Dairy Sci. Technol. 90 (2010) 399–412

Supplementary Material

1. Primer design

Primer length was fixed to 22 nucleotides. The primer melting point under RAP-PCR conditions (2 $\mu mol \cdot L^{-1}$ of primers and 1.67 $mmol \cdot L^{-1}$ of $MgSO_4$) was designed around 60 °C. The PatScan software [2] was used to design the primer 3'-end with a decameric sequence frequently found in the genome of *Lactococcus lactis* subsp. *lactis* IL1403 [1] under a three-mismatch condition (Table SI). The primer 5'-end was a dodecamer used as a 5'-clamp allowing the use of high stringency PCR. This dodecamer was formed by concatenating two different hexameric sequences “CACTCT” and “CACAGA”. Each of these two hexamers is rarely found in the genome of *L. lactis* subsp. *lactis* IL1403 under a three-mismatch condition, so the head-to-tail link of these two hexamers gave a 12-mer that was more rarely represented than the two 6-mers alone. The rare occurrence of the 5' part sequence in the genome of *Lactococcus lactis* subsp. *lactis* IL1403 allowed the introduction in this dodecamer with the software PerlPrimer [4] of single nucleotide variations to decrease homodimerization.

Primer hybridization with its template at 35 °C was oriented to a hybridization of the primer 3' part, reducing nonefficient binding. For fluorescent RAP-PCR on the *Lactococcus* species, 13 arbitrary primers were designed without labeling, and five of these primers were synthesized with a FAM fluorophore at the 5' end (Tab. SI). In the 40 possible pairs, the following eight primer pairs were chosen by their reduced hetero-primer dimerization (Table SII): (STF1, ST8); (STF2, ST13); (STF3, ST7); (STF4, ST11); (STF5, ST9); (STF2, ST12); (STF3, ST6) and (STF4, ST10). Primers synthesized with a fluorophore have the same sequence, but “F” is added to the name**.**

2. Predicted transcriptome coverage

The maximum number of genes expressed in lactococci range from one to two thousand [1, 6] in the mid-exponential growth. According to procaryote RNA operon organization, nearly 400 operons are transcribed simultaneously [5] with an average of two mRNA molecules per operon [3]. Fluorescent RAP-PCR used eight primer pairs that give, on electropherograms, a total of around 1000 peaks. For all the peaks of an electropherogram, each peak was a datum taken randomly in a constant pool of independent operons. As the number of peaks was superior to the number of operons, the Poisson law (Equation S1) which uses a simplification of the multinomial distribution could be applied for calculating transcriptome coverage [7]. The coverage is the noncoverage complementary event (k = 0) of a thousand peaks taken in 400 operons and can be estimated with the simplified formula of the Poisson law (Equation S2). With λ=2.5, the coverage is around 92%.

$$P(\lambda, k) = \frac{\left(\lambda^k * e^{-\lambda}\right)}{k!} \text{ with } \lambda = \frac{Number\ of\ peaks}{Total\ of\ operons\ expressed}$$

Equation S1. Statistical coverage using Poisson law (k is the order of coverage for each operon and λ the global probability for each operon to be represented by a peak).

$$\overline{P}_{k=0}(\lambda) = 1 - e^{-\lambda}$$

Equation S2. Simplified coverage using Poisson law.

Dairy Sci. Technol. 90 (2010) 399–412

Table SI. Number of primer hits with three mismatches, self primer dimer enthalpies and arbitrary primer melting point temperature.

