The Gene Encoding the Glutamate Dehydrogenase in Lactococcus lactis Is Part of a Remnant Tn3 Transposon Carried by a Large Plasmid

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The gene responsible for the uncommon glutamate dehydrogenase (GDH) activity of Lactococcus lactis was identified and characterized. It encodes a GDH of family I that is mainly active in glutamate biosynthesis, is carried by a large plasmid, and is included, with functional cadmium resistance genes, in a remnant Tn3-like transposon.

Lactococcus lactis is a lactic acid bacterium widely used as a starter culture in the production of fermented dairy products. Although this bacterium is commonly found in milk, it is also naturally present in other environments.

Previously, we detected glutamate dehydrogenase (GDH) activity in some L. lactis strains isolated from plants and animals (21). This activity could be of interest in the dairy industry, since it simulated conversion of amino acids to aroma compounds by providing, from glutamate, α-ketoglutarate required for amino acid transamination that initiates the conversion. However, no gdh gene was identified in the two L. lactis genomes available (strain IL1403 [GenBank accession no. AE005176]) (4) and SK11 (Joint Genome Institute [http://genome.ornl.gov/microbial/lcre/]), suggesting that GDH activity in L. lactis is carried by a mobile genetic element.

Cloning and characterization of the gene responsible for GDH activity in L. lactis NCDO 1867 isolated from peas. A 1-kb fragment of the gdh gene of L. lactis NCDO 1867 was first amplified by PCR with degenerate oligonucleotides deduced from sequences of known bacterial GDHs (3F and 8R [Table 1]). This fragment was cloned into the cloning vector TOPO-XL (PCR cloning kit, Invitrogen) and sequenced. The oligonucleotides 24R/23R and 25F/26F (Table 1) were amplified by inverse PCR (Takara kit; Takara Shuzo Co., Japan) by using the oligonucleotides 24R/23R and 25F/26F (Table 1). Amplified fragments were purified (Qiagquick kit; QIAGEN) and sequenced. The gdh gene was then disrupted by a single crossover with the pGhost9 vector containing a 965-bp internal fragment of gdh (generated with oligonucleotides 58F and 1023R [Table 1]) as described by Maguin et al. (12). The gene disruption totally suppressed the GDH activity of the strain, indicating that this gene is the only gene responsible for GDH activity in L. lactis. Indeed, the NAD- and NADP-dependent activities in cell extracts of the wild-type strain NCDO 1867 determined as previously described (8) were 1.4 ± 0.3 and 5.7 ± 0.7 nmol min⁻¹ mg protein⁻¹, respectively, while in the gdh mutant TIL487, they were both equal to zero (0 and 0.2 ± 0.1 nmol min⁻¹ mg protein⁻¹).

The sequence surrounding gdh suggests that the gene is monocistronic (Fig. 1b). The gene encodes a 448-amino-acid protein, which is in agreement with the sizes of monomers of hexameric GDHs purified from other bacteria (1, 13). The protein sequence (Fig. 1c) exhibits the putative GDH active site, the NAD(P) coenzyme binding motif, and the three highly conserved domains characteristic of family I of hexameric GDHs (2). This classification was confirmed by the construction of a phylogenetic tree from the sequence alignments with known GDHs (Clustal W, version 1.8 [22]) (Fig. 2).

As are other GDHs of family I, GDH of L. lactis is more active in glutamate biosynthesis (mean activity ± standard deviation, 103 ± 14 nmol min⁻¹ mg protein⁻¹) than in glutamate catabolism (14 ± 2 nmol min⁻¹ mg protein⁻¹) according to the activity determined by a spectrophotometric method (7). Consequently, in addition to its role in glutamate catabolism (21), GDH of L. lactis plays a major role in glutamate biosynthesis, as do other GDHs of family I. Indeed, while the wild-type strain was capable of growing (mean growth rate ± standard deviation, 0.46 ± 0.07 h⁻¹) after a long latency phase (11 h) in a chemically defined medium (20) without glutamate or glutamine, no growth was observed for the gdh mutant strain.

TABLE 1. Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>3F..................5' TTY GAR CAR ATH TTY AAR AAY 3'</td>
<td></td>
</tr>
<tr>
<td>8R..................5' AAC AAT ACC TTG TGC NSW CAT 3'</td>
<td></td>
</tr>
<tr>
<td>13F..................5' TAA AGG CGG TAG TGA TTT 3'</td>
<td></td>
</tr>
<tr>
<td>22R..................5' GCA TCA GCA ACT TTC AAG 3'</td>
<td></td>
</tr>
<tr>
<td>58F..................5' AAC CAA CGA GTG TCT TGT TGA 3'</td>
<td></td>
</tr>
<tr>
<td>1023R..............5' A TTG GCC CCT GCA GCA ACC AC 3'</td>
<td></td>
</tr>
<tr>
<td>23R..................5' CAC CAA TAT CAC CAG CTG GCA CGT CAA 3'</td>
<td></td>
</tr>
<tr>
<td>24R..................5' AGC CAT TTA GAC CTT GGT ATT GAC CA 3'</td>
<td></td>
</tr>
<tr>
<td>25F..................5' GCT TGG TCG TTG CTT GAT GTA GAT CAA CG 3'</td>
<td></td>
</tr>
<tr>
<td>26F..................5' TTT AGG CGA CGA TTA TGT TGC GGG TG 3'</td>
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The location of \textit{gdh} on a large plasmid was demonstrated by the transfer of the \textit{gdh} gene by electroporation in a plasmid-free strain. Indeed, the plasmid DNA of the \textit{gdh}-negative mutant strain TIL487, which contains the pGhost9 (Ery\textsuperscript{r}) inserted in the \textit{gdh} gene, was used to transform the plasmid-free strain MG1363. The transformants (Ery\textsuperscript{r}) were verified for the presence of the \textit{gdh} gene disrupted with pGhost9 by PCR and for the presence of a plasmid of about 70 kb. The plasmid location of \textit{gdh} in \textit{L. lactis} is very original, since \textit{gdh} has always been found in the chromosomes of bacteria, particularly in \textit{Lactobacillus plantarum} strains, streptococci, and \textit{Listeria innocua} strains in which \textit{gdh} genes share the highest identities with the \textit{L. lactis} gene.

