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Identification and Characterization of Genes Involved in Excision of the *Lactococcus lactis* Conjugative Transposon Tn*5276*

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The 70-kb transposon Tn*5276*, originally detected in *Lactococcus lactis* NIZO R5 and carrying the genes for nisin production and sucrose fermentation, can be conjugally transferred to other *L. lactis* strains. Sequence analysis and complementation studies showed that the right end of Tn*5276* contains two genes, designated *xis* and *int*, which are involved in excision. The 379-amino-acid *int* gene product shows high (up to 50%) similarity with various integrases, including that of the Tn*916*-related conjugative transposons. The *xis* gene product, like almost all known excisionase (Xis) proteins, is a small (68-residue), basic protein. Expression of both the Tn*5276 int* and *xis* genes is required for efficient excision of the ends of Tn*5276* in *Escherichia coli* that appeared to be circularized in the excision process. Mutational analysis of the *xis* and *int* genes showed that excision efficiency is dependent on the integrity of the *int* gene but that an intact *xis* gene is also required for efficient excision.

The transposon Tn*5276*, originally detected in the chromosome of *Lactococcus lactis* NIZO R5 (35, 36), can be conjugally transferred to other *L. lactis* strains. This 70-kb element carries the genetic determinants for a number of seemingly unrelated traits, including production of the lantibiotic nisin, sucrose-fermenting ability, and reduced bacteriophage sensitivity (39). It has been shown to integrate in one orientation into a preferred site (designated site 1) on the chromosome of *L. lactis* MG1614, a derivative of the plasmid-free strain MG1363, but also into several secondary sites (36). The ends of Tn*5276* are asymmetric and do not show any homology to the ends of known transposons. No target site duplication was found upon insertion of Tn*5276* (34, 36). A similar element, Tn*5301*, has been described in a transconjugant derived from a mating between the wild-type nisin producer *L. lactis* NCFB 894 and *L. lactis* MG1614 (19, 23).

Before the identification of the *L. lactis* transposons Tn*5276* and Tn*5301*, conjugative transposons were known as carriers of antibiotic resistance genes, and they were originally identified in clinical isolates of several groups of streptococci (28). The 16.4-kb Tn*916* and the 25.3-kb Tn*1545* were the first conjugative transposons to be described, and they are the best-studied representatives of this group (for reviews, see references 17 and 42). These two related elements possess nearly identical ends, which contain a terminal imperfect inverted-repeat sequence (10, 15). Unlike most transposons, they do not generate a target duplication upon insertion. The transposition system of these transposons is related to the excision-insertion system of lambdoid phages (12, 32, 33). Excision and integration occur by reciprocal nonhomologous recombination between DNA sequences of 5 or 6 bp (Tn*916* [12, 42]) or 6 or 7 bp (Tn*1545* [32, 33, 48]). Excision results in the appearance of a free, nonreplicative, covalently closed circular molecule, which may be the intermediate that is transferred during conjugation (43). After conjugal transfer to the recipient, the transposon inserts into a new target. Excision and integration of Tn*1545* have been shown to be mediated by the transposon-encoded integrase (Int) protein, which is stimulated by the excisionase (Xis) protein in excision (32, 33). The *xis* and *int* genes are located near the right end of Tn*1545* (32). Tn*916* contains essentially identical genes in the same location; however, in Tn*916* this has been termed the left end (16). The excisive and integrative activities of Tn*916* Int have been shown to be required for conjugative transposition of Tn*916* (44). Although it was at first reported that for conjugative transposition an active *int* gene in both donor and recipient was required (44), it has recently been shown that a functional *int* gene is required only in the donor (6). From these results, it was concluded that Tn*916* Int is not required for insertion of Tn*916* into the recipient genome or that the Int protein is transferred from the donor to the recipient during conjugation. There are indications that at least one host factor, absent in *L. lactis* MG1363, is involved in excision of Tn*916* (5, 7).

Here, we report the sequencing of two genes, designated *xis* and *int*, that are located near the right end of Tn*5276*. Expression of these genes in *Escherichia coli* resulted in the excision of the ends of Tn*5276* from a plasmid vector. A rejoined excision site and a circular molecule consisting of the excised Tn*5276* termini were formed. The experiments also showed that expression of the *int* gene alone can promote excision of the Tn*5276* ends in *E. coli* but that this activity is strongly stimulated by simultaneous expression of the *xis* gene. The deduced amino acid sequence of the *int* gene shows overall homology to the Int protein encoded by Tn*1545* and to other proteins belonging to the group of site-specific recombinases.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The following *E. coli* hosts were used to propagate plasmids and bacteriophages: strain MC1061 (13) for pNZ774 (36), strain TG1 (21) for M13mp18 and mp19 (50), and strain HB101 (4) for all other plasmids. Growth conditions were as described elsewhere (41). Antibiotics were added at the following concentrations: ampicillin, 50 μg/μl; chloramphenicol, 35 μg/μl.

