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Xavier Bellanger, Adam P Roberts, Catherine Morel, Frédéric Choulet, Guillaume Pavlovic, et al.. Conjugative Transfer of the Integrative Conjugative Elements ICESt1 and ICESt3 from *Streptococcus thermophilus*. *Journal of Bacteriology*, 2009, 191 (8), pp.2764 - 2775. 10.1128/JB.01412-08 . hal-01659303

**HAL Id: hal-01659303**

**<https://hal.science/hal-01659303>**

Submitted on 8 Dec 2017

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## Conjugative Transfer of the Integrative Conjugative Elements ICESt1 and ICESt3 from *Streptococcus thermophilus*<sup>▽†</sup>

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Received 8 October 2008/Accepted 23 January 2009

**Integrative and conjugative elements (ICEs), also called conjugative transposons, are genomic islands that excise, self-transfer by conjugation, and integrate in the genome of the recipient bacterium. The current investigation shows the intraspecies conjugative transfer of the first described ICEs in *Streptococcus thermophilus*, ICESt1 and ICESt3. Mitomycin C, a DNA-damaging agent, derepresses ICESt3 conjugative transfer almost 25-fold. The ICESt3 host range was determined using various members of the *Firmicutes* as recipients. Whereas numerous ICESt3 transconjugants of *Streptococcus pyogenes* and *Enterococcus faecalis* were recovered, only one transconjugant of *Lactococcus lactis* was obtained. The newly incoming ICEs, except the one from *L. lactis*, are site-specifically integrated into the 3' end of the *fda* gene and are still able to excise in these transconjugants. Furthermore, ICESt3 was retransferred from *E. faecalis* to *S. thermophilus*. Recombinant plasmids carrying different parts of the ICESt1 recombination module were used to show that the integrase gene is required for the site-specific integration and excision of the ICEs, whereas the excisionase gene is required for the site-specific excision only.**

Horizontal transfer of genomic islands plays a key role in bacterial evolution, leading to the acquisition of advantageous functions or to major modifications of the bacterial way of life (37, 38). However, the mechanism of their transfer and integration remains generally unknown. Recent in silico analyses revealed that numerous genomic islands could be integrative and conjugative elements (ICEs) or elements deriving from them (18, 30, 62, 67). The ICEs, also called conjugative transposons, were defined as elements which encode their own excision, their transfer by conjugation, and their integration in a replicon of the recipient cell, whatever their specificity and their mechanism of integration and conjugation (18). Most of the ICEs encode a putative integrase belonging to the tyrosine recombinase family. This enzyme, generally associated with an excisionase, catalyzes the recombination between identical sequences carried by the *attL* and *attR* recombination sites flanking the element. This event leads to the excision of a circular form of the ICE harboring an *attI* site, resulting from the recombination between *attL* and *attR* sites, and to a chromosome carrying an empty *attB* site. After conjugative transfer, most of the ICEs integrate by site-specific recombination between identical sequences carried by the *attI* site and the *attB* site. The *attB* sites are generally located in the 3' end of a tRNA gene or a specific open reading frame (ORF). However, some

ICEs, such as Tn916, can integrate in numerous sites (57). Host ranges can be very different in different ICEs. While Tn916, from the firmicute *Enterococcus faecalis*, has a very broad host range (7, 58), the sex factor from the firmicute *Lactococcus lactis* can self-transfer only between strains of this species (32).

The ICEs, like the other transferable elements such as prophages and plasmids, have a modular structure and evolution (18, 19, 70). Each module is composed of all the sequences and genes involved in a biological function, such as site-specific recombination, conjugation, or antibiotic resistance, and can be exchanged between transferable elements.

Stimuli inducing the conjugative transfer of ICEs have been identified in a small number of elements, including Tn916, *clc*, and ICEs from *Bacteroides* (11, 24, 59, 61). Recently, DNA-damaging agents were found to derepress the excision and transfer of ICEBs1 from *Bacillus subtilis* (2) and SXT from *Vibrio cholerae* (3). This is similar to the derepression of numerous prophages by DNA damage that leads to activation of the lytic pathway (29, 45).

Genomic islands related to each other were found to be integrated in the 3' end of *fda* from *Streptococcus thermophilus*, an ORF encoding a putative fructose-1,6-diphosphate aldolase. They carry sequences almost identical to *L. lactis* sequences, including insertion sequences (ISs), a restriction-modification module, and a cadmium resistance module (19, 20, 54). Two of these elements, ICESt1 and ICESt3, harbor very closely related recombination modules that encode a tyrosine integrase and an excisionase. They excise by site-specific recombination between two 27-bp direct repeats included in their two attachment sites, *attL* and *attR* (20, 54). A DNA-damaging agent, mitomycin C (MC), derepresses the site-specific excision of ICESt1 and ICESt3 (4). These elements also carry an almost identical putative conjugative module distantly related to those of two ICEs, Tn916 and ICEBs1, suggesting

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† Supplemental material for this article may be found at <http://jb.asm.org/>.

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▽ Published ahead of print on 30 January 2009.

that these genomic islands are ICEs or derived from ICEs (19, 54). In the present report, we show that these elements are able to transfer by conjugation to other strains of *S. thermophilus* and we characterize the ICE $St3$  host range. In the vast majority of transconjugants, the *S. thermophilus* ICEs were found to be integrated by site-specific recombination into the 3' end of the *fda* gene. Moreover, the transfer of this ICE is derepressed by MC-induced DNA damage. ICE $St3$  is still active in the transconjugants and can transfer again to a new recipient. Finally, recombinant plasmids carrying different parts of the ICE $St1$  recombination module were used to determine the role of the integrase, the excisionase, and the *attI* site in the site-specific recombination events.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and culture conditions.** The original strains and plasmids used for electroporation to obtain recipient or donor strains are listed in Table 1. Strains which were modified or mutated were named according to the modification. For example, *Streptococcus pyogenes* ATCC 12202 Rif<sup>r</sup> corresponds to a mutated derivative of *S. pyogenes* ATCC 12202 resistant to rifampin, and *S. thermophilus* LMG18311/pMG36e (Erm<sup>r</sup>) corresponds to a derivative of *S. thermophilus* LMG18311 carrying the plasmid pMG36e encoding resistance to erythromycin.

*S. thermophilus* strains were grown in reconstituted skim milk (10%, wt/vol), M17 broth supplemented with 0.5% lactose (LM17) (Oxoid), Hogg-Jago broth supplemented with 1% lactose (HJL) (64), or Belliker broth (53) at 42°C under anaerobic conditions (GENbox Anaer atmosphere generators and incubation jars from bioMérieux, France). *L. lactis* strains were grown in M17 broth supplemented with 0.5% glucose (GM17) at 30°C under anaerobic conditions. *Lactobacillus delbrueckii* subsp. *bulgaricus* was grown in MRS broth at 37°C under anaerobic conditions. *S. pyogenes*, *E. faecalis*, *Enterococcus casseliflavus*, and *Clostridium difficile* strains were grown in brain heart infusion (Difco) broth at 37°C without shaking, whereas *Streptococcus agalactiae*, *Bacillus subtilis*, and *Staphylococcus aureus* strains were grown in the same broth and at the same temperature with shaking. These species were spread on brain heart infusion plates supplemented with defibrinated horse blood (5%). *C. difficile* plates were also supplemented with *Clostridium difficile* selective supplement (Oxoid). Cultures were supplemented with the following antibiotic when required: chloramphenicol, 4 µg · ml<sup>-1</sup>; erythromycin, 5 µg · ml<sup>-1</sup>; fusidic acid, 25 µg · ml<sup>-1</sup>; rifampin, 25 µg · ml<sup>-1</sup>; spectinomycin, 50 µg · ml<sup>-1</sup>; or tetracycline, 10 µg · ml<sup>-1</sup>.

*Escherichia coli* strains were grown in Luria-Bertani (LB) broth at 37°C in aerobiosis (56). Recombinant plasmids derived from pG+host9 were transformed into *E. coli* EC101, a strain containing a chromosomal copy of the pWV01 *repA* gene (17), and selected at 37°C on LB containing 150 µg of erythromycin ml<sup>-1</sup>.

**DNA manipulations and recombinant DNA.** Preparation of chromosomal and plasmid DNAs and Southern analysis were performed according to standard protocols (56). Primers used in this study were purchased from Eurogentec (Angers, France) and are listed in Table S1 in the supplemental material. Their location and orientation are indicated in Fig. 1. In the figures and in Table S1 in the supplemental material, each letter (A to H) indicates a class of primers hybridizing on the same locus in different species. For example, the primers O132.3, Lla fba2 MG1363, Lla fba2 IL1403, Efa fba2, Bsu fba2, and Sau fba2, belonging to class A, hybridize on the noncoding region flanking the stop codon of the *fda* ORF in *S. thermophilus*, *L. lactis* MG1363, *L. lactis* IL1403, *E. faecalis*, *B. subtilis*, and *Staphylococcus aureus*, respectively. Most of the primers used are strain specific, species specific, or ICE specific. PCRs and high-fidelity PCRs were carried out according to the instructions with the ThermoPol PCR kit (New England Biolabs, United Kingdom) and with the Triple Master PCR system (Eppendorf, France), respectively. The transformants and the integrants that were characterized by PCR amplification were previously purified by two successive streakings on agar plates. The restriction and modifying enzymes were purchased from New England Biolabs, United Kingdom.

(i) **ICE tagging.** *S. thermophilus* strains carrying a tagged ICE were constructed as previously described by Thibessard et al. (68) with the following modifications. Briefly, the two regions flanking the locus to be tagged were independently amplified by PCR using primers carrying restriction enzymes sites, digested by these enzymes, and joined by ligation together and with pG+host9 digested by the appropriate enzymes. Thereafter, restriction fragments carrying the specti-

nomycin (*spc*) or the chloramphenicol (*cat*) resistance genes from the plasmids pSET4s and pSET5s were ligated within the digested restriction site located between the two PCR fragments. After introduction of the recombinant plasmid into *S. thermophilus*, two crossovers, upstream and downstream of the tagged region, were selected (68). Thus, the locus of interest is replaced by the tagged locus carried by the plasmid.

