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Lactobacillus helveticus: Strain Typing and Genome Size Estimation by Pulsed Field Gel Electrophoresis

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Abstract. Genomic DNAs of 22 strains of *Lactobacillus helveticus* of various geographical origins were analyzed by pulsed-field gel electrophoresis. Two endonucleases, *Sma*I and *Sgr*AI, of the 19 tested produced DNA fragments useful for strain comparison. With the endonuclease *Sma*I, a characteristic restriction pattern was identified for 18 of the 22 strains. The percentage of similarity (Dice coefficient) between the profiles varied between 26% and 100%, and clustering was accomplished by using the unweighted pair group method with arithmetic averages (UPGMA). For the strains showing identical profiles, the high genomic similarity was confirmed when the endonuclease *Sgr*AI was used instead of *Sma*I. From summation of *Sma*I and *Sgr*AI fragments from three *L. helveticus* strains (CNRZ 241, CNRZ 303, and CIP 57.15), the genomic length was estimated at ca. 1.85–2.0 Mb.

Lactobacillus helveticus is used extensively in the manufacture of Swiss type cheeses and Grana Padano [1, 23], and it has also been found in Bulgarian sour milk [5]. Several technological properties of that species have been studied, such as sugar fermentation, proteolytic and lipolytic activity, autolysis, and aroma production [22, 24]. An efficient strain typing tool would be useful for the preparation of well-defined starters, as well as to identify one particular strain in complex ecosystems like cheeses. Until now, only a few typing methods have been proposed for lactobacilli, and in particular for *L. helveticus* [7], including M13 DNA fingerprinting [16] and ribotyping [18]. These two methods have been applied only to one strain of *L. helveticus* (ATCC 15009), and their efficiency in *L. helveticus* strain differentiation has not been checked by these authors. Previously, by comparing the phenotypic and genotypic characteristics of six strains of *L. helveticus*, Manachini and Parini [14] concluded that fingerprinting of total DNA by the restriction enzymes *Eco*RI, *Bam*HI, and *Hin*dIII with conventional electrophoresis might provide a useful tool for strain differentiation in that species. Recently, the DNA fingerprinting by pulsed field gel electrophoresis (PFGE), allowing the comparison of large restriction fragments [15], has been successfully applied to strain typing of various lactic acid bacteria: *Lactococcus lactis* [11, 21], *Leuconostoc oenos* [10], *Lactobacillus acidophilus* group [19], *Lactobacillus plantarum* [4], and *Streptococcus salivarius* subsp. *thermophilus* [3]. In this paper, we describe the efficiency of PFGE of *Sma*I and *Sgr*AI digests for *L. helveticus* strain differentiation. Moreover, the genome size of that species has been estimated in this way.

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Materials and Methods

Bacterial strains and growth conditions. The strains used are listed in Table 1. They were stored at −70°C in MRS [6] supplemented with glycerol (15% wt/vol) and grown at 43°C in MRS broth.

Genomic DNA preparation and pulsed field gel electrophoresis. An overnight culture of *L. helveticus* was diluted (1/100) in fresh MRS broth and grown at 43°C to an OD_{650} of 0.3 (cellular dry weight = 0.03 mg/ml). The cells from 5-ml samples of the culture were harvested for 10 min in a centrifuge (8000 *g*), washed once in 5 ml of TES buffer, and suspended in 400 µl of 50 mM EDTA. The agarose blocks were prepared as described previously [12] with one modification: the temperature of agarose fusion used was 60°C instead of 45°C. The agarose blocks containing the purified DNA were equilibrated overnight in the restriction endonuclease digestion buffer at 4°C, after which they were transferred to 250 µl fresh digestion buffer containing 15 units of *Sma*I endonuclease or 25 units of *Sgr*AI endonuclease (Boehringer, Mannheim, Germany). For the other enzymes tested, a quantity of 20 units was used. The blocks were incubated overnight at 25°C for *Sma*I and at 37°C for *Sgr*AI. Pulsed field electrophoresis was performed on a Biorad CHEF DRII electrophoresis cell. Samples were electrophoresed through