Primer	Sequence (5' 3')	Three mismatch primer hits*		self **	Tm (°C)
		12-mers	10-mers		
ST1	CACTCTCACAGACAAAAGCAAG	1110	32 501	−0.13	64
ST2	CACTCTCACAGACAAAATCAGG	1110	30 369	−0.08	63
ST3	CA**G**TCTCACAGAAAAATTATTG	1085	63 929	−0.08	58
ST4	CACTCTCAC**TC**AAAAACTTCTG	1272	41 308	0	62
ST5	CA**G**TC**CAT**CAGAAGAAGTTGTT	1624	39 800	0	63
ST6	CA**G**T**G**T**TC**CAGAAAAGTTGATG	1485	40 403	−0.63	62
ST7	CA**G**TCTCACAG**T**AAAAATCAGG	1055	38 877	0	61
ST8	CA**G**TCTCACAG**T**AAAAAGCAAG	1055	39 007	−0.13	62
ST9	CA**G**TCTCACAGAAAGAAGCAAT	1085	38 172	−0.13	63
ST10	CACT**G**TCA**T**A**CG**GAAATTGCTG	914	38 224	−0.44	64
ST11	**G**A**TG**CTCACAA**C**ACAACTTCTG	1330	29 480	−0.13	65
ST12	CA**G**TCTCACAGAAAGAAGCAAG	1085	27 495	−0.13	64
ST13	CACTCTCACAGAAAGAAGCACT	1110	26 411	−0.13	65

In bold: added nucleotide changes.

***** Using the *Lactococcus lactis* subsp. *lactis* IL1403 genome as target.

****** In kcal·mol^{-1}.

Table SII. Enthalpy of primer dimers at 35 °C (kcal·mol^{-1}).

Fluorescent primer	Cold primer							
	ST 6	ST 7	ST 8	ST 9	ST 10	ST 11	ST 12	ST 13
STF 1	−1.75	−0.39	**−0.39**	−0.39	−3.35	−0.74	−0.39	−0.25
STF 2	−1.75	−0.39	−0.39	−0.39	−2.46	−0.63	**−0.39**	**−0.25**
STF 3	**−0.56**	**0**	−0.17	−0.90	−1.75	−2.65	−0.25	−0.39
STF 4	−1.75	−0.49	−0.39	−2.48	**−0.17**	**0**	−2.30	−2.48
STF 5	−2.51	−0.56	−0.56	**0**	−2.16	−7.05	−0.56	−0.39

In bold: Primer pairs used in this study.

3. Pearce test

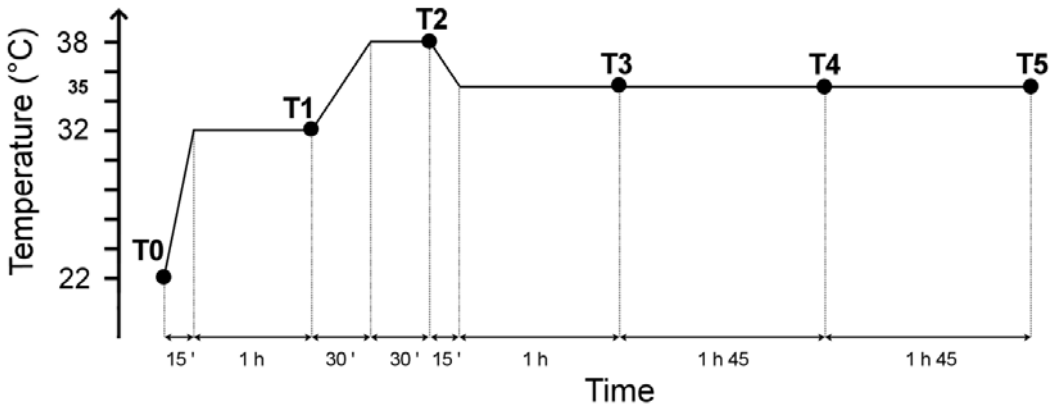

Figure S1. Temperature cycling and sampling points of the modified Pearce test.

