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Rapid identification of *Lactobacillus nantensis, Lactobacillus spicheri* and *Lactobacillus hammesii* species using species-specific primers

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Abstract

Based on the 16S–23S ribosomal DNA (rDNA) intergenic spacer region (ISR), an identification tool for rapid differentiation of *Lactobacillus nantensis*, *Lactobacillus spicheri* and *Lactobacillus hammesii*, species isolated recently from French sourdough was developed. The DNA fragments containing ISRs were amplified with primers pairs 16S/p2 and 23S/p7. Clone libraries of the PCR-amplified rDNA with these primers were constructed using a pCR2.1 TA cloning kit and sequenced. The DNA sequences obtained were analyzed and species-specific primers were designed from these sequences. Two PCR amplicons, which were designated small ISR (S-ISR) and large ISR (L-ISR), were obtained for all *Lactobacillus* species studied. The L-ISR sequence reveale[2]d the presence of two tRNA genes, $tRNA^{Ala}$ and $tRNA^{Ile}$. Species-specific primers designed allowed rapid identification of these species. The specificity of these primers was positively demonstrated as no response was obtained for more than 200 other species tested.

Keywords: *Lactobacillus nantensis*; *Lactobacillus hammesii*; *Lactobacillus spicheri*; 16S–23S rDNA; Intergenic spacer region; Species-specific primers

1. Introduction

The sourdough microflora is composed of lactic acid bacteria (LAB) that represent the prevailing microflora, and yeast existing in symbiotic relationships (Di Cagno et al., 2002; Mäntynen et al., 1999; Ganzle et al., 1998; Corsetti et al., 1996). Numerous genera and species of LAB have been identified in sourdough (Valcheva et al., 2006; Valcheva et al., 2005; Meroth et al., 2004; Ehrmann et al., 2003; De Vuyst et al., 2002; Corsetti et al., 2001; Müller et al., 2000; Herrero et al., 1996; Ottogalli et al., 1996; Wiese et al., 1996). The dominant *Lactobacillus* species in Italian wheat sourdoughs have been reported to be *Lactobacillus sanfranciscensis*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus fructivorans, Lactobacillus plantarum, Lactobacillus alimentarius, Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus farciminis* (Corsetti et al., 2003; Corsetti et al., 2001). Some described species such as *Lactobacillus rossiae* sp. nov (Corsetti et al., 2005), *Lactobacillus siliginis* sp. nov. (Aslam et al., 2006), *Lactobacillus spicheri* (Meroth et al., 2004), *L. plantarum* (Ottogalli et al., 1996), *Lactobacillus mindensis* (Ehrmann et al., 2003), *Lactobacillus frumenti* (Müller et al., 2000) and *Lactobacillus paralimentarius* (Cai et al., 1999) were also isolated from sourdough.

Recently, in our laboratory, *Lactobacillus nantensis* (Valcheva et al., 2006), *L. spicheri* and *Lactobacillus hammesii* (Valcheva et al., 2005) were isolated from French sourdough. They are heterofermentative LAB species that can produce large amount of lactic and acetic acid. They are hence responsible for the souring activity in the sourdough bread, and they help in dough leavening by gas production (Valcheva et al., 2007; Valcheva et al., 2006; Valcheva et al., 2005). It is desirable to develop a molecular approach for their clear and reliable identification. The molecular typing methods developed to identify *Lactobacillus* species are based on randomly amplified polymorphic DNA (Lai et al., 2000), sequencing of the 16S *rrn* gene (Wallbanks et al., 1990),

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Table 1
Bacterial strains used for this study

