Mechanism for transfer of transposon Tn2010 carrying macrolide resistance genes in Streptococcus pneumoniae and its effects on genome evolution

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Objectives: The objective of this study was to identify the mechanism responsible for the horizontal transfer of transposon Tn2010 in Streptococcus pneumoniae, and the genomic alterations introduced by the transfer process.

Methods: Tn2010 was identified using PCR in 15 clinical isolates of S. pneumoniae with erythromycin resistance. S. pneumoniae and Enterococcus faecalis isolates were used as recipient cells in mating and transformation experiments to test the conjugative transferability and transformability of Tn2010. Whole-genome sequencing was used to assess the effects of the Tn2010 transfer on recipient genomes. The biological cost of the horizontal acquisition of Tn2010 and additional genomic changes was investigated by growth competition experiments.

Results: Tn2010 was transformed at a frequency of $3 \times 10^{-7}$ transformants per cfu, whereas no transconjugants were detected using S. pneumoniae or E. faecalis as recipient cells. Genome analysis showed that many other recombinations were scattered throughout the genome of the transformants in addition to transposon Tn2010. The transformants demonstrated a negligible fitness cost compared with the wild-type strain.

Conclusions: Tn2010 tended to be transferred by transformation rather than conjugation in S. pneumoniae, and the spread of Tn2010 could have a profound effect on the evolution of the genome. The acquisition of Tn2010 with negligible fitness cost may facilitate spread of the transposon.

Keywords: transformation, recombination, fitness cost

Introduction

The widespread dissemination of pathogen antibiotic resistance is a clinical challenge.1 Horizontal gene transfer (HGT) has been responsible for the development of resistance.2,3 Mobile genetic elements are vehicles for HGT. These vehicles can enter the recipient cell through transformation, bacteriophage-mediated transduction and plasmid- or transposon-mediated conjugation.4 Bacteria utilize all HGT mechanisms to acquire mobile elements; however, species tend to specialize in the mechanism(s) they use most frequently.5 Streptococcus pneumoniae is the most common bacterial pathogen of the respiratory tract, and is a naturally transformable bacterium able to take up environmental DNA and incorporate it into its chromosome.6 Transformation is believed to contribute to the genetic plasticity of S. pneumoniae and to play a central role in the adaptation of this pathogen to the immune system, vaccines and antibiotics.7 However, S. pneumoniae also contains conjugative transposons.

The conjugative transposon Tn916 carries a tetracycline resistance gene. The Tn916 family of transposons includes mobile elements with the general organization of Tn916 and similar sequence and structure of the conjugation and regulation module to Tn916 at the DNA level.8 Tn6002, Tn2009 and Tn2010, which are members of the Tn916 family of transposons, are prevalent in S. pneumoniae. Tn6002 harbours an accessory gene encoding macrolide phosphotransferase [erm(B)].9 Tn2009 includes the mef(E)-carrying element mega (macrolide efflux genetic assembly 5511 bp).10 Tn2010 contains both erm(B) and mega genetic elements.11 Tn6002 can be transferred to the recipient cell by conjugation.12 However, it has been reported that isolates carrying Tn2010 are not able to transfer macrolide resistance by...
The crossed box indicates an ORF fragment encoding the putative bacteriocin-associated integral membrane protein. The insertion site of Tn in PGX1416 has been described previously. The mechanism of HGT for the Tn2010 transposon in S. pneumoniae, and the effect of transposon transfer on genome evolution, are still unclear. The objective of this study was to investigate the mechanism of dissemination for Tn2010 in S. pneumoniae and understand the effect of Tn2010 transfer on genome evolution.