(ii) **Construction of plasmids carrying fragments of the ICE $St1$  recombination module.** Fragments of the ICE $St1$  recombination module, i.e., the *int* and *xis* genes and the *attI* site, were amplified by high-fidelity nested PCRs using primers carrying a SalI or a XhoI restriction enzymes site and then digested by these enzymes. The amplicons were introduced into the plasmid pTRK398/0H digested by the same enzymes. SalI and XhoI produce compatible cohesive ends that allow the selection of the ligation of the ICE $St1$  integration module in both orientations relative to the P6 constitutive promoter from *Lactobacillus acidophilus* ATCC 4356 carried by this plasmid (sense and antisense transcriptions). Thereafter, the P6 constitutive promoter and the integration module fragments in either the sense or antisense orientation were amplified by PCR using primers carrying a PstI or KpnI restriction enzymes site. These two amplicons were digested by PstI or KpnI and joined by ligation with the thermosensitive replication vector pG+host9 digested by the same restriction enzymes (Fig. 2). The different cloned amplicons carry the *int* gene and the *attI* site (plasmids pNST260+ and pNST260-); the *int* gene, the *xis* gene, and the *attI* site (pNST261+ and pNST261-); or the *int* gene, the *xis* gene, and the *attR* site (pNST262+ and pNST262-).

**Electroporation procedure.** *S. thermophilus*, *L. lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *B. subtilis* strains were electroporated as previously described by Tyvaert et al. (71), O'Sullivan and Klaenhammer (52), Serror et al. (60), and Matsuno et al. (48), respectively. *E. faecalis* and *Staphylococcus aureus* strains were electroporated as described by Okamoto et al. (50). The introduced plasmids carry the thermosensitive replication origin from plasmid pVE6002 (46). Plasmids harboring this replication origin can replicate in numerous gram-positive bacteria, including lactococci (8), streptococci (55, 69), enterococci (25, 49), staphylococci (39, 65), and bacilli (51).

**Filter matings.** The filter mating protocol was modified from that described by Wang et al. (73) as follows. Both donor and recipient strains were grown for 15 h. A 15-ml culture of the relevant broth was inoculated with 150 µl of overnight culture of the recipient or the donor strain. The cultures were grown at the relevant temperature until mid-exponential phase (optical density at 600 nm of 0.4). Cultures of the donor and recipient were mixed and centrifuged for 15 min in a prewarmed centrifuge at 4,500 × g to form a cell pellet. The pellet was resuspended in 1 ml of LM17 broth with or without 50 µg/ml DNase I (23), and 150-µl aliquots were spread on 0.45-µm-pore-size nitrocellulose filters (Sartorius) on LM17 agar plates which were incubated for 20 h at 42°C (intraspecies transfer) or at 37°C (interspecies transfer). The filters were removed from the agar plates, placed in 50-ml bottles containing 10 ml of sterile LM17 broth, and vortexed for 30 s. Various dilutions were spread on agar plates supplemented with the appropriate antibiotics, and the plates were incubated for 24 h to 48 h in order to count CFU of the donor, the recipient, and the transconjugants.

**Integration frequencies of the recombinant plasmids.** The recombinant plasmids created in this work carry a thermosensitive replication origin. The permissive temperature of the plasmids carrying this replication origin is 30°C, whereas temperatures greater than or equal to 35°C are restrictive (46). The plasmids were introduced by electroporation into the bacteria of interest. The transformants were cultivated at 30°C, and various dilutions were spread on plates both with and without erythromycin and incubated at a restrictive temperature. The integration frequency was calculated as the number of CFU resistant to erythromycin divided by the total number of CFU. The integration of the plasmids was verified by PCR amplifications of the resulting attachment sites.

**Induction of ICE transfer by MC.** *S. thermophilus* LMG18311/pMG36e was used as the recipient in the following filter mating experiments. The *S. thermophilus* donor strains carrying ICE $St1$  or ICE $St3$  were grown in LM17 at 42°C to an optical density at 600 nm of 0.4. They were then diluted 10-fold in 10 ml of prewarmed LM17 containing MC at a concentration equal to one-half or one-quarter of the MIC, i.e., 0.2 and 0.1 µg/ml in LM17. A 10-fold dilution without MC was used as a control. After 1 hour of culture, the cells were harvested by centrifugation in a prewarmed centrifuge and washed once with 10 ml of warmed LM17. The cells treated with MC were mixed with exponentially growing recipients (optical density at 600 nm of 0.4) and centrifuged for 15 min in a prewarmed centrifuge to form a cell pellet. The pellet was resuspended in 1 ml of LM17 broth, and 150 µl was spread on 0.45-µm-pore-size nitrocellulose filters on LM17 agar plates, which were incubated for 3 h at 42°C in anaerobiosis. The filters were then treated as in other filter matings.

TABLE 1. Strains and plasmids used in this work

Strain or plasmid	Relevant phenotype or genotype <sup>a</sup>	Source or reference
<b>Strains</b>		
<i>S. thermophilus</i>		
LMG18311	Wild-type strain, no element integrated in the <i>fda</i> gene	BCCM/LMG
CNRZ368	Wild-type strain carrying ICES <i>St1</i>	INRA-CNRZ
CNRZ385	Wild-type strain carrying ICES <i>St3</i>	INRA-CNRZ
CNRZ368 ICES <i>St1cat</i>	<i>S. thermophilus</i> CNRZ368 carrying ICES <i>St1</i> tagged with the <i>cat</i> gene inserted in a noncoding region flanked by fragments of IS1192 and of IS981, Cm <sup>r</sup>	This work
CNRZ368 ICES <i>St1spc</i>	<i>S. thermophilus</i> CNRZ368 carrying ICES <i>St1</i> tagged with the <i>spc</i> gene inserted in a noncoding region flanked by fragments of IS1192 and of IS981, Spc <sup>r</sup>	This work
CNRZ385 ICES <i>St3cat</i>	<i>S. thermophilus</i> CNRZ385 carrying ICES <i>St3</i> tagged with the <i>cat</i> gene inserted in the pseudogene $\Psi$ orf385J, Cm <sup>r</sup>	This work
CNRZ385 ICES <i>St3spc</i>	<i>S. thermophilus</i> CNRZ385 carrying ICES <i>St3</i> tagged with the <i>spc</i> gene inserted in the pseudogene $\Psi$ orf385J, Spc <sup>r</sup>	This work
<i>S. pyogenes</i> ATCC 12202	Wild-type strain, no element integrated in the <i>fda</i> gene	ATCC
<i>S. agalactiae</i>		
COH1	Wild-type strain, no element integrated in the <i>fda</i> gene	74
539-22	Wild-type strain, no element integrated in the <i>fda</i> gene	16
<i>L. lactis</i> subsp. <i>cremoris</i>	Wild-type strain, no element integrated in the <i>fda</i> gene	31
MG1363		
<i>L. lactis</i> subsp. <i>lactis</i> IL1403	Plasmid-free strain derived from <i>L. lactis</i> subsp. <i>lactis</i> IL594, no element integrated in the <i>fda</i> gene	22
<i>E. faecalis</i> JH2-2	No element integrated in the <i>fda</i> gene, Rif <sup>r</sup> Fus <sup>r</sup>	40
<i>E. casseliflavus</i> 664.1H1	Wild-type strain, oral isolate from monkey with amalgam fillings, presence of an element integrated in the <i>fda</i> gene not determined, Tc <sup>r</sup>	A.O. Summers (Athens, GA)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> VI104	Wild-type strain, presence of an element integrated in the <i>fda</i> gene not determined	Collection Génétique Microbienne INRA
<i>B. subtilis</i>		
168	Wild-type strain, no element integrated in the <i>fda</i> gene	43
CU2189	Wild-type strain, presence of an element integrated in the <i>fda</i> gene not determined	23
<i>Staphylococcus aureus</i> RN4220	Restriction-deficient derivative of phage group III strain 8325-4, presence of an element integrated in the <i>fda</i> gene not determined	42
<i>C. difficile</i>		
CD37	Wild-type strain, presence of an element integrated in the <i>fda</i> gene not determined, Tc <sup>r</sup>	63
R20291	Wild-type strain, presence of an element integrated in the <i>fda</i> gene not determined	Anaerobe Reference Laboratory, Cardiff, United Kingdom
<i>E. coli</i>		
EC101	<i>supE hsd-5 thi (lac-proAB) F (traD6 proAB lacF<sup>a</sup> lacZ M15) repA</i> , derivative of strain TG1 (S6)	44
DH5 $\alpha$	<i>supE44 lacU169 (<math>\phi</math>80 lacZ M15) hsdR17 endA1 gyrA96 thi-1 relA1</i>	56
HB101	<i>supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1</i> , no element integrated in the <i>fda</i> gene	47
<b>Plasmids</b>		
pG+host9	3.8 kb, pWV01-type thermosensitive replication origin from pVE6002, Erm <sup>r</sup>	46
pSET4s	4.5 kb, pWV01-type thermosensitive replication origin from pVE6002, <i>lacZ'</i> , Spc <sup>r</sup>	66
pSET5s	4.4 kb, pWV01-type thermosensitive replication origin from pVE6002, <i>LacZ'</i> , Cm <sup>r</sup>	66
pMG36e	3.4 kb, replication origin from pWV01, Erm <sup>r</sup>	72
pTRK398/0H	10.8 kb, P6 constitutive promoter from <i>Lactobacillus acidophilus</i> ATCC 4356 upstream of the <i>llaIR</i> restriction cassette, Erm <sup>r</sup>	27
pNST260+ and pNST260–	6.2 kb, plasmid derived from pG+host9 carrying the <i>int</i> gene and the <i>attI</i> site from ICES <i>St1</i> cloned downstream from the P6 constitutive promoter from pTRK398/0H; + and – indicate that the <i>int</i> gene is in forward or reverse orientation compared to the promoter, respectively	This work
pNST261+ and pNST261–	6.5 kb, plasmid derived from pG+host9 carrying the <i>xis</i> and the <i>int</i> genes and the <i>attI</i> site from ICES <i>St1</i> cloned downstream from the P6 constitutive promoter from pTRK398/0H; + and – indicate that the <i>int</i> gene is in forward or reverse orientation compared to the promoter, respectively	This work
pNST262+ and pNST262–	6.1 kb, plasmid derived from pG+host9 carrying the <i>xis</i> and <i>int</i> genes and the <i>attR</i> site from ICES <i>St1</i> cloned downstream from P6 constitutive promoter from pTRK398/0H; + and – indicate that the <i>int</i> gene is in forward or reverse orientation compared to the promoter, respectively	This work

<sup>a</sup> Abbreviations: Cm<sup>r</sup>, chloramphenicol resistance; Ery<sup>r</sup>, erythromycin resistance; Fus<sup>r</sup>, fusidic acid resistance; Rif<sup>r</sup>, rifampin resistance, Spc<sup>r</sup>, spectinomycin resistance; Tc<sup>r</sup>, tetracycline resistance.