Table 1. Origin of the strains

Strain	Designation(s) in other collection(s)	Source	Country	Year of isolation
CNRZ 32[a]		Artisanal starter, Comté	France	1960
CNRZ 223[T]	ATCC 15009, CIP 103146, NCDO 2712	Emmental	Switzerland	<1919
CNRZ 241		Artisanal starter, Comté	France	1963
CNRZ 243		Artisanal starter, Comté	France	1963
CNRZ 303		Artisanal starter, Comté	France	
CNRZ 414		Koumis (cow milk)	URSS	1971
CNRZ 493		Commercial starter, Emmental	Finland	1978
CNRZ 834	NZ DRI 5001	Emmental	Switzerland	<1918
CNRZ 1094	NCDO 766	Starter, Emmental	Finland	<1955
CNRZ 1102		Artisanal starter, Grana Padano	Italy	1988
CNRZ 1110	(IL 590)	Commercial starter	France	1970
CIP 57.15[b]	ATCC 12046, NCDO 1829, NCIB 8333			1956
ISLC5[c]		Artisanal starter, Grana Padano	Italy	
NCFB 384[d]		Rennet	France	<1954
B 832[e]			Netherlands	
A[f]		Industrial starter, Emmental	France	
B		Industrial starter, Emmental	France	
C		Industrial starter, Emmental	France	
D		Industrial starter, Emmental	France	
E		Industrial starter, Emmental	France	
F		Industrial starter, Emmental	France	
CP615[g]			Japan	

[a] CNRZ, Centre National de Recherche Zootechnique, Collection, 78352 Jouy-en-Josas, France.
[b] CIP, Collection de l'Institut Pasteur.
[c] Kindly provided by E. Neviani, Istituto Sperimentale Latiero-Caesario.
[d] NCFB, National Collection of Food Bacteria.
[e] Kindly provided by the Nizo Institute, Netherlands.
[f] Industrial strains, confidential origin.
[g] Kindly provided by Dr. Yamamoto, Japan.

1% (wt/vol) agarose gels (ultrapur, Gibco-BRL, Paisley, Scotland), in a running buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) at 200 V and at 14°C with pulsed times and total run time indicated in the text. The photographs of the gels were scanned with a XRS laser densitometer, and the image was analyzed by the Bio-Image system (Millipore). The % of similarity [S(%)] (commonly referred to as the Dice coefficient) was calculated for each pair of A and B patterns according to the equation $S(\%) = [2 \times N/N_A + N_B] \times 100$, where N is the number of matched bands, N_A and N_B the number of bands in pattern A and B respectively. Clustering was accomplished by using the unweighted pair group method with arithmetic averages (UPGMA) and a standard deviation of 3.3%.

Determination of the genome size. The *Sma*I and *Sgr*AI restriction patterns (obtained with an increasing pulse time of 3–40 s, for 22 h) were used for the genome size determination of three different strains (CNRZ 241, CNRZ 303, and CIP 57.15). The following markers were loaded on the gels: concatemers λ (Boehringer) as well as a marker named TL that has been developed in our laboratory. The TL was obtained by cutting the genomic DNA of a particular propionibacteria strain with the restriction enzyme *Xba*I. The 13 largest fragments were regularly spaced in the profile, allowing their use as marker. The size of each fragment has been determined (2, 6.2, 21.3, 45.4, 60.8, 78, 108, 154, 217, 257, 278, 460, and 530 kb; A.F. de Carvalho, personal communication) by comparison with three other commercial markers: CHEF DNA (Biorad), λDNA-PFGE (Pharmacia), and DNA size standard yeast chromosomal (Biorad). The fragments 2 and 6.2 are often not visible.

