Polish Journal of Microbiology
2005, Vol. 54, No 3, 183–189

Genetic Diversity among *Lactococcus* sp. and *Leuconostoc* sp. Strains Using PCR-RFLP of Insertion Sequences IS*S1-type*, IS*904*, and IS*982*

PIOTR WALCZAK*, MIROSŁAWA KONOPACKA and ANNA OTLEWSKA

Institute of Fermentation Technology and Microbiology, Technical University of Łódź, Łódź, Poland

Received 3 February 2005, received in revised form 23 June 2005, accepted 24 June 2005

A b s t r a c t

PCR-RFLP analysis of commonly occurring insertion sequences IS*S1*-type, IS*904* and IS*982* in *Lactococcus* sp. and *Leuconostoc* sp. was used for the genetic differentiation of 17 strains of lactic acid bacteria. IS*S1*-type and IS*982* were found in all analysed strains while IS*904* was present exclusively in strains belonging to *Lactococcus* sp. Amplification of IS*S1*-type IS sequences resulted in formation of about 820 bp long amplicons, except of strains *Lactococcus lactis* ssp. *lactis* E and *Leuconostoc lactis* R where extra DNA bands about 370 bp long were observed. Similarly for strains of *Leuconostoc lactis* M and N, additional DNA bands about 280 bp long were present. *Taq*I digestion of IS*S1*-type amplicons revealed that all analysed sequences belonged to the restriction type (ii) or (iii) for which major restriction products were 543 and 147 bp long. Amplification of IS*904* from all strains of *Lactococcus* sp. generated amplicons about 1260 bp long. In three strains of *Leuconostoc* sp. M, N and R, shorter amplicons about 880 bp were observed whereas strains O and P did not contained IS*904*. Amplification of IS*982* resulted in formation of amplicons about 1000 bp long and no extra bands were observed for all tested strains. *Taq*I digestion of amplification products showed that for strains C, I and F, G, H, belonging to *Lactococcus* sp. smaller DNA bands were visible suggesting that they contain two different types of IS*982*.

K e y w o r d s: PCR-RFLP, insertion sequences, *Lactococcus* sp., *Leuconostoc* sp.

Introduction

Published data for genomes of Lactic Acid Bacteria (LAB) shows that insertion sequences are very common movable genetic elements present in the chromosome of bacteria as well as in plasmids. Common situation in LAB is that single strain carries from 3 to 8 different IS sequences, and each one can be present even in several copies (Klaenhammer *et al.*, 2003). They contribute to genetic diversity of Lactic Acid Bacteria due to the conservative and replicative transpositions within the chromosome, causing negative or positive changes of bacterial phenotype. Bongers *et al.* (2003) observed that transposition of IS*981*, recovered activity of lactate production in a *ldhB* deficient strain of *Lactococcus lactis* by activation of transcription of that gene. Transposition of IS*S1* into chromosome of *Lactococcus lactis* resulted in mutation manifested with increased sensitivity of mutants to UV light (Duwat *et al.*, 1997). Presence of at least two identical IS sequences in bacterial chromosome can lead to large inversions of chromosomal DNA caused by homologous recombination between them (Daveran-Mingot *et al.*, 1998). Activation or silencing of genes by spontaneous transposition of insertion sequences within bacterial chromosome may serve as a mechanism of strain adaptation to environmental changes (Duval-Valentin *et al.*, 2001). The presence of IS sequences can also contribute to the genetic stability of particular strain since it may generate spontaneous mutations in growing bacterial population. Stability of biochemical profiles of LAB, especially lactose fermentation and protease activity, is very important in dairy industry where bacterial starters are used for the preparation of dairy products. IS mediated transposition leading to the induction of prophage excision

* Corresponding author. Mailing address: Institute of Fermentation Technology and Microbiology, Technical University of Łódź, Wólczańska 171/173, 90-530 Łódź, Poland. E-mail address: pwalczak@p.lodz.pl