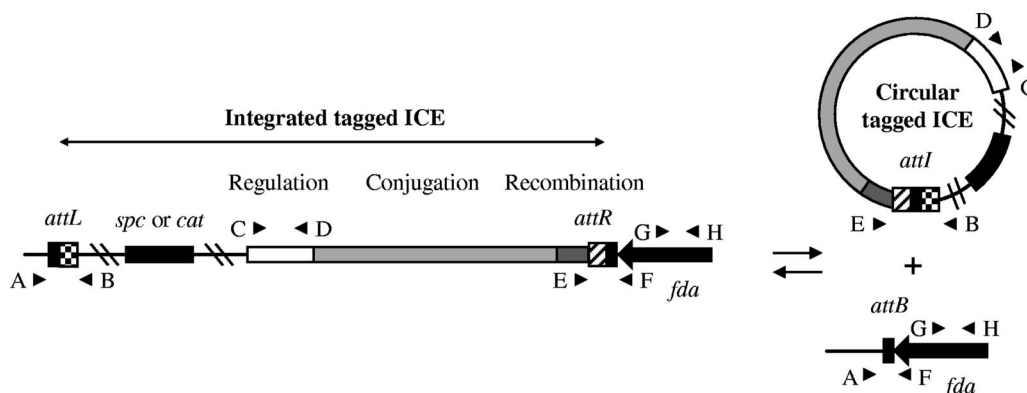


FIG. 1. Schematic representation of the locations and orientations of the primers used in this work to analyze the transconjugants. The locations of the recombination, conjugation, or regulation modules of the *S. thermophilus* ICES are indicated by gray and white rectangles. Recombination sites are drawn as vertical rectangles: black, sequence identical in *attL*, *attR*, *attI*, and *attB* sites; checkerboards, arm of *attR* sites and related arm of *attI* sites; hatched boxes, arm of *attL* sites and related arm of *attI* sites. The recombination sites are magnified. The locations and orientations of the primers hybridizing into the ICES (*ICES<sub>St1</sub>* or *ICES<sub>St3</sub>*) and into the flanking sequences are indicated by black arrowheads. Each letter (A to H) indicates a class of primers hybridizing on the same locus in different species (see Table S1 in the supplemental material). The primer pairs A-B, E-F, A-F, and E-B allow the amplification of fragments carrying the *attL*, the *attR*, the *attB*, and the *attI* sites, respectively. The primer pair C-D allows the amplification of an internal fragment of the regulation module of *ICES<sub>St1</sub>* or *ICES<sub>St3</sub>*. The amplification of an internal fragment of the *fda* gene using the species-specific primers G and H allows the identification of the species. The spectinomycin (*spc*) or the chloramphenicol (*cat*) resistance genes introduced in order to tag the ICES are indicated by a large black rectangle. The sequences between the regulation module and the *attI* site are specific to each *S. thermophilus* ICE. The accession number of the untagged *ICES<sub>St1</sub>* and *ICES<sub>St3</sub>* nucleotide sequences are AJ278471 and AJ586568, respectively.

**Statistical analysis.** Statistical analysis was performed as described by Georgin and Mouet (33) and Cumming et al. (26). The means and standard errors from at least five independent experiments are indicated.

## RESULTS

***ICES<sub>St1</sub>* and *ICES<sub>St3</sub>* intraspecies transfer.** Genes encoding resistance to spectinomycin (*spc*) or chloramphenicol (*cat*) were introduced into loci that should not be involved in the ICE excision or transfer, i.e., the noncoding sequence flanked by IS1192 and IS981 fragments in *ICES<sub>St1</sub>* and a pseudogene (*Ψorf385J*) in *ICES<sub>St3</sub>* (Fig. 1 and Table 1).

Reconstituted skim milk and the rich media LM17, HJL, and Belliker were used during the development of a mating protocol for *S. thermophilus* ICES. The mating attempts were performed using liquid cultures or nitrocellulose filters placed on agar plates. The only conditions which allowed putative transconjugants to be recovered were filter matings on skim milk or LM17 agar plates. The numbers of putative transconjugants per donor were similar with both media whether the filter matings were performed in aerobiosis or in anaerobiosis (data not shown). All of the following filter matings were performed on LM17 agar plates. When the cell-cell contacts between the donor and the recipient strains were prevented by use of a filter, no putative transconjugants were recovered in spite of satisfactory growth. Moreover, the numbers of putative transconjugants per donor were similar with or without DNase I (data not shown). Together, these data strongly suggest that the putative transconjugants were obtained following conjugation events.

*S. thermophilus* LMG18311/pMG36e, a strain devoid of an element integrated in the 3' end of the *fda* gene and carrying the plasmid pMG36e, encoding erythromycin resistance, was used as the recipient in the intraspecies filter mating experiments. *ICES<sub>St3</sub>* tagged with a chloramphenicol resistance gene

(*ICES<sub>St3cat</sub>*) was transferred from strain *S. thermophilus* CNRZ385 *ICES<sub>St3cat</sub>*. The number of putative transconjugants per donor resistant to erythromycin and chloramphenicol is  $3.4 \times 10^{-6} \pm 0.5 \times 10^{-6}$ . The frequencies of spontaneous mutations leading to the appearance of *S. thermophilus* strains resistant to erythromycin or chloramphenicol are less than  $10^{-9}$  and thus do not interfere with the calculation of the transfer frequencies. Putative transconjugants were isolated and tested by PCR. The strain identity was confirmed by the amplification of the central part of the *cse* gene (Fig. 3A). This gene, which is specific to *S. thermophilus*, carries a central part that is highly variable in size and sequence between *S. thermophilus* strains (12; S. Layec, personal communication). Each pair of donor and recipient strains used have different *cse* alleles allowing their identification. These amplifications have shown that all of the putative transconjugants analyzed (>100) were derived from the recipient strain and carry *ICES<sub>St3</sub>*. For all of these clones, the ICE detection was confirmed by the amplification of an internal fragment of the *ICES<sub>St3</sub>* regulation module (Fig. 3A). Therefore, *ICES<sub>St3</sub>* is a functional *S. thermophilus* ICE.

Fragments carrying the *attL* and the *attR* sites resulting from the *ICES<sub>St3</sub>* site-specific integration into the *fda* 3' region were amplified in all 50 transconjugants analyzed, showing that *ICES<sub>St3</sub>* integrates in the *fda* 3' end with a high specificity in *S. thermophilus* (Fig. 3B). Moreover, fragments carrying the *attB* and the *attI* sites resulting from the site-specific excision of *ICES<sub>St3</sub>* were also detected in these transconjugants (Fig. 3C). Therefore, this element excises in the *S. thermophilus* transconjugants, suggesting that it is capable of transfer.

The strain *S. thermophilus* CNRZ368 carrying *ICES<sub>St1</sub>* tagged with the *cat* gene was used as the donor in intraspecies filter mating. Only one putative *ICES<sub>St1</sub>* transconjugant resistant to erythromycin and chloramphenicol was obtained. This clone was confirmed by PCRs as being a transconjugant, i.e.

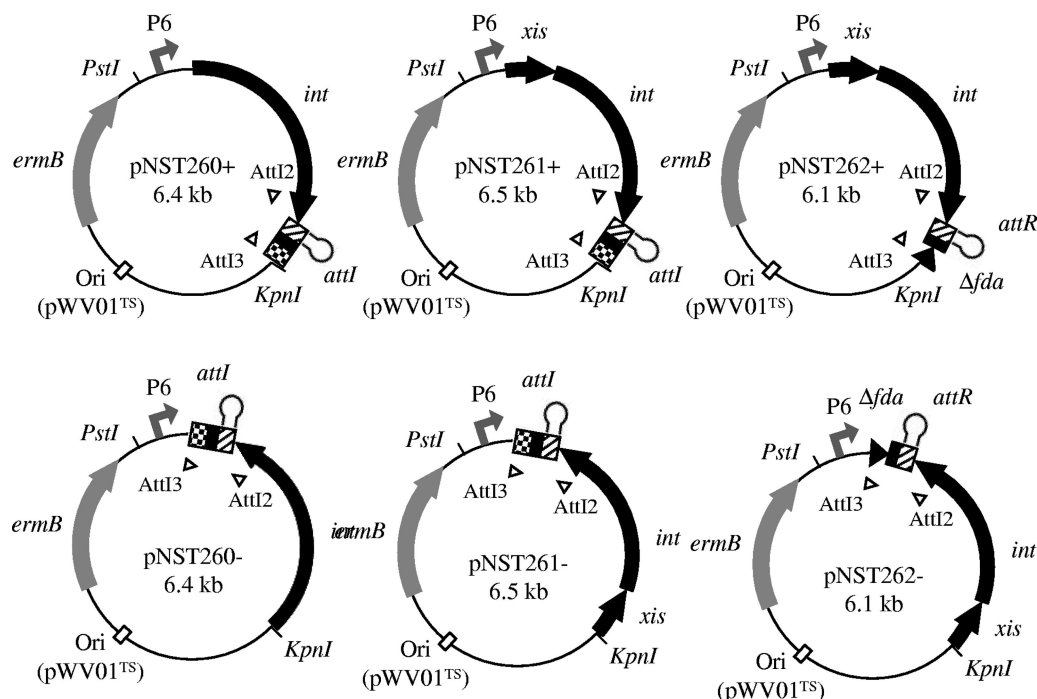


FIG. 2. Map of the thermosensitive replication plasmids carrying the P6 constitutive promoter and the various fragments of the ICES*St1* recombination module. The location of the thermosensitive replication origin [Ori(pWV01<sup>TS</sup>)] is indicated by a white rectangle. The location and the orientation of the *ermB* gene, encoding resistance to erythromycin, is indicated by a gray arrow. The *Pst*I and *Kpn*I restriction sites used for molecular cloning are indicated. The location and the orientation of the P6 constitutive promoter from *Lactobacillus acidophilus* ATCC 4356 are indicated by a right-angle arrow. Black arrows indicate the locations and orientations of the *int* and *xis* genes, encoding the integrase and the excisionase, respectively. The location and the orientation of a fragment corresponding to the last 60 nucleotides of the *fda* gene is indicated by a black triangle. Recombination sites are drawn as rectangles: black, sequence identical in *attL*, *attR*, and *attI* sites; checkerboards, arm of *attL* sites and related arm of *attI* sites; hatched boxes, arm of *attR* sites and related arm of *attI* sites. The recombination sites are magnified. The putative rho-independent terminator downstream from the *int* gene is indicated by a lollipop. The locations and orientations of the primers *AttI2* and *AttI3* are indicated by white arrowheads (see Table S1 in the supplemental material).

deriving from the recipient *S. thermophilus* LMG18311/pMG36e and carrying ICES*St1* (data not shown). Moreover, the ICE was found to be integrated in the *fda* locus and to be still able to excise. This suggests that ICES*St1* is, like ICES*St3*, an *S. thermophilus* ICE but transfers at a very low frequency ( $<10^{-9}$ ) to other *S. thermophilus* strains. ICES*St3* and ICES*St1* are the two first conjugative elements (ICEs or conjugative plasmids) identified and characterized in *S. thermophilus*. Moreover, ICES*St1* and ICES*St3* integrate with a high specificity

in the *fda* 3' end in *S. thermophilus* and are still able to excise in the transconjugants.