Results

Restriction endonuclease digestion patterns of *L. helveticus* ATCC 12046 genomic DNA. The DNA base composition of *L. helveticus* has been reported to be in the range of 37–40 mol% G+C [8, 9]. The genomic DNA of *L. helveticus* ATCC 12046 was digested by 19 endonucleases with recognition sequences that are rich in G+C and/or including CTAG, CGG, and CCG, sequences that have been reported to be rare in prokaryotes DNA [15]. *Apa*I, *Sac*I, *Not*I, *Sfi*I, *Sma*I, *Nar*I, *Ava*II, *Msp*I, *Bgl*I, *Ksp*I, *Sty*I, *Hpa*II, *Nae*I, *Hae*II, *Cfo*I, *Xho*I, *Hin*dIII, *Sgr*AI, *Rsr*II were assayed under the following electrophoresis conditions: pulse time 1–13 s for 20 h, with the buffers and concentrations indicated by the suppliers. The endonucleases *Hin*dIII, *Ava*II, *Bgl*I, *Ksp*I, *Sty*I, *Hpa*II, *Nae*II, *Hae*II, *Cfo*I, *Xho*I led to a very high number of fragments below 100 kb and for that reason were not suitable for strain comparison or genome size determination (data not shown). By contrast, the endonucleases *Not*I, *Sac*I, *Msp*I, and *Sfi*I produced too few fragments (data not shown). The more informative pat-

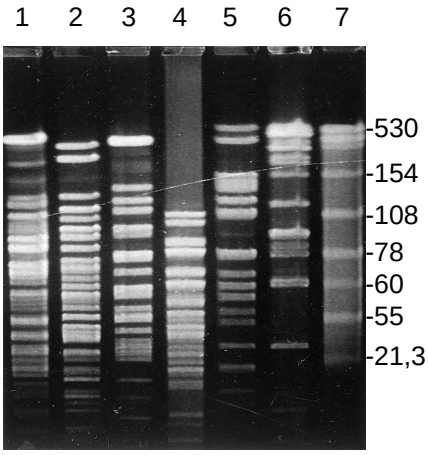

Fig. 1. PFGE patterns of several restriction enzyme digests of genomic DNA from *L. helveticus* CIP 57.15. Electrophoresis was performed with a pulse time ramped from 1 s to 13 s for 20 h at 200 V. Lane 1, *Nar*I; lane 2, *Apa*I; lane 3, *Sma*I; lane 4, *Xho*I; lane 5, *Sgr*AI; lane 6, *Rsr*II; lane 7, TL marker (kb indicated on the right). The enzymes *Sma*I and *Sgr*AI were chosen for further optimization of the electrophoresis parameters.

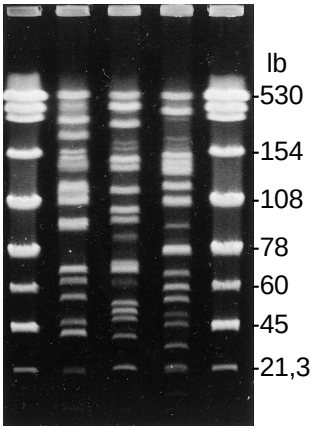

Fig. 2. PFGE patterns of *Sgr*AI digests of genomic DNA separated with a pulse time ramped from 2 s to 13 s for 22 h. Lanes 1 & 5, TL marker; lane 2, strain CNRZ 241; lane 3, strain CNRZ 303; lane 4, strain CIP 57.15.

terns (Fig. 1) were obtained by using *Sma*I, *Sgr*AI, and *Rsr*II, and to a lesser extent *Apa*I. *Sma*I [CCC ↓ GGG] and *Sgr*AI [C(A or G) ↓ CCGG(T or C)G] were retained for further optimization of the pattern.

Optimal separation of *Sma*I and *Sgr*AI digests and genome size determination. Several parameters can modify the pattern obtained with a given restriction enzyme, in particular the switch time, the agarose and buffer concentrations, the total run time, the voltage, and the temperature of the electrophoresis performed [2]. In order to improve the patterns obtained in Fig. 1, we changed the switch time and the total run time as follows: constant switch time (5 s/10 s/30 s) for 22 h, and increasing switch time (2–13 s for 18 h and 22 h; or 3–40 s for 22 h).

For *Sgr*AI, the more convenient patterns for strain comparison were obtained with an increasing switch time of 2–13 s for 22 h (Fig. 2), and for *Sma*I a constant switch time of 5 s for 22 h (Fig. 3). For genome size determination, and for these two endonucleases, the largest bands were better separated with an increasing pulse time of 3–40 s for 22 h (Fig. 4). In these pulse conditions, the molecular size of the genome was estimated by adding the size of all the restriction fragments generated by *Sgr*AI (except for a few particularly weak bands indicated on the figure) as well as by adding the size of all the fragments generated by *Sma*I. The total values ranged from 1.85 Mb with *Sma*I patterns to 2 Mb with *Sgr*AI patterns (Table 2). The three strains led to very close values, and the result depended mainly on the enzyme used.

