

CLUSTAL O(1.2.4) multiple sequence alignment

<i>L. sakei</i> strain FAM18311	GTTTGTCCAAACATCAAGTGTTAACTTGTCTGAAATCGTTACGACGACCAACACGTGTACT	60
<i>L. sakei</i> laboratory collection strain	GTTTGTCCAAACATCAAGTGTTAACTTGTCTGAAATCGTTACGACGACCAACACGTGTACT	60
<i>L. curvatus</i> laboratory collection strain	-----ACATCAAGTGTTAACTTGTCTGAAATCGTTACGACGACCAACACGTGTACT	50
<i>L. curvatus</i> strain MRS6	GTTTGTCCAAACATCAAGTGTTAACTTGTCTGAAATCGTTACGACGACCAACACGTGTACT	60

	<u>LscHRM-R</u>	
<i>L. sakei</i> strain FAM18311	TTCTACTTGATAGTTGACACGACTGATTGGGGTATAAATTGAGTCGATTGGTAAAACACC	120
<i>L. sakei</i> laboratory collection strain	TTCTACTTGATAGTTGACACGACTGATTGGGGTATAAATTGAGTCGATTGGTAAAACACC	120
<i>L. curvatus</i> laboratory collection strain	TTCTACTTGATAGTTGACACGACTGATTGGGGTATAAATTGAGTCGATTGGTAAAACACC	110
<i>L. curvatus</i> strain MRS6	TTCTACTTGATAGTTGACACGACTGATTGGGGTATAAATTGAGTCGATTGGTAAAACACC	120

	<u>LscHRM-F</u>	
<i>L. sakei</i> strain FAM18311	AATTGGCATATCGTCTGACTTGTGTTTGAACAGCAGCAACATAAACCACGGCCTTTTTTAAC	180
<i>L. sakei</i> laboratory collection strain	AATTGGCATATCGTCTGACTTGTGTTTGAACAGCAGCAACATAAACCACGGCCTTTTTTAAC	180
<i>L. curvatus</i> laboratory collection strain	AATTGGCATATCGTCTGACTTGTGTTTGAACAGCAGCAACATAAACCACGGCCTTTTTTAAC	170
<i>L. curvatus</i> strain MRS6	AATTGGCATATCGTCTGACTTGTGTTTGAACAGCAGCAACATAAACCACGGCCTTTTTTAAC	180

<i>L. sakei</i> strain FAM18311	TGTCATTGACACGTGAAATGCGCCTTCAGCTAC	216
<i>L. sakei</i> laboratory collection strain	TGTCATTGACACGTGAAATGCGCCTTC-----	210
<i>L. curvatus</i> laboratory collection strain	TGTCATTGACACGTGAAATGCGCCTTCAGCTAC	206
<i>L. curvatus</i> strain MRS6	TGTCATTGACACGTGAAATGCGCCTTCAGCTAC	216

Fig. S1. Alignment of the sequences of *Lactobacillus sakei* and *L. curvatus* laboratory strains with the respective sections of the *rpoA* gene from the strains in the NCBI database (*L. sakei* FAM18311 strain and *L. curvatus* MR56 strain) (33). SNPs are highlighted in red, primer (LscHRM-F and LscHRM-R) positions are marked

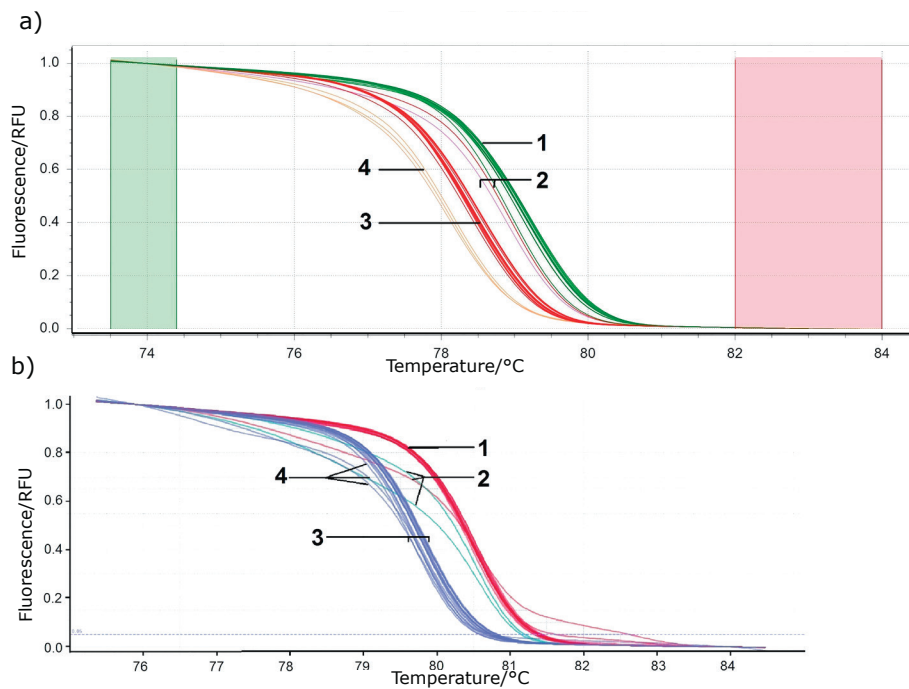


Fig. S2. Normalized melting curves analysed by: a) Precision Melt Analysis software (40), and b) LightCycler® 96 software (39). PCR was performed using LightCycler 480 HRM MasterMix. Samples: 1=1-10³ dilutions of *Lactobacillus curvatus*, 2=10⁴ dilution of *L. curvatus*, 3=1-10³ dilutions of *L. sakei*, and 4=10⁴ dilution of *L. sakei*. RFU=relative fluorescence unit