can be even more dangerous since it can stop fermentation process. IS*S1*-type insertion sequences belonging to IS*6* family such as IS*946*, IS*S1*, iso-IS*S1*, IS1216, are usually associated with plasmids replicating *via* theta mechanism and therefore they are widely distributed among different bacterial genera, due to the plasmid exchange caused by conjugal transfer or plasmid uptake by competent cells (Romero and Klaenhammer, 1990; Schaefer *et al.*, 1991). Insertion sequences of IS*S1*-type can be found in large plasmids of *Bacillus thuringiensis* (pBtoxis) (Berry *et al.*, 2002), *Listeria inocua* (pLI100) (Glaser *et al.*, 2001), as well as in *Lactococcus lactis* (pMRC01, pK214, pIL105) (Dougherty *et al.*, 1998, Teuber *et al.*, 1999, Anba *et al.*, 1995). Plasmids containing IS*S1*-type insertion sequences can transpose to bacterial chromosome and form cointegrates. The resolution of cointegrate leads to the excision of plasmid containing IS*S1* and the second copy of IS is left in the chromosome. This feature was used for the construction of vectors for insertional mutagenesis of pGh9:IS*S1* type (Maguin *et al.*, 1996). The second very common insertion sequence usually present in the chromosome or plasmid DNA of LAB is IS*904* belonging to the IS*3* family. Its iso- forms are IS*1076* (L, R) (Huang *et al.*, 1990), IS*1069* (Rauch *et al.*, 1994) iso-IS*904* (Rauch, 1990). In the chromosome locations of *Lactococcus lactis* IL1403, this sequence accompanies another one IS*1077* (Bolotin *et al.*, 2001). IS*904* can also be found in lactococcal plasmids such as pK214, pNZ4000 (Teuber *et al.*, 1999; van Kranenburg and de Vos 1998; van Kranenburg *et al.*, 1999; 2000) as well as in nisin sucrose conjugative lactococcal transposons Tn*5276* and Tn*5301* (Rauch and De Vos, 1992; Rauch *et al.*, 1994; Horn *et al.*, 1991). Transposons Tn*5276* and Tn*5301* belonging to the large conjugative transposons of LAB, which contains IS*904* and genes responsible for nisin A or Z biosynthesis, sucrose metabolism, biosynthesis of N^5-(L-1-carboxyethyl)-L-ornithine (gene *ceo*) as well as genes coding excision/integration system (Xis/Int) of the transposon (Dodd *et al.*, 1982; 1990). Chromosome of the *Lactococcus lactis* IL1403 contains only one copy of IS*982* which transposase is inactive due to the mutation prematurely terminating transcription of this gene (Bolotin *et al.*, 2001). IS*982* sequence belonging to the separate family often resides on plasmid DNA such as pNZ4000 and pCI658 and is associated with *eps* gene cluster responsible for production of extracellular polysaccharides (van Kranenburg *et al.*, 1997; Forde and Fitzgerald, 2003). However pCI658 contains iso-IS*982* which is about 50 bp shorter than the normal sequence and most likely its transposase is also inactive. Lactococcal citrate plasmid such as pKR223 from strain of *Lactococcus lactis* ssp.*lactis* var *diacetylactis* KR2 also contained IS*982* sequence closely linked with restriction modification gene cluster (Twomey *et al.*, 1998; 2000). Also citrate utilization genes found in other strain of *Lactococcus lactis* ssp. *lactis* var. *diacetylactis* CRL264 were located closely to the IS*982* sequence (Lopez de Felipe *et al.*, 1995). Knowledge about presence of particular insertion sequences and their stability in the chromosome of LAB is therefore of vital interest from the point of view of industrial application of certain strains. Distribution of IS sequences in strains of LAB may also contribute to better characterization of them and elimination of duplicates from strain collections.

Experimental

Materials and Methods

Bacterial strains used in this study. Bacterial strains used in this study were originated from the collection of the Institute of Fermentation Technology and Microbiology, Technical University of Lodz or were isolated from kefir grains of polish origin (Table I).

DNA preparation. Plasmid and chromosomal DNA was isolated according to the method described by Anderson and McKay (1983).

PCR amplification of IS sequences. Primer sequences for amplification of IS*S1*-type, IS*904* and IS*982* were derived from data records published in NCBI Database (Table II). Underlined parts of oligonucleotides were identical to the beginning fragments of inverted repeats of corresponding IS sequences. In case where IRL and IRR sequences of IS were identical, only one primer was sufficient for the PCR procedure. Primer fragments located upstream of IR, marked in boldface, containing restriction sites for *Bam*HI or *Eco*RI, were introduced additionally to facilitate possible cloning of PCR products. Amplification of IS*S1* type IS sequences was performed in the following manner. About 20 ng of DNA template, 40 pmol of primer ISS1FR, 12.5 µl Red-Taq ReadyMix DNA polymerase (Sigma-Aldrich) were mixed together and supplemented with PCR grade water to a total volume of 25 µl. The amplification procedure consisted of one cycle of 2 min at 94°C, followed by 34 cycles for 1 min at 94°C, 1 min at 45°C and 3 min at 72°C with final extension cycle for 2 min at 72°C was performed using Uno II thermocycler, Biometra, with tube lid heating block set for 105°C. No overlay oil was added to the tubes. The reaction mix for amplification of IS*904* was the same except of primers replaced by IS904F and IS904R in the concentration of 20 pmol each. Similarly for amplification of IS*982*, 40 pmol of primer IS982FR was used. The amplification procedure for IS*904* and IS*982* consisted of one cycle of 3 min at 94ºC, followed by 29 cycles for 1 min at 94°C, 1 min at 40°C and 3min at 70°C with final extension cycle for 3 min at 70°C.

