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Short communication

Identification of *Lactobacillus brevis* using a species-specific AFLP-derived marker

Vincenzina Fusco [a,*], Grazia Marina Quero [a], Daniele Chieffi [a], Charles M.A.P. Franz [b]

[a] National Research Council of Italy, Institute of Sciences of Food Production (CNR-ISPA), Bari, Italy
[b] Max-Rubner-Institut, Department of Microbiology and Biotechnology, Hermann-Weigmann-Straße 1, 24103 Kiel, Germany

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ABSTRACT

A simple and specific method for the rapid detection and identification of *Lactobacillus brevis* was developed. A fAFLP (Fluorescent Amplified Fragment Length Polymorphisms) marker for *L. brevis* was used to design oligonucleotide primers for a species-specific PCR assay, targeting a 125 bp fragment of the gene encoding the aldo/keto reductase of the diketogulonate-reductase family of *L. brevis*. This assay resulted in 100% inclusivity and exclusivity of assignment of strains to the species *L. brevis*. The analytical specificity of this assay was successfully tested to identify *L. brevis* isolates from sourdoughs.

1. Introduction

Lactobacillus brevis is a heterofermentative and lactic acid bacterium (LAB) species that can be isolated from many different environments, including fermented foods and beverages such as wine, beer, sourdoughs, meat and dairy products (Altay et al., 2013; Behr et al., 2015; Bessmeltseva et al., 2014; Carafa et al., 2015; Chen et al., 2015; Kandler and Weiss, 1986; Kántor et al., 2015; Ramos et al., 2013). Strains of this species have probiotic potential and some are widely used as starter cultures (Annuk et al., 2003; Gerez et al., 2010; Jeyaram et al., 2010; Mugula et al., 2003; Ramos et al., 2013; Seseña et al., 2005; Takii et al., 2013), as well as for biotechnological purposes (Falck et al., 2013; Guo et al., 2010; Yue et al., 2013; Zhang et al., 2012). On the detrimental side, *L. brevis* is a common beer spoilage microorganism (Behr et al., 2015), and some strains have also been found to produce biogenic amines (Coton and Coton, 2009; Romano et al., 2014) Therefore, the detection and identification of this species is crucial to predict and verify the successful progression of a fermentation process. Furthermore, it would be important to ascertain the dominance of the selected strains on the autochthonous microbiota and, in addition, it could also assure the certification and traceability of final products. The latter is especially important when considering probiotics, which are highly defined in terms of strain-specificity and functional attributes (http://www.fao.org/es/ESN/food/foodandfood_ probio_en.stm). Such a species-specific identification methods is also important in food processing, in case that *L. brevis* needs to be identified as an undesired contaminant. In this study, an AFLP-derived marker was developed and used to identify and characterize *L. brevis* strains of different origins. Furthermore, the marker was applied for the specific detection of *L. brevis* strains among *Lactobacillus* isolates from wheat sourdoughs.

2. Materials and methods

2.1. Bacterial strains and growth medium

The bacterial strains used in this study are listed in Table 1. All wild strains were previously identified and characterized by conventional and/or molecular microbiological methods (Fusco et al., 2011a). Working cultures were prepared as reported by Di Lena et al. (2015) and Fusco et al. (2011a). Moreover, to validate the implemented species-specific PCR assay, 37 lactobacilli isolates from sourdoughs, stemming from previous research in our department, were included in the analysis. All isolates were stored in MRS broth (Oxoid) with 20% glycerol at −80 °C.

2.2. DNA extraction

DNA isolation was carried out as previously reported (Fusco et al., 2011a). DNA quality and quality was evaluated as described by Fusco et al. (2011a).

2.3. Isolation of AFLP fragments

The protocol described by Fusco et al. (2011a) was followed to isolate the fragment specific for *L. brevis*.

* Corresponding author at: National Research Council of Italy, Institute of Sciences of Food Production, via Amendola 122/o, 70126, Bari, Italy.
E-mail address: vincenzina.fusco@ispa.cnr.it (V. Fusco).

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Table 1
Species, origin, source and results of the species-specific PCR of the strains used in this study. ATCC14869[T].