Comparison of *Sma*I restriction patterns of 22 *L. helveticus* strains of various origins. When genomic DNA from 22 strains was digested by *Sma*I and examined by PFGE (Fig. 3), 18 different restriction patterns resulted. As shown in the dendrogram established by the average linkage method (UPGMA), the average percentage of similarity between the profiles varied from 44% to 100% (Fig. 3), and several groups (at least two) can apparently be defined. The first group is composed of the majority of the strains (18 of the 22 tested), for which the average percentage of similarity was higher than 50%. A second distinct group was constituted by four less related strains: CP615 from Japan, B832 from the Netherlands, and two industrial strains (B and E) from France. Such a dendrogram should now be confirmed by another genomic strain typing method, in order to establish whether these four strains are really less related.

The genomic DNA of the strains showing 100% similarity (D # CNRZ 32; CNRZ 243 # CNRZ 414; CNRZ 1102 # ISLC 5; and B # E) were analyzed with the second endonuclease *Sgr*AI. Again the profiles were identical, indicating that these strains were, genetically speaking, very closely related (data not shown). Regarding the industrial strains (indicated by letters), apart from strains B and E which showed identical restriction patterns, the other industrial strains A, C, D, and F were apparently not related. It could be noticed that one of them (strain D) showed 100% similarity with the collection strain CNRZ 32.

Discussion

The restriction of genomic DNA of *Lactobacillus helveticus* with rare-cutting enzymes (*Sma*I and *Sgr*AI) de-