Agarose gel analysis. PCR products of IS amplification were analysed on 1% [w/v] agarose gel in TBE buffer. Amplified PCR products of IS sequences in amount of 10 µl were digested by *Taq*I (MBI Fermentas) for 1 h at 65°C according to the product

Table I
Bacterial strains and their properties

Strain symbol	Strain name	Description / genotype
A	*Lactococcus lactis* ssp. *lactis var. diacetylactis*	Industrial strain / lac$^+$, cit$^+$
B	*Lactococcus lactis* ssp. *lactis var. diacetylactis*	Industrial strain / lac$^+$, cit$^+$
C	*Lactococcus lactis* ssp. *lactis*	Industrial strain / lac+, cit$^-$
D	*Lactococcus lactis* ssp. *lactis*	Industrial strain / lac$^+$, cit$^-$
E	*Lactococcus lactis* ssp. *lactis*	Industrial strain / lac$^+$, cit$^-$
F	*Lactococcus lactis* ssp. *lactis*	Derivative of ATCC 11454
G	*Lactococcus lactis* ssp. *lactis*	Kefir isolate / lac$^+$, cit$^-$
H	*Lactococcus lactis* ssp. *lactis*	Kefir isolate / lac$^+$, cit$^-$
I	*Lactococcus lactis* ssp. *lactis*	Kefir isolate / lac$^+$, cit$^-$
J	*Lactococcus lactis* ssp. *lactis*	Kefir isolate lac$^+$, cit$^-$
K	*Lactococcus lactis* ssp. *lactis*	Kefir isolate / lac$^+$
L	*Lactococcus lactis* ssp.*lactis*	Kefir isolate / lac$^+$
M	*Leuconostoc lactis*	Industrial strain / lac$^+$, cit$^+$
N	*Leuconostoc lactis*	Industrial strain / lac$^+$, cit$^+$
O	*Leuconostoc mesenteroides*	Kefir isolate / lac$^+$
P	*Leuconostoc mesenteroides*	Kefir isolate / lac$^+$
R	*Leuconostoc lactis*	Kefir isolate / lac$^+$, cit$^+$

Note. lac (+/–) – lactose fermentation, cit (+/–) citrate fermentation

Table II
Primers used for PCR amplification of IS sequences

IS sequence	Name	Primer	Sequence source
IS*S1*-type	ISS1FR	5'-GCGGATCCGGTTCTGTTGCAAAGTTT-3'	X62737, AF116286, AF036485
IS*904*	IS904F	5'-GCGGATCCTGGAAAGGTTATAATAAA-3'	M27276, X52273,
	IS904R	5'-CGAATTCTGGAAAGTCAACGAAAAA-3'	X78469, X92946
IS*982*	IS982FR	5'-GCGGATCCAWACCCGAATTGCTAGTT-3'	L34754, AF036485

instruction. Restriction fragments were analysed on 2% [w/v] agarose gel in TBE buffer. Gells placed on UV transiluminator were photographed with digital camera through yellow filter and obtained pictures were electronically inverted for better visualisation of separated DNA bands.

Results

Insertion sequences IS*S1*, iso-IS*S1* and IS*946* are very common in Lactic Acid Bacteria as well as in other gram positive organisms including *Listeria*, *Staphylococcus*, and *Bacillus* sp. Computer analysis of published sequences of IS*S1* type, revealed their genetic diversity in respect to size (807–809 bp), nucleotide sequence of inverted repeats IR and transposase gene *tnpA* as well as presence of restriction sites for different enzymes. Insertion sequences IS*S1* and IS*946* contain 1, 3 or 4 restriction sites for *Taq*I and this enzyme generates fragments with the following length: (i) – 661 and 147 bp; (ii) – 543, 147, 72 and 48 bp; (iii) – 543, 147, 48, 42 and 30 bp. Restriction enzyme *Eco*RV may cut some of IS*S1* type molecules in one site generating fragments of 377 and 431 bp whereas other types are not cut. Therefore, RFLP analysis of IS*S1* type insertion sequences digested with one of these enzymes may be a simple method of their differentiation. Figure 1A shows results of agarose gel electrophoresis of PCR amplified IS*S1*-type insertion sequences from all tested strains of *Lactococcus* sp. and *Leuconostoc* sp. In one strain of *Lactococcus lactis* ssp. *lactis* (E) and in three strains of *Leuconostoc* sp. (M, N, R), normal size of IS*S1*-type amplicons as well as different shortened amplicons were observed. Size comparison of shortened amplicons revealed that strain *Lactococcus lactis* ssp. *lactis* (E) and *Leuconostoc lactis* (R) possessed extra amplicon of the same size (about 370 bp), whereas strains of *Leuconostoc lactis* (M) and (N) contained shorter fragments of the

