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Characterization of *Lactobacillus helveticus* strains isolated from cheeses by distribution studies of insertion sequences

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Abstract

A collection of 38 *Lactobacillus helveticus* strains, isolated from a number of different artisan Italian cheeses, and 4 reference strains were studied with respect to the presence of insertion sequences and their distribution and abundance. The mobile genetic element ISLh1, that contains one open reading frame coding for a putative transposase of the IS982 family, was used for DNA fingerprinting, together with IS1201 and ISL2, previously isolated from *L. helveticus*. The number of insertion sequences per strain and the size of DNA restriction fragments containing them, was variable and allowed the discrimination at the strain-level. The genomic distribution of the three unrelated insertion sequences showed significant correlations and allowed the differentiation of the strains also with regard to the specific ecological niche of origin of the isolates. Consequently, insertion sequences comparison may be useful in determining the history of a group of strains known to be related because of identity and offers a further parameter for evaluating the population polymorphism in *L. helveticus*.

Keywords: *Lactobacillus helveticus*; Mobile genetic elements; Distribution; Typing

1. Introduction

The important contribution of mobile genetic elements (MGE) to bacterial adaptability and diversity has been recently appreciated (Arber, 2000; Turner et al., 2002; van Elsas and Bailey, 2002). In this context, studies on MGE distribution and diversity should be considered as an essential research phase to explore microbial diversity, as well as to understand the plasticity of bacterial genomes. Insertion sequences (IS) are the shortest autonomously MGE. They consist of a unique functional module which catalyses transposition, and its cognate sites of action, the element ends (Gasson and Fitzgerald, 1994; Mahillon and Chandler, 1998). Many IS elements form an integral part of the chromosomes of most bacterial species, where they can participate in chromosome rearrangements and in plasmid integration. In certain cases, the localization of different specific IS elements at definite places in the chromosome is sufficiently stable to allow them to be used as markers in studies for species typing and for epidemiological purposes. The restriction fragment length polymorphism (RFLP) associated with the presence of multiple IS elements has been applied successfully for the differentiation of strains of a variety of bacterial species (Bik et al., 1996; Westberg et al., 2002; Ravin and Alatossava, 2003), but not for *Lactobacillus helveticus*, the predominant species in natural starters used in the production of aged Italian cheeses (Torriani et al., 1994; Cogan, 1996). Previous studies have shown that *L. helveticus* species is composed of different biotypes that can be grouped in relation to their phenotypic and genotypic characteristics, sometimes associated with the source of isolation (Fortina et al., 1998; Giraffa et al., 1998, 1999, 2000). Studies regarding the occurrence and distribution of IS elements can represent a further species-specific approach to evaluate the genetic diversity among related strains. To date, there are two IS elements described for *L. helveticus*, ISL2 (Zwahlen and Mollet, 1994) and IS1201 (Taillez et al., 1994). ISL2 belongs to the IS5 family, and it is able to inactivate the β-galactosidase genes by insertional mutagenesis.

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IS1201 belongs to the IS256 family and was shown to be specific for the species and different from those of the other bacterial IS elements from the same class. Mahillon and Chandler (1998) cite in their database the pLH3-designated plasmid of the type strain of *L. helveticus* as host of an IS element, named ISLh1 and belonging to IS982 family. Although the cryptic plasmid pLH3 has been previously studied and its complete sequence determined (Fortina et al., 1993; Pridmore et al., 1994), in literature no studies referring to the characteristics and distribution of this IS in *L. helveticus* have been reported. Recently, a homologue of the ISLh1, named ISLhe1, has been found in *L. helveticus* commercial strains, upstream of the genes encoding the β-galactosidase enzyme, together with a larger novel IS, ISLhe15 (Callanan et al., 2005). Also in this case, no distribution studies have been done.

In this paper, we report the structural organization of ISLh1 and its occurrence in other lactic acid bacteria species. We also report the distribution of ISLh1, IS1201 and ISL2 in plasmids and chromosomal DNAs of a collection of 38 natural *L. helveticus* strains, isolated from different artisan Italian cheeses, and 4 reference strains. Using this approach, we demonstrated that strain-specific distribution of IS elements in the chromosome of *L. helveticus* can be used for distinguishing related strains coming from different ecological niches.

2. Materials and methods

2.1. Strains and culture conditions

L. helveticus strains and other lactic acid bacteria used in this study are listed in Table 1. The strains were cultivated in MRS (Difco, Detroit, USA) agar or broth at their optimal temperature and maintained by weekly transfers.

