

Insertion of *avPAL/ermB* Transposon in *Lactobacillus reuteri* 100-23C (pHENOMMenal)

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Abstract:

Phenylketonuria (PKU) is an autosomal recessive disease that prevents metabolic breakdown of L-phenylalanine into L-tyrosine. If left untreated, PKU can result in severe neurological defects. An effective treatment is a diet in which phenylalanine intake is restricted. However, compliance to the diet is difficult due to its unpleasant taste and cost of supplemental medical foods. A possible alternative therapy is the use of a genetically engineered probiotic that could degrade ingested phenylalanine in the gut prior to its absorption. Preliminary work using *Lactobacillus reuteri* 100-23C carrying a plasmid borne copy of the *avPAL* gene encoding a modified phenylalanine ammonia lyase (PAL) enzyme capable of metabolizing phenylalanine resulted in reduced blood phenylalanine levels in a mouse model of PKU when the bacterium and gene were delivered as a probiotic. Due to issues related to plasmid transfer/loss and antibiotic resistance, a chromosomally inserted copy of *avPAL* is desired in an effort to move forward to human therapeutic trials. To this end, *L. reuteri* 100-23C cells were grown in MRS broth for 48 hours and prepared for electroporation with a transposon encoded copy of *avPAL*. After electroporation, the *L. reuteri* 100-23C cells were allowed to recover and then transformants were selected by playing on media containing erythromycin. DNA was then extracted from the resultant colonies and quantified. Primers were designed to amplify the regions of the transposon containing *ermB* and *avPAL* genes, and DNA from clones of *L. reuteri* 100-23C were screened by PCR. Gel electrophoresis of PCR amplicons was used to detect successful insertion of *ermB* and *avPAL* genes in the DNA of isolated colonies. Three *L. reuteri* 100-23C clones were found to contain *avPAL* and *ermB*, indicating that erythromycin resistance was not spontaneous. Future efforts will determine functional expression of the *avPAL* enzyme and map the insertion sites.

Keywords:

Phenylketonuria (PKU), Phenylalanine, Probiotic, Polymerase chain reaction (PCR), Electroporation, Gel Electrophoresis, Transposon, Lactobacillus reuteri 100-23C