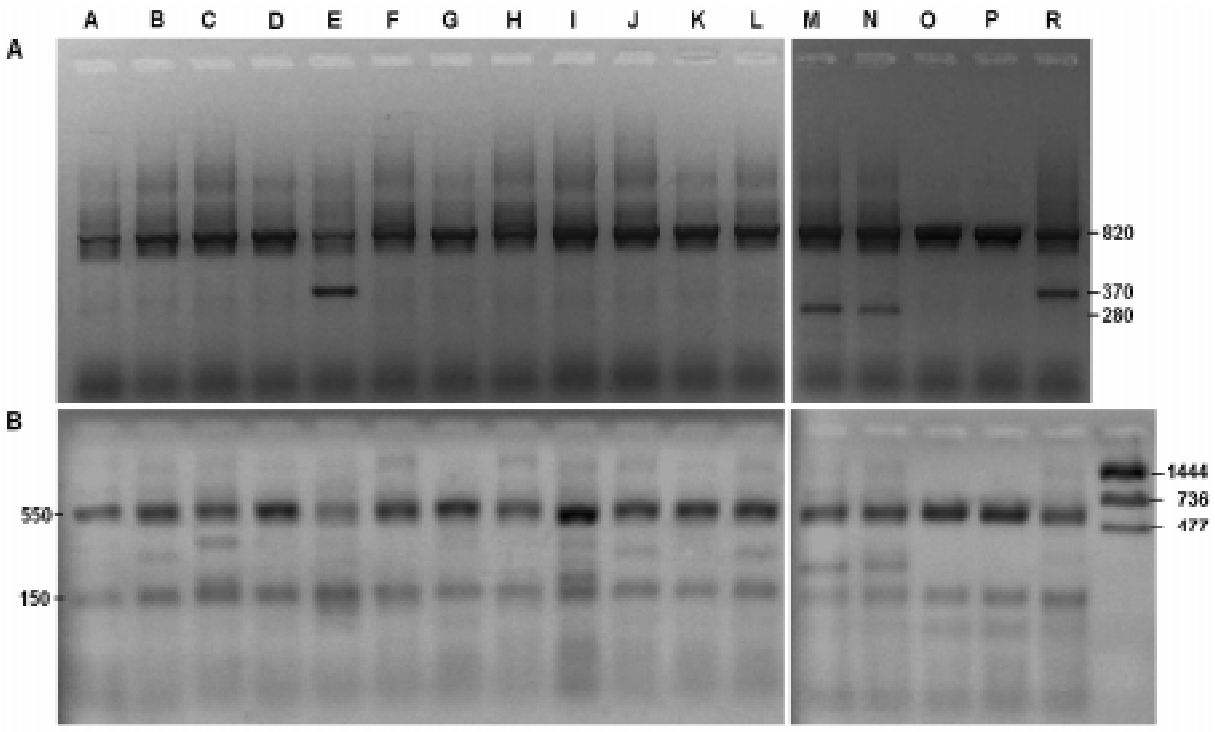

Fig. 1. A – Agarose gel electrophoresis of amplification products with primer ISS1FR specific for IS*S1* type IS sequences.
B – Agarose gel electrophoresis of *Taq*I digested amplification products of IS*S1*-type IS sequences.
A, B, C, D, E, F, G, H, I, J, K, L – strains of *Lactococcus* sp., M, N, O, P, R – strains of *Leuconostoc* sp.

same length being about 280 bp. Size of short amplicons were calculated form the separate agarose gel electrophoresis experiment carried out in the presence of molecular size marker (BTL cat. No G004, pUC19 digested with *Rsa*I, *Hinf*I and *Pvu*II). Presence of such shorter than normal IS*S1*-type sequence in a particular strain may be a unique marker differentiating it from other strains. However the nature and possible function of shorten amplicons is not known. They can represent truncated forms of IS*S1* type sequences transpositionaly not functional due to the deletion of *tnpA* gene. This view strongly supports finding from the DNA sequence (Accession Number Z98171) published by Bourgoin (2002) for exopolysaccharide synthesis genes and insertion sequences of *Streptococcus thermophilus*. In the variable region of exopolysaccharide synthesis genes, three IS*S1*-type sequences (IS*S1SA*, IS*S1SB* and IS*S1SC*) were found of which IS*S1SB* was truncated form containing 62 first nucleotides and 270 last nucleotides of normal type IS*S1* sequence. This truncated form being 332 bp long was named delta-IS*S1SB*. Figure 1B shows results of electrophoretic analysis of *Taq*I digested amplification products. RFLP analysis revealed presence of two major DNA bands with size of 550 and 150 bp which is very close to the expected values of 543 and 147 bp characteristic to the (ii) or (iii) restriction pattern. Fragments smaller than 100 bp were not visible. All analyzed IS sequences belonged to the restriction type (ii) or (iii) according to the proposed classification, for which the largest fragment had 543 bp. DNA bands with size between 543 and 147 bp visible in lanes B, C, E, I, J, L, M, N and R were most likely originated from digestion of "upper" amplicons visible above IS*S1* bands or from shorten amplicons. It is also possible that those bands, are results of presence of two kinds of IS *S1*-type IS sequences in the same strain. Such situation was demonstrated by Bourgoin *et al.* (1996) for strain of *Streptococcus thermophilus*.

