A Multi-resistance Plasmid Isolated from Commensal Neisseria Species is Closely Related to the Enterobacterial Plasmid RSF1010

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pFM739, an R plasmid from Neisseria sicca that encodes penicillin, streptomycin and sulphonamide resistance, and the enterobacterial IncQ(P-4) plasmid RSF1010, which encodes streptomycin and sulphonamide resistance, were incompatible, and were mobilized by the same conjugative plasmids. Restriction mapping confirmed a high degree of similarity between both R plasmids; pFM739 carried DNA fragments corresponding to the known replication and resistance regions of RSF1010. pFM739 also carried an extra segment with the same restriction map as that described for the β-lactamase-coding region of transposon Tn3. It is suggested that the R plasmids isolated from commensal Neisseria sp. could have resulted from transposition of a Tn3-like genetic element to an RSF1010-like plasmid, and that they contain deletion derivatives of transposon Tn3.

INTRODUCTION

The recent isolation, in our laboratory, of commensal Neisseria spp. strains carrying multi-resistance plasmids (Rotger et al., 1981; Pintado et al., 1985) raised questions about the origin, transfer mechanisms and possible spread of these plasmids to pathogenic bacteria colonizing the same mucosal surfaces. The problem of the introduction of R plasmids into the genus Neisseria is of increasing concern in view of the possibility of commensal strains becoming a reservoir of R plasmids (Genco et al., 1984) and the fact that R plasmids seem to have reached strains of Neisseria meningitidis (Dillon et al., 1983). The plasmids we have isolated from strains of Neisseria sicca, Neisseria subflava and Neisseria mucosa had the same molecular size (9.45 kb) and restriction patterns, and coded for a TEM-type β-lactamase, a streptomycin phosphotransferase and a sulphonamide-resistant dihydropteroate synthetase (Pintado et al., 1985). They were also stably maintained in Escherichia coli and Pseudomonas putida (Rotger et al., 1981). These properties suggested the possibility of such R plasmids from Neisseria spp. being related to R plasmids of the IncQ(P-4) group. Such plasmids are of similar size and host range and encode the same mechanisms of streptomycin and sulphonamide resistance (Heffron et al., 1977; Barth et al., 1981). In this communication we show that a close relationship exists between pFM739, from N. sicca, and RSF1010, a representative IncQ(P-4) plasmid encoding streptomycin and sulphonamide resistance.

METHODS

Bacterial strains, plasmids and culture media. Strains and plasmids used in this investigation are listed in Table 1. E. coli was grown in Luria Bertani (LB) broth containing 0.5% (w/v) yeast extract, 1% (w/v) tryptone (Difco) and 1% (w/v) NaCl, or in the same medium solidified with 2% (w/v) agar (Difco). This medium was also used for selection in all genetic transfer experiments, except when sulphamethoxazole was the selective agent. In this case Mueller-Hinton agar (BBL) supplemented with 5% (v/v) lysed horse blood was used. Incubations were always done at 37°C.

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Table 1. Bacterial strains and plasmids used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant characteristics*</th>
<th>Origin†</th>
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<tbody>
<tr>
<td><strong>E. coli</strong> K12 HB101</td>
<td>ara-14 galK2 hsdS20 lacY1 leu mil-1 proA2 recA13 rpsL20 supE44 thi xyl-5</td>
<td>V. Rubio</td>
</tr>
<tr>
<td><strong>E. coli</strong> K12 NEM259</td>
<td>hsdR&lt;sub&gt;4&lt;/sub&gt; hsdM&lt;sub&gt;E&lt;/sub&gt; met supE supF</td>
<td>M. Vicente</td>
</tr>
<tr>
<td><strong>E. coli</strong> K12 185</td>
<td>Nal&lt;sup&gt;R&lt;/sup&gt;</td>
<td>J. M. Ortiz</td>
</tr>
<tr>
<td>Plasmid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pFM739</td>
<td>Ap&lt;sup&gt;R&lt;/sup&gt; Sm&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This laboratory; Pintado et al. (1985)</td>
</tr>
<tr>
<td>pFM202</td>
<td>Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This laboratory; Rotger &amp; Nombela (1983)</td>
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<tr>
<td>RSF1010</td>
<td>Sm&lt;sup&gt;R&lt;/sup&gt; Su&lt;sup&gt;R&lt;/sup&gt; IncQ(P-4)</td>
<td>J. Jofre</td>
</tr>
<tr>
<td>R124</td>
<td>Tra&lt;sup&gt;+&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt; IncFIV</td>
<td>J. Jofre</td>
</tr>
<tr>
<td>pIP113</td>
<td>Tra&lt;sup&gt;+&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt; IncN</td>
<td>J. Jofre</td>
</tr>
<tr>
<td>RP4</td>
<td>Tra&lt;sup&gt;+&lt;/sup&gt; Ap&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt; Nm&lt;sup&gt;R&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt; IncP(P-1)</td>
<td>J. Jofre</td>
</tr>
<tr>
<td>Rts1</td>
<td>Tra&lt;sup&gt;+&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt; IncT</td>
<td>J. Jofre</td>
</tr>
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* Ap, ampicillin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Km, kanamycin; Nm, neomycin.
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**RESULTS**