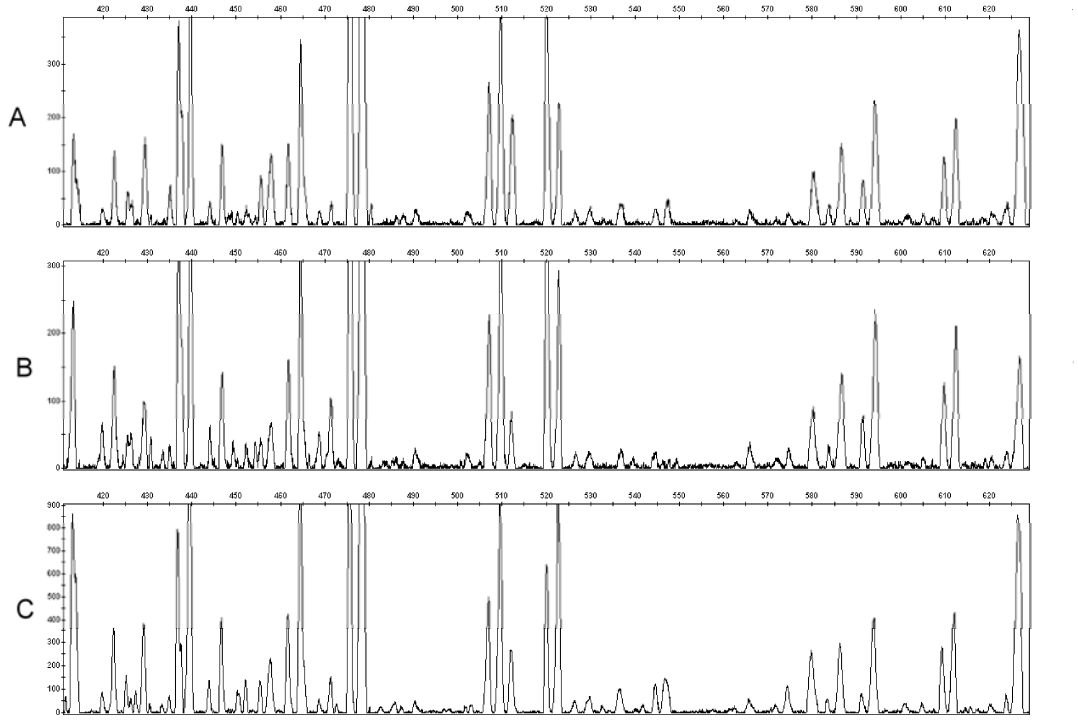

Figure S2. Fluorescent RAP-PCR amplimers from 400 to 650 nucleotides obtained using primers STF4 with ST11. Y-axis: fluorescence units. A, B, C: triplicate experiments using microfiltered milk.

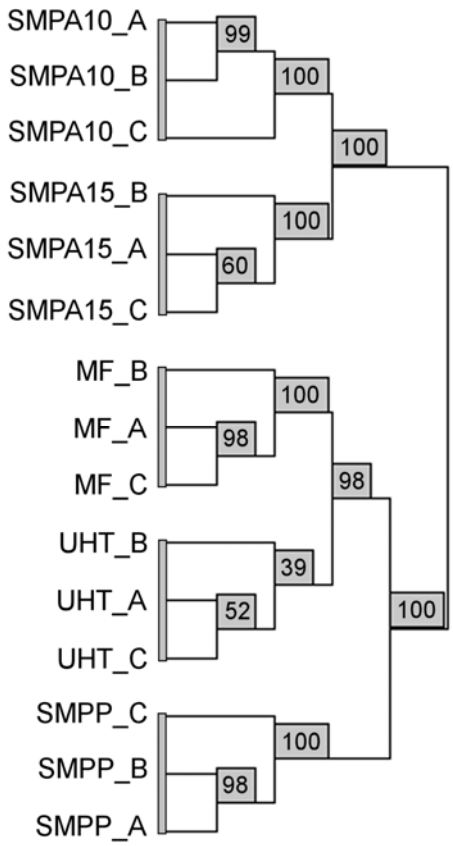

Figure S3. Hierarchical clustering of fluorescent RAP-PCR amplicon profiles obtained from RNA extracted at T1 and T2 of the Pearce test. SMPA10: skim milk powder autoclaved 10 min at 120 °C, SMPA15: skim milk powder autoclaved 15 min at 120 °C, SMPP: skim milk powder pasteurized 30 min at 65 °C, MF; microfiltered milk, UHT: commercial ultra high temperature treated milk.

