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Diversity of stress tolerance in *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum*: A multivariate screening study

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ABSTRACT

Sixty-three strains of the taxonomically related species *Lactobacillus plantarum* subsp. *plantarum*, *L. plantarum* subsp. *argentoratensis*, *L. paraplantarum* and *L. pentosus* isolated from sourdoughs and other food and non-food sources and 14 strains of other members of the genus *Lactobacillus* were screened for their tolerance of acid, alkaline, heat, oxidative, osmotic, detergent and starvation stresses in order to evaluate the diversity of stress response. Most strains of the *L. plantarum* group were highly tolerant of acid, alkaline and osmotic stress and highly sensitive to detergent stress, while a larger diversity was found for other stress. Multivariate analysis allowed grouping the strains in clusters with similar response patterns. Stress response patterns in the *L. plantarum* group were similar to those of species of the *L. casei*/*L. paracasei* group but clearly different from those of other mesophilic *Lactobacillus*. No relationship was found between grouping obtained on the basis of stress response patterns and by genotypic fingerprinting (rep-PCR), nor with the taxonomic position or isolation source of the strains. Further experiments with selected strains showed that exponential phase cells were generally but not always more sensitive than stationary phase cells. The ability to grow under stressful conditions showed a slightly better correlation with the ecological conditions prevailing in the isolation niches of the strains.

This study will be the basis for further investigations to identify and exploit the basis of diversity in the stress response of lactic acid bacteria.

1. Introduction

Lactobacillus plantarum is an industrially important lactic acid bacterium (LAB) which can be found in indigenous fermented foods (Kostinek et al., 2005; Mathara et al., 2008; Tanganurat et al., 2009), sourdoughs (Corsetti et al., 2003; Catzeddu et al., 2006; van der Meulen et al., 2007), olives (Maldonado et al., 2002; Chamkha et al., 2008), fermented vegetables (Lu et al., 2003; Pérez Pulido et al., 2007; Plengvidhya et al., 2007; Seseña and Palop, 2007), fermented sausages (Tyopponen et al., 2002; Ammor and Mayo, 2007), cheese (Fitzsimons et al., 1999; Milesi et al., 2008; Gómez-Ruiz et al., 2008), wine (Alegría et al., 2004; Spano and Massa, 2006) and silage (Rossi and Dellaglio, 2007). A number of *L. plantarum* strains have probiotic properties and this species has been used for the development of functional and therapeutic foods and potential live oral vaccines (Siragusa et al., 2007; Shah, 2007).

In most of these environments, *L. plantarum* shares its ecological niche with the closely related species *Lactobacillus pentosus* and *Lactobacillus paraplantarum* and with other facultatively heterofermentative members of the genus *Lactobacillus* (Stiles and Holzapfel, 1997). *L. plantarum*, *L. pentosus* and *L. paraplantarum* show highly similar phenotypes and are genotypically closely related since their rRNA exhibits more than 99% sequence identity (Torriani et al., 2001a; Torriani et al., 2001b; Bringel et al., 2005). For this reason, these three species cannot be distinguished by 16 S rRNA gene sequence analysis (Bringel et al., 2005) and the *recA* gene has been used as a phylogenetic marker (Torriani et al., 2001b).

L. plantarum has a relatively large genome (*L. plantarum* WCFS1 genome is 3.30 Mbp, coding for 3009 proteins; Kleerebezem et al., 2003; *L. plantarum* JDM1 genome is 3.20 Mbp and codes for 2948 proteins; Zhang et al., 2009) and displays unusually diverse metabolic abilities compared to other LAB (Molenaar et al., 2005). The proteome is well characterized (Cohen et al., 2006; Koistinen et al., 2007) and significant strain-to-strain variation has been found (Koistinen et al., 2007). The species has a large genomic diversity, most of which is concentrated in some regions of the chromosome which are directly connected to lifestyle adaptation (Molenaar et al., 2005; Siezen et al., 2010).

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Table 1
List of bacterial strains used in this study. The strains used to evaluate the effect of growth phase on stress tolerance are in bold.

