NEW PROPHAGE FROM AN INDUSTRIAL LACTOBACILLUS RHAMNOSUS STRAIN PRODUCING L-LACTIC ACID

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Introduction. Sequencing and annotation of industrial *Lactobacillus rhamnosus* strain used for highly efficient production of L-lactic acid revealed presence of unknown prophage DNA sequence inserted between rRNA_B and rRNA_C operons. This phenomenon has not been observed yet in the known strains of *L. rhamnosus*.

The objective of work was identification of prophage gene functions and the regulatory sequences controlling its lytic and lysogenic life cycle as well as other functions contributing to the stability of the strain in large scale L-lactic acid fermentation process.

Methods. Bacterial DNA from *Lactobacillus rhamnosus* strain was extracted using Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer instruction. The sequencing-ready libraries were prepared with Nextera® XT DNA Sample Preparation Kit for the Miseq illumina sequencing platform at Genomed S. A., Warsaw Poland. Manual primary annotation of DNA contigs were determined by comparing the predicted ORFs with the public protein sequence database using the BLASTX with the default parameter settings [1]. Sequence analysis was made with Vector NTI Express v.1.1.1 software "Life Technologies Corporation".

Results. Analysis of the DNA contig containing *L. rhamnosus* prophage revealed a genome size of 43,948 bp and a G+C content of 45.07% whereas host DNA contained 46.79% of G+C. Off the 68 identified open reading frames (ORFs), 46 shared homology with known bacteriophage genes. Another 20 ORFs were found to be hypothetical proteins and two were of bacterial origin. Bacterial attachment site was located at the tRNA-Leu gene of the rRNA_B operon. Prophage organization was not compatible with known phages of closely related organisms such as A2, ΦAT3, Lca1 and Lrm1 of *L. casei* and *L. rhamnosus* [2]. However, similarly arranged prophage genome has been found in the chromosome of *L. rahmnosus* Lc705, not sharing homology at the nucleotide level [3, 4]. Genetic switch with two outward oriented promoters as well as cI and Cro homologs were identified. The continuity of lytic module was broken due to the insertion of two genes of bacterial origin in an opposite orientation directly after prophage antirepressor protein. Most likely, this insertion inactivated bacteriophage lytic cycle making it stable in its lysogenic form. The phage genome contained integrase, three replication associated genes, small and large subunits of terminase, one protease gene, major capsid protein and two head-tail joining proteins, four tail building proteins, portal protein, one holin and two endolysin proteins. Additionally, genes encoding type I restriction/ modification system comprising of specific methylases and restriction endonuclease were also present.

Conclusions. Presence of two DNA methylases together with restriction endonuclease makes this strain resistant to any invading DNA, phage or plasmid, thus contributing to the strain stability in large scale fermentation.

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