DISTRIBUTION AND PROPERTIES OF LACTATE DEHYDROGENASE GENES IN AN INDUSTRIAL LACTOBACILLUS RHAMNOSUS STRAIN PRODUCING L-LACTIC ACID

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Introduction. Production of biodegradable polylactide (PLA) requires optically pure stereoizomers of lactic acid which may be economically produced by large scale fermentation. We have isolated and characterized new *Lactobacillus rhamnosus* strain synthesizing exclusively L-lactic acid with high sugar conversion rate and final concentration ranging of 150 g/l after 60 h of fermentation.

The aim of work was genomic and proteomic analysis of lactate dehydrogenase genes involved in lactic acid fermentation process and find reason why this strain is producing only L-lactate.

Methods. Bacterial DNA from *Lactobacillus rhamnosus* strain was extracted using Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland). 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. Obtained DNA contigs were analyzed for the presence of lactate dehydrogenase genes with Vector NTI Express v.1.1.1 software “Life Technologies Corporation”.

Results. Seven lactate dehydrogenase genes with different stereospecificity were found in the genome of *L. rhamnosus* strain. They were named according to the corresponding genes present in the type strain *L. rhamnosus* ATCC53103. Four genes were L-lactate specific, two D-lactate specific and one malate/D-lactate specific. Genetic analysis of their promoter regions revealed that five genes were monocistronic and possessed their own promoters. Genes *ldhL*_0684 and *ldhD*_2080 were the second in the operon structure and preceded only with the rbs site (table 1). Three genes *ldhL*_0585, *ldhL*_2266 *and* *ldhL*_2421 possessed putative promoters contained -10 promoter recognition site resembling canonical -10 extended sequence 5'-TGNTATAAT-3'.

In *Bacillus subtilis* and other gram positive bacteria, RNA polymerase with σ^A subunit recognizing vegetative promoters does not require presence of -35 region [1]. The -35 promoter regions of these genes were also close to the canonical sequence of vegetative promoters. Most likely these genes mainly contribute to the exclusive synthesis of L-lactate.

Table 1. Genes responsible for the synthesis of α-hydroxyacis in genome of *L. rhamnosus* and their transcription and translation signals.

Gene symbol	Putative promoter(s)	rbs
ldhD_0158	ttaaacgttctattttacgagcataat ctattttacgagcataatcttgtatact	aaggaga
ldhL_0585	ctgaaagcctacacctctgttacaat	aaggagggg
ldhL_0684*	acgataattccatttgtgaagccctatact	gaaaggaaa
mal/lac	ttgtcttaagattgcgcttccgctaaaat	gaggaaggg
ldhD_2080*	ttgactgtgtggctggaatcaggagaat	aaaggagaa
ldhL_2266	aagtgctacacttataagtgttctaat	aggagg
ldhL_2421	ttgtttcagtgatttgataatgtgttatact	aagaaagga

*promoter of the preceding gene forming operon structure with lactate dehydrogenase. -35 and -10 sites were underlined.

Gene *ldhD*_0158 is α-hydroxy-isocaproate specific dehydrogenase and is inactive in pyruvate reduction to D-lactate. Gene *ldhD*_2080 preceded by aromatic amino acid aminotransferase and followed with putative membrane protein in the three component operon is possibly involved in amino acid metabolism and does not contribute to the formation of D-lactate.

Conclusions. Stereospecificity of produced L-lactic acid mainly depends on the activity of *ldhL*_0585, *ldhL*_2266 *and* *ldhL*_2421 genes.

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References.

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