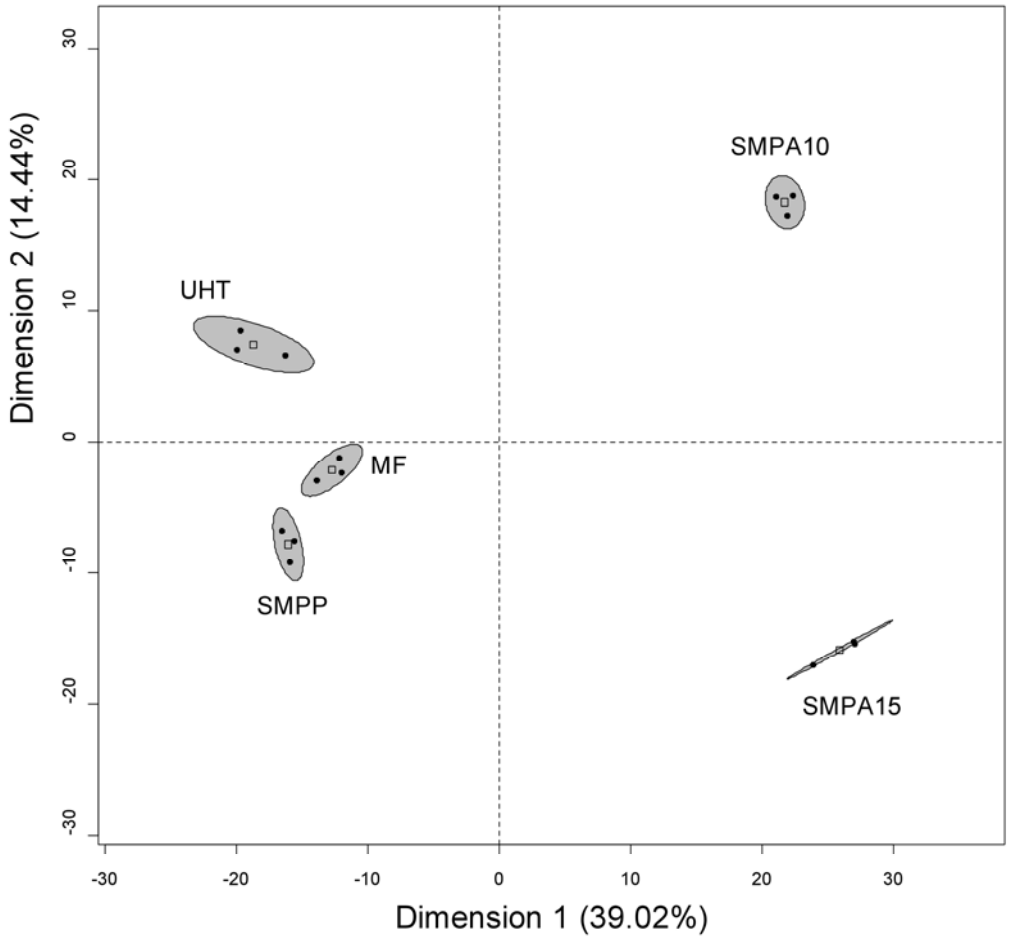

Figure S4. Principal component analysis of fluorescent RAP-PCR amplicon profiles obtained at T1 and T2 of the Pearce test using different types and thermal treatments of milk. SMPA10: skim milk powder autoclaved 10 min at 120 °C, SMPA15: skim milk powder autoclaved 15 min at 120 °C, SMPP: skim milk powder pasteurized 30 min at 65 °C, MF; microfiltered milk, UHT: commercial ultra high temperature treated milk. Gray shading represents the clustering of the three experimental replicates (black dots) inside an ellipse which represents a confidence level of 95 %. □: cluster's barycenter.

