Tn5253 Family Integrative and Conjugative Elements Carrying \textit{mef}(I) and \textit{catQ} Determinants in \textit{Streptococcus pneumoniae} and \textit{Streptococcus pyogenes}

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The linkage between the macrolide efflux gene \textit{mef}(I) and the chloramphenicol inactivation gene \textit{catQ} was first described in \textit{Streptococcus pneumoniae} (strain Spn529), where the two genes are located in a module designated IQ element. Subsequently, two different defective IQ elements were detected in \textit{Streptococcus pyogenes} (strains Spy029 and Spy005). The genetic elements carrying the three IQ elements were characterized, and all were found to be Tn5253 family integrative and conjugative elements (ICEs). The ICE from \textit{S. pneumoniae} (ICE\textit{Spn529IQ}) was sequenced, whereas the ICEs from \textit{S. pyogenes} (ICE\textit{Spy029IQ} and ICE\textit{Spy005IQ}, the first Tn5253-like ICEs reported in this species) were characterized by PCR mapping, partial sequencing, and restriction analysis. ICE\textit{Spn529IQ} and ICE\textit{Spy029IQ} were found to share the \textit{int}_{5253} integrase gene and an identical Tn916 fragment, whereas ICE\textit{Spy005IQ} has \textit{int}_{5252} and lacks Tn916. All three ICEs were found to lack the linearized pC194 plasmid that is usually associated with Tn5253-like ICEs, and all displayed a single copy of a toxin-antitoxin operon that is typically contained in the direct repeats flanking the excisable pC194 region when this region is present. Two different insertion sites of the IQ elements were detected, one in ICE\textit{Spn529IQ} and ICE\textit{Spy029IQ}, and another in ICE\textit{Spy005IQ}. The chromosomal integration of the three ICEs was site specific, depending on the integrase (\textit{int}_{5253} or \textit{int}_{5252}). Only ICE\textit{Spy005IQ} was excised in circular form and transferred by conjugation. By transformation, \textit{mef}(I) and \textit{catQ} were cotransferred at a high frequency from \textit{S. pyogenes} Spy005 and at very low frequencies from \textit{S. pneumoniae} Spn529 and \textit{S. pyogenes} Spy029.
ferences in the abilities of the three strains to transfer the mef(1) and catQ genes.

MATERIALS AND METHODS

Bacterial strains. Three Streptococcus isolates (one of S. pneumoniae and two of S. pyogenes) carrying mef(1) and catQ were studied. The S. pneumoniae isolate (strain Spn529) was the one in which the mef(1)-catQ linkage and the IQ element were originally described (1). The two S. pyogenes isolates were those for which two different types of defective IQ elements were later described in that species: Spy005 (formerly called MB56Spy005) and Spy005 (formerly called MB56Spy005) (6). Spn529, Spy029, and Spy005 exhibited the M phenotype of macrolide resistance, with erythromycin MICs of 8, 16, and 16 μg/ml, respectively. The chloramphenicol MICs for the same strains were 16, 64, and 64 μg/ml, respectively.

PCR amplification experiments. DNA preparation and amplification and the electrophoresis of PCR products were carried out by established procedures and according to the recommended conditions for the use of individual primer pairs. The Ex Taq system (Takara Bio, Shiga, Japan) was used in the amplification experiments that were expected to yield PCR products of >3 kb in size. All primers used are listed in Table 1.

Detection and characterization of genetic elements. The demonstration of Tn5253-like ICEs was obtained by the detection of the integrase gene (int252 or int23FST81) and other functional genes involved in ICE maintenance and conjugative transfer, using previously described PCR-based strategies (16). The mef(1)- and catQ-carrying ICE of Spn529 was completely sequenced. To characterize the two S. pyogenes ICEs, the primary approach was usually based on PCRs and mapping. DNA sequencing, or, in some instances, restriction analysis, were resorted to when special issues had to be elucidated or when amplification reactions yielded unexpected amplicon sizes.

