Transposition of IS1181 in the genomes of Staphylococcus and Listeria

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Abstract

The recombinant plasmid pIP1713 was constructed to analyse the transpositional activity of the insertion sequence IS1181 in Staphylococcus aureus RN4220, Staphylococcus carnosus TM300 and Listeria monocytogenes EGD. This 11.3-kb plasmid contains two genetically different elements: (i) a pE194ts-derived replicon, the ermC gene of which confers resistance to erythromycin in Gram-positive bacteria of several species, and (ii) a copy of IS1181, cloned from S. aureus BM3121, in which the tetracycline resistance gene, tet(T), has been inserted between the transposase-encoded gene and the downstream inverted repeat. When introduced by electroporation into the three bacterial hosts, pIP1713 delivered IS1181tet(T) to various chromosomal sites. Cointegrate structures between pIP1713 and the host chromosome were occasionally detected. Transposition was associated with 8-bp repeats at the insertion sites. IS1181tet(T) could be used for random mutagenesis in Gram-positive bacteria. ß 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Staphylococcus aureus; Staphylococcus carnosus; Listeria monocytogenes; Mutagenesis; Insertion sequence; Shuttle vector

1. Introduction

Insertion sequences (IS) are the simplest transposable elements known [1]. Five have been described in Staphylococcus (IS256, IS257/431, IS1272, IS1181, IS1182) and several variants of each have been sequenced [2–11]. Most were identified as putative transposable elements because repeated sequences were found at the insertion sites of the IS or the composite transposon they form [9–12]. The mobility of IS257 has been demonstrated in vivo [13].

IS1181 is 1512 bp long [10]. It was initially identified in a methicillin-resistant S. aureus strain, BM3121, which has at least eight copies scattered along the chromosome. No sequences homologous to IS1181 have been detected in coagulase-negative staphylococci (26 strains belonging to nine species) or in streptococci-enterococci (35 strains tested). Three of the four cloned copies of IS1181 have perfect 8-bp direct repeats at their ends, suggesting that they were formed by a transposition event. This study analysed the transpositional activity of IS1181 in S. aureus, S. carnosus and Listeria monocytogenes.

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2. Materials and methods

2.1. Bacterial strains, plasmids and media

The Gram-positive recipients were *S. aureus* RN4220 [14], *S. carnosus* TM300 [15] and *L. monocytogenes* EGD [16]. The bacterial host for plasmid constructions was *Escherichia coli* TG1 [18] supE hsdS thi Δ(lac-proAB) F' (traD36 proAB lacF lacZΔM15). Culture media were either Luria-Bertani (LB) medium (Diﬀco) or brain heart infusion (BHI, Diﬀco). The selection media contained 100 mg l⁻¹ ampicillin (Ap), 10 mg l⁻¹ tetracycline (Tc) or 10 mg l⁻¹ erythromycin (Em). X-gal (Ap-pliåne') was used at 40 mg l⁻¹ in agar media. Purple agar base (Diﬀco) medium was supplemented with 1% (w/v) galactose (Sigma) as previously described [17]. The recombinant plasmids pIP1682 [10] and pIP1697 [18] were used as sources of the DNA fragments carrying IS1181 (pIP1682) and the tet(T) gene (pIP1697). The recombinant plasmids pIP1603 [10] and pIP1695 [18] carried DNA fragments from within IS1181 (pIP1603) and the tet(T) gene (pIP1695). The vector pOX7 [19] was a hybrid plasmid containing both pUC18 and pE194ts sequences.

2.2. Plasmid DNA techniques

Recovery of the plasmids from *E. coli* cells was done with the QIAPrep Spin Miniprep Kit (Qiagen). DNA fragments used as probes were puriﬁed with the GeneClean II Kit (Bio 101) after 0.7% (w/v) agarose gel electrophoresis in TAE buffer. Recombinant DNA was introduced into *Staphylococcus* and *Listeria* by electroporation [20]. Site-directed mutagenesis was performed using the Sculptor in vitro mutagenesis system (Amersham). Sequencing reactions were carried out on double-stranded templates with the T7 sequencing kit (Pharmacia).