Materials and methods

Key strains are listed in Table S1 (available as Supplementary data at JAC Online). Pneumococci were grown in brain heart infusion broth or tryptic soy agar plates containing 5% defibrinated sheep's blood, as described previously. Two donor strains (A021 and A026) and seven recipient strains were used in mating experiments to test the conjugative transferability of Tn2010 (Table S1). Mating experiments were carried out as described previously. Briefly, donor and recipient bacteria were mixed at a 1:10 ratio after having been grown separately to the mid-log phase, plated onto blood agar plates with or without 50 μg/mL DNase I and incubated overnight. After incubation, the cells were plated onto selection plates with erythromycin and streptomycin or chloramphenicol. Mating experiments were performed at least in triplicate.

A transformation assay was used to determine the transformability of Tn2010. Genomic DNA of strain A026 was introduced into S. pneumoniae R6 in which competence had been induced using a competence peptide following the procedure described by Joloba et al. Transformants were selected in medium containing 2 mg/L erythromycin.

S. pneumoniae clinical isolate A026 and three transformants (Sp-Z1, Sp-Z2 and Sp-Z3) were sequenced with the Illumina HiSeq2000 system. The complete genome of A026 was sequenced. The SOAP program was utilized to align the sequencing reads onto the reference genome and identify the single nucleotide polymorphisms (SNPs).

Competition assays were carried out as described previously. Four transformants (Sp-Z1, Sp-Z2, Sp-Z3 and Sp-Z4) and R6 were grown to OD600 ~0.2, and the cultures were diluted 2000-fold. Equal densities of each transformant and R6 were mixed and incubated in antibiotic-free brain heart infusion medium for 6 h. The number of viable cells was counted at 0 and 6 h. The relative fitness of each transformant was determined from the ratio of the number of generations of the transformant to that of strain R6. Four replicate competition assays were performed.

Results and discussion

Thirteen isolates of ST271, one of ST320 and one of ST6993 were recovered from patients with S. pneumoniae infections in China (Table S1). Isolate A026 (ST271) was selected for genome sequencing and its genome was found to consist of 2091879 nucleotides and have a GC content of 39.76% (GenBank: CP006844.1). The isolate carried Tn2010 sharing 100% identity with the reported sequence (AB426620.1). Analysis of the regions flanking Tn2010 revealed that the transposon was inserted at the 3’ end of spr1764 of the R6 genome and the 5’ end of spr1772 (Figure 1). Interestingly, the segment between spr1764 and spr1772 not only included Tn2010 but also other sequences (1966 bp). The 256 bp block downstream of Tn2010 showed 92% identity to the non-coding region between spr1774 and spr1775. Next, sequences encoding the putative bacteriocin-associated integral membrane protein were located upstream of spr1772. Another 14 isolates were also identified carrying Tn2010 located at the same site as in A026. However, this result was different from previous reports that the Tn2010 insertion site was located in spr1764 of the S. pneumoniae R6 genome and the sequence between spr1774 and spr1775.

Two clinical isolates of S. pneumoniae carrying Tn2010 were used as donors for mating experiments; S. pneumoniae strains SPR1, SPR2 and SP6B1, and Enterococcus faecalis strains P149, P078, K484 and H049, were used as recipients. No transfer from any donor was detectable using E. faecalis P149, P078, K484 or H049 as the recipient (<10^-9 transconjugants per donor). Tn2010 was transferred at a frequency of 2 x 10^-8 transconjugants per donor using S. pneumoniae strains SPR1 and SP6B1 as recipients. However, no transfer (<10^-9 transconjugants per donor) was detected when matings were performed in the presence of DNase I or using as the recipient SPR2 in which the comC gene encoding the competence-stimulating peptide was deleted. We transformed genomic DNA of A026 into S. pneumoniae susceptible strain R6, and a higher transfer frequency (3 x 10^-7 transformants per cfu) was obtained. This result suggests that Tn2010
Figure 2. Recombination across the genomes of three transformants. A simplified annotation of the S. pneumoniae R6 genome (carrying Tn2010) is displayed across the top with the capsule biosynthesis (cps) locus. The black blocks represent the recombination events predicted to have occurred in the genomes of the transformants.

is transferred by transformation rather than conjugation. A high transformation efficiency for S. pneumoniae has been detected in the nasopharynx; the highest efficiency reported is 10−2.21 Therefore, Tn2010 could be easily transferred by in vivo transformation.