DNA sequencing and sequence analysis. PCR products were purified using Montage PCR filter units (Millipore Corporation, Bedford, MA). Sequencing was carried out, bidirectionally or by primer walking, using ABI Prism (PerkinElmer Applied Biosystems, Foster City, CA) with dye-labeled terminators. The sequences were analyzed using the Sequence Navigator software package (PerkinElmer). Open reading frames (ORFs) were predicted using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and NEBuilder version 2.0 software programs. The criteria to design a potential new ORF were the existence of a start codon and a minimum coding size of 50 amino acids. Sequence similarity and conserved domain searches were carried out using tools (BLAST and CDART) available online at the National Center for Biotechnology Information of the National Library of Medicine (Bethesda, MD) (see http://www.ncbi.nlm.nih.gov).

Experiments using restriction enzymes. Some details about the structures of the genetic elements investigated were determined by PCR-restriction fragment length polymorphism (RFLP) analysis. Different endonucleases (HindIII, XbaI, EcoRI, and BamHI; Roche Applied Science, Basel, Switzerland) were used, depending on the region analyzed. Two primer pairs (INTF/13R and 28F/21R) were used in the amplification experiments that were expected to yield PCR products of >3 kb in size. All primers used are listed in Table 1.

Transformation experiments. The laboratory strain S. pneumoniae Rx1 was used as the recipient in the transformation assays. The experimental procedure, including the preparation of competent cells, was as described previously (23). The transforming DNA was a crude lysate or was extracted using the GenElute bacterial genomic DNA kit (Sigma-Aldrich). Transformants were selected by plating the transformation mixture onto selective plates containing erythromycin (1 μg/ml) or chloramphenicol (5 μg/ml). The transfer of mef(1) and catQ and their linkage were verified by PCR. The transformation frequency was expressed as the number of CFU of the transformants per recipient.

Nucleotide sequence accession numbers. The complete nucleotide sequence of ICESpn529IQ was submitted to the EMBL sequence database and assigned accession no. HG965092. The accession no. of the sequences referred to in this study are as follows: S. pneumoniae strain R6 genome, AE007317; S. pneumoniae strain TIGR4 genome, AE005672; S. pneumoniae strain Spn529, AJ971089; S. pneumoniae strain ATCC 700669 genome harboring ICESp23FST81, FM211187; Tn5253 from S. pneumoniae Spn529, DP1322, EU351020; S. pyogenes MGAS2096 genome, CP000026; and the Streptococcus parasanguinis FW213 genome harboring pathogenicity island FWisland_1, CP003122.

RESULTS

Evidence of Tn5253-like ICEs containing the IQ element in S. pneumoniae strain Spn529 and S. pyogenes strains Spy029 and Spy005. A number of indications suggested that the mef(1)- and catQ-carrying elements were likely to be Tn5253 family ICEs. Three strains being studied were thus subjected to PCR analysis for integrase and other scaffold genes of Tn5253 family ICEs and for their linkage with the respective IQ element. Positive PCRs for int23FST81 were obtained with S. pneumoniae Spn529 and S. pyogenes Spy029, whereas strain Spy005 yielded a positive reaction for int252. A linkage between the IQ element and a scaffold gene was demonstrated by positive PCRs using the primer pairs R18/ CATQ1 (strains Spn529 and Spy029) and MEF2/NEWORF6 (strain Spy005) (Table 1). These results established that in all three strains, the IQ element was contained in a Tn5253-like ICE. The three ICEs were designated ICESpn529IQ, ICESpyp029IQ, and ICESpy005IQ for strains Spn529, Spy029, and Spy005, respectively.

Characterization and organization of ICESpn529IQ. ICESpn529IQ is the complete organization, revised in the light of new pieces of knowledge and the new notion of ICE, of the mef(1)- and catQ-carrying structure harbored by S. pneumoniae Spn529. In this isolate, early studies led to the identification of the 5216IQ complex (1), which is in fact a part of ICESpn529IQ. ICESpn529IQ was completely sequenced; it was found to be 59,466 bp in size and have a G+C content of 36%, and its sequence analysis disclosed 66 ORFs. Its genetic organization was characterized using ICESp23FST81 (13) as the genetic reference (Fig. 1A). While the two elements had virtually identical scaffolds, the cargo genes of ICESp23FST81, such as a putD helicase gene involved in the SOS response, a lantibiotic synthesis gene cluster, and pC194 plasmid, were missing in ICESpn529IQ, whose genetic organization is summarized below.