**ICES*St3* interspecies transfer.** Various *Firmicutes* that are close to, distant from, or very distant from *S. thermophilus* and a gammaproteobacterium, *Escherichia coli*, were used as recipients in order to determine the ICES*St3* host range (Table 1). The following species were used as recipients: *Streptococcus pyogenes*, *Streptococcus agalactiae* (two strains), *Lactococcus lactis* (two strains), *Enterococcus faecalis*, *Enterococcus cas-*

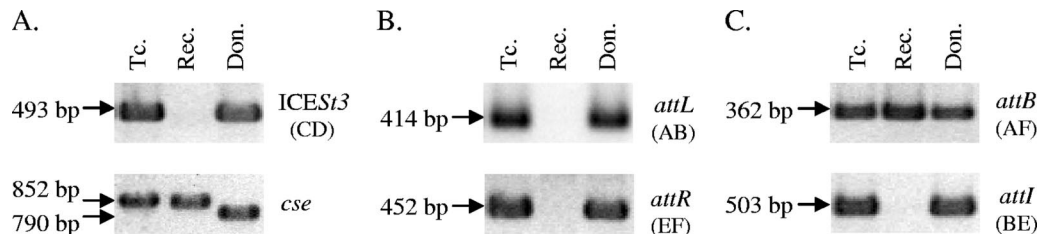


FIG. 3. Characterization by PCR of an intraspecies ICES*St3* transconjugant. CNRZ385 ICES*St3cat* and LMG18311/pMG36e were used as the donor and the recipient, respectively. Don, Rec, and Tc indicate donor, recipient, and transconjugant, respectively. (A) ICES*St3*, amplification of a fragment of the regulation module. *cse*, amplification of the central part of the *cse* gene. (B and C) *attR*, *attL*, *attB*, and *attI*, amplifications of fragments carrying these attachment sites. The sizes of the PCR fragments are deduced from sequence analysis and confirmed by comigration with a DNA ladder. The classes of primer pairs used for these amplifications are indicated in parentheses, and their localizations are indicated in Fig. 1.

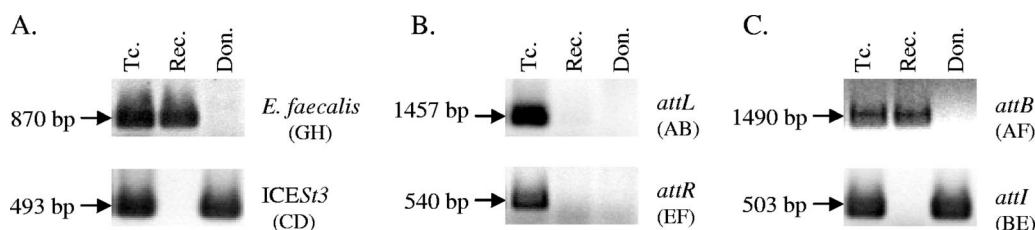


FIG. 4. Characterization of an ICES<sub>3</sub> transconjugant of *E. faecalis* by PCR. *S. thermophilus* CNRZ385 ICES<sub>3</sub>*spc* and *E. faecalis* JH2-2 Rif<sup>r</sup> Fus<sup>r</sup> were used as the donor and the recipient, respectively. Don, Rec, and Tc indicated donor, recipient, and transconjugant, respectively. (A) *E. faecalis* and ICES<sub>3</sub>, amplification of an internal *fda* fragment specific to *E. faecalis* and amplification of a fragment of the ICES<sub>3</sub> regulation module, respectively. (B and C) *attR*, *attL*, *attB*, and *attI*, amplifications of fragments carrying these attachment sites. The sizes of the PCR fragments are deduced from sequence analysis and confirmed by comigration with a DNA ladder. The classes of primer pairs used for these amplifications are indicated in parentheses, and their localizations are indicated in Fig. 1.

*seliflavus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bacillus subtilis* (two strains), *Staphylococcus aureus*, *Clostridium difficile* (two strains), and *E. coli* (strain HB101). *S. thermophilus* strain CNRZ385 carrying ICES<sub>3</sub> tagged with the *spc* gene was used as the donor in all the interspecies filter matings. The frequencies of spontaneous mutations leading to the appearance of donor or recipient strains resistant to the antibiotics used are less than  $10^{-9}$  and thus do not interfere with the calculation of the transfer frequencies. Some of these filter matings were done in aerobiosis, but the intraspecies ICES<sub>3</sub> transfer frequencies resulting from intraspecies filter matings in aerobiosis and anaerobiosis were found to be similar (data not shown).

Numerous putative transconjugants of *S. pyogenes* ATCC 12202 Rif<sup>r</sup> and *E. faecalis* JH2-2 Rif<sup>r</sup> Fus<sup>r</sup> were obtained (Fig. 4). Some putative transconjugants were purified and analyzed by PCR. All the primers hybridizing to the chromosomes of the recipient strains (primers belonging to the classes A, F, G, and H) hybridize to a species-specific sequence (Fig. 1; see Table S1 in the supplemental material). Therefore, PCR products obtained with these primers are also species specific. The amplification of an *fda* fragment specific to each species and of an internal fragment of the ICE regulation module has shown that the putative ICES<sub>3</sub> transconjugants of *E. faecalis* JH2-2 were derived from the recipient and that they carried the ICE (Fig. 1 and 4A). Similar amplifications by PCR have confirmed that transconjugants of *S. pyogenes* were obtained (data not shown). The hemolytic property of *S. pyogenes* was also used to differentiate *S. pyogenes* from *S. thermophilus*. The numbers of transconjugants per donor for *S. pyogenes* and *E. faecalis* were  $3.0 \times 10^{-6} \pm 1.5 \times 10^{-6}$  and  $3.9 \times 10^{-7} \pm 0.9 \times 10^{-7}$ , respectively. Therefore, ICES<sub>3</sub> transfers to various *Firmicutes*.

Fragments carrying the *attL* and the *attR* sites resulting from the site-specific recombination between the *fda* 3' end from *E. faecalis* and the ICES<sub>3</sub> *attI* site were amplified from 40 transconjugants (Fig. 1 and 4B). These sites were also amplified in 25 transconjugants of *S. pyogenes* ATCC 12202 (data not shown). Therefore, ICES<sub>3</sub> integrates in the *fda* 3' end with a high specificity in these species. The *attB* site and the *attI* site resulting from the site-specific excision of ICES<sub>3</sub> were amplified from 40 *E. faecalis* transconjugants (Fig. 4C) and from 25 *S. pyogenes* transconjugants (data not shown), indicating that this element is still able to excise in the transconjugants of these species.

One putative ICES<sub>3</sub> transconjugant of *L. lactis* MG1363/pMG36e was also recovered. This clone was analyzed by PCR

like the previous interspecies transconjugants. These amplifications confirmed that this clone is *L. lactis* MG1363 carrying ICES<sub>3</sub>. This shows that ICES<sub>3</sub> transfers to this species at low frequency ( $<10^{-9}$ ). However, in this transconjugant, ICES<sub>3</sub> did not enter the *fda* locus. Additionally, the detection of a chromosomal *attI* site in the transconjugant strongly suggests that this site was not used as a substrate for the integration of the element in a putative *attB'* site (data not shown).

After the incubation of *S. thermophilus* carrying ICES<sub>3</sub> and one of the *S. agalactiae* recipient strains on a filter, no viable *S. thermophilus* colonies were recovered. Therefore, the conditions allowing the coculture of these two species on a filter could not be determined.

In spite of satisfactory growth conditions during cocultures, no transconjugants were recovered after at least three filter matings using recipients from the other species (*Lactobacillus delbrueckii* subsp. *bulgaricus*, *B. subtilis*, *Staphylococcus aureus*, *C. difficile*, and *E. coli*). This suggests that ICES<sub>3</sub> is poorly able to establish a functional mating apparatus with these species and/or is poorly able to integrate into the genomes of these strains.

**ICES<sub>3</sub> transfers from *E. faecalis*.** The ability of the newly incoming ICES<sub>3</sub> to transfer from a transconjugant was analyzed. *E. faecalis* JH2-2 carrying ICES<sub>3</sub>*spc* and *S. thermophilus* LMG18311/pMG36e were used in filter mating as donor and recipient, respectively. Some putative transconjugants were purified and analyzed by PCR as described above. Thirteen ICES<sub>3</sub>-containing transconjugants of *S. thermophilus* were obtained, at a frequency of  $3.2 \times 10^{-9} \pm 0.8 \times 10^{-9}$  per donor. PCR amplification of a fragment of the ICES<sub>3</sub> regulation module and an *fda* fragment showed that all 13 transconjugants were derived from the recipient and carried the ICE (data not shown). The PCR amplification of fragments harboring the specific *attR* and *attL* sites showed that ICES<sub>3</sub> is integrated in the *fda* 3' ends of all the transconjugants. Moreover, the amplification of fragments carrying the *S. thermophilus attB* site and the ICES<sub>3 attI</sub> site in the transconjugants showed the site-specific excision of the ICE in the transconjugants. Therefore, ICES<sub>3</sub> is still able to transfer from *E. faecalis* transconjugants. The amplification of the *attB* and the *attI* sites in the transconjugants of *S. pyogenes* and *S. thermophilus* strongly suggests that ICES<sub>3</sub> could also transfer again from these strains.