2.3. Cellular DNA analysis

Whole-cell DNA was extracted from *Staphylococcus* and *Listeria* strains using the QIAamp tissue kit (Qiagen) according to the manufacturer’s instructions, with optimised lysis buﬀers containing 40 μg ml⁻¹ of either lysostaphin (Ambi) or lysozyme (Sig-

ma). The yield of puriﬁed DNA was higher when the 95°C incubation step was omitted and a ﬁnal RNase treatment was done. Southern blotting of restricted DNA was conducted onto Hybond-N+ membranes (Amersham) by standard protocols [20]. 32P-labelled DNA probes were prepared using the Megaprime DNA labelling system (Amersham). Hybridisation was performed under stringent conditions [21].

2.4. Polymerase chain reaction (PCR)

All the PCR experiments, including inverse PCR procedure [22], were carried out in a Robocycler Gradient 96 temperature cycler (Stratagene) using Ready-To-Go PCR beads (Pharmacia) as reagents and oligodeoxynucleotides (Eurogentec) described below as primers. Time and temperature conditions were 5 min at 94°C, 2 min at 60°C, then 30 cycles of 72°C (3 min), 92°C (1 min), 60°C (1 min), and ﬁnally 10 min at 72°C.

Ren1: 5'-ACATATGAAGCTGCCATGCAC from nt 2741 to nt 2762 of the tet(T) coding sequence
Ren2: 5'-CCTTTAGTTTGGGTTTGGTCAC from nt 432 to nt 410 of the IS1181 reverse sequence
Ren3: 5'-CCCTTAACCTTTTTTTGCTGTGAC from nt 1980 to nt 1957 of the pE194 reverse sequence

2.5. Enzymes and chemicals

Restriction endonucleases, T4 DNA ligase, and alkaline phosphatase were purchased from Pharmacia. Radioactive deoxynucleotides were supplied by Amersham. Antibiotics were kindly provided by the following manufacturers: Bristol-Myers Squibb (ampicillin), Abbott France (erythromycin) and Hoechst Marion Roussel (tetracycline).

3. Results

3.1. Construction of pIP1713

Plasmid pIP1682, containing a copy of IS1181
from *S. aureus* RN4220 [10], was cut with *Xba*I and *Hind*III. The resulting 2.1-kb DNA piece carrying *IS*1181 was inserted into M13mp18 cleaved with *Xba*I and *Hind*III. A *Sph*I restriction site was created 25 nt downstream of the transposase gene, *tnp*, at the end of *IS*1181, using a 23-mer oligodeoxynucleotide 5′-AACTCTAAAAGTTGCTGCTAAAAATAGTTCTT, three bases of which (underlined) were different from the original sequence [10]. The *Sph*I fragment of pIP1697 [18], carrying the *tet*(T) gene, was inserted into the *Sph*I site, within *IS*1181, in both orientations. The structure of each of the two constructs was first verified by restriction analysis and then by DNA sequencing. Only the construct bearing the *tnp* and *tet*(T) genes in tandem was used for further cloning experiments. Digestion with *Sma*I and *Hind*III generated a 4.9-kb restriction fragment carrying *IS*1181*tet*(T). This fragment was inserted into *Sma*I and *Hind*III sites of pOX7 to give pIP1713 (Fig. 1).

### 3.2. Integration assays

pIP1713 was introduced by electroporation into *S. aureus* RN4220, *S. carnosus* TM300 and *L. monocytogenes* EGD. Transformants were selected on Em at 30°C. The plasmid content of three EmR transformants was analysed: BM12312 (*S. aureus*), BM12484 (*S. carnosus*) and BM3782 (*L. monocytogenes*). All harboured an 11.3-kb plasmid, the restriction map of which was identical to that of pIP1713.

A single colony from each transformant was grown overnight at 30°C in 10 ml BHI with Em. Stationary-phase cultures were diluted 1:100 in antibiotic-free BHI, and were incubated for 1 h at 43°C. Samples were spread onto BHI agar with Tc. Colonies growing at 43°C were counted as putative integrants.