The region orf1 to orf19 (bp 1 to 20061) includes genes usually
### TABLE 1 Oligonucleotide primer pairs used

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<tr>
<th>Procedure gene</th>
<th>Primer designation</th>
<th>Sequence (5' to 3')</th>
<th>Reference or source</th>
<th>Product size a</th>
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<td></td>
<td>NewORF6x</td>
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<tr>
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<td>6,3 kb</td>
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<td>ICESpyp005IQ junctions</td>
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<td>1,735</td>
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<tr>
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<tr>
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<td>2.5 kb</td>
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<tr>
<td>Circular form of the IQ elements from ICESpyp029IQ and ICESpyp005IQ</td>
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<td></td>
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</tr>
<tr>
<td>catQ</td>
<td>catQ1</td>
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<td>16</td>
<td>NO f</td>
</tr>
<tr>
<td>mef[I]</td>
<td>MEFA2</td>
<td>TTCTCTGGTACAAAGTGCG</td>
<td>42</td>
<td></td>
</tr>
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</table>

(Continued on following page)
found in the scaffolds of Tn5253-like pneumococcal elements. In particular, orf1 encodes the integrase, having 99% amino acid identity to the site-specific integrase of ICEsp23FST81. orf2 is identical to the second ORF of ICEsp23FST81. orf3 encodes a transcriptional regulator having 99% amino acid identity to orf3 of Tn5252, involved in the regulation of the conjugal transposition of the element (24). The orf4-orf6 cluster is formed by three conjugative machinery genes encoding relaxase (orf4) and mobilization proteins (orf5 and orf6), whose deduced amino acid sequences show the highest identities (96%, 93%, and 99%, respectively) to the corresponding ORFs of ICEsp23FST81. In S. pneumoniae ATCC 700669 and in ICEsp23FST81, the same cluster is found in the pathogenicity island PPI-1 (13). The product of orf7 shows 99% amino acid identity to a protein encoded by a gene of the pathogenicity island FWIsland_1 of S. parasanguinis (25). orf8 and orf9 code for a toxin-antitoxin (TA) system. The two genes are closely related to the bicistronic operon pezT-pezA, with 92% and 98% identities between the respective deduced amino acid sequences, described in the pathogenicity island PPI-1 of S. pneumoniae TIGR4 (26). ORFs from orf10 to orf19 encode proteins with various degrees of amino acid identity (70% to 98%) to the ICEsp23FST81 ORFs. The region orf20 to orf21 (bp 20062 to 22775) is formed by two adjacent genes whose deduced amino acid sequences show 99% and 98% identities, respectively, to the adjacent genes spr0602 and spr0601 of the S. pneumoniae R6 genome and SP0687 and SP0686 of the S. pneumoniae TIGR4 genome. The duplication of these two chromosomal genes in ICEspn529IQ is one reason why during an investigation of the 5216IQ complex environment, this area was thought to belong to the chromosome (1). The region orf22 to orf36 (bp 22776 to 37886) corresponds to the IQ element previously described in the same pneumococcal isolate. In the ORF order of ICEspn529IQ, the two tsp1 transposase genes, located at either end of the IQ element, are orf22 and orf36, catQ is orf23, and mef1 is orf28. The region orf37 to orf45 (bp 37887 to 43889) corresponds to the Tn916 fragment previously described in the same isolate. The silent tet(M) gene, which is unexpressed because it lacks the promoter, the ribosome-binding site, and part of the leader peptide (1), is orf37, and intg16 is orf45. The region orf46 to orf55 (bp 43890 to 53150) is the cluster originally regarded as the Tn5252 fragment of the 5216IQ complex. It corresponds to the conjugal transfer-related functional module (CTR) of Tn5252 involved in the production of a type IV secretion system, delivering protein and DNA substrates to target cells, generally by a contact-dependent mechanism (27).