DNA techniques. Standard procedures were used for DNA

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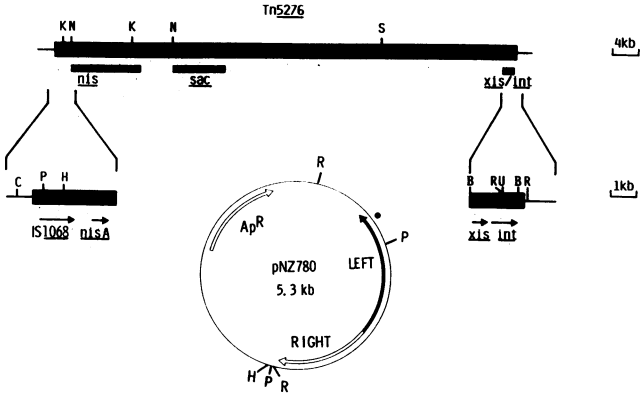

FIG. 1. Construction of pNZ780. The Tn*5276* map was taken from Rauch and De Vos (36). The solid bars below Tn*5276* indicate the positions of the nisin (*nis* [25, 36, 39]), sucrose (*sac* [37]), and transposition (*xis/int*) gene clusters. Arrows indicate the positions and the directions of the putative transposase of iso-IS*904* (35), *nisA* (36), and *sacA* (37). Restriction enzyme abbreviations: B, *Bsp*HI; C, *Sca*I; H, *Hin*dIII; K, *Kpn*I; N, *Nci*I; P, *Pst*I; R, *Eco*RI; S, *Sst*II; U, *Pvu*II.

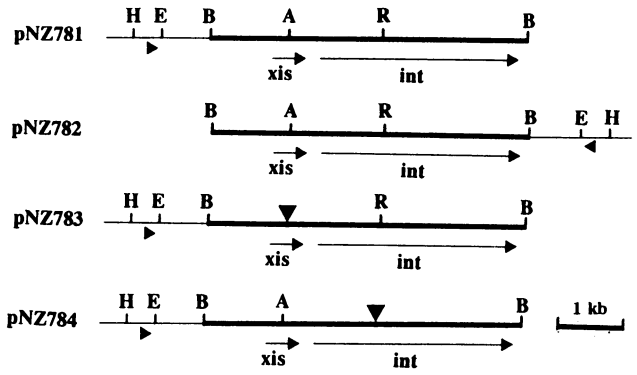

FIG. 2. Plasmids carrying the Tn*5276 xis* and *int* genes. The 1.9-kb *Bsp*HI fragment of the left end of Tn*5276* (see Fig. 1) is indicated by thick lines, and the relevant part of the vector pACYC184 is indicated by a thin line. The positions and directions of the promoter of the tetracyclin resistance gene of pACYC184 are indicated by the arrowhead. Restriction enzyme abbreviations are as described in the legend to Fig. 1 (E, *Eco*RV; A, *Afl*III). Only unique sites are shown, except for *Eco*RI, which also cuts in the part of pACYC184 that is not shown. Filled triangles indicate the frameshift mutations.

isolation and manipulations (41), agarose gel electrophoresis (41), and Southern blot analysis (36).

Hybridization probes. As probe for the left end of Tn*5276*, an oligonucleotide with the sequence 5′-GCTGTATAGTTT TGCTTTGG, designated Tn*5276*-L and complementary to position 107 to 126 in the nucleotide sequence of the left end of Tn*5276* (GenBank accession numbers M84769 and M84770) (36), was used at a hybridization temperature of 52°C.

Nucleotide sequencing. Subclones of pNZ774 in M13mp18 and mp19 were sequenced by the dideoxy chain termination method adapted for Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, Ohio) with either the M13 universal primer or synthesized primers. The sequence data were assembled and analyzed by using the PC/Gene program version 6.6 (Genofit, Geneva, Switzerland).

Plasmid constructions. The following plasmids were constructed to study the action of the *xis* and *int* gene products in *E. coli* (see Fig. 1).