Table S1. Melting temperature (T_m) and threshold cycle values of positive controls and their dilutions obtained by CFX96 (40)

<i>Lactobacillus sakei</i>			<i>Lactobacillus curvatus</i>		
Dilution	T_m /°C	Threshold cycle	Dilution	T_m /°C	Threshold cycle
LightCycler 480 HRM master kit					
1	(78.5±0.1) ^a	(21.7±0.2) ^a	1	(79.1±0.1) ^a	(21.55±0.07) ^a
10 ⁻¹	(78.4±0.0) ^a	(25.09±0.03) ^a	10 ⁻¹	(79.0±0.0) ^a	(25.23±0.08) ^a
10 ⁻²	(78.4±0.0) ^a	(28.47±0.09) ^a	10 ⁻²	(79.1±0.1) ^a	(28.52±0.08) ^a
10 ⁻³	(78.4±0.0) ^a	(32.2±0.2) ^a	10 ⁻³	(79.0±0.0) ^a	(32.32±0.03) ^a
10 ⁻⁴	(78.2±0.0) ^a	(35.9±0.6) ^a	10 ⁻⁴	(79.0±0.0) ^a	(35.8±0.6) ^a
Total	(78.4±0.1) ^b		Total	(80.45±0.03) ^b	
Real-time PCR reagent					
1	(79.4±0.0) ^a	(20.3±0.1) ^a	1	(80.0±0.0) ^a	(20.21±0.04) ^a
10 ⁻¹	(79.3±0.1) ^a	(24.07±0.08) ^a	10 ⁻¹	(79.8±0.0) ^a	(24.15±0.05) ^a
10 ⁻²	(79.2±0.0) ^a	(28.5±0.1) ^a	10 ⁻²	(79.8±0.0) ^a	(27.8±0.1) ^a
10 ⁻³	(79.2±0.0) ^a	(31.6±0.3) ^a	10 ⁻³	(79.8±0.0) ^a	(31.15±0.04) ^a
10 ⁻⁴	(79.2±0.0) ^a	(35.3±0.2) ^a	10 ⁻⁴	(79.8±0.0) ^a	(34.5±0.4) ^a
Total	(79.25±0.09) ^b		Total	(79.84±0.08) ^b	

Data represent the mean value±standard deviation (^aN=3, ^bN=15). PCR=polymerase chain reaction

Table S2. The comparison of high-resolution melting analysis (HRM) and biochemical identification results of samples

Sample code	Species included in the composition of the starter culture	Microbiological identification*	PCR with LscHRM primers	HRM differentiation
Starter culture				
A-1	<i>Lactobacillus sakei</i> , <i>Debaromyces hansenii</i> , <i>Pediococcus pentosaceus</i> , <i>Staphylococcus carnosus</i> , <i>S. xylosum</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-2	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-3	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-4	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-5	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-6	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-7	<i>L. sakei</i> , <i>S. carnosus</i> , <i>S. xylosum</i> , <i>P. pentosaceus</i> , <i>Candida famata</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-8	<i>L. sakei</i> , <i>S. carnosus</i>	<i>L. sakei</i>	+	<i>L. sakei</i>
A-9	<i>L. curvatus</i> , <i>S. carnosus</i> , <i>S. xylosum</i> , <i>Lactococcus lactis</i> , <i>D. hansenii</i>	<i>L. curvatus</i>	+	<i>L. curvatus</i>
A-10	<i>L. curvatus</i>	<i>L. curvatus</i>	+	<i>L. curvatus</i>
A-11	<i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>Kocuria varians</i>	<i>L. plantarum</i> (5 colonies) <i>L. rhamnosus</i> (1 colony)	-	
Fermented sausage				
B-1		<i>L. sakei</i>	+	<i>L. sakei</i>
B-2		<i>L. sakei</i>	+	<i>L. sakei</i>
B-3		<i>L. sakei</i>	+	<i>L. sakei</i>
B-4		<i>L. sakei</i>	+	<i>L. sakei</i>
B-5		<i>L. sakei</i>	+	<i>L. sakei</i>
B-6		<i>L. sakei</i>	+	<i>L. sakei</i>
B-7		<i>L. sakei</i>	+	<i>L. sakei</i>
B-8		<i>L. sakei</i>	+	<i>L. sakei</i>
B-9		<i>L. sakei</i>	+	<i>L. sakei</i>
B-10		<i>L. curvatus</i>	+	<i>L. curvatus</i>
B-11		<i>L. curvatus</i>	+	<i>L. curvatus</i>
B-12		<i>L. curvatus</i>	+	<i>L. curvatus</i>
B-13		<i>L. plantarum</i>	-	
B-14		<i>L. plantarum</i>	-	
B-15		<i>P. pentosaceus</i>	-	

*According to API 50 CHL test system result of 6 isolated colonies from the Petri dishes of the last dilutions in which growth was observed; +=positive result, -=negative result, PCR=polymerase chain reaction