Electrophoresis of fluorescent RAP-PCR amplimers

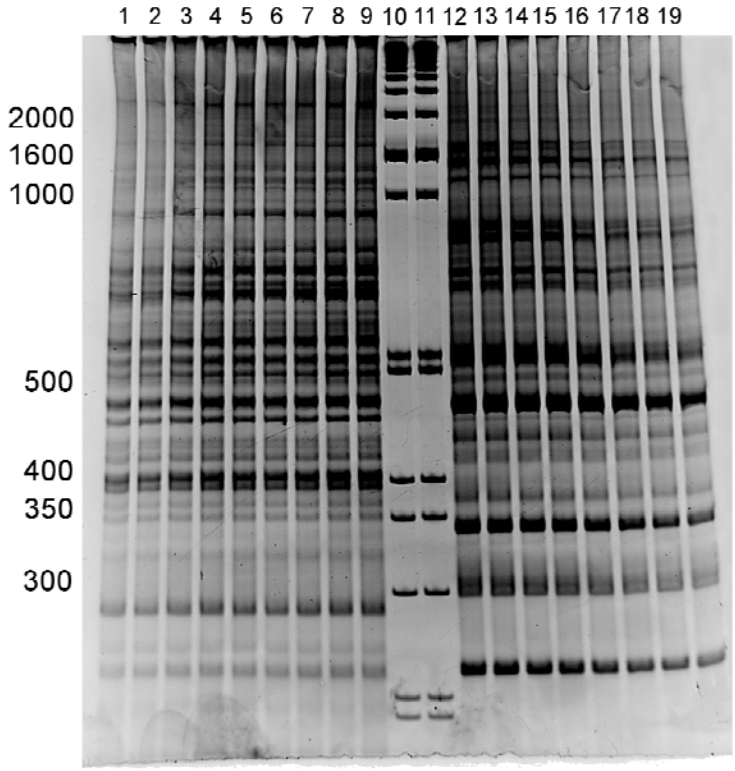

Figure S5. RAP-PCR profiles of defined mixed starter fermentations with skim milk were electrophoresed on a 6% acrylamide gel. Two different RAP-PCR profiles using primers STF4 with ST11 (lanes 1 to 9) or primers STF3 with ST7 (lanes 12 to 19) are shown for skim milk (lanes 7 to 9 and 18, 19), CO_2 treated skim milk not neutralized (lanes 1 to 3 and 12 to 14) and CO_2 treated skim milk neutralized by agitation (lanes 4 to 6 and 15 to 17). Each group of three lanes represents replicates A, B and C. Molecular marker 1 kb DNA ladder (Invitrogen, Burlington, Ontario, Canada) are in lanes 10 and 11. Size in nucleotides is indicated on the left.

4. References

[1] Bolotin A., Wincker P., Mauger S., Jaillon O., Malarme K., Weissenbach J., Ehrlich S.D., Sorokin A., The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403, Genome Res. 11 (2001) 731−753.

[2] Dsouza M., Larsen N., Overbeek R., Searching for patterns in genomic data, Trends Genet. 13 (1997) 497−498.

[3] Fislage R., Berceanu M., Humboldt Y., Wendt M., Oberender H., Primer design for a prokaryotic differential display RT-PCR, Nucleic Acids Res. 25 (1997) 1830−1835.

[4] Marshall O.J., PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR, Bioinformatics. 20 (2004) 2471−2472.

[5] Neidhardt C.F., Ingraham J.C.I., Schaechter M., Physiology of the bacterial cell, a molecular approach, Sinauer Associates Inc., Sunderland, USA, 1990.

[6] Raynaud S., Perrin R., Cocaign-Bousquet M., Loubiere P., Metabolic and transcriptomic adaptation of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* in response to autoacidification and temperature downshift in skim milk, Appl. Environ. Microbiol. 71 (2005) 8016−8023.

[7] Venkatesh B., Hettwer U., Koopmann B., Karlovsky P., Conversion of cDNA differential display results (DDRT-PCR) into quantitative transcription profiles, BMC Genomics 6 (2005) 1−12.