Insertion sequence IS*904* formerly named as IS*1069* or IS*1076* is very common in the chromosome of many lactococcal strains (7 copies in *Lactococcus lactis* IL1403), (Bolotin *et al.*, 2002). Figure 2A shows results of agarose gel electrophoresis of PCR amplified IS*904* insertion sequences from all tested strains. IS*904* was present in all strains belonging to the genus *Lactococcus* and the size of obtained PCR amplicons were the same, being about 1260 bp. In two strains of *Leuconostoc* sp. (O, P) IS*904* was not present and in the remaining ones (M, N, R), amplified sequence was shorter than that from strains of *Lactococcus* sp. Figure 2B shows results of electrophoretic analysis of *Taq*I digested amplification products. RFLP analysis of *Taq*I digestion products from strains of *Lactococcus* sp. showed their size of about 736 and 477 bp. Computer modeled restriction analysis of 5 published IS*904* sequences or its iso- forms digested with *Taq*I,

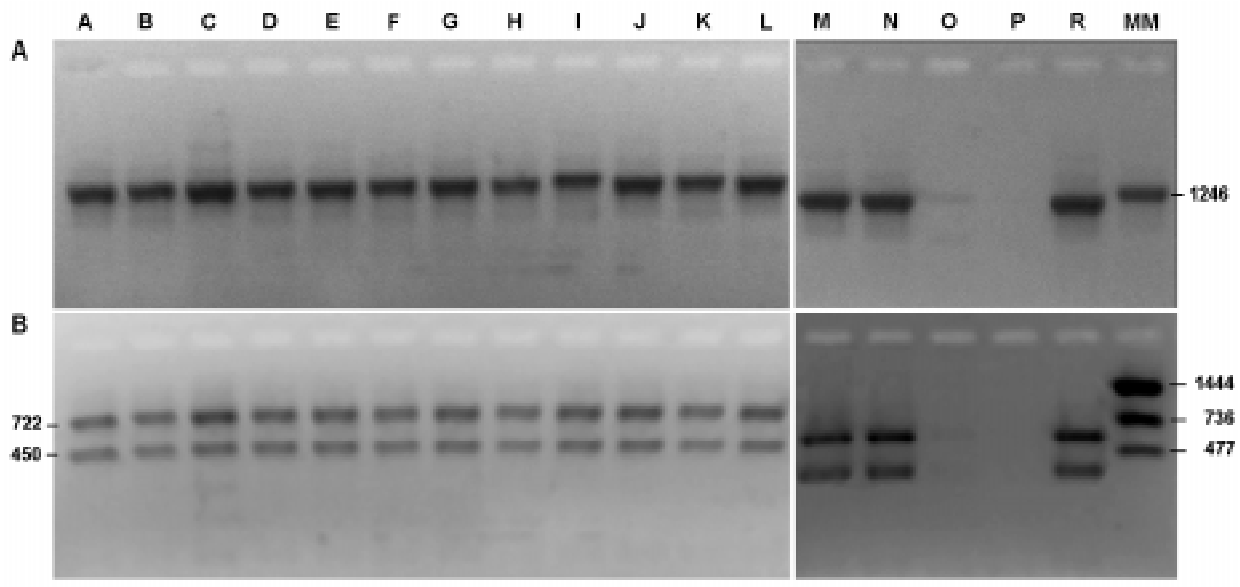

Fig. 2. A – Agarose gel electrophoresis of amplification products with primer IS904F and IS904R specific for IS*904* sequences.
B – Agarose gel electrophoresis of *Taq*I digested amplification products of IS*904* sequences.
A, B, C, D, E, F, G, H, I, J, K, L – strains of *Lactococcus* sp., M, N, O, P, R – strains of *Leuconostoc* sp.

revealed presence of two recognition sites for that enzyme located at positions 75 and 797 of 1245 bp long entire IS sequence. Taking into account extra nucleotides present in primers IS904F and IS904R, *Taq*I restriction fragments of PCR amplified sequences should have the following length 722, 456 and 82 bp. However the smallest fragment can be hardly visible when digestion products are analysed on 2% agarose gel. *Taq*I digestion of PCR products from strains of *Leuconostoc* sp. (M, N and R) were shorter than for original IS*904* sequence (about 550 and 330 bp). This results indicate that analysed strains of *Leuconostoc* sp. possessed most probably a different type of an insertion sequence with front parts of inverted repeats having the same oligonucleotide sequence. The length of the new sequence was estimated to be about 300–400 bp shorter than IS*904*. Question whether it is a new sequence or only deletion form of IS*904* remains still opened. The lack of IS*904* in strains of *Leuconostoc* sp. or the presence of its shorter form, may be diagnostic feature used for differentiation of *Leuconostoc* sp. and *Lactococcus* sp. associated with milk products.

