transcriptase fusion protein, capable of selectively identifying a genetic target and replacing it with a desired mutation. The system will target two genes, the pili-related *spaC* gene and the nucleotide related *thyA* gene, resulting in easily screenable phenotypes. These genes will be knocked out with the introduction of in-frame stop codons.

Results: First, the nCas9-reverse transcriptase fusion gene was codon optimized for lactobacilli, and synthetically produced. The fusion gene was cloned into an expression vector for lactobacilli. As a proof of concept, this system will now be introduced in *Lacticaseibacillus rhamnosus* GG, the type strain of the genus and one of the best studied probiotics.

Discussion: After successful introduction in this type strain, the system will be introduced in other non-model strains isolated from food and the human vaginal niche in order to facilitate functional molecular studies.