The region orf56 to orf66 (bp 53151 to 59466) includes a number of genes with high identities (>90%) to the chromosomal genes of S. pneumoniae R6 and/or TIGR4. Their detection was one reason why this area was considered to fall within the chromosome during the investigation of the 5216IQ complex environment (1). In fact, in a later study, the same chromosomal genes were reported to be duplicated in an ICE remnant in the TIGR4 genome (13). orf64 and orf65 (the third and second to last ORFs, respectively) are two adjacent and conserved genes that are consistently found in pneumococcal ICEs: they code for a cytosine methyltransferase and a replication initiator protein, respectively, which show 97% and 100% identities, respectively, to the deduced amino acid sequences of the corresponding ORFs (again, the third and second to last) of ICEsp23FST81. In parallel, we determined the chromosomal integration site of ICEspn529IQ. By combining PCR and sequencing assays, the integration site was found to be the same as that of ICEsp23FST81, i.e., near the 3’ end of gene rplL (13), which is consistent with the site specificity of the intsp23FST81-encoded integrase (16). Structure of ICEsp029IQ. ICEsp029IQ displayed a structure closely comparable to that of ICEspn529IQ (Fig. 1A). This was first shown by PCR mapping; further confirmation was provided by partial sequencing of the amplicons containing major functional genes, such as those coding for the integrase, the TA operon, and the replication initiator protein. Moreover, the region of ICEsp029IQ where the defective IQ element is inserted was analyzed by partial sequencing of the amplicon obtained by pairwise primers R18 and catQ1 (Table 1).

The product of intsp23FST81 from ICEsp029IQ showed 99% and 98% identities to the deduced amino acid sequences of the corresponding integrase genes of ICEspn529IQ and ICEsp 23FST81, respectively. The products of the TA-coding genes displayed 100% identities to the deduced amino acid sequences of ICEspn529IQ orf8 and orf9. The defective IQ element was inserted

<table>
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<th>Procedure gene</th>
<th>Primer designation</th>
<th>Sequence (5’ to 3’)</th>
<th>Reference or source</th>
<th>Product sizea</th>
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</tr>
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<td>AAGCGAACATTGTCAAGAGC</td>
<td>This study</td>
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<td>ICESpy005IQ circular form</td>
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<td>ACTACTGGGTAGTAAGCCG</td>
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<td>ICE-RepA</td>
<td>AAGCGAACATTGTCAAGAGC</td>
<td>This study</td>
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</tbody>
</table>

TABLE 1 (Continued)

a Unless otherwise specified, values are reported as bp.
b Originally proposed for mef(A) and mef(E) (42) and likewise targeting mef(1) (2).
c Except for integrase and resistance genes, and unless otherwise specified, the designations are according to the ORF numbering of ICEspn529IQ.
d Except for the integrase gene, and unless otherwise specified, the designations are according to the ORF numbering of Tn5253.
e Amplicon also used for RFLP analysis.
f The first value was expected from the reported sequence of Tn5253, and the second value was consistent with the structure of ICEsp005IQ.
g According to the ORF numbering of Tn5253.
h From the S. pyogenes MGAS2096 genome.
i From the S. pneumoniae R6 genome.
j NO, not obtained.
in the same position as the pneumococcal IQ element, i.e., next to a couple of genes corresponding to \textit{orf20} and \textit{orf21} of ICE\textsubscript{Spn}529IQ. Aside from the shorter IQ element, the only noticeable difference from ICE\textsubscript{Spn}529IQ was the presence of a \textit{tnp1} transposase gene adjacent to \textit{intSp}23FST81, so that ICE\textsubscript{Spy}029IQ carried a third \textit{tnp1} in addition to the two found at the ends of the defective IQ element.