Insertion sequences IS*982* has been found in both already sequenced chromosomes of *Lactococcus lactis* strains IL1403 and MG 1363 (Klaenhammer *et al.*, 2002). Also *Lactococcus lactis* ssp. *cremoris* SK11 contains this sequence (Yu *et al.*, 1995). Nothing is known about presence of IS*982* sequence in strains of

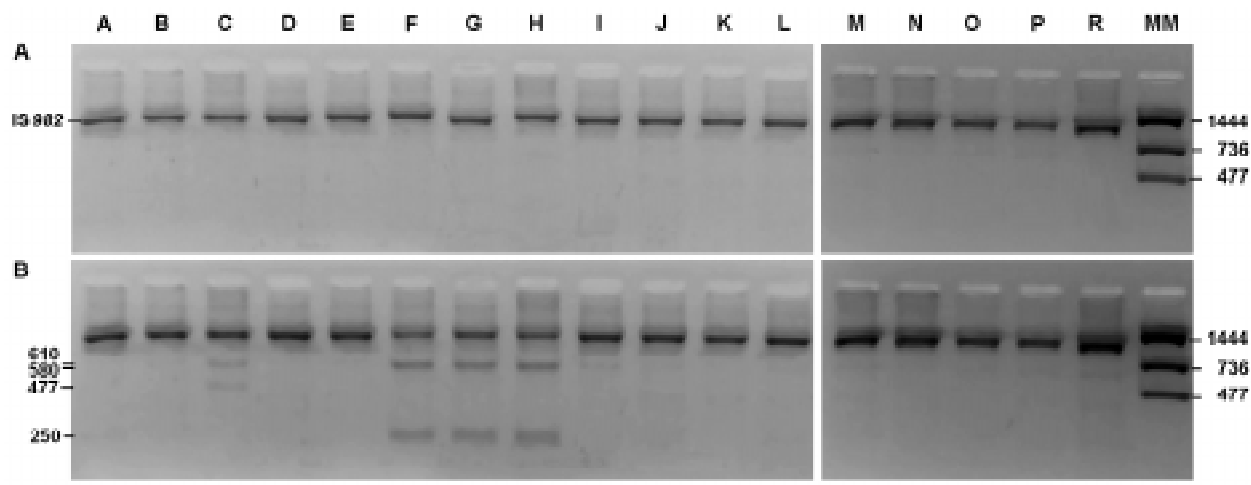

Fig. 3. A – Agarose gel electrophoresis of amplification products with primer IS982FR specific for IS*982* sequences.
B – Agarose gel electrophoresis of *Taq*I digested amplification products of IS*982* IS sequences.
A, B, C, D, E, F, G, H, I, J, K, L – strains of *Lactococcus* sp., M, N, O, P, R – strains of *Leuconostoc* sp.

Leuconostoc sp. The typical length of this sequence varies from about 950 to 1011bp. Published sequences contain a single restriction site for *Eco*RV and are not digested with *Taq*I enzyme. Figure 3A shows results of agarose gel electrophoresis of PCR amplified IS*982* insertion sequences from all tested strains of *Lactococcus* sp. and *Leuconostoc* sp. This sequence was present in all strains and obtained amplicons were uniform with respect to their size. No extra bands were observed. Figure 3B shows results of electrophoretic analysis of *Taq*I digested amplification products. RFLP analysis showed in some strains of *Lactococcus* sp. (C, I) and (F, G, H,) that obtained amplicons were partially digested with *Taq*I enzyme, what may suggest the presence of two kinds of that sequence in one strain. Strains (C) and (I) had similar restriction pattern and strains (F), (G), (H) were characterized by the other type of restriction pattern. So far IS*982* sequences published in the data bases do not contain *Taq*I recognition sites and therefore detected paralogs are still waiting for the recovery and sequencing.

Discussion

Detection of insertion sequences IS*S1*-type, IS*904*, and IS*982* in the chromosome and plasmid DNA of LAB combined with RFLP analysis of *Taq*I digests of amplified sequences can be a valuable tool for analysis of genetic differences among closely related strains. Structural diversity of IS*S1*-type insertion sequences may be used for grouping strains according to their RFLP profile. The presence of certain type of IS*S1* sequences as well as their degenerated shortened forms in particular strain is often associated with the specific plasmid which may function in strains of *Lactococcus* sp. (E) as well as in *Leuconostoc* sp. (R). Absence of IS*904* or its presence in shorter form in strains of *Leuconostoc* sp. may be a method of strain differentiation and allows to distinguish them from strains of *Lactococcus* sp. RFLP analysis of *Taq*I digestion of IS*982* amplification product revealed the existence in certain strains of *Lactococcus lactis*, the new type of such element which DNA is digested with this enzyme whereas, already known sequences, are not. It seems possible that such *Taq*I digested IS*982* sequences are associated with the ability of strains to synthesize lantibiotic bacteriocin nisin. This view supports observation that strain of *Lactococcus lactis* ssp. *lactis* (H) having the same restriction pattern for *Taq*I digested IS*982* as known nisin producer *L. lactis* ATCC 11454 (F) was also able to produce bacteriocin (data not published). However, the nature and structure of bacteriocin produced by strain *Lactococcus lactis* ssp. *lactis* (H) remains unknown. Also strain (G) represented the same *Taq*I restriction pattern of IS*982* as (F) and (H) and therefore most probably it belongs to the same group. It seems possible that *Taq*I digested IS*982* is therefore characteristic of nisin producing strains having nisin-sucrose transposons such as Tn*5276* and Tn*5301* carrying nisin biosynthesis operons. Similarity between strains (F), (G) and (H) and fact that last two of them were isolated from the kefir grains suggests that kefir microflora may produce nisin contributing to the probiotic properties of kefir. However these suggestions has to be confirmed experimentally.