### 3.3. Characterisation of the integrants

A total of 800 putative integrants from each experiment were replica-plated onto tetracycline- and erythromycin-containing BHI agar. The colonies that grew at 43°C were either TcR-EmR or TcR-EmS. Those that were TcR-EmS were presumed to harbour the entire plasmid, inserted into the host chromosome, whereas those that were TcR-EmR were presumed to have lost pIP1713. The proportion of the TcR-EmS colonies obtained from *S. carnosus* BM12484 and from *L. monocytogenes* BM3782 was significantly higher (87 and 85.5%, respectively) than that obtained from *S. aureus* BM12312 (18%). Cellular DNA of putative integrants from each antibiotic type was digested with *Sma*I and *Cla*I used separately. Both these enzymes recognise single sites in pIP1713 (Fig. 1). Southern analysis was performed using three probes that derived from different portions of pIP1713: the *tnp* and *tet*(T) genes correspond to the shuttle vector, pOX7. Open arrows and boxes indicate the orientation of the genes and the location of the functionally important regions, respectively. Arrows inside the circle designate the three primers used in PCR experiments. Only the relevant restriction sites are shown. Abbreviations: *bla*, β-lactamase resistance gene from pUC18; *erm*, erythromycin resistance gene from pE194; *rep*, thermosensitive RepF protein; *S. aureus* ori, replicative origin in *S. aureus*; *E. coli* ori, replicative origin in *E. coli*; *tnp*, transposase gene of *IS*1181; IRL and IRR, left and right inverted repeats of *IS*1181; *tet*(T), tetracycline resistance gene.

Fig. 1. Schematic diagram of pIP1713. Solid black lines represent the DNA fragment carrying *IS*1181*tet*(T), and grey lines correspond to the shuttle vector, pOX7. Open arrows and boxes indicate the orientation of the genes and the location of the functionally important regions, respectively. Arrows inside the circle designate the three primers used in PCR experiments. Only the relevant restriction sites are shown. Abbreviations: *bla*, β-lactamase resistance gene from pUC18; *erm*, erythromycin resistance gene from pE194; *rep*, thermosensitive RepF protein; *S. aureus* ori, replicative origin in *S. aureus*; *E. coli* ori, replicative origin in *E. coli*; *tnp*, transposase gene of *IS*1181; IRL and IRR, left and right inverted repeats of *IS*1181; *tet*(T), tetracycline resistance gene.
DNA fragments carrying the resident copies of IS1181 in S. aureus RN4220 were detected in the hybridisation patterns of all the TcR-EmS clones derived from S. aureus BM12312 (Fig. 2A). The DNA fragment that varied in size with the IS1181 probe was the only one that hybridised with the tet(T) probe. This DNA fragment probably resulted from IS1181 tet(T) transposition. At least 21 separate IS1181 tet(T) insertion sites were found for the 25 TcR-EmS S. aureus colonies that were investigated by both enzymes. Similar diversity in the chromosomal location of the IS1181 tet(T) copies was observed among the TcR-EmS colonies derived either from S. carnosus BM12484 (12 different sites for the 12 colonies tested) (Fig. 2B) or from L. monocytogenes BM3782 (11 different sites for the 12 colonies tested) (Fig. 2C). Two chromosomal sites to which the IS1181 tet(T) had transposed were cloned by inverse PCR using Ren1 and Ren2 as primers from the two S. aureus clones, BM12376 and BM12382, harbouring the smallest HindIII restriction fragments that hybridised to both the IS1181 and tet(T) probes (Fig. 2A, lanes 1 and 7). The corresponding recombinant plasmids, pIP1796 and pIP1797, carried DNA pieces of 1.5 kb and 0.3 kb, respectively, whose sequences each contained 8-bp direct repeats (Fig. 3).

Hybridisation experiments carried out with the

Clone BM12376:

\[
\text{IS1181} \quad \text{CGTAAACCTAATGAAATGCCggttct}-/-\text{agaaccTGAAGTGCCCTACCTATC} \quad 3'
\]

Clone BM12382:

\[
\text{IS1181} \quad \text{TCTCCGCTTTATTTATAGggttct}-/-\text{agaaccTTTTTTATATAAAATTCC} \quad 3'
\]

Fig. 3. DNA sequences at the junctions between the transposable element IS1181 tet(T) (lowercase) and the chromosome of two TcR-EmS derivative clones from S. aureus BM12312 (uppercase). The 8-bp direct repeats are underlined.
TcR-EmR S. aureus clones suggested that pIP1713 was inserted into either one of the two resident copies of IS1181. Each TcR-EmR S. aureus clone carried three HindIII or ClaI fragments that hybridised to the tet(T) probe, one of which also hybridised to the IS1181 probe. Within the host chromosomes devoid of any resident copies of IS1181, pIP1713 integrated at various sites: 12 different sites for the 12 colonies analysed that were derived from S. carnosus BM12484 and 12 different sites for the 12 colonies analysed that were derived from L. monocytogenes BM3782. Two HindIII or ClaI fragments that hybridised to both the tet(T) and IS1181 probes were detected in all these TcR-EmR clones. Both ClaI fragments also hybridised to the pOX7 probe, whereas only one of the two HindIII fragments gave a detectable signal with this probe. Such results are consistent with there being a copy of the pOX7 vector...
flanked by two copies of the transposable element, IS1181Ωtet(T). The PCR analysis of five TcR-EmR clones using Ren2 and Ren3 as primers confirmed the existence of this putative DNA inserted structure (Fig. 4).