The chromosomal integration site of ICE\textsubscript{Spy}029IQ was near the 3' end of gene \textit{rplL}, in line with the site specificity of the \textit{intSp}23FST81-encoded integrase.
Structure of ICESpy005IQ. Given the presence of int5252,
ICESpy005IQ was characterized using Tn5253 as the genetic ref-
ence (Fig. 1B). Compared to Tn5253, ICESpy005IQ carried a
defective IQ element [reduced to the mef(I)-catQ fragment] but
lacked both cargo regions represented by Tn916/Tn5251 and the
linearized pC194 plasmid. This was first demonstrated by PCR
mapping; further confirmation was provided by partial sequenc-
ing of all relevant PCR products. The amplicons obtained by pair-
ing primers INTF and 13R and 28F and 21R (Table 1) were also
examined by RFLP analysis.

The products of int5252 and xis5252, from ICESpy005IQ showed
99% and 100% identities, respectively, to the deduced amino acid
sequences of the corresponding genes from Tn5253. The products
of TA-coding genes displayed 92% and 96% identities to the
deduced amino acid sequences of ICESpy005IQ orf8 and orf9, re-
spectively.

By PCR mapping, the amplicon obtained using primer pair
CTRfor/NewORF66x, targeting orf12 and orf2, respectively, of
Tn5253 (Table 1), was much larger (>10 kb) than expected (5,562
bp). This was due to the insertion of the defective IQ element into
the distal region of the Tn5252-like structure of ICESpy005IQ, as
confirmed by positive PCRs using the primer pairs CTRfor/
CATQ1 and MEF2/NewORF66x (Table 1).

In S. pneumoniae, the site-specific integration of Tn5253 is in the
rbgA gene (spr1043, according to ORF numbering in the S.
pneumoniae R6 genome) (11, 16, 22, 28). Therefore, we investi-
gated whether the same site specificity also applied to
ICESpy005IQ. A homolog of the pneumococcal rbgA gene was
identified in all S. pyogenes genomes available in GenBank: its
highest DNA identity was to gene Spy0956 of S. pyogenes
MGAS2096 (29), whose product, a GTP-binding protein, is the
same as that of gene spr1043 of S. pneumoniae R6. The MGAS2096
genome was thus used as the reference sequence to determine the
chromosomal junctions of ICESpy005IQ. The primer pairs
Spy0956L/INTR and ICERepA/Spy0955R (Table 1) were used to
detect the left and right junctions, respectively. The sequencing of
the resulting amplicons (1,679 bp and 1,735 bp, respectively) dis-
closed that ICESpy005IQ was integrated in a chromosomal gene
corresponding to the Spy0956 gene of the MGAS2096 genome (at
base 917261).

Search for IQ element and ICE circular forms. PCR experi-
ments using outward-directed primer pairs (CATQ1/NF2 for S.
pneumoniae Spn529 and CATQ1/MEFA2 for S. pyogenes Spy029
and S. pyogenes Spy005) (Table 1) were performed to seek IQ
element circular forms in the relevant strains. A circular form was
detected only for the IQ element of S. pneumoniae Spn529.

The circular forms of ICESpy529IQ, ICESpy029IQ, and
ICESpy005IQ were sought by a similar approach using outward-
directed primer pairs: ICE-5'/ICERepA for the first two ICEs and
INTR/ICERepA for the third (Table 1). A circular form was de-
tected for ICESpy005IQ, whereas no circular forms were detected
for the other two ICEs.

Transferability of the mef(I) and catQ determinants. In con-
jugative transfer experiments, mef(I) and catQ proved to be non-
transferable to any recipient from S. pneumoniae Spn529 and S.
pneumoniae Spy029, in line with the early negative results obtained
in intraspecies mating assays (1, 6). In contrast, ICESpy005IQ was
consistently transferred to all recipients, usually at high frequen-
cies, from S. pyogenes Spy005 (Table 2).

In the transformation experiments, the transformants were
obtained from both S. pyogenes donors, although at a much higher
frequency from Spy005 (usually around 10^{-6}) than from Spy029
(around 10^{-4}). Using S. pneumoniae Spn529 as the donor, the
transformants were obtained at a low frequency (around 10^{-3}),
and only using a crude lysate as the transforming DNA. All trans-
formants were resistant to erythromycin (M phenotype) and
chloramphenicol, and PCR experiments confirmed that they bore
mef(I) and catQ, linked at the expected distance.