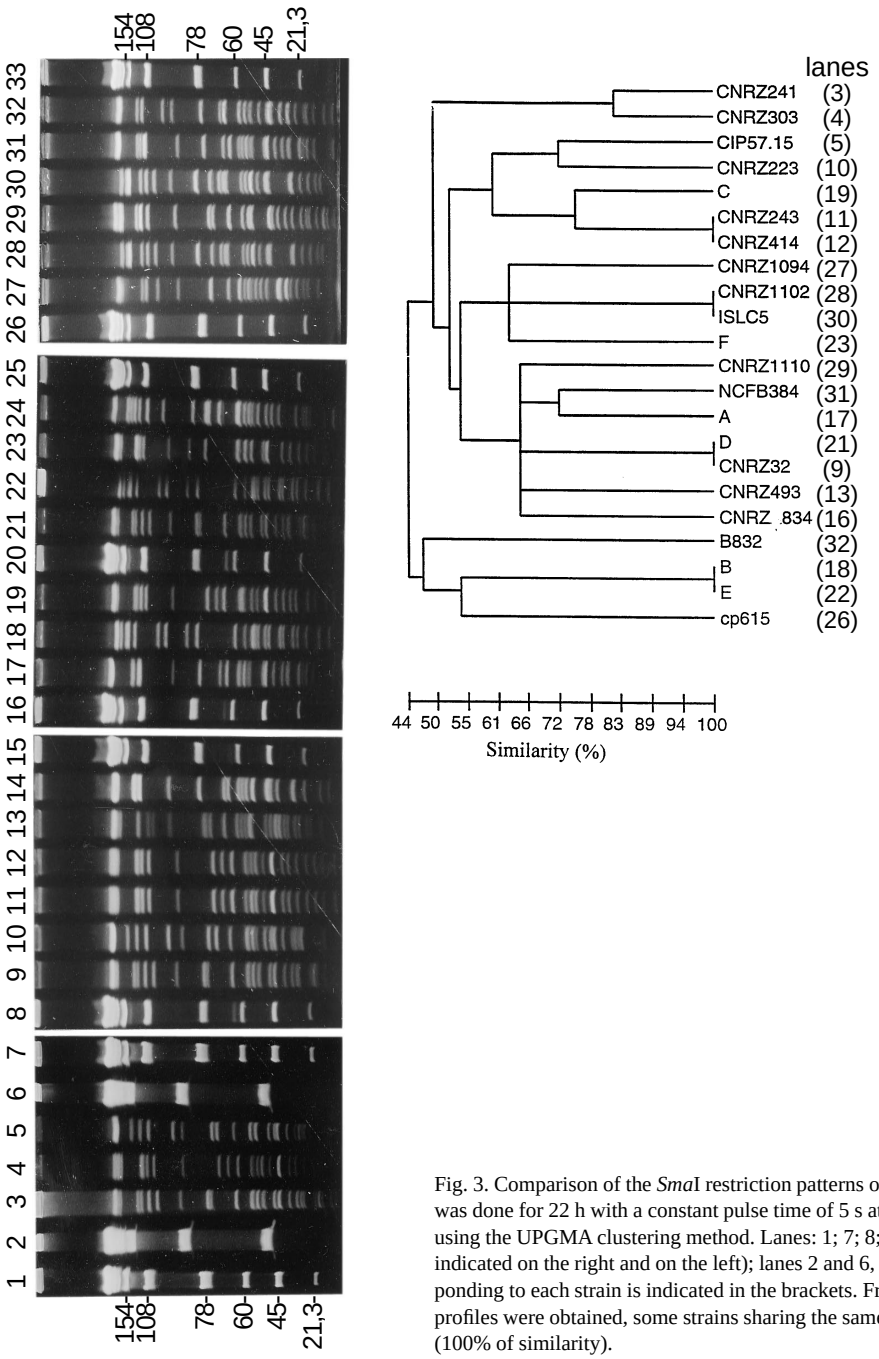

Fig. 3. Comparison of the *Sma*I restriction patterns of 22 *L. helveticus* strains. Electrophoresis was done for 22 h with a constant pulse time of 5 s at 200V; the dendrogram was obtained by using the UPGMA clustering method. Lanes: 1; 7; 8; 15; 16; 20; 25; 26; 33, TL marker (kb indicated on the right and on the left); lanes 2 and 6, concatemers lambda; the lane corresponding to each strain is indicated in the brackets. From the 22 strains tested, 18 different profiles were obtained, some strains sharing the same profile as highlighted by the dendrogram (100% of similarity).

scribed in this work has allowed the genomic typing of 22 strains of various origins as well as genome size estimation. The fact that *Sma*I could be a convenient enzyme for strain typing was not surprising since similar conclusions have been drawn for species closely related, and with a similar G+C content: *L. acidophilus* (36%) [19] and *Listeria monocytogenes* (37–39%) [17]. By contrast, this is, to our knowledge, the first time that *Sgr*AI was shown to be potentially as efficient as *Sma*I for strain typing purpose in lactic acid bacteria. Comparison of the *Sma*I restriction patterns indicated that the majority of strains had unique restriction patterns, and this result supports the idea that DNA restriction pattern can be a useful epidemiological tool for *L. helveticus*. However, strains with identical patterns were also identified, and this high genomic similarity has been confirmed by comparing the *Sgr*AI restriction patterns of these strains, which were again identical. It could be highlighted that strains B and

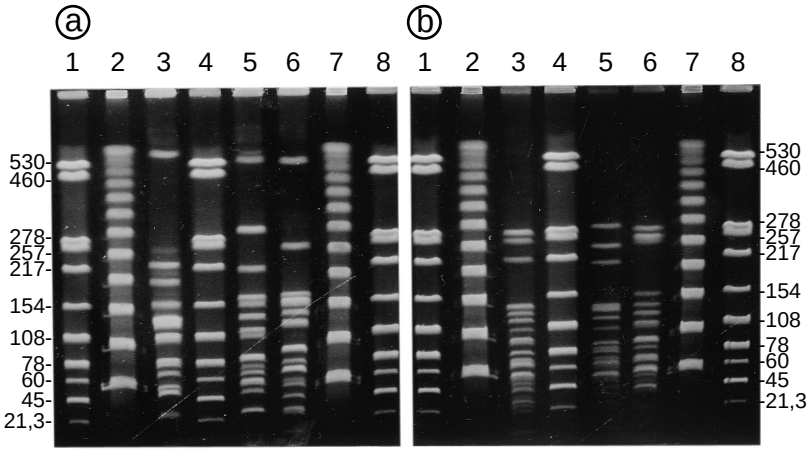

Fig. 4. For genome size determination, the largest fragments of the PFGE patterns of *Sgr*AI (a) and *Sma*I (b) digests were separated with a pulse time ramped from 3 s to 40 s for 22 h. Lanes 1, 4, and 8, TL marker (kb indicated on the left); lanes 2 and 7, concatemers lambda; lane 3, strain CNRZ 241; lane 5, strain CNRZ 303; lane 6, strain CIP 57.15.