Literature

Anba J., E. Bidnenko, A. Hillier, D. Ehrlich and M.C. Chopin. 1995. Characterization of the lactococcal abiD1 gene coding for phage abortive infection. *J. Bacteriol.* **177**: 3818–3823.

Anderson D.G. and L.L. McKay. 1983. Simple and rapid method for isolating large plasmid DNA from Lactic Streptococci. *Appl. Environ. Microbiol.* **46**: 549–552.

Berry C., S. O'Neil, E. Ben-Dov, A.F. Jones, L. Murphy, M.A. Quail, M.T.G. Holden, D. Harris, A. Zaritsky and J. Parkhill. 2002. Complete Sequence and Organization of pBtoxis, the Toxin-Coding Plasmid of *Bacillus thuringiensis* subsp. *Israelensis*. *Appl. Environ. Microbiol.* **68**: 5082–5095.

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

Bongers R.S., M.H.N. Hoefnagel, M.J.C. Starrenburg, M.A.J. Siemerink, J.G.A. Arends, J. Hugenholtz and M. Kleerebezem. 2003. IS*981*-Mediated Adaptive Evolution Recovers Lactate Production by *ldhB* Transcription Activation in a Lactate Dehydrogenase-Deficient Strain of *Lactococcus lactis*. *J. Bacteriol.* **185**: 4499–4507.

Bourgoin F., G. Guedon, M. Pebay, Y. Roussel, C. Panis and B. Decaris. 1996. Characterization of a mosaic IS*S1* element and evidence for the recent horizontal transfer of two different types of IS*S1* between *Streptococcus thermophilus* and *Lactococcus lactis*. *Gene* **178**: 15–23.

Bourgoin F. 2002. *Streptococcus thermophilus* variable locus including exopolysaccharide synthesis genes and insertion sequences. NCBI Accession Z98171.

Daveran-Mingot M.-L., N. Campo, P. Ritzenthaler and P. Le Bourgeois. 1998. A natural Large Chromosomal Inversion in *Lactococcus lactis* Is Mediated by Homologous Recombination between Two Insertion Sequences. *J. Bacteriol.* **180**: 4834–4842.

Dodd H.M., N. Horn and M.J. Gasson. 1990. Analysis of the genetic determinant for production of the peptide antibiotic nisin. *J. Gen. Microbiol.* **136**: 555–566.

Dodd H.M., N. Horn and M.J. Gasson. 1982. A lactococcal expression system for engineered nisins. *Appl. Environ. Microbiol.* **58**: 3683–3693.

Duval-Valentin G., Ch. Normand, V. Khemici, B. Marty and M. Chandler. 2001. Transient promoter formation: a new feedback mechanism for regulation of IS*911* transposition. *The EMBO J.* **20**: 5802–5811.

Dougherty B.A., C. Hill, J.F. Weidman, D.R. Richardson, J.C. Venter and R.P. Ross. 1998. Sequence and analysis of the 60 kb conjugative, bacteriocin-producing plasmid pMRC01 from *Lactococcus lactis* DPC3147. *Mol. Microbiol.* **29**: 1029–1038.

Duwat P., A. Cochu, S.D. Ehrlich and A. Grus. 1997. Characterization of *Lactococcus lactis* UV-Sensitive Mutants Obtained by IS*S1* Transposition. *J. Bacteriol.* **179**: 4473–4479.

Forde A. and G.F. Fitzgerald. 2003. Molecular organization of exopolysaccharide (EPS) encoding genes on the lactococcal bacteriophage adsorption blocking plasmid pCI658. *Plasmid* **49**: 130–142.

Glaser P., L. Frangeul, C. Buchrieser, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Duchaud, L. Durand, O. Dussurget, K.-D. Entian, H. Fsihi, F. Garcia-Del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L.-M. Jones, U. Karst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueno, A. Maitournam, J. Mata Vicente, E. Ng, G. Nordsiek, S. Novella, B. de Pablos, J.-C. Perez-Diaz, B. Remmel, M. Rose, C. Rusniok, T. Schlueter, N. Simoes, A. Tierrez, J.-A. Vazquez-Boland, H. Voss, J. Wehland and P. Cossart. 2001. From the pathogenic to the innocuous: comparison of the *Listeria monocytogenes* and the *Listeria innocua* genomes. NCBI Accession NC_003383.