(i) Construction of pNZ780. A 1.7-kb *Sca*I-*Hin*dIII fragment containing the left junction of Tn*5276* in site 1 (0.6 kb of site 1 and 1.1 kb of the Tn*5276* left end) was isolated from *L. lactis* T165.6 (36) and cloned into *Sma*I- and *Hin*dIII-digested M13mp19, resulting in mpNZ776. The insert of mpNZ776 was isolated by digestion with *Hin*dIII and then by filling in of the 3′-recessed ends with the large fragment of DNA polymerase I, deproteinization, and digestion with *Eco*RI. The right end of Tn*5276* was isolated as a 0.9-kb *Pvu*II-*Hin*dIII fragment from mpNZ775/2 (36), which contains the right junction of Tn*5276* from *L. lactis* NIZO R5 cloned as a 1.0-kb *Eco*RI fragment in *Eco*RI-linearized M13mp19 (0.9 kb of the Tn*5276* right end and 0.1 kb of NIZO R5 DNA). The 1.7-kb *Eco*RI-blunt fragment (containing 1.1 kb of the left end) and the 0.9-kb *Pvu*II-*Hin*dIII fragment (containing 0.8 kb of the right end) were ligated into pUC19 (50) digested with *Eco*RI and *Hin*dIII (Fig. 1).

(ii) Construction of pNZ781 and pNZ782. A 1.9-kb *Bsp*HI-fragment containing the *xis* and *int* genes (Fig. 1) was isolated from pNZ774 (36) and ligated into *Bsp*HI-digested pACYC184 (14). One of the resulting plasmids, designated pNZ781, contains the *xis* and *int* genes in a clockwise orientation with respect to the promoter of the Tc^r gene, while in the plasmid designated pNZ782 these genes are in a counterclockwise orientation (Fig. 2).

(iii) Construction of pNZ783 and pNZ784. The *xis* gene was inactivated in pNZ781 by digestion with *Afl*III and then by filling-in of 3′-recessed ends with the large fragment of DNA polymerase I and religation, resulting in pNZ783. The *int* gene was inactivated in pNZ781 by partial digestion with *Eco*RI and then by filling in of recessed ends with the large fragment of DNA polymerase I and religation, resulting in pNZ784 (Fig. 2).

Data base searches. The EMBL (release 31.0), Swiss Prot (release 22.0), and NBRF/PIR (release 27.0) data bases were searched using the program FASTA (31) through the facilities of the CAOS/CAMM Center, Nijmegen, The Netherlands.

Nucleotide sequence accession number. The sequence described in this paper has been assigned GenBank accession number L27649.

RESULTS

The nucleotide sequences of the *xis* and *int* genes located in the right end region of Tn*5276*. The highly homologous *xis* and *int* genes of the conjugative transposons Tn*916* and Tn*1545* are located at comparable positions near one of the ends (16, 32). When the recently reported nucleotide sequence of the right end of Tn*5276* (36) was analyzed for the presence of open reading frames (ORFs), we found a 1.9-kb *Bsp*HI-fragment to contain the 3′ end of an ORF that stopped 152 nucleotides upstream from the hexanucleotide TTTTTG located at the terminus. The amino acid sequence deduced from this ORF contained a stretch of residues that fit the consensus sequence of domain II of the integrase family of site-specific recombinases (2). Therefore, the complete nucleotide sequence of the 1.9-kb *Bsp*HI fragment was determined (Fig. 3) and was found to contain two complete genes, designated *xis* and *int* (see below). Both genes start with ATG initiation codons that are preceded by potential lactococcal ribosome-binding sites (18). A promoter-like sequence (18, 49) was found upstream from the *xis* gene and is located in the 3′ part of another ORF, which is designated ORF1 (Fig. 3). The amino acid sequences deduced from ORF1 and the *xis* and *int* genes are shown in Fig.

