Lait (1998) 78, 53-58

Does gene horizontal transfer occur in lactic acid bacteria co-cultures?

Gérard Guédon*, Florence Bourgoin, Bernard Decaris

Laboratoire de Génétique et Microbiologie, U.A. Inra 952, Université Henri-Poincaré-Nancy I, Faculté des Sciences, B.P. 239, 54506 Vandœuvre-lès-Nancy, France

Abstract — Four types of insertion sequences, IS*1191*, IS*981*, IS*S1* and IS*1194*, were identified in *Streptococcus thermophilus* CNRZ368. The IS*S1* elements can be divided into three subgroups, α, β and γ sharing 75–85% nucleotide sequence identity. Mosaic elements resulting from recombination between IS*S1* belonging to different subgroups were also identified. The IS*1191*, IS*981*, α and β IS*S1* nucleotide sequences from *S. thermophilus* CNRZ368 were found to be almost identical to insertion sequences from *Lactococcus lactis*, suggesting that horizontal transfers have recently occurred between these distantly related lactic acid bacteria. IS distribution indicates that horizontal transfers have probably occurred from *S. thermophilus* to *L. lactis* and from *L. lactis* to *S. thermophilus* during cocultures used for cheese manufacture. Furthermore, IS*1194* distribution also suggests that some strains of *S. thermophilus* and *L. lactis* have acquired this IS by horizontal transfer(s) from an unidentified bacterium. The three IS*S1* copies, two of the four IS*981* copies and the unique IS*1194* copy of *S. thermophilus* CNRZ368 are clustered in the same 12 kb chromosomal region which could have been acquired from *L. lactis*. Furthermore, other sequence comparisons showed that horizontal transfers of IS and other genes like *citP* encoding citrate permease occurred between various species belonging to five genera of lactic acid bacteria, *Lactococcus*, *S. treptococcus*, *Lactobacillus*, *Leuconostoc* and *Enterococcus*. © Inra/Elsevier, Paris.

horizontal transfer / *Streptococcus thermophilus* / *Lactococcus lactis* / lactic acid bacteria / insertion sequence

Résumé — Des transferts horizontaux d'information génétique se produisent-ils lors des cocultures de bactéries lactiques ? Quatre types différents de séquences d'insertion, IS*1191*, IS*981*, IS*S1* et IS*1194* ont été caractérisés chez *S. thermophilus* CNRZ368. Les éléments IS*S1* peuvent être répartis en trois sous-groupes, α, β et γ, dont les séquences divergent de 15 à 25 % et des éléments mosaïques résultant de la recombinaison d'IS*S1* de sous-groupes différents. Les séquences nucléotidiques de types IS*1191*, IS*981*, IS*S1* α et β de *S. thermophilus* CNRZ368 sont presque identiques à celles des éléments homologues de *L. lactis,* suggérant que des transferts horizontaux se sont produits récemment entre ces deux espèces bactériennes. La distribution de ces éléments suggère que des transferts horizontaux se seraient produits dans les deux sens lors des cocultures de ces deux espèces utilisées dans les fabrications fromagères. De plus, IS*1194* aurait

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* Correspondence and reprints

également été acquise chez les deux espèces par des transferts horizontaux à partir d'une espèce bactérienne non identifiée. Les trois copies d'IS*S1*, deux des quatre copies d'IS*981* et l'unique copie d'IS*1194* de *S. thermophilus* CNRZ368 sont regroupées dans un fragment chromosomique de 12 kb qui pourrait avoir été acquis par transfert horizontal à partir de *L. lactis*. Les transferts d'IS et d'autres gènes comme *citP* codant la citrate perméase concernent des espèces appartenant à cinq genres différents de bactéries lactiques, *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* et *Enterococcus*. © Inra/Elsevier, Paris.

transfert horizontal / *Streptococcus thermophilus* / *Lactococcus lactis* / bactéries lactiques / séquence d'insertion

1. INTRODUCTION

Dissemination of genes encoding resistance to antibiotics or heavy metals and comparison of their nucleotide sequences have provided ample circumstantial evidences for recent horizontal transfer of genetic information between distantly related pathogenic bacteria (i. e. belonging to different genera) in the environment. However, until recently, good evidence of recent intergeneric transfer was only found for resistance genes and for plasmids and transposons which contain these genes.