DISCUSSION

The special feature shared by the three Tn5253 family ICEs of this
study, and the reason why they were examined together, is carriage
of the resistance genes mef(I) and catQ, which in the original re-
port were described as being linked in the so-called IQ element
(15.1 kb) in S. pneumoniae Spn529 (1). In the early descriptions
of S. pyogenes Spy029 and Spy005 (6), the two resistance genes
were linked in two different IQ elements, both of which were
defective compared to the original pneumococcal IQ element. In
this study, all three IQ elements were found to be carried by
Tn5253 family ICE. This finding strengthens and expands our
understanding of such ICEs. To our knowledge, this is the first
time that Tn5253 family ICEs, which are very common in S. pneu-
moniae, were described in natural isolates of S. pyogenes. The con-
jugal transfer of Tn5253 (or related variants) from S. pneumoniae
and S. pyogenes and other Streptococcus species recipients has been
obtained in the laboratory, as documented in previous reports
(11, 22, 28). Remarkably, the two integrate genes known to be
associated with the Tn5252-like moiety of such ICEs in S. pneu-
moniae, i.e., int 5252 and int_{5252}^{SFST181}, were both detected in the S.
pneumoniae ICEs, with int 5252 in ICESpy005IQ and int_{5252}^{SFST181}
ICESpy029IQ.

A conserved mef-catQ fragment, most often with the mef gene

---

### TABLE 2 Conjugal transfer of ICESpy005IQ from the S. pyogenes donor Spy005 to erythromycin- and chloramphenicol-susceptible recipients

<table>
<thead>
<tr>
<th>Streptococcus species</th>
<th>MIC (µg/ml)</th>
<th>Transfer frequency</th>
<th>Transconjugants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Resistance genotype</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td></td>
<td></td>
<td>ERY</td>
</tr>
<tr>
<td>12RF</td>
<td>≤0.125</td>
<td>2.8 × 10^{-6}</td>
<td>mef(I) catQ</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>0.25</td>
<td>7.6 × 10^{-3}</td>
<td>mef(I) catQ</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>R6F</td>
<td>1.7 × 10^{-4}</td>
<td>mef(I) catQ</td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>5381RF</td>
<td>4.0 × 10^{-6}</td>
<td>mef(I) catQ</td>
</tr>
<tr>
<td>S. gordoni</td>
<td>1435RF</td>
<td>7.2 × 10^{-3}</td>
<td>mef(I) catQ</td>
</tr>
<tr>
<td>S. oralis</td>
<td>1235RF</td>
<td>2.8 × 10^{-3}</td>
<td>mef(I) catQ</td>
</tr>
</tbody>
</table>

ERY, erythromycin; CHL, chloramphenicol.

* The reported transfer frequencies are those obtained by selecting with chloramphenicol; comparable values were obtained with erythromycin.

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**Note:** This text is a transcription of a scientific document discussing the transfer of elements from one bacterium to another, focusing on the structure and transferability of a specific integron (ICESpy005IQ). It involves genetic mapping, PCR experiments, and conjugative transfer studies, with a particular emphasis on resistance determinants (mef and catQ) and their transfer to various Streptococcus species.
subclass mef(E) instead of mef(I), was recently detected in a number of variously defective IQ elements from different species (Streptococcus mitis, S. oralis, Streptococcus sanguinis, and S. parasanguinis) of viridans group streptococci (30). In studies currently in progress in our laboratory, the same mef(I)-catQ fragment has also been detected in other Streptococcus species (M. Mingoia, unpublished data). This suggests that the DNA fragment spanning from the mef gene [mef(I) or possibly mef(E)] to the catQ gene (six ORFs, ~5.8 kb) is a very conserved region, a sort of mef-catQ cassette that may be found alone or inside a variable structure represented by a more or less defective IQ element in different Streptococcus species, but that so far has not been reported outside this genus. Although apparently confined to streptococci, mef(I) (2, 31, 32) and catQ (4, 5) do not appear to be widespread in these organisms.