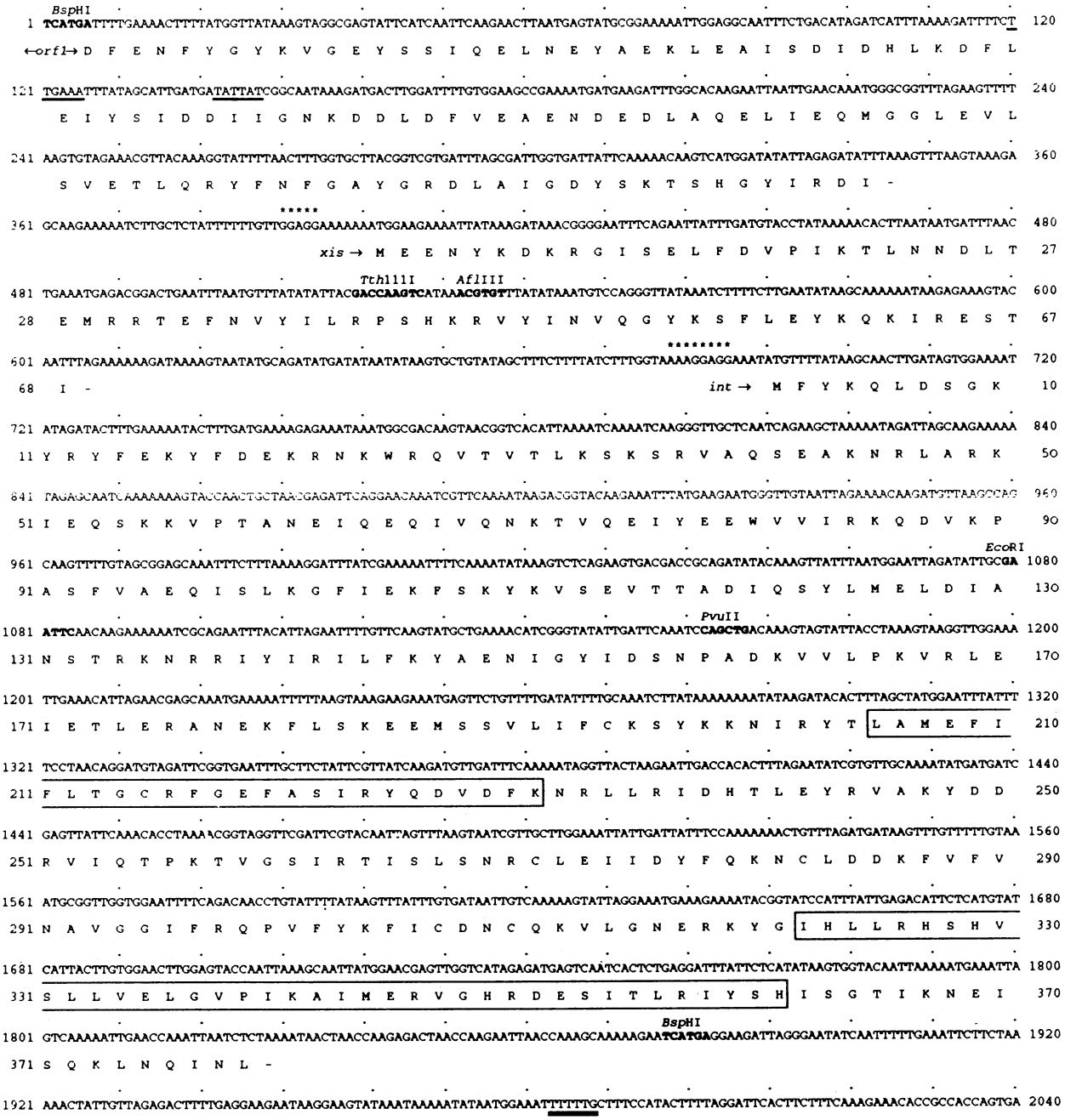

FIG. 3. Nucleotide sequence of the right junction of Tn*5276* in *L. lactis* T165.1 site 1. The amino acid sequences deduced from the ORFs present in the sequence are given below the nucleotide sequence. The amino acid stretches in the Int sequence which correspond to the consensus domains I and II of the Int-family of site-specific recombinases proposed by Argos et al. (2) are boxed. Potential ribosome-binding sites are denoted by stars over the bases that are complementary to the 3′ end of *L. lactis* 16S rRNA (27). Relevant restriction sites are in boldface. A putative promoter sequence is underlined. The TTTTTG hexanucleotide flanking (or part of) the right end of Tn*5276* in site 1 (36) is doubly underlined.

3. The *xis* gene could encode a polypeptide of 68 amino acids with a predicted molecular weight of 8,292. The *int* gene could encode a polypeptide of 379 amino acids with a predicted molecular weight of 44,621. An alternative GTG start codon for the *int* gene is located at position 648 to 650, but it is not preceded by a consensus lactococcal ribosome-binding site. The *int* gene is not followed by an inverted repeat that could serve as a terminator of transcription. The region between the *xis* and *int* genes is almost identical in size (85 bp) to the corresponding 84-bp regions of Tn*916* and Tn*1545*, which have identical sequences (16, 32).