**Integration and excision of recombinant plasmids harboring the ICES<sub>3</sub> recombination module.** All the attachment sites

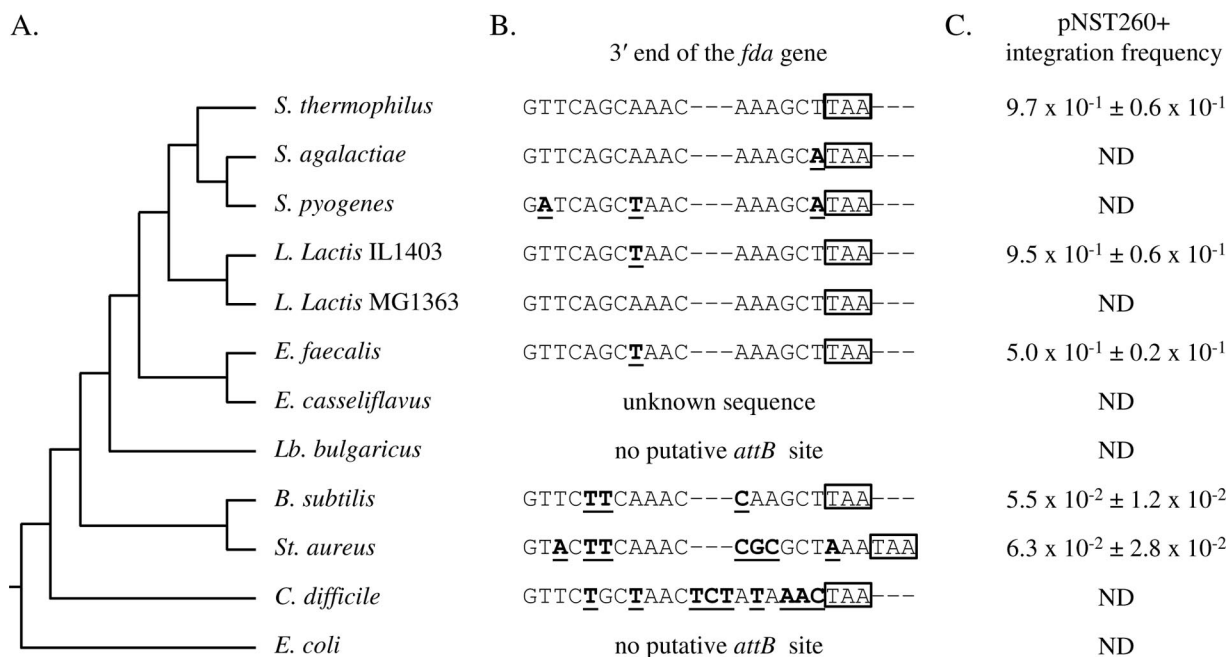


FIG. 5. Sequences of the *fda* 3' ends of the strains used as recipients and integration frequencies of pNST260+ in some of these strains. (A) Cladogram of the species used as recipients in this work. (B) The stop codon of the *fda* ORFs is enclosed by a rectangle. The bold and underlined nucleotides indicate the differences from the *S. thermophilus* sequence. The sequences in this figure correspond to those found in the sequenced strains. Only one of the two *Staphylococcus aureus* alleles, the most common in databases, is indicated. The second *Staphylococcus aureus* allele is more different from the *S. thermophilus* sequence: the adenine at the position 8 is replaced by a thymine. The *fda* 3' end sequences available in the databases are identical for all the strains of the other species. The sequence of the *E. casseliflavus fda* 3' end is not available in databases. The *fda* 3' end sequences from *Lactobacillus delbrueckii* subsp. *bulgaricus* and *E. coli* do not display a significant identity with that of *S. thermophilus*. (C) pNST260+ integration frequencies in five strains used as recipients in intra- and interspecies filter mating. *S. thermophilus* LMG18311 and *B. subtilis* 168 were used in this experiment. ND, not determined.

of ICES*t1* and ICES*t3* contain, in *S. thermophilus*, a 27-bp sequence identical to the last 20 nucleotides of the *S. thermophilus fda* gene and the 7 nucleotides downstream the stop codon of this gene. Only the 20 nucleotides belonging to the *fda* ORF are similar in members of the *Firmicutes* (Fig. 5). Moreover, the *S. thermophilus* ICEs were shown in this work to integrate overwhelmingly in the *fda* 3' end. Therefore, this sequence is the putative *attB* site of the *S. thermophilus* ICEs. Recombinant thermosensitive plasmids were created in order to investigate the ability of the *S. thermophilus* ICEs to integrate in the *attB* sites of various *Firmicutes* and to excise. Different parts of the ICES*t1* recombination module, i.e., the integrase gene (*int*), the excisionase gene (*xis*), and the *attI* site of this element, were cloned in both orientations downstream from the P6 constitutive promoter, which is active in *L. lactis*, *E. faecalis*, and *B. subtilis* (27), in the vector pG+host9 (Fig. 2). In some plasmid constructs, the promoter fusions are expected to drive expression of the cloned gene(s), and in others there should be no expression (recombinant plasmid names end with a + or -, respectively). The plasmids pNST260+ and pNST260- carrying the *int* gene and the *attI* site were introduced in *S. thermophilus* LGM18311, *L. lactis* IL1403, *E. faecalis* JH2-2, *B. subtilis* 168, and *Staphylococcus aureus* RN4220. The integration frequencies of pNST260+ in *S. thermophilus* LGM18311 and *L. lactis* IL1403 are  $9.7 \times 10^{-1} \pm 0.6 \times 10^{-1}$  and  $9.5 \times 10^{-1} \pm 0.6 \times 10^{-1}$  (Fig. 6), respectively. Therefore, pNST260+ has a very high ability to integrate in the chromosomes of *S. thermophilus*

and *L. lactis*, whereas no ICES*t3* transconjugant of *L. lactis* integrated in the *fda* locus was recovered. The integration frequencies of pNST260+ in *E. faecalis* JH2-2, *B. subtilis* 168, and *Staphylococcus aureus* RN4220 are  $5.0 \times 10^{-1} \pm 0.2 \times 10^{-1}$ ,  $5.5 \times 10^{-2} \pm 1.2 \times 10^{-2}$ , and  $6.3 \times 10^{-2} \pm 2.8 \times 10^{-2}$ , respectively (Fig. 5). Hence, pNST260+ integrates in the *E. faecalis* genome with an integration efficiency approximately two times lower than that in *S. thermophilus* or in *L. lactis*, whereas it does not integrate efficiently in *B. subtilis* 168 and *Staphylococcus aureus* RN4220. The integration frequencies of pNST260- in all these strains are less than  $10^{-3}$ , strongly suggesting that the integration of pNST260+ was achieved through the expression of the *int* gene (data not shown). Four purified *E. faecalis* transformants carrying pNST260+ were characterized by PCR using species-specific and ICE-specific primers. The amplification of the *attR* and *attL* sites resulting from the site-specific recombination between the *attI* site carried by pNST260+ and the *attB* site was obtained in the four integrants (Fig. 6). Thus, the plasmid pNST260+ integrates by site-specific recombination in the *fda* 3' end in *E. faecalis*. Moreover, a fragment carrying the *attB* site could be amplified in the *E. faecalis* wild-type strain but not in the integrant, showing that the plasmid cannot excise (Fig. 6). This strongly suggests that the integrase alone cannot catalyze the excision of the plasmid. Four purified pNST260+ transformants of *S. thermophilus*, *L. lactis*, *B. subtilis*, and *Staphylococcus aureus* were also characterized by PCRs using species-specific and ICE-specific



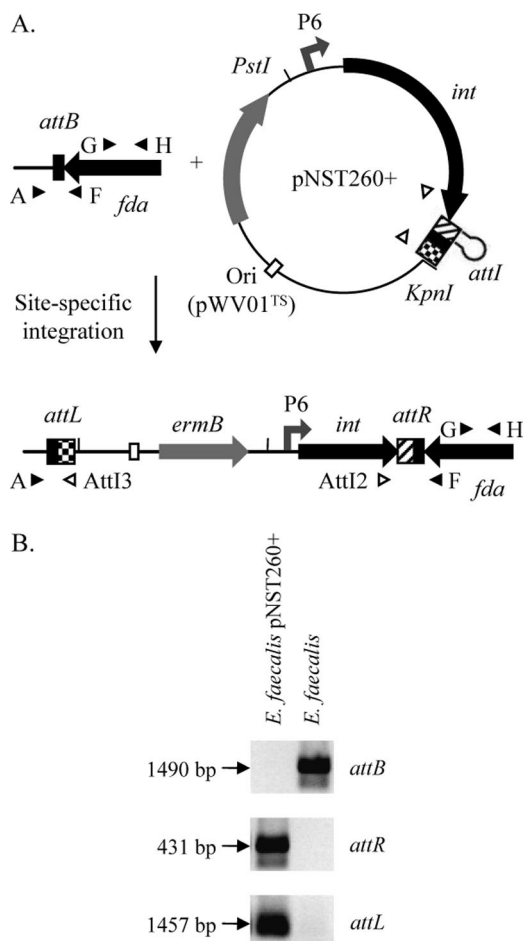


FIG. 6. Schematic representation of the site-specific integration of plasmid pNST260+ into the *fda* 3' end and characterization of an *E. faecalis* transformant carrying this plasmid. (A) The locations and orientations of primers hybridizing to the chromosome and to the plasmid are indicated by black and white arrowheads, respectively. The recombination sites are magnified. The primer pairs A-AttI3, AttI2-F, A-F, and AttI3-AttI2 allow the amplification of fragments carrying the *attL*, the *attR*, the *attB*, and the *attI* sites, respectively (see Table S1 in the supplemental material). The classes of primer pairs used for these amplifications are indicated. The symbols used to represent plasmid pNST260+ are identical to those used in Fig. 3. (B) Amplifications by PCR using the DNA of *E. faecalis* JH2-2 and a clone of *E. faecalis* JH2-2 carrying plasmid pNST260+. *attR*, *attL*, and *attB*, amplifications of fragments carrying these attachment sites.

primers. Transformants of *S. thermophilus* and *L. lactis* were similar to those of *E. faecalis*. However, PCRs failed to detect the integration of pNST260+ in the *fda* locus in *B. subtilis* and in *Staphylococcus aureus* (data not shown).

The plasmids pNST261+ and pNST261-, carrying the *int* and the *xis* genes and the *attI* site, were introduced in *S. thermophilus* LGM18311. Whereas pNST261- integrants were not recovered, integrants of pNST261+ were isolated at a frequency of  $4.6 \times 10^{-2} \pm 2.2 \times 10^{-2}$ . The *attR* and the *attL* sites resulting from the site-specific integration of pNST261+ into the *fda* 3' end were detected by PCR (data not shown). Moreover, the amplification of the *attI* site of the plasmid and the chromosomal *attB* site from a purified pNST261+ integrant showed that this plasmid is also able to excise by site-

specific recombination. Therefore, while the expression of the integrase gene is necessary and sufficient to obtain site-specific integration, the expression of the excisionase is required for site-specific excision. Previous results showed that the integrase gene is involved in the ICES*St1* site-specific excision (20). Together, these data strongly suggest that the expression of the integrase and the excisionase is required for the pNST261+ site-specific excision.

The plasmids pNST262+ and pNST262-, carrying the *int* and the *xis* genes and the *attR* site, were also introduced in *S. thermophilus* LGM18311; however, no integrants were isolated (Fig. 2). Therefore, while the integrase and the excisionase are expressed in pNST262+ (data not shown), the plasmid does not integrate in the *fda* 3' end. Thus, the site-specific recombination between the *attB* and the *attR* sites cannot occur, suggesting that the presence of *attI* is also required for the site-specific integration of a circular molecule in the *attB* site.