**Incompatibility of plasmids pFM739 and RSF1010**

Plasmid pFM739, from *N. sicca* 739, has been described as an extrachromosomal element encoding a triple resistance phenotype. In order to examine compatibility relationships between pFM739 and the enterobacterial plasmid RSF1010, the former was transformed into *E. coli* K12 HB101(RSF1010) by selecting for carbenicillin resistance. Several of the corresponding transformant clones were analysed by agarose gel electrophoresis in order to examine the resulting plasmid complement. The experiment was repeated three times with the same results.
IncQ plasmids from Neisseria

Table 2. Mobilization of plasmids pFM739 and RSF1010 by pIP113 and RP4

After mating, cells were plated on media containing: nalidixic acid, to determine the number of recipients; nalidixic acid with tetracycline (conjugative plasmid marker), to determine the number of transconjugants; or streptomycin (mobilized plasmid marker), to measure mobilization of the non-conjugative plasmid.

<table>
<thead>
<tr>
<th>Conjugative plasmid</th>
<th>Conjugation per recipient</th>
<th>Mobilized plasmid</th>
<th>Mobilization frequency</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Per transconjugant</td>
</tr>
<tr>
<td>pIP113</td>
<td>0.4</td>
<td>RSF1010</td>
<td>1.0 × 10⁻⁶</td>
</tr>
<tr>
<td>pIP113</td>
<td>0.3</td>
<td>pFM739</td>
<td>4.1 × 10⁻⁶</td>
</tr>
<tr>
<td>RP4</td>
<td>0.4</td>
<td>RSF1010</td>
<td>1.0</td>
</tr>
<tr>
<td>RP4</td>
<td>0.8</td>
<td>pFM739</td>
<td>0.9</td>
</tr>
</tbody>
</table>

and RSF1010 stably coexisted in the same cells. These results clearly showed that pFM739 displaced RSF1010 upon appropriate selection. We concluded that pFM739, a plasmid isolated from a commensal strain of N. sicca, belongs in the IncQ(P-4) incompatibility group, which has been classically regarded as a group of Pseudomonas and enterobacterial plasmids (Guerry et al., 1974).

Genetic mobilization

N. sicca 739 can transfer pFM739 to E. coli in an apparently conjugal process, although no plasmid DNA large enough to encode sex factor activity has been detected in the donor strain (Pintado et al., 1985). In order to compare plasmid pFM739 and RSF1010 further with regard to their mobilization by conjugative plasmids between E. coli strains, we transferred plasmids R124 (IncFIV), pIP113 (IncN), RP4 (IncP) and Rts1 (IncT) to E. coli K12 HB101 carrying either pFM739 or RSF1010. Plasmids were transferred by conjugation, and transconjugants were selected and shown to maintain both plasmids stably. Mobilization of pFM739 and RSF1010 was studied by mating with E. coli K12 185 (nalidixic acid resistant) and by selecting for either the mobilized or the conjugative plasmid independently. It is known that RSF1010 can be efficiently mobilized by IncP plasmid but very poorly or not at all by others (Barth et al., 1981). Our results confirmed this, since RSF1010 was mobilized at a high frequency by RP4 (IncP) and much less efficiently by pIP113 (IncN). Other conjugative plasmids gave no detectable mobilization (Table 2). The behaviour of pFM739 was again identical to that of RSF1010 in this respect (Table 2), suggesting a high degree of similarity.

Restriction endonuclease analysis

The comparison between the N. sicca plasmid pFM739 and the enterobacterial plasmid RSF1010 was extended by analysis with several restriction endonucleases. This analysis enabled us to estimate a size of 9-45 kb for pFM739, by addition of the sizes of its fragments. This value is more accurate than the approximate value of 6-0 MDa that was estimated by agarose gel electrophoresis of the plasmid (Pintado et al., 1985). Both plasmids had unique restriction sites for EcoRI (data not shown) and pFM739 had an additional unique site for BamHI.