The first step in the conjugal transfer of Tn916 is to excise the molecule to form a circular intermediate molecule.22 A circular form of Tn2010 was detected by PCR in our experiment. A Tn2010 without extra sequence is therefore believed to integrate into the chromosome by target-site recombination through conjugation. However, the Tn2010 integration analysis of positive clones demonstrated that Tn2010 and 1966 bp were inserted between spr1764 and spr1772 in the R6 genome. Analysis of the reported genome of S. pneumoniae demonstrated that Tn916 and Tn6002 insert at different sites on the chromosome, whereas most Tn2009 and Tn2010 were located between spr1764 and spr1772 of the R6 genome according to our results (Table S2, available as Supplementary data at JAC Online). Furthermore, a 1.9 kb segment was present downstream of the transposon in sequenced strains. Taken together, these results suggest that Tn2009 and Tn2010 are more likely to be integrated into recipients through homologous recombination by transformation.

The genomes of three transformants (Sp-Z1, Sp-Z2 and Sp-Z3) were sequenced to understand the effect of natural transformation of Tn2010 on genome evolution. Mapping the reads of transformants to the recipient genome R6 revealed 631 SNPs in three transformants in addition to the Tn2010 region. No SNPs were found to be recurrent in the transformants. We reasoned that recurrent SNPs might increase the likelihood that they are associated with Tn2010, whereas unique SNPs may simply be an unselected recombination. The unselected recombinations were scattered throughout the genome. The proportion of the recipient genome found to have undergone recombination was 3.00% for Sp-Z1, 0.44% for Sp-Z2 and 1.67% for Sp-Z3. Conservative estimates suggested that a range of 4–13 recombination events occurred across Sp-Z1 to Sp-Z3 in addition to the region covering Tn2010 (Figure 2). Analysis of the distribution of SNPs according to their biological process or function of the clusters of orthologous groups showed that the genes with SNPs were classified into 15 groups (Figure S1, available as Supplementary data at JAC Online).23 SNPs were most abundant in genes belonging to the transcription group, and a considerable proportion (~10%) of the genes belonged to defence mechanisms. In addition, recombination during transformation may influence bacterial virulence and infection, including capsular switch (cps locus) and mutations in genes involving the regulation of various virulence factors (LytR) or evasion of the host immune response (PpiA) (Tables S3, S4 and S5, available as Supplementary data at JAC Online). Transformation provides more variation on which natural selection can act and contribute to the evolution of bacteria. The spread of Tn2010 by transformation under macrolide pressure has a profound effect on the evolution of the S. pneumoniae genome.

The biological cost of Tn2010 carriage was determined for transformants Sp-Z1, Sp-Z2, Sp-Z3 and Sp-Z4. Sp-Z4 was obtained by transforming Sp-Z2 chromosomal DNA to R6, and the SNPs discovered in strain Sp-Z2 were not detected in Sp-Z4, except for Tn2010 and its homologous recombination region. The fitness assays indicated no significant fitness cost for the four transformants, and all assays yielded relative fitness values of 0.99–1.04. These results suggest that the horizontal acquisition of transposon Tn2010 (including the co-transferred and often extensive additional DNA regions) did not lead to significant biological costs for transformant cells. These findings are consistent with the observation that Tn2010 in S. pneumoniae is distributed widely in China.20

Conclusions

Our observations indicate that Tn2010 is more likely to be transferred by transformation than by conjugation in S. pneumoniae. Furthermore, the spread of Tn2010 by transformation has resulted in multiple integrations throughout the chromosome under macrolide pressure, and had a profound effect on the S. pneumoniae genome. Finally, the negligible fitness cost of acquiring Tn2010 may facilitate the spread of Tn2010.

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Transparency declarations

None to declare.
Supplementary data

Figure S1 and Tables S1 to S5 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References