Table 2. Genome size (kb) of *L. helveticus* CNRZ 303, CNRZ 241, and CIP 57.15 estimated by summation of the *Sma*I or *Sgr*AI digests shown in Fig. 4 (except for the few weak bands indicated by white stars)

	CNRZ 241		CNRZ 303		CIPH 57.15	
	*Sma*I	*Sgr*AI	*Sma*I	*Sgr*AI	*Sma*I	*Sgr*AI
	271	594	282	540	268	529
	252	221	237	291	250	249
	217	188	205	208	240	163
	136	154	133	161	148	153
	125	132	127	150	131	145
	115	122	115	130	119	134
	102	109	100	113	107	120
	90	100	84	104	100	114
	86	80	79	80	86	87
	77	76	75	71	78	74
	69	66	68	64	66	66
	64	48	63	48	48	48
	48	46	47	46	47	47
	47	26	46	28	45	42
	46	16	41	15	40	26
	42		32		34	23
	28		22		25	18
	23		20		20	15
	21		18			
	16		16			
Total size (kb)	1875	1976	1810	2051	1853	2054
Nb of fragments	20	15	20	15	18	18

E came from French dairy products and are used as dairy starters, and that strains ISLC5 and CNRZ 1102 were both isolated from Italian artisanal starter of Grana Padano. By contrast, the 100% similarity between strain CNRZ 414 isolated from Russian Koumiss and CNRZ 243 isolated from French Comté artisanal starter was more surprising. In a more general view, it would now be interesting to observe to which extent the strains showing identical restriction patterns differ in their technological properties (such as acidification, proteolysis, autolysis, phage resistance). Regarding, for example, the phage resistance, Séchaud [20] characterized the sensitivity of 65 strains of *L. helveticus* (including most of the strains studied in this work) towards 35 bacteriophages, and we have noted that the strains CNRZ 243 and 414 (showing identical restriction patterns) do not share identical bacteriophage sensitivity. Indeed, the strain CNRZ 243 was sensitive to four bacteriophages, whereas strain CNRZ 414 was sensitive to only one, which was, moreover, different of the four infecting CNRZ 243. Furthermore, our own unpublished observations regarding autolysis revealed a different level of activity between strains sharing identical restriction profiles. It would probably be far from evident, or maybe impossible, to predict any technological behavior from these macro-restriction profiles. To our mind, their main interest is to give the ability to follow a particular strain in a complex ecosystem. Moreover, it can be used to apply technological screenings (in general, heavy and time consuming) to a reduced number of strains, which would be chosen, not randomly, but more rationally on the basis of their genomic profiles.

With slightly different pulse conditions to separate the largest bands, the restriction patterns were used to determine the genomic length in that species. For three strains of *L. helveticus*, it was estimated to be near 1.85 Mb with the summation of *Sma*I fragments and near 2.0 Mb with the summation of *Sgr*AI fragments. The validity of this result was difficult to assess, since the genome size of *L. helveticus* has not yet been estimated by another method. Nevertheless, it was in agreement with the genome size of *L. acidophilus*, the most phylogenetically related species of *L. helveticus*, which was estimated to be near 1.85 Mb [19]. For other lactobacilli like *L.*

plantarum and *L. delbrueckii* subsp. *bulgaricus*, it was shown to be respectively 2.8 Mb [4] and 2.3 Mb [13].