Several bands were common to both plasmids after digestion with restriction endonucleases and electrophoresis of the fragments: four after digestion with HinfI, three with AvaI, two with HincII and one with PstI (Fig. 1). Partial and double digestions were also done and all these data enabled us to prepare a restriction map of pFM739 (Fig. 2); this was compared with the published restriction map of RSF1010 (Bagdasarian et al., 1981). The restriction patterns of sulphonamide and streptomycin resistance determinants as well as the replication region of RSF1010 appeared to be shared by pFM739. Notable differences were observed between the 2.44 kb AvaI fragment of pFM739 and the corresponding fragment of RSF1010; the former was
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Fig. 1. Agarose (lanes a to h) or polyacrylamide (lanes i to l) gel electrophoresis of fragments produced by treatment of plasmids pFM739 and RSF1010 with various restriction endonucleases. (a) pFM739 treated with EcoRI plus BamHI; (b) pFM739 treated with AvaI; (c) RSF1010 treated with AvaI; (d) λ phage DNA treated HindIII (sizes of fragments in kb are 23-7, 9-46, 6-61, 4-26, 2-26, 1-98); (e) pFM739 treated with PstI plus BamHI; (f) pFM739 treated with PstI; (g) RSF1010 treated with PstI; (h) λ phage DNA treated with EcoRI plus HindIII [sizes of fragments in kb are 21-8, 5-24 and 5-05 (appearing in one band in this gel), 4-21, 3-41, 1-98, 1-9, 1-57, 1-32, 0-83, 0-84]; (i) pFM739 treated with Hinfl; (j) RSF1010 treated with Hinfl; (k) pFM739 treated with HincII; (l) RSF1010 treated with HincII.

Fig. 2. Restriction maps of plasmids pFM739 and RSF1010. The location of resistance genes and the replication origin in RSF1010 has been described by Badgasarian et al. (1981), Barth et al. (1981) and Sakaguchi (1982). The dotted line below pFM739 indicates the region which does not appear in RSF1010. The 0-2 kb AvaI fragment of RSF1010 and pFM739 was not detected in our electrophoretic system, but could be deduced from the difference with other endonuclease restriction treatments and from the previously published analysis of RSF1010. A, AvaI; B, BamHI; E, EcoRI; H, HincII; P, PstI.

0·85 kb longer, and carried the single BamHI site of pFM749 as well as PstI and HincII restriction sites, all of which are absent in RSF1010. The restriction map of this extra region is very similar to that of the β-lactamase-encoding region of Tn3 (Heffron et al., 1981). This observation is in agreement with the ability of pFM739 to code for a TEM-type β-lactamase (Rotger et al., 1981; Cenamor et al., 1986).

DISCUSSION

The discovery of multi-resistance plasmids in commensal Neisseria spp. (Pintado et al., 1985) represents a clear demonstration that these bacteria can harbour and maintain resistance plasmids that have arisen in nature. The results presented above clearly show that a high degree
of similarity exists between pFM739, a plasmid isolated from \textit{N. sicca}, and RSF1010, a typical IncQ\(\text{(P-4)}\) R plasmid that was originally isolated from enteric bacteria (Guerry \textit{et al.}, 1974), and that codes for streptomycin and sulphonamide resistance. Both plasmids were incompatible and were mobilized by the same conjugative plasmids. Their restriction maps showed that pFM739 could have resulted from the transposition of a fragment of a Tn3-like genetic element to a plasmid of the RSF1010 type. Consistent with this interpretation, pFM739 DNA, labelled with \(\text{\textit{\text{-32P}}}\text{GTP}\) by nick-translation, hybridized with the DNA fragments obtained by \textit{AvaI} digestion of RSF1010 (data not shown). In fact, the transposition of Tn3-like genetic elements to RSF1010-related plasmids, which acquire the capacity to code for a TEM-type \(\beta\)-lactamase in this way, has been shown to occur in nature as well as in the laboratory (Heffron \textit{et al.}, 1977; Yamada \textit{et al.}, 1979). Plasmids that arose in this way, were very similar to pFM739, but carried the whole transposon. On the other hand, gonococcal \(\beta\)-lactamase plasmids have been shown to carry a Tn3 fragment, with a restriction map very similar to that of the Tn3 fragment that seems to be part of pFM739, and these are considered to be deletion derivatives of plasmid bearing the entire Tn3 transposon. The possession of this incomplete and therefore non-transposable Tn3-like segment seems to be the only similarity between pFM739 and the gonococcal penicillinase plasmids, which so far do not encode any other resistances and which have restriction patterns and incompatibility groups very different from those of the plasmid described here (Fayet \textit{et al.}, 1982).

The spread of IncQ plasmids to other, possibly pathogenic bacterial species was first reported by Albritton \textit{et al.} (1982), who identified sulphonamide-resistance plasmids in \textit{Haemophilus ducreyi}. The presence of pFM739-like plasmids in several strains of commensal \textit{Neisseria} spp. points to the possibility of further spread of such nonconjugative multiple resistance plasmids to other pathogenic or opportunistic bacteria.

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\textbf{REFERENCES}


