Insertion sequence analysis of protoplast fused strains of *Lactococcus lactis* ssp. *cremoris*

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**Abstract:** The use of insertion sequence probes for the analysis of fusants obtained following protoplast fusion is described. Hybridization of both total and plasmid DNA from parent and fusant strains with probes to IS904 and ISS1 showed that of the four protoplast fusions examined, three appeared to involve a rearrangement of genetic material while in the fourth the fusant appeared similar to one of the parental strains. This method of analysis provides more information about the changes induced by protoplast fusion than that obtained by monitoring the acquisition or loss of individual characteristics.

**Key words:** Insertion sequence; Protoplast fusion; *Lactococcus; Strain identification*

**Introduction**

*Lactococcus lactis* ssp *cremoris* and *Lactococcus lactis* ssp *lactis* are important organisms in dairy fermentations. Improvement of industrial strains, including selection for desirable characteristics such as bacteriophage resistance and acid production is an ongoing requirement. Protoplast fusion of lactococci to produce strains with improved characteristics has been reported [1–3]. Protoplast fusion requires removal of the cell wall from cells of two or more strains, fusion of the cells, regeneration of the fusants and finally selection of fusants with the desired characteristics. Conditions enabling removal of the cell wall, fusion of the cells and the subsequent exchange of genetic material have been established for lactococci [1–3]. The exchange of genetic information has been monitored by following the acquisition of an indicator plasmid [1,5] and/or the recombination of chromosomal markers [1]. These markers have limited use in monitoring the fusion of industrial strains since plasmids with known characteristics are not always present and chromosomal markers may not differentiate between strains. In addition it is difficult with such markers to tell whether the fusant is really a hybrid strain or is just one of the parent strains. Insertion sequences are small transposable elements which are present in multiple copies in the genome of most lactococcal strains [6–9]. The pattern of hybridization with insertion sequence probes in different strains may provide information on the relatedness of similar strains. During
experiments with lactococcal strains using protoplast fusion a number of fusants were isolated that showed altered bacteriophage resistance. The purpose of this investigation was to determine if the fusants were genetically altered or whether a phage resistant variant of one of the parent strains had been selected.

Both total and plasmid DNA were hybridized with the lactococcal insertion sequences ISS1 [6] and IS904 [10]. The position of the insertion sequences in the parent and fusant strains provides an indication of the genetic rearrangement which has been achieved by the protoplast fusion. This method of analysis will also be applicable to strains altered by other methods.

Materials and Methods

Strains and media

Bacterial strains are listed in Table 1. All strains were propagated on M17 media [11].

Protoplast fusion

The method used was essentially that described by Gasson [1]. Exponential phase cells (30 ml) were washed in distilled water and resuspended in 10 ml of protoplast buffer (0.04 M ammonium acetate pH 7.0, 0.001 M magnesium acetate and 0.5 M sucrose). Lysozyme (1 mg/ml final concentration) was added to the protoplast buffer and incubation carried out at 37°C for 1–3 h. During this time the formation of protoplasts was followed by phase-contrast microscopy. After incubation, cells were washed, mixed and resuspended in the same medium; 30% polyethylene glycol was added and the cells left at room temperature for 20 min. The suspension was then diluted with three volumes of protoplast buffer and centrifuged. After resuspending, appropriate dilutions were incorporated into soft agar overlays and spread on regeneration media. For regeneration of protoplasts, M17 agar containing 0.5 M sucrose was used. Incubation was at 30°C for 3–5 days.

DNA methodology

Total and plasmid DNA was extracted from strains essentially by the method of Anderson and McKay [12]. DNA was digested with EcoRI (BRL) according to the manufacturer’s instructions. Agarose gels (1.0% for total DNA and 0.7% for plasmid DNA) were transferred to Hybond N+ membrane (Amersham) by the method of Southern [13]. The enhanced chemiluminescent method (ECL, Amersham) was used for DNA hybridization and detection according to the manufacturer’s protocols.