Both the *xis* and the *int* genes are required for efficient excision of Tn*5276* ends in *E. coli*. To assess the functionality of the Tn*5276* *xis* and *int* genes, we determined whether their expression in *E. coli* would result in excision of the ends of Tn*5276* present in pNZ780 (Fig. 1). For this purpose, the 1.9-kb *Bsp*HI fragment containing both genes was cloned in two orientations in the low-copy-number plasmid pACYC184,

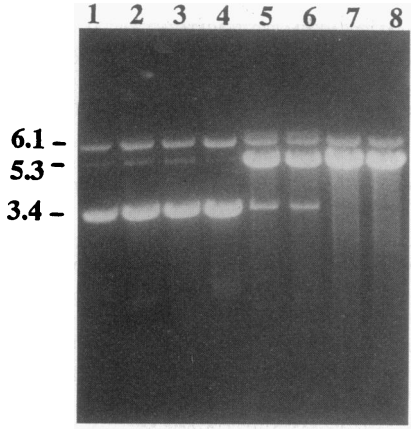

FIG. 4. Detection of excision products of pNZ780 by agarose gel electrophoresis. Plasmid DNAs from two representative transformants of *E. coli* HB101 harboring pNZ780 and either pNZ781 (lanes 1 and 2), pNZ782 (lanes 3 and 4), pNZ783 (lanes 5 and 6), or pNZ784 (lanes 7 and 8) were digested with *Hin*dIII and separated by agarose gel electrophoresis. Sizes are indicated in kilobases at the left.

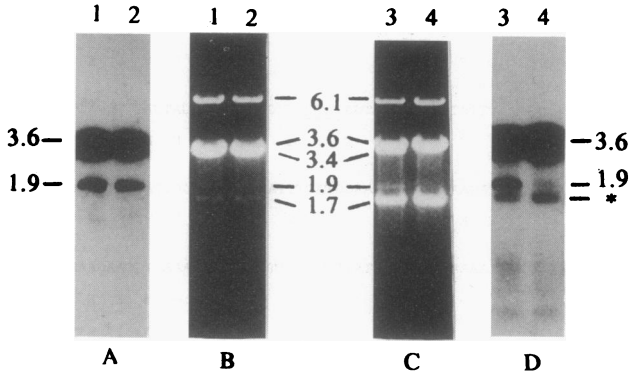

FIG. 5. Detection of excision products of pNZ780 by hybridization and circularization of Tn*5276* ends in *E. coli* HB101 harboring pNZ780 and transformed with different plasmids containing the *xis* and *int* genes of Tn5276. (A and B) Transforming plasmids were pNZ781 (lanes 1) and pNZ782 (lanes 2). (C and D) Transforming plasmids were pNZ783 (lanes 3) and pNZ784 (lanes 4). Plasmid DNAs were digested with *Eco*RV and *Pst*I and electrophoresed on a 1.0% agarose gel (B and C, respectively). Major bands are indicated between the panels and include the 6.1-kb *Eco*RV-linearized pNZ781, pNZ782, pNZ783, or pNZ784, the 3.6- and 1.7-kb *Pst*I-fragments from pNZ780 containing the Tn*5276* ends, the 3.4-kb *Pst*I fragment resulting from pNZ780 without Tn*5276* ends, and the *Pst*I-linearized 1.9-kb circle consisting of the excised Tn*5276* ends. Subsequently, Southern blots of the gels were hybridized to the oligonucleotide Tn*5276*-L specific for the left end of Tn*5276* (A and D). Autoradiography of the blot shown in panel A was approximately five times longer than that of the blot shown in panel D. The probe hybridized to the 3.6-kb *Pst*I fragment of pNZ780 containing the Tn*5276* left end and to the 1.9-kb circle consisting of the Tn*5276* ends. The star indicates the background hybridization to the 1.7-kb *Pst*I fragment from pNZ780 that is abundant in lanes 3 and 4.