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Literature Cited

1. Auclair J, Accolas JP (1983) Use of thermophilic lactic starters in the dairy industry. Antonie Leeuwenhoek 49:313–326
2. Birren BW, Lai E, Clark SM, Hood L, Simon MI (1988) Optimized conditions for pulsed field gel electrophoretic separations of DNA. Nucleic Acids Res. 16:7563–7582
3. Boutrou R, Thuault D, Bourgeois CM (1995) Identification and characterization of *Streptococcus thermophilus* strains by pulsed-field gel electrophoresis. J Appl Bacteriol 79:454–458
4. Daniel P (1995) Sizing of the *Lactobacillus plantarum* genome and other lactic acid bacteria species by transverse alternating field electrophoresis. Curr Microbiol 30:243–246
5. Dellaglio F, Torriani S, Stefanova-Kondratenko M, Vlaikovska K, Ivanova P, Mustafova K (1986) Presence of *Lactobacillus helveticus* and an L(+)lactic acid former in Bulgarian sour milk. Syst Appl Microbiol 8:223–225
6. De Man JC, Rogosa M, Sharpe E (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135
7. Dykes GA, von Holy A (1994) Strain typing in the genus Lactobacillus. Lett Appl Microbiol 19:63–66
8. Gasser F, Mandel M (1968) Desoxyribonucleic acid composition of the genus Lactobacillus. J Bacteriol 96:580–588
9. Kandler O, Weiss N (1986) Regular, non sporing gram positive rods. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, Vol 2. Baltimore, Md: Williams and Wilkins, pp. 1208–1234
10. Kelly WJ, Huang CM, Asmundson RV (1993) Comparison of *Leuconostoc oenos* strains by pulsed-field gel electrophoresis. Appl Environ Microbiol 59:3969–3972
11. Le Bourgeois P, Mata M, Ritzenthaler P (1989) Genome comparison of *Lactococcus* strains by pulsed-field gel electrophoresis. FEMS Microbiol Lett 59:65–70
12. Lemée R, Rouault A, Guezenec S, Lortal S (1994) Autolysis of 57 strains of dairy propionibacteria. Lait 76:241–251
13. Leong-Morgenthaler P, Ruettener C, Mollet B, Hottinger H (1990) Construction of the physical map of *Lactobacillus bulgaricus*. In: Abstracts of the third symposium on lactic acid bacteria, 17–21 September 1990, Wageningen, The Netherlands. FEMS Microbiol Lett 87:A28, p 19
14. Manachini PL, Parini C (1983) DNA restriction endonuclease cleavage patterns, DNA sequence similarity and phenotypical characteristics in some strains of *L. helveticus* and *L. jugurti*. Antonie Leeuwenhoek 49:143–152
15. McClelland M, Jones R, Patel Y, Nelson M (1987) Restriction endonucleases for pulsed field mapping of bacterial genomes. Nucleic Acids Res 15:5985–6005
16. Miteva VI, Abadjieva AN, Stefanova TzT (1992) M13 DNA fingerprinting, a new tool for classification and identification of Lactobacillus spp. J Appl Bacteriol 73:349–354
17. Moore MA, Datta AR (1994) DNA fingerprinting of *Listeria monocytogenes* strains by pulsed-field gel electrophoresis. Food Microbiol 11:31–38
18. Rodtong S, Tannock GW (1993) Differentiation of Lactobacillus strains by ribotyping. Appl Environ Microbiol 59:3480–3484
19. Roussel Y, Colmin C, Simonet JM, Decaris B (1993) Strain characterization, genome size and plasmid content in the *Lactobacillus acidophilus* group (Hansen and Mocquot). J Appl Bacteriol 74:549–556
20. Séchaud L (1990) Ph.D. thesis. University of Paris VII & XI, France
21. Tanskanen E, Tulloch DL, Hillier AJ, Davidson BE (1990) Pulsed-field gel electrophoresis of *Sma*I digests of Lactococcal genomic DNA, a novel method of strain identification. Appl Environ Microbiol 56:3105–3111
22. Torriani S, Vescovo M, Scolari G (1994) An overview on *Lactobacillus helveticus*. Ann Microbiol Enzymol 44:163–191
23. Turner KW, Morris HA, Martley FG (1983) Swiss type cheese: II. The role of thermophilic lactobacilli in sugar fermentations. N Z J Dairy Sci Tech 18:117–123
24. Valence F, Lortal S (1995) Zymogram and preliminary characterization of *L. helveticus* autolysins. Appl Environ Microbiol 61:3391–3399