Strain	Genus	Origin	Source	*L. brevis* species-specific PCR
10A	*L. brevis*	ISPA	Sourdough	+
ATCC 14869[T]	*L. brevis*	ATCC	Human faeces	+
DPPMA 33	*L. brevis*	DPPMA	Unknown	+
LMG 11435	*L. brevis*	LMG	Silage	+
LMG 11436	*L. brevis*	LMG	Unknown	+
LMG 11437	*L. brevis*	LMG	Silage	+
LMG 11495	*L. brevis*	LMG	Unknown	+
LMG 11774	*L. brevis*	LMG	Unknown	+
LMG 11992	*L. brevis*	LMG	Hard cheese	+
LMG 11993	*L. brevis*	LMG	English hard cheese	+
LMG 11969	*L. brevis*	LMG	Marinated fish	+
LMG 12023	*L. brevis*	LMG	Human intestine	+
LMG 18022	*L. brevis*	LMG	Zabady (yoghurt)	+
LP5-6	*L. brevis*	ISPA	Raw milk	+
P2	*L. brevis*	ISPA	Wheat flour	+
ATCC29643	*L. alimentarius*	ATCC	Marinated fish products	−
LMG14527[T]	*L. brevisimilis*	LMG	Beer	−
DPPMA155	*L. buchneri*	DPPMA	Sourdough	−
DSM20057	*L. buchneri*	DSM	Tomato pulp	−
RN30	*L. casei*	ISPA	Cheese	−
ATCC27216	*L. casei* subsp. *paracasei*	ATCC	Infant saliva	−
MY165	*L. casei* subsp. *paracasei*	ISPA	Fermented milk	−
ATCC25601	*L. curvatus*	ATCC	Milk	−
ATCC7469[T]	*L. rhamnosus*	ATCC	Unknown	−
ATCC53103	*L. rhamnosus*	ATCC	Human faeces	−
My167	*L. delbrueckii* subsp. *bulgaricus*	ISPA	Fermented milk	−
DSM20072	*L. delbrueckii* subsp. *lactis*	DSM	Emmenthal	−
ATCC29644	*L. farciminis*	ATCC	Salame	−
DSM20052	*L. fermentum*	DSM	Fermented beet	−
ATCC8288	*L. fructivorans*	ATCC	Unknown	−
LMG23074	*L. hammesii*	LMG	Sourdough	−
MY179	*L. helveticus*	ISPA	Fermented milk	−
LMG6895	*L. hilgardii*	LMG	Wine	−
LP17-1	*L. johnsonii*	ISPA	Raw milk	−
LMG11455[T]	*L. malefermentas*	LMG	Beer	−
LMG11494	*L. parabrevis*	LMG	Artisanal Chesire cheese	−
LMG16673	*L. paraplantarum*	LMG	Beer	−
ATCC8041	*L. pentosus*	ATCC	Unknown	−
ATCC10012	*L. plantarum*	ATCC	Unknown	−
LB2	*L. plantarum*	DPPMA	Sourdough	−
ATCC51518	*L. pontis*	ATCC	Sourdough	−
LB1	*L. rossiae*	DPPMA	Sourdough	−
DPPMA70	*L. rossiae*	DPPMA	Sourdough	−
13F	*E. faecalis*	ISPA	Raw milk	−
127GT0	*E. faecium*	ISPA	Fresh cheese	−
DSM20686	*Lc. plantarum*	DSM	Forzen peas	−
DSM20069	*Lc. cremoris*	DSM	Unknown	−
DSM20684	*Lc. garvieae*	DSM	Mastic cow	−
DSM20450	*Lc. lactis* subsp. *hordniae*	DSM	Cicadellide (Hordniacircellata)	−
DSM4644	*Lc. lactis*	DSM	Unknown	−
L01-6	*Lc. lactis*	ISPA	Raw milk	−
DSM20481	*Lc. lactis* subsp. *lactis*	DSM	Unknown	−
DSM6634	*Lc. piscium*	DSM	Unknown	−
DSM20443	*Lc. raffinolactis*	DSM	Raw milk	−
DSM8582	*Leuc. argentinum*	DSM	Raw milk	−
DSM5576	*Leuc. carnosum*	DSM	Under vacuum meat	−
DSM20188	*Leuc. citreum*	DSM	Unknown	−
DSM5577	*Leuc. citreum*	DSM	Ambrosia	−
ATCC20189	*Leuc. fallax*	ATCC	Crauti	−
DSM20349	*Leuc. fructosum*	DSM	Flowers	−
DSM29346	*Leuc. mesent.* subsp. *cremoris*	DSM	Lyophilized starter (Christian Hansen)	−
DSM20343	*Leuc. mesent.* subsp. *mesenteroides*	DSM	Fermented olives	−
DSM20193	*Leuc. pseudomesenteroides*	DSM	Cane juice	−
ATCC9135	*Leuc.mesenteroides*	ATCC	Unknown	−
MC	*Mc. caseolyticus*	ISPA	Cured meat	−
11GT5	*Mc. caseolyticus*	ISPA	Fresh cheese	−
SX180	*S. xylosus*	ISPA	Cured meat	−
62GT0	*Strept. macedonicus*	ISPA	Fresh cheese	−
DSM15879	*Strept. macedonicus*	DSM	Kasseri fresh cheese	−
6GT5	*Strept. parauberis*	ISPA	Fresh cheese	−
DSM20617	*Strept. thermophilus*	DSM	Pasteurized milk	−
DSM15830	*W koreensis*	DSM	Kimchii	−
DSM15878	*W. cibaria*	DSM	Chili bo	−
DSM20196[T]	*W. confusa*	DSM	Sugar cane	−
DSM20190	*W. halotolerans*	DSM	Cured meat	−
DSM20593	*W. kandleri*	DSM	Unknown	−