Cocultures of various lactic acid bacteria are used in manufacture of dairy products. Cocultures of *S. thermophilus* and various other bacteria including *L. lactis* are used as starters in the production of some cheeses. However, until recently, no genetic exchange was observed between these bacterial species.

2. COMPARISON OF INSERTION SEQUENCES (IS) FROM *S. THERMOPHILUS* AND OTHER LACTIC ACID BACTERIA

The study of a chromosomal polymorphic region of *S. thermophilus* CNRZ368 has led to the characterization of an insertion sequence (IS), IS*1191*, which belongs to the IS*256* family [4]. At the same time, IS*905*, an IS which is almost identical to IS*1191* (98.3% identity in nucleotide sequence) was found in *L. lactis* [3]. Furthermore, one of the IS*1191* copies is inserted in a truncated copy of an unrelated IS, IS*981* [4] which belongs to the IS*3* family. This type of IS was previously found in *L. lactis* [6]. Nucleotide sequences of IS*981* from the two species share 99.3% identity.

Moreover, one of the IS*981* copies of *S. thermophilus* CNRZ368 is interrupted by a third type of IS, IS*S1*SA, which belongs to IS*6* family [1]. The nucleotide sequences of IS*S1*SA and of an IS*S1* previously identified in *L. lactis*, IS*S1*N, share 98.6% identitity. A phylogenetic tree deduced from the nucleotide sequences of IS*S1*SA and 21 other IS*S1* elements (reviewed in [1, 8]) clearly showed that the IS group is divided into three subgroups which we have named α, β and γ (*figure 1*). IS*S1*SA belongs to the α subgroup. Nucleotide sequences of elements belonging to the same subgroup share more than 97% identity. α and β elements share about 85% identity whereas γ sequences share about 75% identity with α or β elements.

Some strains of *L. lactis* (CNRZ270 and MG1820) contain both α and β elements. Furthermore, the nucleotide sequences of IS*S1*SA and of another IS*S1* copy from *S. thermophilus* CNRZ368, IS*S1*SC, share only 89% identity. A detai-