\textit{gdh} is part of a remnant transposon of the Tn3 family. The study of the genetic environment of \textit{gdh} revealed that it is part of a structure clearly related to a complex transposon of the Tn3 family (Fig. 1a). The putative transposon (6.5 kb) is delimited by two imperfect inverted repeats of 28 bp with a GGGG terminal sequence characteristic of Tn3 transposons (19). It also includes a resolvase of 197 amino acids (ORF5) that contains the invariant serine in the N-terminal part and the C-terminal DNA binding domain characteristic of Tn3 resolvase (19) and is similar to the resolvase of Tn552 of \textit{Staphylococcus aureus} (80% identity) (15). Moreover, we identified a putative resolution site sharing 70% identity with site I of Tn3 transposons (10, 15, 18). The putative transposon also

\texttt{FIG. 1.} (a) Schematic representation of the transposon of \textit{L. lactis} NCDO 1867 and its flanking regions (7,380 bp). Open reading frames 1 through 5 show high levels of identity with \textit{gdh}, transposase IS\textsubscript{1216}, cadmium resistance gene \textit{cadA}, cadmium resistance gene \textit{cadC}, and resolvase \textit{tnpR}, respectively. The resolution site (res site) is represented by a triangle. Inverted repeats at the left (IR\textsubscript{L}) or at the right (IR\textsubscript{R}) of the transposon are indicated by hachured triangles. The G+C contents of the transposon and flanking regions are indicated at the top of the figure. (b) Partial nucleotide sequence of the fragment containing the \textit{gdh} gene of \textit{L. lactis} NCDO 1867. In the upstream region, the two 10 extended consensus sequences are boxed and the ribosome binding site sequence is in a grey-tinted box. The ATG start codon and TAA stop codon are in bold, with the coding region designated by dots. Arrows show the potential terminator. (c) The deduced amino acid sequence of \textit{gdh} shows conserved hexameric family I GDH regions (in bold and in grey-tinted boxes), the GDH active site (in bold and italics), and the NAD or NADP binding site (in bold and underlined).
encodes two proteins of 105 and 705 amino acids highly similar to known cadmium resistance proteins. In particular, they are 100% identical to functional CadA and CadC found in other *L. lactis* plasmids (AF243383 and U78967) (11, 14), in a *Listeria innocua* plasmid (NC_003383), and in the chromosomes of *Listeria monocytogenes* (EAL08508.1) and *Streptococcus thermophilus* (AJ315964) (16). They also exhibited 70 and 54% identities with CadA and CadC carried by the Tn\(^3\) transposon in *L. monocytogenes* (L28104) (9). Finally, ORF2 of the putative transposon encodes a transposase of 200 amino acids which is different from and much shorter than the 1,000-amino-acid transposases (TnpA) usually carried by Tn\(^3\) transposons (3). This transposase shared 98% identity with IS1216, widespread in composite transposons of gram-positive bacteria. This suggests that the original Tn\(^3\) transposase has been deleted and IS1216 inserted and that the putative transposon is not functional. Indeed, we have no evidence that this element transposes. Firstly, we did not identify direct repeats, which are normally the result of Tn\(^3\) transposon insertion, at each end (3, 19). Secondly, attempts to transpose by mating procedures resulted in the transfer of the whole plasmid (data not shown). Due to its location on a mobilizable or conjugative plasmid, the initial Tn\(^3\) transposon may have evolved into an immobilized element.

The characteristics of the remnant transposon suggest that it originates from a bacterium other than *Lactococcus* sp. Indeed, transposons of the Tn\(^3\) family, as well as the *gdh* gene, have never been reported in *Lactococcus* sp., while they are frequently found in other gram-positive bacteria. The percentages of identity of *cadA/cadC* and *gdh* genes with homologous...
genes of other bacteria suggest that this transposon could originate from Streptococcus strains, which are often found in the same biotopes (16, 17).

Interestingly, this remnant transposon carries, in addition to heavy metal resistance genes which are usually carried by Tn7 transposons, a functional gdh gene. Generally, auxiliary genes of transposons confer a selective advantage to the host. The gdh gene confers to the strains the ability to biosynthesize glutamate from \( \alpha \)-ketoglutarate and ammonia and, therefore, the ability to assimilate ammonia (2). This capacity could be an advantage in certain environments, such as vegetal environments which contain high levels of ammonia and are poor in glutamate and other free amino acids. However, \( L. \text{lactis} \) GDH seems also to be partly involved in cadmium resistance, since the gdh mutant is more sensitive to \( \text{CdCl}_2 \) than the wild-type strain (Table 2). This impact of GDH on cadmium resistance may be related to a better elimination of ammonia generated by cell protein degradation resulting from cell damages, as suggested for GDH in tomato plants under cadmium stress (5).

### Nucleotide sequence accession number
The nucleotide sequence reported in this paper appears in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession number AB849557.

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