Horn N., S. Swindell, H. Dodd, M. Gasson. 1991. Nisin biosynthesis genes are encoded by a novel conjugative transposon. *Mol. Gen. Genet.* **228**: 129–35.

Huang D.C., M. Novel, G. Novel. 1990. A transposon like element on the lactose plasmid of *Lactococcus lactis* subsp. *lactis* Z270. *FEMS Microbiol. Lett.* **61**: 101–106.

Klaenhammer T., E. Alterman, F. Arigoni, A. Bolotin, F. Breidt, J. Broadbent, R. Cano, S. Chaillou, J. Deutscher, M. Gasson, M. van de Guchte, J. Guzzo, A. Hartke, T. Hawkins, P. Hols, R. Hutkins, M. Kleerebezem, J. Kok, O. Kuipers, M. Lubbers, E. Maguin, L. McKay, D. Mills, A. Nauta, R. Overbeek, H. Pel, D. Pridmore, M. Saier, W. de Vos, B. Weimer, M. Zagorec and R. Siezen. 2002. Discovering lactic acid bacteria by genomics. *Antonie van Leeuwenhoek* **82**: 29–58.

Lopez de Felipe F., C. Magni, D. de Mendoza and P. Lopez. 1995. Citrate utilization gene cluster of the *Lactococcus lactis* biovar *diacetylactis*: organization and regulation of expression. *Mol. Gen. Genet.* **246**: 590–599.

Maguin E., H. Prevost, S.D. Ehrlich and A. Gruss. 1996. Efficient insertional mutagenesis in lactococci and other gram-positive bacteria. *J. Bacteriol.* **178**: 931–935.

Rauch P.J.G. 1990 *Lactococcus lactis* insertion element iso-IS*904* coding for a transposase. NCBI Accession: X52273.

Rauch P.J., W.M. De Vos. 1992. Characterization of the novel nisin-sucrose conjugative transposon Tn*5276* and its insertion in *Lactococcus lactis*. *J. Bacteriol.* **174**: 1280–1287.

Rauch P.J., M.M. Beerthuyzen and W.M. de Vos. 1994. Distribution and evolution of nisin-sucrose elements in *Lactococcus lactis. Appl. Environ. Microbiol.* **60**: 1798–1804.

Romero D.A. and T.R. Klaenhammer. 1990. Characterization of insertion sequence IS*946*, an Iso-IS*S1* element, isolated from the conjugative lactococcal plasmid pTR2030. *J. Bacteriol.* **172:** 4151–4160.

Schaefer A., A. Jahns, A. Geis and M. Teuber. 1991. Distribution of the IS elements IS*S1* and IS*904* in lactococci. *FEMS Microbiol. Lett.* **80:** 311–318.

Teuber M., L. Meile, F. Schwarz. 1999. Acquired antibiotic resistance in lactic acid bacteria from food. *Antonie van Leeuvenhoek* **76**: 115–137.

Twomey D.P., L.L. McKay and D.J. O'Sullivan. 1998. Molecular characterization of the *Lactococcus lactis* LlaKR2I restriction-modification system and effect of an IS*982* element positioned between the restriction and modification genes. *J. Bacteriol.* **180**: 5844–5854.

Twomey D.P., De P.J. Urraza, L.L. McKay and D.J. O'Sullivan. 2000. Characterization of AbiR, a novel multicomponent abortive infection mechanism encoded by plasmid pKR223 of *Lactococcus lactis* subsp. *lactis* KR2. *Appl. Environ. Microbiol.* **66**: 2647–2651.

van Kranenburg R. and W.M. de Vos. 1998. Characterization of multiple regions involved in replication and mobilization of plasmid pNZ4000 coding for exopolysaccharide production in *Lactococcus lactis*. *J. Bacteriol.* **180**: 5285–5290.

van Kranenburg R., J.D. Marugg, I.I. van Swam, N.J. Willem and W.M. de Vos. 1997. Molecular characterization of the plasmid-encoded eps gene cluster essential for exopolysaccharide biosynthesis in *Lactococcus lactis. Mol. Microbiol.* **24**: 387–397.

van Kranenburg R., I.I. van Swam, J.D. Marugg, M. Kleerebezem and W.M. de Vos. 1999. Exopolysaccharide biosynthesis in *Lactococcus lactis* NIZO B40: functional analysis of the glycosyltransferase genes involved in synthesis of the polysaccharide backbone. *J. Bacteriol.* **181**: 338-340.

van Kranenburg R., M. Kleerebezem and W.M. de Vos. 2000. Nucleotide sequence analysis of the lactococcal EPS plasmid pNZ4000. *Plasmid* **43**: 130–136.

Yu W., I. Mierau, A. Mars, E. Johnson, G. Dunny and L.L. McKay. 1995. Novel insertion sequence-like element IS*982* in lactococci. *Plasmid* **33**: 218–225.