resulting in plasmids pNZ781 and pNZ782. In addition, plasmids pNZ783 and pNZ784, which contained frameshift mutations in the *xis* and *int* genes, respectively, were constructed (Fig. 2). Each of these plasmids was introduced into the *recA E. coli* strain HB101 harboring the compatible plasmid pNZ780. Excision promoted by members of the integrase family is a strand exchange process in which the excision site is resealed and the excised DNA is circularized (40). Since pNZ780 contains 1.1 kb of the left end of Tn*5276* and 0.8 kb of its right end, excision of the termini of Tn*5276* from pNZ780 would generate a 3.4-kb derivative of pNZ780 lacking the Tn*5276* ends and a 1.9-kb nonreplicative covalently closed circular form of the Tn*5276* ends. Overnight cultures of 12 ampicillin- and chloramphenicol-resistant colonies of each transformation were grown in the presence of the two antibiotics, and total plasmid DNA was isolated. Analysis of the multiplasmid DNA by agarose gel electrophoresis showed an identical plasmid profile for colonies obtained from the same transformation. However, in addition to a constant amount of pNZ781, pNZ782, pNZ783, or pNZ784 (6.1 kb), variable amounts of pNZ780 (5.3 kb) were found, while in some cases a new 3.4-kb plasmid could be observed (data not shown). Subsequently, the multiplasmid DNA from two independent transformants was digested with *Hin*dIII, resulting in linearization of all replicating plasmids. Upon staining of agarose gels on which the digested DNA was separated, the 6.1-kb bands of linearized plasmids carrying the wild-type or mutated *xis* and *int* genes were clearly detectable (Fig. 4). However, only in cells harboring pNZ781 or pN782 were large amounts of a 3.4-kb band found, together with small amounts of the 5.3-kb form of pNZ780 (lanes 1 to 4). Since the 3.4-kb band represents the linearized derivative of pNZ780 lacking the Tn*5276* ends (Fig. 1), this result indicates that both pNZ781 and pNZ782 promote excision at a similar, high frequency. In contrast, when the *int* or *xis* gene was disrupted such as in pNZ783 or pNZ784, large amounts of the expected 5.3-kb linearized pNZ780 were found in addition to the 6.1-kb band of linearized pNZ784 (lanes 5 to 8). Only small amounts of the 3.4-kb pNZ780 derivative lacking the Tn*5276* ends were observed when pNZ783 was present; however, this plasmid species was not detectable in cells harboring pNZ784. This indicates that intact *xis* and *int* genes are required for efficient excision and recircularization events.

In the stained gel, the expected 1.9-kb nonreplicative covalently closed circular form of the Tn*5276* ends was not observed as a consequence of its small amount (see below) and its heterogeneity, since it is not cut by *Hin*dIII. Therefore, representative multiplasmid DNA obtained from each transformation was digested with *Pst*I and *Eco*RV. Plasmid pNZ780 contains no *Eco*RV but two *Pst*I sites, yielding fragments of 3.6 and 1.7 kb (Fig. 1). The *Pst*I sites are located in such a way that the 1.9-kb plasmid containing the nonreplicating circular ends of Tn*5276* and the complementary 3.4-kb part of pNZ780 are each linearized. The 6.1-kb plasmids carrying the *xis* and *int* genes do not contain *Pst*I sites but are linearized by digestion with *Eco*RV (Fig. 2). Agarose gel electrophoresis showed the expected 6.1-kb *Eco*RV fragment of these helper plasmids (Fig. 5B and C). In addition, the 3.6-kb and 1.7-kb *Pst*I fragments from pNZ780 are abundant when pNZ783 and pNZ784 are present (Fig. 5C) but are found only in small amounts in the presence of pNZ781 and pNZ782 (Fig. 5B). In cells harboring pNZ781 or pNZ782, the excised 3.4-kb fragment is dominant; however, no 1.9-kb *Pst*I fragment from the excised nonreplicating ends could be found upon staining. However, after blotting of these gels followed by hybridization with probe Tn*5276*-L, which is specific for this part of pNZ780 (Fig. 1), it was possible to detect small amounts of the 1.9-kb fragment in cells harboring pNZ781 or pNZ782 (Fig. 5A). The strongly hybridizing band with a size of 3.6-kb is due to the small amount of intact pNZ780 that is still present in those cells (Fig. 4). It is likely that the nonreplicative nature of the

1.9-kb plasmid explains its small amount in those transformants, compared with the complementary 3.4-kb plasmid that contains the pUC19 replicon and is easily detectable. Remarkably, in cells harboring pNZ783 with the mutated *xis* gene that show a greatly reduced excision efficiency (Fig. 4, lanes 6 and 7), a considerable amount of the 1.7-kb *Pst*I fragment could be detected, even in the stained digest (Fig. 5C and D). In contrast, cells harboring pNZ784 with the mutated *int* gene do not contain detectable amounts of the plasmid carrying the Tn*5276* ends (Fig. 4, lanes 7 and 8) and the 1.9-kb *Pst*I fragment cannot be seen in the stained digest (Fig. 5, lane 4). However, a faintly hybridizing band of 1.9 kb is detectable in the blot (Fig. 5D). Since the complementary excised circle was not detected in these cells (Fig. 4), the simplest explanation for the presence of this diffuse band is that it actually represents some aspecific hybridization, as found at the bottom of this gel. Thus, in the presence of the *int* gene alone (as in pNZ783), the ends of Tn*5276* in pNZ780 are excized and recircularized, but the additional presence of a functional *xis* gene (as in pNZ781 or pNZ782) strongly enhances the excision efficiency.