Species and strain	Source	Isolation source
Lactobacillus plantarum subsp. *plantarum*		
DSM20174 ^{T}	DSM	Pickled cabbage
ISLCPT57	ISLC	Cheese, Italy
FSL720, FSM170, B15, C17, S12	DBPZ	Cheese, Italy
MT2A11S, MT2D12L, MT2D6S, MT2D20S, MT2D7S, MTNTA3S, MTC13L, MT2D25L, MTD2S, MTF13S, MTF1L, MTF26S, MTF5S, MTF9L, MTG8L, MTI8L, MT2A21L	DBPZ	Sourdough (Cornetto di Matera, Italy)
NCIMB8826	NCIMB	Human saliva
S85	DBVR	Fresh vegetable products
DCU101	DBVR	Silage
NCFB340	NCIMB	Silage, UK
38AA	DBVR	Cassava, Colombia
4TP	DPPMA	Olive fermentation brine
UT2.1, UBS3, US3.1	DOFATA	Wine
P1.5	DOFATA	Olives brine
LM3	DSANA	Human saliva
1505, 1513	PCC	Sourdough (Zichi bread, Italy)
895, 872, 1069, 1089	PCC	Sourdough (Carasau bread, Italy)
947, 954	PCC	Sourdough (Moddizzosu bread, Italy)
L. plantarum subsp. argentoratensis		
DKO22^{T}	DBVR	Sour cassava, Nigeria
NCIMB1220	NCIMB	Fermented cereals (ogi), Nigeria
CNRZ1890	CNRZ	Fermented millet (baba), Nigeria
DK36	DBVR	Tapioca, Nigeria
L. paraplantarum		
LMG16673^{T1}	LMG	Beer, France
DSM10667^{T}	DSM	Beer
MTI23S, MTF28L, MTG30L	DBPZ	Sourdough (Cornetto di Matera, Italy)
F10, B7N26,	DBPZ	Caciocavallo cheese, Italy
L. pentosus		
LPL	DBPZ	Fermented sausages
LMG10775^{T2}	LMG	Unknown
DSM20314^{T}	DSM	Unknown
MTD23S	DBPZ	Sourdough (Cornetto di Matera, Italy)
1034	PCC	Sourdough (Carasau bread, Italy)
5TP, P13.3, 2TG, 4TG ,2TP	DPPMA	Olive fermentation brine
Other species		
L. alimentarius DSM20249^{T}.	DSM	Marinated fish
L. casei DSM20011^{T}	DSM	Cheese
L. casei DMS20178 (former type strain of '*Lactobacillus zeae*'; Judicial Commission of the International Committee on Systematics of Bacteria, 2008)	DSM	Corn steep liquor
L. coryniformis subsp. *coryniformis* DSM20001^{T}	DSM	Silage
L. coryniformis subsp. *torquens* DSM 20004 ^{T}	DSM	Air of cow shed
L. curvatus DSM20010	DSM	Air of cow shed
L. curvatus DSM20019^{T}	DSM	Milk
L. paracasei subsp. *paracasei* DSM5622^{T}	DSM	Unknown
L. paracasei subsp. *paracasei* DSM4905	DSM	Oral cavity
L. paracasei subsp. *tolerans* DSM20258^{T}	DSM	Pasteurized milk
L. rhamnosus DSM 20021 ^{T}	DSM	Unknown
L. sakei subp. *sakei* DSM20017^{T}	DSM	Sake
L. sakei subp. *sakei* DSM6333	DSM	Pork
L. sakei subp. *carnosus* DSM15831^{T}	DSM	Fermented meat

T type strain. CNRZ INRA culture collection Dairy Research and Applied Genetics Unit, Jouy-en-Josas Research Centre, France; DBPZ our culture collection; DBVR Prof. S. Torriani, Department of Biotecnology, University of Verona, Italy; DOFATA, Prof. C. Caggia, University of Catania; DPPMA, Dr. M. De Angelis, Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Italy; DSM Deutsche Sammlung fur Mikroorganismen und Zellkulturen, Germany; DSANA, Dipartimento di Scienze Ambientali, 2° Università di Napoli, Italy; ISLC Istituto Sperimentale Lattiero-Caseario, CRA-FLC, Italy; LMG BCCM/LMG Laboratorium voor Microbiologie, Universiteit Gent; NCIMB National Collection of Industrial, Marine and Food Bacteria, UK; PCC, Porto Conte Ricerche, Italy. 1 LMG16673 and DSM10667 are identical strains maintained in two different collections. 2 LMG10775 and DSM20314 are identical strains maintained in two different collections.

Food processing subjects microorganisms to adverse environmental conditions (acid, heat and cold, oxidative, osmotic, starvation, toxic compounds, etc.; van de Guchte et al., 2002), to which they respond by activating adaptive mechanisms which in turn affect their survival and technological performances. Because of its industrial relevance, a better understanding of the stress response of *L. plantarum* is of great importance (De Angelis et al., 2004). *L. plantarum* has been shown to respond to stress factors such as heat shock (De Angelis et al., 2004), the presence of lactic acid (Pieterse et al., 2005), bile (Bron et al., 2006), oxidative stress (Serrano et al., 2007), low pH and ethanol (Alegría et al., 2004). Even if the stress response of some strains of *L. plantarum* (with several studies on the probiotic strain WCFS1; Pieterse et al., 2005; Bron et al., 2006; Serrano et al., 2007; Ingham et al, 2008) is relatively well characterized, studies using several strains (Alegría et al., 2004; Mathara et al., 2008) are rare.