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Characterization of IS*Lpl4*, a functional insertion sequence in *Lactobacillus plantarum*

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Abstract

A *Lactobacillus plantarum* strain, CECT 4645, was found to have insertions of a sequence (985 bp in length) at least in eight loci in its genome. The prototype copy (Lp1) of this insertion sequence (named IS*Lpl4*) has one open reading frame encoding a putative protein that is 292 amino acids in length with significant levels of similarity with IS*982* family transposases. Perfect 16-bp inverted repeats were found at its termini. Upon transposition, generates 8-bp direct repeats of the target sequence, but no consensus sequences could be identified at either insertion site. The IS*Lpl4* pattern changed over many generations on the CECT 4645 strain. This finding strongly supports our hypothesis that IS*Lpl4* is a functional element in *L. plantarum*. Some of these elements may be cryptic, since point mutation or 1-nucleotide deletions were found in their transposase encoding genes. IS*Lpl4* copies have been detected in *Leuconostoc mesenteroides*, *Oenococcus oeni*, and *Lactobacillus sakei*. An IS*Lpl4* copy of *O. oeni* contained a +1 nucleotide insertion on its transposase encoding gene and, by using an experimental system, we were able to demonstrate that this specific sequence originates a +1 programmed translational frameshifting. Although the frameshifting process reported here operates at a low rate, this description might represent the first case of a functional +1 frameshifting among IS.

Keywords: Lactic acid bacteria; Transposase; Frameshift; Mobile element

1. Introduction

Insertion sequences (ISs) are small mobile genetic elements that are found in the genomes of numerous bacteria. They only contain genes involved in their transposition. ISs usually have inverted repeats (IRs) at their termini and duplicate a sequence consisting of several base pairs at the target site upon transposition (for reviews, see Mahillon and Chandler, 1998; Nagy and Chandler, 2004). Insertion of an IS can cause gene disruption or activation due to the creation or insertion of upstream promoters, and this contributes significantly to the plasticity of the host genome. Furthermore, a critical role of ISs in bacterial virulence is currently being recognized (Brynestad et al., 1997; Collins and Gutman, 1992; Hacker et al., 1997; Stroeher et al., 1995). IS elements are frequently used as markers for epidemiological purposes (Kivi et al., 2002; Stanley et al., 1993).

Lactobacillus plantarum is a flexible and versatile species that is encountered in a variety of environmental habitats, including some dairy, meat, and many vegetable or plant fermentations. Moreover, *L. plantarum* is frequently encountered as a natural inhabitant of the human gastrointestinal tract. The analysis of the complete genome sequence of *L. plantarum* WCFS1 strain (Kleerebezem et al., 2003) has revealed two classes of transposase encoding regions that are likely to represent mobile genetic elements; and recently, Nicoloff and Bringel (2003) described IS*Lpl1*, the first functional IS described in *L. plantarum*.

In the course of an investigation to identify genes possessing esterase activity, we isolated a segment of *L. plantarum* CECT 4645 DNA that exhibited all of the hallmarks of a bacterial IS. Since this IS was not present in the genome of the *L. plantarum*

Abbreviations: IS, insertion sequence; IR, inverted repeat; DR, directed repeat; ORF, open reading frame; PCR, polymerase chain reaction; RBS, ribosome binding site; CECT, Spanish Type Culture Collection.

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WCFS1 strain (Kleerebezem et al., 2003), here we report the sequence and characterization of IS*Lpl4*, a functional IS found in *L. plantarum* CECT 4645.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

Pure cultures of 33 lactic acid bacterial (LAB) strains were provided by the Spanish Type Culture Collection (CECT). A total of 51 LAB strains isolated from must grape or wine have been described in previous publications (Moreno-Arribas et al., 2003; Vaquero et al., 2004). *Escherichia coli* DH5α (Sambrook et al., 1989) was also used. Plasmid pUC19 (New England Biolabs) and pGEM-T (Promega) were used to clone PCR fragments.

LAB strains were routinely grown in MRS medium (Difco) at 30 °C without shaking. *E. coli* cells were incubated in Luria–Bertani medium (Sambrook et al., 1989) at 37 °C with shaking. When required, ampicillin was added to the medium at 100 μg/ml. Chromosomal DNA, plasmid purification, and transformation of *E. coli* were carried out as described (Sambrook et al., 1989).

The genetic stability of the IS element pattern was evaluated by subculturing a single-colony isolate of *L. plantarum* CECT 4645 strain on MRS broth during approximately 150 generations. Ten colony isolates were selected and chromosomal DNA was prepared as described previously, and hybridized with an IS*Lpl4*-specific DNA probe.