IS1181Ωtet(T) mutagenesis was tested by replicating the 696 TcR-EmR derivative clones found in S. carnosus onto X-gal-containing BHI agar and galactose-containing purple agar base. The parental strain, BM12484, exhibited both β-galactosidase activity, as shown by dark blue colonies in the presence of X-gal, and acid production from galactose, as shown by yellow halos around colonies in the presence of galactose. Two TcR-EmS derivative clones were phenotypically modified: one, BM12545, gave no detectable β-galactosidase activity whereas the other, BM12550, failed to produce acid from galactose. Their respective HindIII restriction fragments that hybridised to the IS1181 probe were different in size (data not shown). The corresponding DNA targets were therefore affected by two independent transposition events. The minimal frequency of IS1181Ωtet(T) transposition events might be estimated as 1.4 × 10⁻⁵ clones in S. carnosus BM12484. No spontaneous mutation was detected when the recipient strain, S. carnosus TM300, was analysed in the same experimental conditions.

4. Discussion

The Staphylococcus and Listeria genera include major pathogenic bacteria (e.g. S. aureus and L. monocytogenes) and non-pathogenic useful bacteria (e.g. S. carnosus, used in the food and pharmaceutical industries). We tested the active mobility of IS1181 in these species by constructing a recombinant plasmid, pIP1713. This plasmid was stable at the permissive temperature, the delivery vector was efficiently eliminated at the non-permissive temperature, and the antibiotic resistance markers were readily selectable, which made it possible to trace the vector and IS element separately (EmR and TcR, respectively).

Tagging IS1181 with a tetracycline resistance marker did not prevent the mobility of the IS element. Transposition of IS1181Ωtet(T) was detected in all the TcR-EmS clones investigated. In all hosts, a great diversity of IS1181Ωtet(T) insertion sites was detected. Eight-bp repeated sequences were evidenced at the ends of two inserted copies of IS1181Ωtet(T). The repeated sequences differed from one another and from all others previously sequenced from the ends of cloned copies of IS1181 [10]. The detection of such repeats suggests that no rearrangements occurred in the vicinity of the insertion sites.

IS1181Ωtet(T) integration events resulted from transposition and/or recombination. The cointegrate structures found in TcR-EmR clones of S. carnosus and L. monocytogenes were either the intermediates of a replicative transposition [1] or the results of transposition of IS1181Ωtet(T) to the chromosome followed by homologous recombination between the copies of this IS element on the plasmid and the chromosome. The cointegrate structures found in TcR-EmR clones of S. aureus were exclusively the products of a recombination process between the IS1181Ωtet(T) copy of pIP1713 and either one of the two resident copies of IS1181 present on the chromosome. The three recipient strains studied are recombinant-proficient; they were chosen because they are frequently used in microbial genetics [23–25]. Thus, the effect of the host recombination system on the mechanism of transposition, particularly on the resolution of the cointegrates, could not be evaluated. The recA null mutant derivative of S. aureus RN4220, KB103 [26], would allow such studies in the same genetic background.

Transpositional mutagenesis experiments with IS1181Ωtet(T) may extend the range of applications of pIP1713. There are very few mobile elements that are suitable for such a purpose in the world of the Gram-positive bacteria [27]. Tn917, similar in sequence and genetic organisation to Tn551, is the most extensively used, with delivery vectors consisting of pE194ts [28] or pWV01 derivatives [29]. This study has clearly demonstrated that the transposable element IS1181Ωtet(T), delivered by pIP1713, fulfills the requirements for a satisfactory mutagenic tool in any host that does not contain sequences homologous to IS1181. The dissemination of IS1181 in staphylococci was thought to be confined to S. aureus strains [10]. Some epidemic clones of S. aureus are devoid of any resident copies [30]. If it turns out that IS1181 is not widely distributed among any oth-
er Gram-positive bacteria, IS1181/Ωtet(T) may be a valuable mutagenic element of the unexplored genomes.

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References