It is intriguing that all three Tn5253 family ICEs investigated, in which chloramphenicol resistance is provided by catQ, lack the linearized pC194 plasmid carrying the chloramphenicol resistance determinant cat_{pc194}, which is commonly regarded as typical cargo of the Tn5252-like moiety of Tn5253 family ICEs. As shown by early studies (10, 33), the cat_{pc194}-containing DNA region is flanked by direct repeats, the recombination of which may lead to spontaneous curing of the region. Very recently, the issue was thoroughly investigated by Lannelli et al. (11), who described an Õcat(pC194) (7,627 bp), flanked by two 1,169-bp direct repeats. This region has also been reported as an example of the so-called unconventional circularizable structures, i.e., particular genetic structures which, though lacking their own recombinase genes, can be excised in circular form thanks to extensive flanking direct repeats (34).

The direct repeats flanking the cat_{pc194}-containing region in Tn5253 family ICEs generally contain the genes coding for a TA system (11, 34). This TA operon thus appears to be typically associated with the Tn5252-like moiety of Tn5253 family ICEs. The single copy of the TA operon found in ICESpn529IQ (orf8-orf9), ICESpy029IQ, and ICESpy005IQ is likely to represent the recombination site of Õcat(pC194) and might be the outcome of a repair after pC194 excision (11). Several TA systems are found in streptococcal genetic elements. In S. pneumoniae, the TA operon of ICESpn529IQ has been described and characterized in the pathogenicity island PPI-1 of strain TIGR4 (26), but several additional TA systems, most not yet characterized in terms of structure and function, have recently been recognized in this species (35). In S. pyogenes, the best investigated TA system is the one harbored by the pSM19035 plasmid (36), of which a TA-containing fragment is also found in an ICE (ICESp1116) that is responsible for emm(B)-mediated inducible resistance to erythromycin in this species (37). Another S. pyogenes TA system has been detected in a prophage (Pm46.1) carrying the mef gene subclass mef(A) and tet(O) (38).

Transfer experiments demonstrated significant differences among the three strains and relevant ICEs. While ICESpy005IQ was transferred at high frequencies in both intra- and interspecies matings, no conjugal transfers were obtained with the closely related ICESpn529IQ and ICESpy029IQ. These differences were consistent with the finding that only ICESpy005IQ was excised in circular form, a critical condition for an ICE to undergo conjugal transfer (9). An excision defect might be the reason why ICESpn529IQ and ICESpy029IQ are incapable of conjugal transfer. A parallel dissimilarity between S. pyogenes Spy005, on the one hand, and S. pyogenes Spy029 and S. pneumoniae Spn529, on the other, was also observed in transformation experiments, since transformants carrying the mef(I)-catQ cassette were obtained at a high frequency from S. pyogenes Spy005 but at very low frequencies from the two other strains. The fact that transformants were obtained from S. pneumoniae Spn529 using only a crude lysate as the transforming DNA suggests possible alterations of Spn529 genomic DNA during the extraction process and is likely to account for early negative results (1). In another study, mef(I) and catQ were also cotransferred from a pneumococcus by transformation but not by conjugation (32). Transformation might be important in IQ element spread: in S. pneumoniae, Tn1207.1 (39) and the mega element (40), both transferable by transformation rather than by conjugation, play comparable roles in the spread of other mef determinants, i.e., mef(A) and mef(E), respectively. Whereas Tn1207.1 is integrated at a specific site of the pneumococcal chromosome (into the celB gene), and mef(A) dissemination is mainly clonal, the mega element has a variety of insertion sites into the pneumococcal chromosome, and its dissemination is more erratic (41). There are no data about the chromosomal insertion of IQ-like elements, which are however characterized by two different insertion sites in the three ICEs investigated here. Nonclonal dissemination of IQ-like elements is suggested by the fact that the pneumococcal isolates so far shown to be mef(I) positive all belonged to different serotypes (2, 31, 32); on the other hand, S. pyogenes Spy029 and Spy005 belong to different emm types (6). In these respects, the spread of mef(I) appears to be more reminiscent of mef(E) than of mef(A).

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