2.2. DNA purification and hybridization

Restriction endonucleases, T4 ligase, and the Klenow fragment of the DNA polymerase were obtained commercially and used according to the recommendations of the suppliers. Gel electrophoresis of plasmids, restriction fragments, and PCR products was carried out in agarose gels as described previously (Sambrook et al., 1989). DNA was recovered from gel slices with the QUIAquick gel extraction kit (Quiagen). An internal fragment of IS*Lpl4* from *L. plantarum* CECT 4645 was PCR amplified and used as a probe. DNA was digoxigenin-labeled and chemiluminescently detected by using the DIG High Prime DNA labeling and detection Starter Kit (Roche) according to the manufacturer's instructions. DNA sequencing was carried out by using an Abi Prism 377™ DNA sequencer (Applied Biosystems). Sequence similarity searches were carried out using BLAST. Computer promoter predictions were carried out at http://www.fruitfly.org/seq_tools/promoter.html. Multiple alignment was done using CLUSTAL W at the EBI site (http://www.ebi.ac.uk).

2.3. Standard and inverse PCR amplification

PCR amplifications (25 μl) were performed with 2 U of AmpliTaq Gold DNA polymerase, 10 ng of template DNA, 200 μM of each dNTP, and 1 μM of each synthetic oligonucleotide primer in the buffer recommended by the manufacturer. The following primers, based on the nucleotide sequence of the prototype copy of the IS*Lpl4* element of *L. plantarum* CECT 4645, the Lp1 copy (accession no. AJ968657) (see Sections 3.1 and 3.2), were used: 124 (876), 5′-TGAAACTAACTTAACGCGGAGTG; 141 (377/c), 5′-GCGCGACGGTTGAACCGTGAC; 147 (392), 5′-CGTGGTCAATGCGATCCGTACTG; and 168 (1 and 985/c), 5′-ACGCGTGGTGCTAGTT. The numbers in parentheses indicate the positions of the first nucleotide of the number in the sequence of Lp1, and c means that the corresponding sequence is on the complementary strand. The following primers were used based on the *L. plantarum* WCFS1 strain complete sequence (accession NC_004567): 181 (315949/c), 5′-CAATAGCCGGTACATCTAACG; 182 (315699), 5′-GGTTATCTTAATACTAGAGC;183 (736741/c), 5′-TCAGAGTAAAGGTAGCCACG; 184 (736449), 5′-CTTAGCTGGTAATTATGTTAGC; 185 (2260012), 5′-CGTTTTCTTGTAACATTACACC; and 186 (2260291/c), 5′-TTTCGTCAAGTAACATTACACC.

To localize some of the IS*Lpl4* integration sites in the hybridization positive strains, inverse PCR amplifications were performed. Chromosomal DNA was digested with *Cla*I, which cuts within the insertion element, and religated. The ligation mixture was used as template for inverse PCR. The PCR fragments were purified and sequenced. When the inverse PCR amplified DNA fragment was a mix of two or more different fragments, it was cloned into pGEM-T vector (Promega).

2.4. Construction of β-galactosidase reporter plasmids

The β-galactosidase reporter plasmids pISO, pISO+1, and pISO−1 were constructed by inserting into the polylinker of pUC19 a 66-bp DNA fragment, from nucleotide 169 to 234 of the IS*Lpl4* element of *Oenococcus oeni* RM83 (referred to as the Oo copy) identified in this study (accession no. AJ781303; see Section 3.3). This DNA fragment includes the putative slippery sequence and the putative hairpin loop structure containing the stop codon (see Section 3.5 and Fig. 4). DNA fragments were inserted by using oligonucleotides ISO-up, 5′-TTTATAAGCTTTGCCAGTCGCTTTAC; ISO+1-up, 5′-TTTATAAGCTTTTGCCAGTCGCTTTAC; ISO−1-up, 5′-TTTATAAGCTTGCCAGTCGCTTTAC; and ISO-down, 5′-GTCATCGAATTCACGTGCGCCAC. The sequences underlined indicate introduced restriction sites. β-Galactosidase activity was detected in cell extracts by using *O*-nitro-phenyl-β-D-galactoside (Sigma) as substrate (Sambrook et al., 1989).

3. Results

3.1. Identification of ISLpl4

In the course of a research to identify *L. plantarum* CECT 4645 proteins showing esterase activity, we constructed a *L. plantarum* genomic library in *E. coli*. When the insert of one recombinant clone from the library possessing esterase activity was sequenced, a DNA sequence that exhibited all the hallmarks of a bacterial IS was found close to the putative esterase gene (unpublished results). This IS was located in an