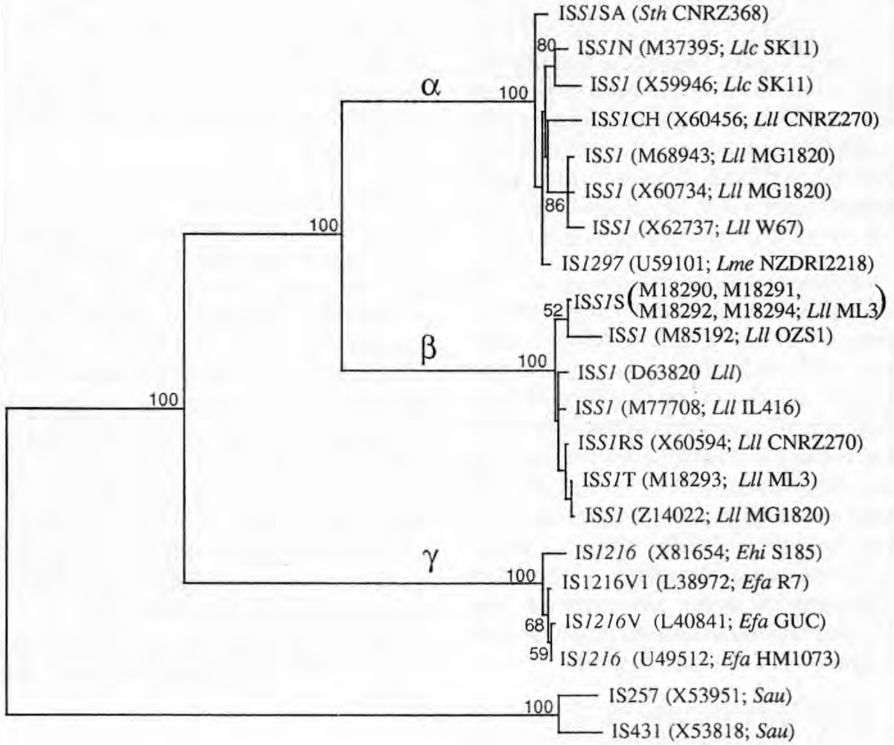

Figure 1. Dendrogram of the IS*S1* group. The dendrogram was produced by unordered character state parsimony using PAUP program and Heuristic algorithm to find the shortest tree. Nucleotide sequences of IS*257* and IS*431* from *Staphylococcus aureus* were used as a monophyletic outgroup. The numbers correspond to the percentage of support for individual nodes on the consensus tree based on 300 bootstrapped runs. Only bootstrapped values greater than 50% are indicated. The length of the bar corresponds to 10 substitutions. The bacterial host species, subspecies and strains from which each IS was isolated are given: *Efa, Enterococcus faecium*; *Ehi, Enterococcus hirae*; *Llc, Lactococcus lactis* subsp. *cremoris*; *Lll, Lactococcus lactis* subsp. *lactis*; *Lme, Leuconostoc mesenteroides*; *Sau, Staphylococcus aureus*; *Sth, Streptococcus thermophilus*.

Figure 1. Dendrogramme du groupe IS*S1*. Le dendrogramme a été produit par la méthode du maximum de parcimonie en utilisant le programme PAUP (algorithme « Heuristic ») pour trouver l'arbre le plus court. Les séquences nucléotidiques d'IS*257* et d'IS*431* de *Staphylococcus aureus* ont été utilisées comme groupe monophélétique extérieur. Les nombres correspondent au pourcentage de 300 répliques de bootstrap soutenant chaque nœud individuel de l'arbre consensus. Seules les valeurs de bootstrap supérieures à 50 % sont indiquées. La longueur du trait isolé correspond à 10 substitutions. Les espèces, sous-espèces et souches bactériennes hôtes d'où ont été isolées chacune des IS sont indiquées : *Efa, Enterococcus faecium* ; *Ehi, Enterococcus hirae* ; *Llc, Lactococcus lactis* subsp. *cremoris* ; *Lll, Lactococcus lactis* subsp. *lactis* ; *Lme, Leuconostoc mesenteroides* ; *Sau, Staphylococcus aureus* ; *Sth, Streptococcus thermophilus*.

led comparison of the IS*S1*SC sequence with those of all the other IS*S1* elements showed that IS*S1*SC is a mosaic of two α and two β regions suggesting that this element results from recombinations between α and β IS*S1* elements [1]. The β regions of IS*S1*SC and the corresponding regions of IS*S1*S, a β IS*S1* element from *L. lactis* subsp. *lactis* ML3, are almost identical (98.7% nucleotide sequence identity).

However, the sequences of the 16S and 23S rRNA of *L. lactis* and *S. thermophilus* share only 89.1–89.2% identity. According to [5], such divergence suggests that the last ancestor of the two species lived about 6×10^8 years ago. All other nucleotide sequences available for the two species (5S rRNA, tRNA^{asn}, *recA* and the gene encoding lactate deshydrogenase) have only 71.6–85.1% identities. Therefore, the quasi-identities of ISs from *L. lactis* and *S. thermophilus* strongly suggest that horizontal transfers have recently occurred between these species.

Sequence analysis also showed that two iso-IS*S1* elements from *L. lactis* are mosaics of α and β regions [1]. IS*S1*W from *L. lactis* Wg2 is a mosaic of a γ region and a region sharing only 76–81% identity with α, β and γ elements. In the same way, a truncated IS*S1* element from *L. lactis* UC653 (GenBank n° U60336) is a mosaic of a region sharing 84–88% identity with α and β elements (about 40 bp left sequence), an α region (about 90 bp), and a γ region (about 260 bp right region). Furthemore, a 76 bp region of an iso-IS*S1* element from *Enterococcus hirae* S185 (GenBank n° X81654) shares only 91% identity with the corresponding sequence of γ IS*S1* elements whereas the remaining sequences share 99.2–100% identity.

α IS*S1* sequences were found in *S. thermophilus*, *L. lactis* and *Leuconostoc mesenteroides* (*figure 1*). In the same way, complete, truncated or partially sequenced γ elements sharing more than 99% identity were found in *E. hirae*, *E. faecium* (*figure 1*), *E. faecalis* (GenBank n° U17153), *L. lactis* (GenBank n° U60336) and *Lactobacillus sake* (GenBank n° Z54312). The 16S rRNAs of *Enterococci*, *Lactococci*, *Lb. sake* and *Lc. mesenteroides* share less than 85% nucleotide identity.