The amino acid sequences of the proteins encoded by the *int* and the *xis* genes from ICES*St1* and ICES*St3* exhibit a high degree of identity of over 99% of their length. The 27-bp sequences of the *attI* sites from the *S. thermophilus* ICES into which the site-specific recombinations occurred are identical, whereas the nucleotide sequences carrying these sites (285 bp) show more than 98% identity. These data strongly suggest that the site-specific recombinations of ICES*St1* and ICES*St3* are nearly identical.

**ICES*St3* transfer to recipients carrying pNST260+.** *S. thermophilus* LGM18311, *L. lactis* IL1403, and *L. lactis* MG1363 harboring pNST260+ integrated in the *fda* locus were used as recipients in filter mating in order to determine if the overexpression of the *int* gene in these strains could have an impact on the ICES*St3* transfer frequency. The recovering of transconjugants was confirmed by PCRs as described above (data not shown). The number of ICES*St3* transconjugants of LGM18311/pNST260+ per donor,  $3.8 \times 10^{-6} \pm 1.5 \times 10^{-6}$ , is similar to that obtained with LGM18311/pMG36e ( $3.4 \times 10^{-6} \pm 0.5 \times 10^{-6}$ ); therefore, the overexpression of the *int* gene in the recipient strain LGM18311 does not have an obvious impact on ICES*St3* transfer frequency. No ICES*St3* transconjugants of *L. lactis* IL1403 or *L. lactis* MG1363 carrying pNST260+ was obtained. Hence, the overexpression of the *int* gene in this species cannot complement a hypothetical incoming ICES*St3* that could not express the ICE integrase.

**Derepression of the conjugative transfer.** Previous results have shown that MC, a DNA-damaging agent, derepresses the excision of ICES*St1* and ICES*St3* at least 10-fold (4). The MC concentration providing the highest derepression factor was equal to half of the MIC. *S. thermophilus* strains harboring ICES*St1* or ICES*St3* treated with MC and *S. thermophilus* LGM18311/pMG36e were used in filter matings as donor and recipient, respectively. The donor cells were treated with various concentrations of MC. The purified transconjugants were characterized by PCR as described above (data not shown). The transfer frequencies are expressed as the number of transconjugants per recipient instead of the number of transconjugants per donor because of the mortality of the donor caused by MC (2, 3). When the donor cells were not treated with MC, the number of ICES*St3* transconjugants per recipient was  $3.8 \times 10^{-7} \pm 1.3 \times 10^{-7}$ . The numbers of transconjugants per recipient were  $1.0 \times 10^{-5} \pm 0.4 \times 10^{-5}$

and  $7.6 \times 10^{-6} \pm 2.6 \times 10^{-6}$  after treatment with MC concentrations equal to one-fourth and one-half of the MIC, respectively. Therefore, MC treatment of cells carrying ICES $t3$  leads to a derepression of the conjugative transfer of this ICE by almost 25-fold. Whereas MC derepresses the excision of ICES $t1$  and ICES $t3$ , no ICES $t1$  transconjugants of LMG18311 were characterized after MC treatment of the donor cells carrying this element, suggesting that MC does not have a detectable impact on ICES $t1$  transfer frequency.

## DISCUSSION

Many ICES $t3$  transconjugants of *S. thermophilus*, *S. pyogenes*, and *E. faecalis* and one ICES $t1$  transconjugant of *S. thermophilus* were isolated and characterized in this work. Furthermore, the presence of DNase does not alter the transfer frequency, and ICES $t3$  transfer is not observed if the donor and recipient strains are separated by a filter. Therefore, ICES $t3$  is a functional conjugative element, the first one proven and characterized in *S. thermophilus*. Moreover, its retransfer from *E. faecalis* to *S. thermophilus* has shown that this element is able to transfer from different species. The observation of a single ICES $t1$  transfer event can leave doubt as to the functionality of this ICE and its ability to transfer in the environment.

The late competence genes were found to be functional in some strains of *S. thermophilus*, *S. pyogenes*, and *E. faecalis*, and sequence data suggest that intergenomic recombinations have occurred. However, natural transformation was nevertheless never reported for these species (6, 9, 10, 14, 41). Furthermore, the chromosomal sequences near the *attL* site from the *S. thermophilus* ICEs, i.e., the sequence downstream from the *fda* gene in an empty *attB* site, are completely different in the various species used as recipient or donor. Therefore, the acquisition of an *S. thermophilus* ICE by natural transformation is very unlikely in interspecies matings, since no RecA-dependent recombination could occur in the chromosomal sequence near *attL*. During intraspecies matings, the donor and the recipient are two *S. thermophilus* strains. Therefore, the integration by homologous recombination of a linear DNA fragment carrying an ICE could happen if this transforming fragment is internalized in the recipient. Nevertheless, no ICE transfer was recovered in liquid cultures, which are the conditions used for the artificial induction of *S. thermophilus* competence (9). Therefore, the acquisition of an *S. thermophilus* ICE by natural transformation is also very unlikely in intraspecies matings.

The nucleotide sequences of the recombination and the conjugation modules of ICES $t1$  and ICES $t3$  are almost identical (95%) (54). Moreover, in spite of the high identity between the recombination modules of the *S. thermophilus* ICEs, the excision frequencies of ICES $t1$  and ICES $t3$  are very different:  $1 \times 10^{-6}$  and  $0.9 \times 10^{-3}$ , respectively (54). The regulation modules are more distantly related than the recombination and the conjugation modules. They shared three genes whose nucleotide sequences are 82 to 85% identical and which encode two putative regulatory pathways (5, 54). These pathways involved two ORFs named *arp1* and *arp2*, belonging to the *cI* and the *cI*-like repressor families, respectively. However, numerous particularities such as specific putative regulatory genes or

differences in the sequences of the promoters and ribosome binding sites have been previously described in the regulation modules (5). We have proposed that the differences between the regulation modules of ICES $t1$  and ICES $t3$  could be responsible for the dissimilar excision frequencies of these elements. Together, these data suggest that these differences could also be involved in the very distinct transfer frequencies of the *S. thermophilus* ICEs.

In this work, the derepression by MC of the ICES $t3$  conjugative transfer by a factor of 25 was demonstrated. Previous results have shown that MC treatment of cells harboring ICES $t1$  or ICES $t3$  led to a derepression of the excision of these elements by at least a factor of 10 (4). Thus, although the excision frequencies of ICES $t1$  and ICES $t3$  are very different and these elements seem to be differently regulated, MC treatments cause similar derepression of their excision frequencies. Nevertheless, no ICES $t1$  transconjugant was recovered after such treatments.

While the role of the *arp1* gene, encoding a *cI* homolog, has been previously determined in ICES $t1$ , the role of the *arp2* gene, encoding a *cI*-like homolog, has not yet been investigated (4). Furthermore, some recent results strongly suggest that the *arp1* gene is also functional in ICES $t3$  (unpublished results). The role of the *arp2* gene encoding a *cI*-like homolog is also undetermined in this ICE. To our knowledge, the *S. thermophilus* ICEs are the only integrative elements (i.e., phages, ICEs, and related elements) carrying both a *cI* repressor and a *cI*-like repressor. Although these two repressor families are unrelated, they are both inactivated in response to DNA damage, which leads to a derepression of the target genes of the repressors (1, 13, 29, 45). MC-induced DNA damage is also known to derepress the conjugative transfer of others ICEs, such as SXT from *Vibrio cholerae* and ICEBs1 from *B. subtilis*, which carry a *cI* homolog and a *cI*-like homolog, respectively (2, 3).

ICES $t3$  from *S. thermophilus* was characterized in this work as able to transfer to the other *Firmicutes* *S. pyogenes* and *E. faecalis*, whereas no ICES $t3$  transconjugants of *S. agalactiae*, *E. casseliflavus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *B. subtilis*, *Staphylococcus aureus*, *C. difficile*, and *E. coli* were recovered. If optimal coculture conditions of the donor and the recipient strains were used, some traits specific to one of these strains were able to interfere with the ability of ICES $t3$  to transfer. For example, the coculture of *S. thermophilus* and each of the two *S. agalactiae* recipient strains led to the death of the donor cells. This impossible coculture could be due to the synthesis by these *S. agalactiae* recipients of a compound such as a bacteriocin that is active against *S. thermophilus*.

Many studies with *att* site mutations have demonstrated that efficient recombination occurs only when the two attachment sites have very closed direct repeats. The plasmid pNST260+ was created in order to investigate the ability of the integrase to catalyze site-specific integrations into different *fda* 3' ends, i.e., the putative ICES $t3$  *attB* site of the recipient strains. This plasmid carrying the *int* gene under the control of the P6 promoter and the *attI* site integrates efficiently in the *fda* 3' ends from *S. thermophilus*, *L. lactis*, and *E. faecalis* but does not in *B. subtilis* and *Staphylococcus aureus*. The activity of the P6 promoter carried by the recombinant plasmids was shown in *B. subtilis* (27). Therefore, this strongly suggests that the integrase is expressed from pNST260+ but cannot catalyze the site-

specific integration of the plasmid in this species. *B. subtilis* and *Staphylococcus aureus* are related species, and therefore we hypothesize that pNST260+ should have behaved the same in these two species. The integration range of plasmid pNST260+ and the ICESt3 host range are similar except for *L. lactis* (Fig. 5). Together these data suggest that although the promoters controlling the expression of the integrase in the plasmid and the ICE are not the same, a defective ICESt3 integration similar to that observed for pNST260+ would be a limiting step of their transfer and another part of the explanation of their host range.

*L. lactis* is a species close to *S. thermophilus* and carries a putative *attB* site identical or almost identical to the *attB* site of *S. thermophilus* (Fig. 5). ICESt3 transfers easily to *E. faecalis*, a species more distant from *S. thermophilus* than *L. lactis* and harboring the same putative *attB* site as *L. lactis* IL1403. Thus, the recovery of numerous ICESt3 transconjugants of *L. lactis* integrated in the *fda* 3' end was expected. Surprisingly, only one transconjugant of *L. lactis* was isolated and characterized. Analyses by PCR have confirmed that this clone derives from the recipient strain and that it carries the ICE, i.e., that this clone is an ICESt3 transconjugant. Nonetheless, the amplification of the *attL* and the *attR* sites resulting from the site-specific integration of the ICE in the *fda* 3' end of the recipient cell was not obtained. Together these data suggest that ICESt3, which cannot replicate itself and does not share any sequence identity with the *L. lactis* genome, was transferred and integrated in the chromosome of this transconjugant by a mechanism of illegitimate recombination. This integration in a locus other than *fda* could result from a defective integration process. Nevertheless, pNST260+, which overexpresses the *int* gene, integrates with a high efficiency in *L. lactis*. Differences in the integrase transcription from the P6 promoter of pNST260+ and from the natural integrase promoter in ICESt3 could lead to this defective integration. However, no ICESt3 transconjugant of *L. lactis* was recovered when a strain carrying pNST260+ was used as the recipient. Thus, pNST260+ cannot complement a hypothetical incoming ICESt3 that could not express the ICE integrase. This suggests that ICESt3 is poorly able to establish a functional mating apparatus with *L. lactis*.