2.2. Taxonomic identification

The taxonomic position of *L. helveticus* strains used in this study was performed by species-specific PCR assay, according to protocols previously described (Fortina et al., 2001).

2.3. β-galactosidase assays

Strains were grown in MRS broth prepared with 1% glucose or 1% lactose at 42 °C for 10 to 12 h. Cells were harvested, resuspended in 0.2 ml of potassium phosphate buffer (PPB) (0.1 M, pH 7.5), containing 0.1% Triton X-100, incubated for 30 min at room temperature, centrifuged and resuspended in PPB (Hickey et al., 1986). The reaction mixture (1 ml) contained 4 mM of *o*-nitrophenyl-β-galactopyranoside (ONPG) (Sigma, St. Louis, Mo.) in PPB and a volume of permeabilized cell suspension to reach, in the reaction mixture, a cellular concentration corresponding to an optical density (OD600 nm) of 1.0. The assay was carried out at 37 °C. Color development was stopped by adding 1 ml of cold 0.5 M sodium carbonate to the reaction mixture. Cells were removed by centrifugation and the OD of the supernatant was measured at 420 nm. β-galactosidase activity was calculated as follows: activity = (OD420 nm × 1000)/(3.5 × *t*), where 3.5 is the millimolar extinction coefficient of the *o*-nitrophenol and *t* is the reaction time.

Table 1
Bacterial strains used in this study

Strains	Origin and/or reference
Lactobacillus helveticus	
ATCC 15009^T	ATCC [a]
CNRZ 303	CNRZ [b]
NCDO 348, NCDO 30	NCDO [c]
ILC 2, ILC 4, ILC 10, ILC 15, ILC 18, ILC 23, ILC 30, ILC 31	ILC — from Grana cheese starters [d]
G5, G7, G8, G9, G10, G11, G13, G14, G16, G19, G20, G22	From Grana cheese starters [e]
ILC 38, ILC 41, ILC 59, ILC 60, ILC 62, ILC 68, ILC 74	ILC — from Provolone cheese starters
GR1, GR2, GR3, GR4	From Robiola cheese [f]
GR5, GR6, GR7	From Bra cheese [f]
GR8, GR9, GR10, GR11	From Bra tenero cheese [f]
Lactobacillus delbrueckii subsp. *lactis*	
ILC3	ILC
Lactobacillus delbrueckii subsp. *bulgaricus*	
ATCC 11842^T	ATCC
ILC12	ILC
Lactobacillus acidophilus	
DSM 20079^T, DSM9126, DSM20242	DSM [g]
Lactobacillus paracasei subsp. *paracasei*	
DSM 5622^T	DSM
TO1.1, TO18	From Toma cheese [f]
Lactobacillus crispatus	
DSM 20584^T	DSM
Lactobacillus gasseri	
DSM 20243^T	DSM
Lactobacillus jonsonii	
DSM 10533^T	DSM
Lactobacillus sakei	
DSM 6333	DSM
Lactobacillus plantarum	
ATCC 4008	ATCC
Lactococcus lactis subsp. *lactis*	
DSM 20481^T	DSM
TO1.15, TE3, A1.18	From Toma cheese [f]
Lactococcus lactis subsp. *cremoris*	
DSM 20069^T	DSM
TO1.12, TE12, TO1.18	From Toma cheese [f]
Lactococcus garvieae	
DSM 20684^T	DSM
TE1.7, TE16, TB2.5, TD6, TC3	From Toma cheese [f]
Streptococcus thermophilus	
DSM 20617^T	DSM
A2.3, B1.18	From Toma cheese [f]
Enterococcus faecium	
ATCC 19434^T	ATCC
TB1.1, TB1.3, TB1.5, TB1.10	From Toma cheese [f]

[a] ATCC, American Type Culture Collection, Rockville, Md., USA.
[b] CNRZ, Centre National de Recherches Zootechniques, Institut National de la Recherche Agronomique, Jouy en Josas, France.
[c] NCDO, National Collection of Dairy Organisms, Reading, United Kingdom.
[d] ILC, laboratory culture collection of Istituto Lattiero—Caseario, Lodi, Italy.
[e] Strains kindly provided by Prof. A. Galli Volonterio, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione di Microbiologia Agraria, Alimentare ed Ecologica, University of Milano, Italy.
[f] Our collection.
[g] DSM, Deutsche Sammlung von Mikroorganismen und Zelkulturen, Braunschweig, Germany.