The polymorphism and quasi-identities of IS*S1* from various hosts suggest a three-step evolution. In a first step, the IS*S1* subgroups have probably appeared by separate evolution of initially identical IS*S1* elements in different bacteria which did not exchange genes in their natural environment. In a second step, numerous horizontal transfers have occurred between species belonging to five different genera of lactic acid bacteria probably during dairy cocultures. Presence of IS*S1* belonging to different subgroups in the same strain probably resulted from this second step. In a third step, mosaic elements appeared by recombinations between IS*S1* belonging to different subgroups.

3. IS DISTRIBUTION

IS*1191* related elements were detected by hybridization in all the 28 *S. thermophilus* strains tested but only in 5 out of the 15 strains of *L. lactis* tested [3, 4] suggesting that IS*1191* was transferred from *S. thermophilus* to *L. lactis*.

On the contrary, one to four IS*981* copies were found by hybridization in only 15 out of 28 strains of *S. thermophilus* tested whereas all the 30 strains of *L. lactis* tested were found to contain IS*981* related elements [4, 6]. One to three α and/or β IS*S1* elements are present in 19 out of the 24 strains of *S. thermophilus* tested whereas α and/or β IS*S1* elements were found, generally in more than 10 copies, in almost all strains of *L. lactis* tested [1]. Therefore, these distributions suggest that

IS*981*, α and β IS*S1* elements were transferred from *L. lactis* to *S. thermophilus*.

S. thermophilus is used as starter in the manufacture of yoghurt and various types of cheeses whereas *L. lactis* is used as starter in the manufacture of most types of cheeses but never in that of yoghurt. Nine of the 10 *S. thermophilus* strains tested which do not possess IS*981* were isolated from yoghurt whilst 12 of the 13 strains tested isolated from cheeses possess IS*981*. Thus, this correlation suggests that IS*981* was transferred from *L. lactis* to some strains of *S. thermophilus* during cheese manufacture.

Another IS, IS*1194*, which belongs to IS*5* family was found downstream from IS*S1*SA. Only one IS*1194* copy was detected by hybridization in 2 out of the 19 *S. thermophilus* strains tested and in 4 out of the 15 strains of *L. lactis* tested. These results suggest that IS*1194* was acquired in both species by horizontal transfers from an unidentified bacterium.

4. HORIZONTAL TRANSFERS OF OTHER SEQUENCES

Sequence analysis showed that a chromosomal 12 kb region of *S. thermophilus* CNRZ368 contains the two α IS*S1* copies (IS*S1*SA and ΔIS*S1*SB), the αβ mosaic copy (IS*S1*SC), two of the four IS*981* copies and the unique IS*1194* copy. This region is included in a variable locus, *varD*. Four probes which are adjacent to the 12 kb region hybridized only with two out of the 19 strains of *S. thermophilus* tested, CNRZ368 and A054. These strains are very closely related and possess the 12 kb region [1]. Therefore, a 23 kb region could result from the integration of a lactococcal DNA fragment into the chromosome of a common ancestor of the two strains.

5. CONCLUSION

Thus, sequence comparison and IS distribution strongly suggest that horizontal transfers have occurred from *S. thermophilus* to *L. lactis* and from *L. lactis* to *S. thermophilus* probably during cocultures used in cheese manufacture. Many horizontal transfers of IS and other genes have probably occurred between various species belonging to five lactic acid bacteria genera which are used as starters in dairy industry. Furthermore, it must be noticed that the *citP* genes from *L. lactis* and *Leuconostoc lactis* share 99.2% nucleotide sequence identity [7] suggesting that this gene has been acquired by a horizontal transfer. This plasmidic gene encodes the citrate permease which allows citrate uptake and its metabolisation in diacetyl, an important flavour compound of butters and creams.

However, the mechanism involved in these transfers remains unknown. The most likely mechanism appears to be conjugative transfer. Some conjugative plasmids like pAMβ1 and conjugative transposons like Tn*916* which have very broad host range transfer were found in pathogenic *Streptococci* and *Enterococci*. Furthermore, a conjugative transposon found in various strains of *L. lactis*, Tn*5307*, was recently transferred by conjugation from *L. lactis* to an *Enterococcus* strain [2]. However horizontal transfers by natural transformation can not be excluded since at least some pathogenic *Streptococci* like *S. pneumoniae* are naturally transformable. Furthermore, it must be noticed that bacteria lysis and therefore DNA release occur during cheese ripening. Transduction seems to be more unlikely since no phage infecting different species or genera of lactic acid bacteria is known.

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