The lactic acid bacterium *S. thermophilus* is extensively used as a starter in the manufacture of dairy products with other lactic acid bacteria, such as *Lactococcus lactis*. Sequence comparisons and hybridizations have revealed horizontal gene transfers between a large array of lactic acid bacteria. The quasi-identities of the IS905 sequence of *L. lactis* (28) and the IS1191 sequence of *S. thermophilus* and the distribution of these ISs suggested that IS905/IS1191 were horizontally transferred from *S. thermophilus* to *L. lactis* (35, 36). In the same way, horizontal transfers of IS981 and ISS1 from *L. lactis* to *S. thermophilus* have recently occurred, probably in cocultures used for cheese manufacture (15, 35, 36). The sequencing of *S. thermophilus* genomes has shown that this species also acquired exogenic DNA from *Lactobacillus delbrueckii* subsp. *bulgaricus* and has suggested DNA exchanges with commensal streptococci (10). However, conjugative plasmids that might be involved in these transfers were never identified in *S. thermophilus*.

The *S. thermophilus* ICEs and the related genomic islands contain many examples of genetic exchange. They contain dif-

ferent types of enterococcal and lactococcal ISs and IS fragments (21, 54, 75). The genomic island  $\Delta$ CIME308, which is related to ICESt1 and ICESt3, carries a 10-kb sequence almost identical to the sequence of the plasmid pAH82 from *L. lactis*. Moreover, ICESt1 and ICESt3 are closely related to the putative ICE RD2 of *S. pyogenes* (34), to the putative ICE SmuE of *Streptococcus mutans* (18), and to six putative ICEs and genomic islands from *S. agalactiae* (16). Finally, one of the genomic islands related to the *S. thermophilus* ICEs carries a cadmium resistance module closely related to plasmids from *L. lactis*, *Listeria innocua*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*; to the putative ICEs ICELm1 from *Listeria monocytogenes*, ICE\_2603\_riL from *S. agalactiae*, and ICESde3396 from *S. dysgalactiae* subsp. *equisimilis*; and to SCCmec genomic islands from staphylococci (15, 50; unpublished data).

The presence of closely related genes in the *S. thermophilus* ICEs and the related genomic islands in various species strongly suggests that all these elements transfer in the environment and that they evolve by cointegration, deletion, and module exchanges. The genetic mixing between ICEs and other elements seems to be an important mechanism allowing the spread of exchanged genes into different ecological niches.

#### ACKNOWLEDGMENTS

X.B. was supported by a grant from the Ministère de l'Éducation Nationale de l'Enseignement Supérieur et de la Recherche and by a grant from the European Molecular Biology Organization (ASTF 279-2006).

#### REFERENCES

1. Auchtung, J. M., C. A. Lee, K. L. Garrison, and A. D. Grossman. 2007. Identification and characterization of the immunity repressor (*ImmR*) that controls the mobile genetic element ICEBs1 of *Bacillus subtilis*. *Mol. Microbiol.* **64**:1515–1528.
2. Auchtung, J. M., C. A. Lee, R. E. Monson, A. P. Lehman, and A. D. Grossman. 2005. Regulation of a *Bacillus subtilis* mobile genetic element by intercellular signaling and the global DNA damage response. *Proc. Natl. Acad. Sci. USA* **102**:12554–12559.
3. Beaber, J. W., B. Hochhut, and M. K. Waldor. 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427**:72–74.
4. Bellanger, X., C. Morel, B. Decaris, and G. Guedon. 2007. Derepression of excision of integrative and potentially conjugative elements from *Streptococcus thermophilus* by DNA damage response: implication of a *ci*-related repressor. *J. Bacteriol.* **189**:1478–1481.
5. Bellanger, X., C. Morel, B. Decaris, and G. Guedon. 2008. Regulation of excision of integrative and potentially conjugative elements from *Streptococcus thermophilus*: role of the *arp1* repressor. *J. Mol. Microbiol. Biotechnol.* **14**:16–21.
6. Beres, S. B., E. W. Richter, M. J. Nagiec, P. Sumby, S. F. Porcella, F. R. DeLeo, and J. M. Musser. 2006. Molecular genetic anatomy of inter- and intraserotype variation in the human bacterial pathogen group A *Streptococcus*. *Proc. Natl. Acad. Sci. USA* **103**:7059–7064.
7. Bertram, J., M. Stratz, and P. Durre. 1991. Natural transfer of conjugative transposon Tn916 between gram-positive and gram-negative bacteria. *J. Bacteriol.* **173**:443–448.
8. Biswas, I., A. Gruss, S. D. Ehrlich, and E. Maguin. 1993. High-efficiency gene inactivation and replacement system for gram-positive bacteria. *J. Bacteriol.* **175**:3628–3635.
9. Blomqvist, T., H. Steinmoen, and L. S. Havarstein. 2006. Natural genetic transformation: a novel tool for efficient genetic engineering of the dairy bacterium *Streptococcus thermophilus*. *Appl. Environ. Microbiol.* **72**:6751–6756.
10. Bolotin, A., B. Quinquis, P. Renault, A. Sorokin, S. D. Ehrlich, S. Kulakauskas, A. Lapidus, E. Goltsman, M. Mazur, G. D. Pusch, M. Fonstein, R. Overbeek, N. Kyprides, B. Purnelle, D. Prozzi, K. Ngui, D. Masuy, F. Hancy, S. Bouteau, M. Boutry, J. Delcour, A. Goffeau, and P. Hols. 2004. Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. *Nat. Biotechnol.* **22**:1554–1558.
11. Bonheyo, G. T., B. D. Hund, N. B. Shoemaker, and A. A. Salyers. 2001. Transfer region of a *Bacteroides* conjugative transposon contains regulatory as well as structural genes. *Plasmid* **46**:202–209.
12. Borges, F., S. Layec, A. Fernandez, B. Decaris, and N. Leblond-Bourget.