When the *xis* gene was inactivated as in pNZ783, an amount of circular intermediate was found that was greater than that found with pNZ781 or pNZ782 containing intact *xis* and *int* genes (compare the autoradiographs in Fig. 5A and D and note the longer period of exposure in Fig. 5A). This could be explained by the inefficiency of the excision process in the absence of functional Xis. In this case, pNZ780 molecules containing the Tn*5276* ends will continuously be present and excision of the (nonreplicating) 1.9-kb circle continues. In contrast, efficient Int-promoted excision in the presence of functional Xis results in a rapid loss of the substrate (intact pNZ780).

Since Int promotes insertion in the absence of Xis in other Xis-Int systems, the possible insertion of the 1.9-kb circle carrying the Tn*5276* ends into new sites on the *E. coli* genome was investigated in transformants containing pNZ780 and pNZ783 by hybridization of total DNA with the Tn*5276*-L probe. No new bands were found (data not shown), indicating the absence of insertion into new sites, although reinsertion of the 1.9-kb circle into the site of excision cannot be excluded.

Features of Int and Xis amino acid sequences. Data bases were searched for the presence of protein sequences that were similar to those deduced from ORF1 and the *xis* and *int* genes. No proteins with significant similarity to the amino acid sequence deduced from ORF1 were found. In addition, no proteins with significant similarity to the small Tn*5276* Xis were found. However, Tn*5276* Xis, like most known excisionases, is a short, basic protein (pI 9.71).

Five proteins showing overall homology to Int were detected in the data bases (Fig. 6). The highest percentages of similar and identical residues were encountered in the C-terminal regions. An alignment of the C-terminal regions of these proteins and Tn*5276* Int is given in Fig. 6. As the five other integrases, Tn*5276* Int is a basic protein with a calculated pI of 9.99.

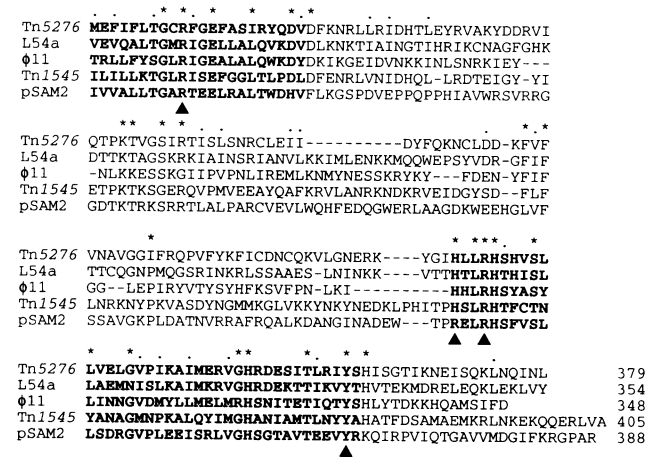

FIG. 6. Amino acid sequence alignments of the C-terminal regions of, from top to bottom, the Int proteins of Tn*5276*, bacteriophage L54a (52), Tn*1545* (32), bacteriophage ϕ11 (51), and pSAM2 (32). Domains I and II (2) are in boldface. Asterisks indicate residues present in at least four sequences, and similar residues (I-L-V-M, D-E, R-K, Q-N, S-T, and F-Y) present in at least four sequences are indicated by points. Triangles indicate the residues conserved in all known integrases (except in pSAM2 Int).

DISCUSSION

Excision of the conjugative transposon Tn*5276* from the donor replicon would be the first step of the transposition process, by analogy with the conjugative transposons Tn*916* and Tn*1545*. Here, we show that Tn*5276* contains two juxtaposed genes, *xis* and *int*, at its extreme right terminus. The location and organization of the *xis* and *int* genes of Tn*5276* are similar to those of the corresponding genes in Tn*1545* (32) and Tn*916* (16). In all cases, the genes are located near one end of the element and the *xis* and *int* genes are separated by a region of 84 (Tn*916* and Tn*1545*) or 85 (Tn*5276*) bp. The functionality of the Tn*5276* *xis* and *int* genes was established by the observation that their expression resulted in excision of the Tn*5276* ends in *E. coli*. Gene disruption studies showed that an intact *int* gene is essential for excision, while a frameshift mutation in the *xis* gene, resulting in a truncated protein lacking the C-terminal 23 amino acid residues, greatly reduced the excision efficiency. Two products are formed in the excision reaction: a deleted plasmid containing a religated excision site and a 1.9-kb covalently closed circular molecule consisting of the ends of Tn*5276*. Recently, we found that the nucleotide sequence of the joint of this 1.9-kb plasmid was identical to that found for the circular intermediate of Tn*5276* in *L. lactis* (34, 38). These results strongly suggest that the *xis* and *int* genes code for proteins that are required for excision of Tn*5276* in *L. lactis*.