Insertion sequence probes

DNA from ISS1 and IS904 was obtained by amplification from lactococcal DNA with primers designed from published sequences (Genbank X62737 (ISS1) and M27276 (IS904)) to the inverted repeat regions of the two insertion sequences. The primer 5'-AAA CTA GAC AC(AG)AG TTA AGA GAA-3' was used for IS904 and 5'-GUT TCT GTT GCA AAG TTT-3' for ISS1. PCR conditions were 35 cycles of 93°C for 30 s, 60°C for 30 s and 72°C for 1 min.

Results

Digestion of total genomic DNA with EcoRI (Fig. 1A) demonstrated differences between all strains except 450 and 2218 which appeared to have identical patterns (Fig. 1A, lanes 4 and 5).

Hybridization of the Southern blot of the total genomic DNA with ISS1 DNA (Fig. 1B) showed that all strains examined contained multiple copies of ISS1. The pattern of hybridizing fragments was different for each of the strains except 450 and 2218 which showed a similar hybridization pattern (Fig. 1B, lanes 4 and 5). Strains 450
and 2218 did not hybridize with the IS904 DNA probe (Fig. 1C) and strain 2128 (Fig. 1C, lanes 1 and 10) showed only weak hybridization with the IS904 probe. Strains 176 and 178 which are the parent strains of fusant 726 showed similar patterns with the IS904 probe but not with the ISS1 probe (Fig. 1B, and C lanes 7–9).

The plasmid profiles of the lactococcal strains used in this study were for the most part different. The exceptions were strains 450 and 2218 (Fig. 2A, lanes 4 and 5) which appeared identical and strains 2208, 726 and 2182 (Fig. 2A, lanes 2, 8 and 11) which had a very similar plasmid profile.

Hybridization of a Southern blot of plasmid DNA with ISS1 (Fig. 2B) indicated that all strains carried copies of ISS1 on one or more plasmids. In some strains all plasmids carried a copy of ISS1, in other strains (for example 2128, Fig. 2B, lanes 1 and 11) only one plasmid had a copy of ISS1. Strains 450 and 2218 (Fig. 2B, lanes 4 and 5) appeared identical. Strains 2208, 726 and 2182 (Fig. 2B, lanes 2, 8 and 11) showed similar patterns of hybridization with ISS1.

Hybridization with the IS904 probe showed that three strains (2128, 450 and 2218) did not contain copies of this insertion sequence on their plasmids. The pattern obtained with IS904 on strains 2208, 726 and 2182 did not allow differentiation of these strains using only the data for this hybridization.

Discussion

Insertion sequences have been shown to be associated in lactococci with industrially important traits such as lactose and sucrose metabolism,
proteinase activity, nisin production and bacteriophage resistance [for a review see 14]. All strains examined in this investigation carried copies of both ISS1 and IS904, in most cases multiple copies. ISS1 was present on plasmids in all strains, however, three strains lacked plasmid copies of IS904.

Of the four protoplast fusion events represented in this investigation, three appear to involve a genuine rearrangement of the DNA as indicated by plasmid profiles and the position of insertion sequences. In the fourth case, that of the fusion of parent strains 450 and 168 to produce the fusant strain 2218 it would appear that strains 450 and 2218 have identical restriction patterns, plasmid profiles and have an identical arrangement of ISS1 and IS904. Hence 2218 is most probably a phage resistant derivative of strain 450 rather than a genuine protoplast fusant. If the pattern obtained for IS904 only is examined strains 176 and 178 may be mistakenly considered as identical. However, this is not the case when the data from the ISS1 hybridization is examined. In this case the fusant (strain 726) does not have a similar pattern to either parent strain.

The work reported here shows the use of insertion sequence analysis of total and plasmid DNA to demonstrate the rearrangement of genetic material when two strains are fused by protoplast fusion. This method of analysis gives more information about the changes in the genome brought about by the fusion event and is more widely applicable to industrial strains than previous methods used to monitor protoplast fusions.

References

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