2006. High genetic variability of the *Streptococcus thermophilus* *cse* central part, a repeat rich region required for full cell segregation activity. *Antonie van Leeuwenhoek* **90**:245–255.
13. Bose, B., J. M. Auchtung, C. A. Lee, and A. D. Grossman. 2008. A conserved anti-repressor controls horizontal gene transfer by proteolysis. *Mol. Microbiol.* **70**:570–582.
  14. Bourgonne, A., D. A. Garsin, X. Qin, K. V. Singh, J. Sillanpaa, S. Yerrapragada, Y. Ding, S. Dugan-Rocha, C. Buhay, H. Shen, G. Chen, G. Williams, D. Muzny, A. Maadani, K. A. Fox, J. Gioia, L. Chen, Y. Shang, C. A. Arias, S. R. Nallapareddy, M. Zhao, V. P. Prakash, S. Chowdhury, H. Jiang, R. A. Gibbs, B. E. Murray, S. K. Highlander, and G. M. Weinstock. 2008. Large scale variation in *Enterococcus faecalis* illustrated by the genome analysis of strain OG1RF. *Genome Biol.* **9**:R110.
  15. Bourgonne, F., G. Guédon, M. Pébay, Y. Roussel, C. Panis, and B. Decaris. 1996. Characterization of a mosaic *ISSI* element and evidence for the recent horizontal transfer of two different types of *ISSI* between *Streptococcus thermophilus* and *Lactococcus lactis*. *Gene* **178**:15–23.
  16. Brochet, M., E. Couve, P. Glaser, G. Guedon, and S. Payot. 2008. Integrative conjugative elements and related elements are major contributors to the genome diversity of *Streptococcus agalactiae*. *J. Bacteriol.* **190**:6913–6917.
  17. Buist, G., J. Kok, K. J. Leenhouts, M. Dabrowska, G. Venema, and A. J. Haandrikman. 1995. Molecular cloning and nucleotide sequence of the gene encoding the major peptidoglycan hydrolase of *Lactococcus lactis*, a muramidase needed for cell separation. *J. Bacteriol.* **177**:1554–1563.
  18. Burrus, V., G. Pavlovic, B. Decaris, and G. Guédon. 2002. Conjugative transposons: the tip of the iceberg. *Mol. Microbiol.* **46**:601–610.
  19. Burrus, V., G. Pavlovic, B. Decaris, and G. Guédon. 2002. The *ICESI* element of *Streptococcus thermophilus* belongs to a large family of integrative and conjugative elements that exchange modules and change their specificity of integration. *Plasmid* **48**:77.
  20. Burrus, V., Y. Roussel, B. Decaris, and G. Guédon. 2000. Characterization of a novel integrative element, *ICESI*, in the lactic acid bacterium *Streptococcus thermophilus*. *Appl. Environ. Microbiol.* **66**:1749–1753.
  21. Burrus, V., and M. K. Waldor. 2004. Shaping bacterial genomes with integrative and conjugative elements. *Res. Microbiol.* **155**:376–386.
  22. Chopin, A., M. C. Chopin, A. Moillo-Batt, and P. Langella. 1984. Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. *Plasmid* **11**:260–263.
  23. Christie, P. J., R. Z. Korman, S. A. Zahler, J. C. Adsit, and G. M. Dunny. 1987. Two conjugation systems associated with *Streptococcus faecalis* plasmid pCF10: identification of a conjugative transposon that transfers between *S. faecalis* and *Bacillus subtilis*. *J. Bacteriol.* **169**:2529–2536.
  24. Clewell, D. B., D. D. Jaworski, S. E. Flannagan, L. A. Zitzow, and Y. A. Su. 1995. The conjugative transposon Tn916 of *Enterococcus faecalis*: structural analysis and some key factors involved in movement. *Dev. Biol. Stand.* **85**:11–17.
  25. Connil, N., Y. Le Breton, X. Dousset, Y. Auffray, A. Rince, and H. Prevost. 2002. Identification of the *Enterococcus faecalis* tyrosine decarboxylase operon involved in tyramine production. *Appl. Environ. Microbiol.* **68**:3537–3544.
  26. Cumming, G., F. Fidler, and D. L. Vaux. 2007. Error bars in experimental biology. *J. Cell Biol.* **177**:7–11.
  27. Djordjevic, G. M., and T. R. Klaenhammer. 1996. Positive selection, cloning vectors for gram-positive bacteria based on a restriction endonuclease cassette. *Plasmid* **35**:37–45.
  28. Dodd, H. M., N. Horn, and M. J. Gasson. 1994. Characterization of IS905, a new multicopy insertion sequence identified in lactococci. *J. Bacteriol.* **176**:3393–3396.
  29. Dodd, I. B., K. E. Shearwin, A. J. Perkins, T. Burr, A. Hochschild, and J. B. Egan. 2004. Cooperativity in long-range gene regulation by the lambda CI repressor. *Genes Dev.* **18**:344–354.
  30. Gaillard, M., T. Vallaes, F. J. Vorholter, M. Minoia, C. Werlen, V. Sentschilo, A. Puhler, and J. R. van der Meer. 2006. The *clc* element of *Pseudomonas* sp. strain B13, a genomic island with various catabolic properties. *J. Bacteriol.* **188**:1999–2013.
  31. Gasson, M. J. 1983. Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. *J. Bacteriol.* **154**:1–9.
  32. Gasson, M. J., J. J. Godon, C. J. Pillidge, T. J. Eaton, K. Jury, and C. A. Shearman. 1995. Characterization and exploitation of conjugation in *Lactococcus lactis*. *Int. Dairy J.* **5**:757–762.
  33. Georgin, P., and M. Mouet. 2000. Statistiques avec Excel, Eyrolles ed. Presses Universitaires de Rennes, Paris, France.
  34. Green, N. M., S. Zhang, S. F. Porcella, M. J. Nagiec, K. D. Barbican, S. B. Beres, R. B. LeFebvre, and J. M. Musser. 2005. Genome sequence of a serotype M28 strain of group A *Streptococcus*: potential new insights into puerperal sepsis and bacterial disease specificity. *J. Infect. Dis.* **192**:760–770.
  35. Guédon, G., F. Bourgonne, and B. Decaris. 1998. Does gene horizontal transfer occur in lactic acid bacteria co-cultures? *Lait* **78**:53–58.
  36. Guédon, G., F. Bourgonne, M. Pébay, Y. Roussel, C. Colmin, J. M. Simonet, and B. Decaris. 1995. Characterization and distribution of two insertion sequences, IS1191 and iso-IS981, in *Streptococcus thermophilus*: does intergeneric transfer of insertion sequences occur in lactic acid bacteria co-cultures? *Mol. Microbiol.* **16**:69–78.
  37. Hacker, J., and E. Carniel. 2001. Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes. *EMBO Rep.* **2**:376–381.
  38. Hacker, J., and J. B. Kaper. 2000. Pathogenicity islands and the evolution of microbes. *Annu. Rev. Microbiol.* **54**:641–679.
  39. Hartford, O., L. O'Brien, K. Schofield, J. Wells, and T. J. Foster. 2001. The Fbe (SdrG) protein of *Staphylococcus epidermidis* HB promotes bacterial adherence to fibrinogen. *Microbiology* **147**:2545–2552.
  40. Jacob, A. E., and S. J. Hobbs. 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. *J. Bacteriol.* **117**:360–372.
  41. Kehoe, M. A., V. Kapur, A. M. Whatmore, and J. M. Musser. 1996. Horizontal gene transfer among group A streptococci: implications for pathogenesis and epidemiology. *Trends Microbiol.* **4**:436–443.
  42. Kreiswirth, B. N., S. Lofdahl, M. J. Betley, M. O'Reilly, P. M. Schlievert, M. S. Bergdoll, and R. P. Novick. 1983. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* **305**:709–712.
  43. Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Conner-ton, A. Danchin, and et al. 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**:249–256.
  44. Leenhouts, K. 1995. Integration strategies and vectors. *Dev. Biol. Stand.* **85**:523–530.
  45. Madsen, P. L., A. H. Johansen, K. Hammer, and L. Brondsted. 1999. The genetic switch regulating activity of early promoters of the temperate lactococcal bacteriophage TP901-1. *J. Bacteriol.* **181**:7430–7438.
  46. Maguin, E., P. Duwat, T. Hege, D. Ehrlich, and A. Gruss. 1992. New thermosensitive plasmid for gram-positive bacteria. *J. Bacteriol.* **174**:5633–5638.
  47. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
  48. Matsuno, Y., T. Ano, and M. Shoda. 1992. High-efficiency transformation of *Bacillus subtilis* NB22, an antifungal antibiotic iturin producer, by electroporation. *J. Ferment. Bioeng.* **73**:261–264.
  49. Nallapareddy, S. R., K. V. Singh, and B. E. Murray. 2006. Construction of improved temperature-sensitive and mobilizable vectors and their use for constructing mutations in the adhesin-encoding *acm* gene of poorly transformable clinical *Enterococcus faecium* strains. *Appl. Environ. Microbiol.* **72**:334–345.
  50. Okamoto, R., T. Okubo, and M. Inoue. 1996. Detection of genes regulating beta-lactamase production in *Enterococcus faecalis* and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **40**:2550–2554.
  51. O'Reilly, M., and K. M. Devine. 1997. Expression of AbrB, a transition state regulator from *Bacillus subtilis*, is growth phase dependent in a manner resembling that of Fis, the nucleoid binding protein from *Escherichia coli*. *J. Bacteriol.* **179**:522–529.
  52. O'Sullivan, D. J., and T. R. Klaenhammer. 1993. High- and low-copy-number *Lactococcus* shuttle cloning vectors with features for clone screening. *Gene* **137**:227–231.
  53. O'Sullivan, T. F., and G. F. Fitzgerald. 1999. Electroporation of industrial strains of *Streptococcus thermophilus*. *J. Appl. Microbiol.* **86**:275–283.
  54. Pavlovic, G., V. Burrus, B. Gintz, B. Decaris, and G. Guédon. 2004. Evolution of genomic islands by deletion and tandem accretion by site-specific recombination: *ICESI*-related elements from *Streptococcus thermophilus*. *Microbiology* **150**:759–774.
  55. Perez-Casal, J., J. A. Price, E. Maguin, and J. R. Scott. 1993. An M protein with a single C repeat prevents phagocytosis of *Streptococcus pyogenes*: use of a temperature-sensitive shuttle vector to deliver homologous sequences to the chromosome of *S. pyogenes*. *Mol. Microbiol.* **8**:809–819.
  56. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
  57. Scott, J. R., F. Bringel, D. Marra, G. Van Alstine, and C. K. Rudy. 1994. Conjugative transposition of Tn916: preferred targets and evidence for conjugative transfer of a single strand and for a double-stranded circular intermediate. *Mol. Microbiol.* **11**:1099–1108.
  58. Scott, J. R., and G. G. Churchward. 1995. Conjugative transposition. *Annu. Rev. Microbiol.* **49**:367–397.
  59. Sentschilo, V., R. Ravatn, C. Werlen, A. J. Zehnder, and J. R. van der Meer. 2003. Unusual integrase gene expression on the *clc* genomic island in *Pseudomonas* sp. strain B13. *J. Bacteriol.* **185**:4530–4538.
  60. Serron, P., T. Sasaki, S. D. Ehrlich, and E. Maguin. 2002. Electroporation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis* with various plasmids. *Appl. Environ. Microbiol.* **68**:46–52.
  61. Sezonov, G., C. Possoz, A. Friedmann, J. L. Pernodet, and M. Guérineau. 2000. KorSA from the *Streptomyces* integrative element pSAM2 is a central



- transcriptional repressor: target genes and binding sites. *J. Bacteriol.* **182**: 1243–1250.
62. Sirand-Pugnet, P., C. Lartigue, M. Marends, D. Jacob, A. Barre, V. Barbe, C. Schenowitz, S. Mangenot, A. Couloux, B. Segurens, A. de Daruvar, A. Blanchard, and C. Citti. 2007. Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. *PLoS Genet.* **3**:e75.
  63. Smith, C. J., S. M. Markowitz, and F. L. Macrina. 1981. Transferable tetracycline resistance in *Clostridium difficile*. *Antimicrob. Agents Chemother.* **19**:997–1003.
  64. Stingle, F., and B. Mollet. 1996. Disruption of the gene encoding penicillin-binding protein 2b (*php2b*) causes altered cell morphology and cease in exopolysaccharide production in *Streptococcus thermophilus* Sf6. *Mol. Microbiol.* **22**:357–366.
  65. Supersac, G., Y. Piemont, M. Kubina, G. Prevost, and T. J. Foster. 1998. Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of *Staphylococcus aureus*. *Microb. Pathog.* **24**: 241–251.
  66. Takamatsu, D., M. Osaki, and T. Sekizaki. 2001. Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. *Plasmid* **46**:140–148.
  67. te Poele, E. M., H. Bolhuis, and L. Dijkhuizen. 2008. Actinomycete integrative and conjugative elements. *Antonie van Leeuwenhoek* **94**:127–143.
  68. Thibessard, A., F. Borges, A. Fernandez, B. Gintz, B. Decaris, and N. Leblond-Bourget. 2004. Identification of *Streptococcus thermophilus* CNRZ368 genes involved in defense against superoxide stress. *Appl. Environ. Microbiol.* **70**:2220–2229.
  69. Thibessard, A., A. Fernandez, B. Gintz, B. Decaris, and N. Leblond-Bourget. 2002. Transposition of pGh9:ISS1 is random and efficient in *Streptococcus thermophilus* CNRZ368. *Can. J. Microbiol.* **48**:473–478.
  70. Toussaint, A., and C. Merlin. 2002. Mobile elements as a combination of functional modules. *Plasmid* **47**:26–35.
  71. Tyvaert, G., C. Morel, J. Joly, B. Decaris, and F. Charron-Bourgoin. 2006. The *eps* locus of *Streptococcus thermophilus* IP6756 is not involved in exopolysaccharide production. *Int. Dairy J.* **16**:467–473.
  72. van de Guchte, M., J. M. van der Vossen, J. Kok, and G. Venema. 1989. Construction of a lactococcal expression vector: expression of hen egg white lysozyme in *Lactococcus lactis* subsp. *lactis*. *Appl. Environ. Microbiol.* **55**: 224–228.
  73. Wang, H., A. P. Roberts, D. Lyras, J. I. Rood, M. Wilks, and P. Mullany. 2000. Characterization of the ends and target sites of the novel conjugative transposon Tn5397 from *Clostridium difficile*: excision and circularization is mediated by the large resolvase, TndX. *J. Bacteriol.* **182**:3775–3783.
  74. Wessels, M. R., R. F. Haft, L. M. Heggen, and C. E. Rubens. 1992. Identification of a genetic locus essential for capsule sialylation in type III group B streptococci. *Infect. Immun.* **60**:392–400.
  75. Willems, R. J., J. Top, N. van den Braak, A. van Belkum, D. J. Mevius, G. Hendriks, M. van Santen-Verheul, and J. D. van Embden. 1999. Molecular diversity and evolutionary relationships of Tn1546-like elements in enterococci from humans and animals. *Antimicrob. Agents Chemother.* **43**:483–491.