Tn*5276* Xis and Int, each in different degrees, are similar to other proteins involved in excision and insertion of genetic elements. The Int-related family (2) is one of the two main groups of site-specific recombinases. The homologous domains I and II (2) in the C-terminal regions of a large number of proteins that belong to the Int-related family have been aligned (1–3, 8, 32). These alignments showed that a histidine, a tyrosine and two arginine residues are conserved in all integrases, except for that of pSAM2. It has been shown that Tyr-342 in domain II of bacteriophage λ Int is the residue that forms an *O*-phosphotyrosine bond with the 3′-phosphate of *att* at the site(s) of strand exchange (30). Thus, it is very likely that the C-terminal region of Tn*5276* Int (which is absent in the mutant Int specified by pNZ784) participates in strand transfer and that the active site is at the corresponding Tyr-359.

A variety of excisionases have been identified thus far from different mobile elements. These elements are the lambdoid phages (26), several *Streptomyces* integrative plasmids (3, 8), the streptococcal conjugative transposons Tn*916* (16) and Tn*1545* (32), and the bacteriophages L54a (51) and ϕ11 (52), which are present as prophages in *Staphylococcus aureus*. The

Tn*5276* Xis protein shares with almost all of these excisionases its small size and high pI. The excisionases of bacteriophages L54a and φ11 are acidic and represent a different class of excisionases (51). The basic character of the excisionases from the first class is thought to reflect their interaction with DNA. In the case of bacteriophage λ, it has been shown that Xis in conjunction with the *E. coli* protein factor for inversion stimulation (FIS) binds to sites in *attR* and enhances excision (9, 29, 47). When λ Xis binds the DNA at its recognition sites in λ *attR*, the DNA is bent (45). The formation or stabilization of this bend is thought to be required for excisive recombination of λ. The involvement of FIS in λ excision couples recombination to the growth phase of the host cell (47). Another host factor involved in λ site-specific recombination is integration host factor (IHF [46]). We cannot exclude the involvement of host factors in excision of Tn*5276*, since the possibility exists that *E. coli* IHF and FIS can substitute for similar lactococcal host factors. Several subsequences conforming to the proposed consensus *E. coli* IHF and FIS binding sites (22, 24) can be found in the ends of Tn*5276* (34). However, excision experiments in *E. coli* strains deficient in the production of IHF and FIS should be carried out to investigate the involvement of *E. coli* host factors in excision of the Tn*5276* ends from pNZ780. It was recently suggested that a host factor that is absent from *L. lactis* MG1363 is required for the conjugative transposition of Tn*916* (5). This putative host factor is evidently not involved in transposition of Tn*5276*, since we have shown that insertion of Tn*5276* into the genome of a derivative of *L. lactis* MG1363 occurs via nonhomologous recombination (36, 38) and not, as for Tn*916* in *L. lactis* MG1363, through homologous recombination (5, 7).

The amino acid sequence of Tn*5276* Int shows an overall (up to 50%) similarity to those of the Int proteins of conjugative transposons from *Streptococcus* species (Tn*1545* and Tn*916*), an integrative plasmid from *Streptomyces ambofaciens* (pSAM2), and bacteriophages carried as a prophage in *S. aureus* (L54a and φ11). In contrast to the overall similarity of the Int proteins (which is highest in their C-terminal regions), other features of these elements, such as the structures of their ends, the amino acid sequences of their Xis proteins, their insertional behaviors, and the requirements of host factors for their excision and insertion, differ strongly from each other. The question arises of how these elements have acquired the genes for the homologous Int proteins. The joining together of distinct modules from different sources as proposed for the evolution of lambdoid phages (11) would be an attractive model for the evolution of Tn*5276* and related transposons, since they carry the genes for a number of very different traits, such as nisin production (36), sucrose fermentation (37), and in some cases the synthesis of N^5-carboxyethyl-ornithine (20). Further investigation of Tn*5276* could contribute to our knowledge of its origin and its evolutionary relationships with conjugative transposons, bacteriophages, and plasmids.

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