Species	Strain designation
Lactobacillus acidophilus	DSM 20079, NBIMCC 504, ATCC 4356, IR 1650, IR1851
Lactobacillus alimentarius	CIP 102986^T, DSM 20181, DSM 20249, NBIMCC 3315, CIP102986^T, TMW 1.418, LTH 954, LTH 691, IFREMER SF767
Lactobacillus amylovorus	DSM 20531^T, ENITIAA LP 41, ENITIAA LP42, ENITIAA LP43, ENITIAA LP44
Lactobacillus brevis	CIP 102806^T, DSM 1286, DSM 20556, TMW 1.57, TMW 1.100, ATCC 14869, LMG 11435, LMG 11438, DSM 6235, DANONE EXT 65, DANONE EXT 69, DANONE EXT 70
Lactobacillus casei	DSM 20011, ENITIAA LP55, ENITIAA LP56, ENITIAA LP57, ENITIAA LP58, ENITIAA LP59, DANONE 1.128, DANONE 1.307
Lactobacillus crispatus	DSM 20584, NCDO 4, ATCC 33199
Lactobacillus curvatus	DSM 20019 ^{T}, ENITIAA M5411, FY 113, J 116, T 402, YMG 348, INRA Theix H382, INRA Theix H383, INRA Theix H648
Lactobacillus curvatus subsp. *curvatus*	cip 102992^T, ENITIAA LP17, ENITIAA LP18
Lactobacillus delb. subsp. *bulgaricus*	CIP 55.1, DSM 20074, NBIMCC 1132, CIP 101027^T
Lactobacillus delb. subsp. *delbrueckii*	DSM 20074, ENITIAA LP03, ENITIAA LP04, ENITIAA LP11, ENITIAA LP13
Lactobacillus delb. subsp. *lactis*	CIP 101028^T, ENITIAA LP02, ENITIAA LP06, ENITIAA LP12
Lactobacillus farciminis	DSM 20184, DSM 20180, LTH 4812, LTH 692, LTH 693, LTH 694, LTH 4812, SIGMO P222
Lactobacillus fermentum	DSM 20052, ATCC 14931, ENITIAA LP21, ENITIAA LP22, ENITIAA LP23, ENITIAA 12A, ENITIAA LP12B
Lactobacillus fructivorans	DSM 20203, ENITIAA LP08
Lactobacillus frumenti	DSM 13145, TMW 1.690, TMW 1.666
Lactobacillus hammesii	DSM 16381, ENITIAA LP38, ENITIAA LP39, ENITIAA LP40, ENITIAA LP41, TMW 1.1236
Lactobacillus helveticus	CIP 103146^T, NBIMCC 1383, ATCC 15009, CNRZ 303
Lactobacillus hilgardii	DSM 20176, ENITIAA LP08, ENITIAA LP09
Lactobacillus johnsonii	DSM 10533
Lactobacillus kimchii	DSM 13961
Lactobacillus mindensis	DSM 6035, DSM 14500, TMW 1.1201
Lactobacillus nantensis	DSM 16982, ENITIAA LP33, ENITIAA LP34, ENITIAA LP35, ENITIAA LP36, ENITIAA LP37
Lactobacillus panis	DSM 6035, JCM 11053
Lactobacillus paraalimentarirus	DSM 13238, ENITIAA LP 31, ENITIAA LP 33,
Lactobacillus paraplantarum	INRA Jouy 1888, INRA Jouy 1885, CNRZ 1885 ^{T}, CNRZ 1886, CNRZ 1887, CNRZ 1888
Lactobacillus plantarum	ATCC 14917 ^{T}, ATCC 8014 ^{T}, NCDO 343, CNRZ 184, CNRZ 424, CNRZ 738, CNRZ 764, CNRZ 1228, CNRZ 1246, INRA Jouy 1228, INRA Jouy 432, INRA Jouy 738, INRA Theix 702, ENITIAA ST 31, DANONE 1.11, DANONE 1.060, DANONE 1.175, DANONE 1.287
Lactobacillus pontis	DSM 8475, DSM 8476, TMW 1.1104, TMW 1.397, ATCC 51519
Lactobacillus reuteri	DSM 20016, ATCC 55730
Lactobacillus sakei	ATCC 15531, NBIMCC 3453, INRA THEIX 240, INRA THEIX 214, INRA THEIX 475, INRA THEIX 381, IFREMER SF 842, IFREMER SF 771, IFREMER SF 841, IFREMER SF 843, IFREMER SF 811, IFREMER SF 812, IFREMER SF C26, IFREMER SF 770, IFREMER SF 697, J 64, J 180, T 240, V 553, YMW 540
Lactobacillus sanfranciscensis	ATCC 43322, CIP 103252, TMW 1.52, TMW 1.1149, ENITIAA LP4, ENITIAA LP5, ENITIAA LP3, ENITIAA LP2, DSM 20451^T,
Lactobacillus spicheri	CIP 5753, ENITIAA LP 26, ENITIAA LP 27, ENITIAA LP 28, ENITIAA LP 29, LTH 5753, LMG 21871
Lactococcus lactis	ATCC 11454, CNRZ 1075, CNRZ 1076
Lactococcus lactis subsp. *cremoris*	ATCC 11454
Leuconostoc lactis	DSM 20192
Leuconostoc sp.	DSM 20186, ENITIAA 58, ENITIAA 59
Leuconostoc mesent subsp. *mesenteroides*	DSM 20240, ENITIAA 61, ENITIAA 62, ENITIAA 63, ENITIAA 64
Weilssella confusa	DSM 20196^T, JCM 1093, ENITIAA ISO1

DSM, Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Braunschweig, Germany; *CIP*, Collection de l'Institut Pasteur, Paris, France; *INRA*, Institut National de Recherche Agronomique; Jouy en Josas, France; *ATCC*, American Type Culture Collection; *NBIMCC*, National Bank for Industrial Microorganisms and Cell Cultures; *ENITIAA*, Ecole nationale d'ingénieurs des techniques des industries agricoles et alimentaires; *CNRZ*, centre national de recherches zootechniques; *IFREMER*, Institut Français de Recherche pour l'Exploitation de la Mer; *SIGMO*, Service Identification Génétiques des MicroOrganismes, Nantes, France; *JCM*, Japan Collection of Microorganisms; *LTH*, Institute of Food Technology, University of Hohenheim, Stuttgart, Germany; IR, Institut Rosel.