

Complete Genome Sequence of *Lactococcus lactis* IO-1, a Lactic Acid Bacterium That Utilizes Xylose and Produces High Levels of L-Lactic Acid

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We report the complete genome sequence of *Lactococcus lactis* IO-1 (= JCM7638). It is a nondairy lactic acid bacterium, produces nisin Z, ferments xylose, and produces predominantly L-lactic acid at high xylose concentrations. From ortholog analysis with other five *L. lactis* strains, IO-1 was identified as *L. lactis* subsp. *lactis*.

Lactococcus lactis IO-1 was isolated from water in the drain pit of a kitchen sink (7). It produces nisin Z (1, 2, 10), ferments xylose, and shows predominant production of L-lactic acid when grown at high xylose concentrations (8, 13), and D-lactic acid was not detected in its culture broth (6). IO-1 was reported to have two different pathways for xylose metabolism, the phosphoketolase (PK) pathway and the pentose phosphate (PP)/glycolytic pathway (13). The former produces 1 mol of lactate from 1 mol of xylose, whereas the latter yields 5 mol of lactate from 3 mol of xylose without carbon loss. Polylactate as a green plastic is a polymer of optically pure lactic acid. Therefore, the use of IO-1 in production is highly desirable, and its genome analysis is necessary for its genetic improvement.

The genome sequence was determined by a whole-genome shotgun strategy. We constructed 3-kb-insert genomic libraries and generated 30,270 sequences using ABI 3730xl (Life Technologies, Carlsbad, CA), giving 8.7-fold coverage from both ends of the genomic clones. Sequence reads were assembled with the Phred-Phrap-Consed program (3–5), and gaps were closed by direct sequencing of clones or PCR products that spanned gaps. The overall accuracy was estimated to have an error rate of less than one per 10,000 bases (Phrap score of ≥ 40). *In silico* Molecular Cloning, Genomic Edition (In Silico Biology, Inc., Yokohama, Japan), was used to predict open reading frames (ORFs) and for ortholog analysis with other five *L. lactis* strains (IL1403, KF147, CV56, MG1363, and SK11). BLAST search identified rRNA genes using the IL1403 genome as a query sequence. tRNAscan-SE (9) (version 1.23, using default setting) detected tRNA genes.

The complete genome of *L. lactis* IO-1 consists of a single, circular chromosome (2,421,471 bp, 35.1% GC content) which contains 2,233 predicted ORFs, 6 rRNA operons, and 65 tRNAs. There are two possible prophage sequences. This strain harbors no plasmid. From the ortholog analysis, IO-1 has high similarity to three *L. lactis* subsp. *lactis* strains, IL1403, KF147, and CV56 ($\geq 97\%$), while two *Lactococcus lactis* subsp. *cremoris* strains, MG1363 and SK11, show homology of $< 90\%$. This indicates that IO-1 belongs to *L. lactis* subsp. *lactis*. Genes involved in nisin Z production and resistance are clustered in the order *nisZBTCIP-RKFEG*, as in other nisin-producing strains (11). Genes involved in xylose utilization are also present: (i) a gene cluster (*xyIRABM-*

xynTB-xyLXT) for xylose transport and xylulose 5-phosphate production, (ii) *pfk*, *tkt*, *fbaA*, *tpi*, *gap*, and *ldh* genes for the PP/glycolytic pathway, and (iii) *ptk*, *ack*, *pta*, and *adh* genes for the PK pathway. Interestingly, no transaldolase gene was observed. Since its product converts sedoheptulose 7-phosphate (S7P) and glyceraldehyde 3-phosphate to erythrose 4-phosphate (E4P) and fructose 6-phosphate in the common PP/glycolytic pathway, IO-1 probably has an alternative PP/glycolytic pathway formed by 6-phosphofructokinase (*pfk* product) and fructose biphosphate aldolase (*fbaA* product), as Nakahigashi et al. showed in *Escherichia coli* (12). That is, the former phosphorylates S7P to sedoheptulose 1,7-bisphosphate (S1,7P), and the latter cleaves S1,7P to E4P and dihydroxyacetone phosphate.

Nucleotide sequence accession number. The complete genome of *L. lactis* subsp. *lactis* IO-1 has been deposited in DDBJ/EMBL/GenBank under the accession number [AP012281](https://www.ncbi.nlm.nih.gov/nuccore/AP012281).

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