Characterization of two different types of resistance genes among producers of fortimicin-group antibiotics

TOSHI OHTA, TOHRU DAIRI and MAMORU HASEGAWA*

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd, 3-6-6 Asahi-machi, Machida-shi, Tokyo 194, Japan

(Received 5 May 1992; revised 8 September 1992; accepted 17 November 1992)

Fortimicin-A (FTM-A; astromicin)-resistance genes (fmr genes) isolated from six producers of the FTM-group of antibiotics were analysed. These genes could be classified into two types by the resistance profiles to aminoglycoside antibiotics and by their DNA homologies. Three genes, fmrT from the istamycin producer Streptomyces tenjimariensis ATCC 31603, fmrS from the sannamycin producer Streptomyces sannanensis IFO 14239 and fmrH from the sporaricin producer Saccharopolyspora hirsuta ATCC 20501, conferred resistance to FTM-A, kanamycin (Km) and neomycin B (Nm-B), but not to gentamicin (Gm). The other three genes, fmrO from the FTM-A producer Micromonaspora olivasterospora ATCC 21819, fmrM from the antibiotic SF-2052 producer Micromonaspora sp. SF-2098 (ATCC 31580) and fmrD from the dactimicin producer Dactyllosporangium matsuzakiense ATCC 31570, conferred resistance to FTM-A, Km and Gm, but not to Nm-B. No DNA homology was detected between the two types of the resistance genes in Southern-blot analysis. The present results revealed that, in spite of the similarity of their biosynthesis genes, there are at least two different types of resistance genes among the FTM-group antibiotic producers.

Introduction

It is interesting, from the point of view of the evolution and distribution of antibiotic biosynthetic genes, that antibiotics with similar structures are often produced by taxonomically distant micro-organisms (Malpartida et al., 1987; Leskiw et al., 1988; Schiffman et al., 1988; Weigel et al., 1988; Burnham et al., 1989). Fortimicin (FTM)-group antibiotics with unique pseudodisaccharide structures are produced by six strains of four genera, namely Micromonaspora olivasterospora ATCC 21819 (FTM-A) (Nara et al., 1977), Micromonaspora sp. SF-2098 ATCC 31580 (SF-2052 compounds) (Ohba et al., 1981), Dactylosporangium matsuzakiense ATCC 31570 (dactimicins) (Inoue et al., 1979), Streptomyces tenjimariensis ATCC 31603 (istamycins) (Okami et al., 1979), Streptomyces sannanensis IFO 14239 (sannamycins) (Watanabe et al., 1979) and Saccharopolyspora hirsuta ATCC 20501 (sporaricins) (Deushi et al., 1979).

The biosynthetic pathway of FTM-A in M. olivasterospora is believed to consist of more than 14 steps (Odakura et al., 1984), and all members of the FTM-group antibiotics are thought to be synthesized via similar biosynthetic pathways (Dairi & Hasegawa, 1989; Hotta et al., 1989). Sequence conservation of the glycytransferase, one of the key enzymes for the biosynthesis of the FTM-group antibiotics, among the FTM-group antibiotic producers suggested that these biosynthesis genes were derived from a common set of ancestral genes (Ohta et al., 1992).

All the FTM-group antibiotic producers exhibit high-level resistance to FTM-A. We previously isolated the FTM-A resistance gene (fmrT) from the istamycin producer S. tenjimariensis, and showed that sequences homologous to fmrT were present in the sannamycin producer S. sannanensis and the sporaricin producer Sac. hirsuta (Ohta & Hasegawa, 1989). These findings seemed to indicate that the resistance genes were also conserved among these antibiotic producers. However, very interestingly, recent preliminary hybridization experiments for an expanded range of strains unexpectedly indicated that this was not the case, suggesting the existence of two or more types of resistance genes. In the present study, we cloned the FTM-A-resistance genes (fmr genes) from the other five FTM-group antibiotics producers in order

*Author for correspondence. Tel. 0427 25 2555 (ext. 261); fax 0427 26 8330.

Abbreviations: FTM-A, fortimicin A; Km, kanamycin; Gm, gentamicin; Nm-B, neomycin B; Sm, streptomycin; CIAP, calf intestine alkaline phosphatase.
to compare them in the same genetic background and screen for sequences similar to *fmr* genes in other actinomycetes.

**Methods**

**Bacterial strains and plasmids.** *S. sannanensis* IFO 14239, *S. hirsuta* ATCC 20501, *M. olierasterospora* ATCC 21819, *Micromonaspora* sp. ATCC 31580 and *D. matsuzakienense* ATCC 31570 were used as sources of total DNA for the shot gun cloning experiments of *fmr* genes. *S. lividans* TK23 was used as a cloning host. Plasmids pIJ702 (Katz et al., 1983), pEN101 (Nagano et al., 1988) and pEN101B in which a *BglII* site of pEN101 was converted to a *BamHI* site using a synthetic DNA linker (unpublished data) were used as cloning vectors.

**Culture conditions and media.** All actinomycetes were grown at 30 °C in SK no. 2 medium (Ohta et al., 1992), or maintained on ATCC no. 5 agar medium. Strains harbouring plasmids carrying *fmr* genes were grown in medium containing 50 µg FTM-A hydrochloride ml⁻¹ (Kyowa Hakko Kogyo).

**Preparation and manipulation of DNA.** Total DNA and plasmid DNA were isolated by the methods described by Chater et al. (1982) and Kieser (1984), respectively. Restriction endonucleases, calf intestine alkaline phosphatase (CIAP) and T4 DNA ligase were purchased from Boehringer Mannheim or Takara Shuzo. They were used under the conditions specified by the suppliers.

**Isolation of *fmr* genes.** Total DNA of *S. sannanensis* IFO 14239 was partially digested with *Sau3AI* and ligated to pIJ702 cut by *BglII*. Total DNA of *M. olierasterospora* ATCC 21819 was partially digested with *Sau3AI* and ligated to pEN101 cut by *BglII*. Total DNA of *Micromonaspora* sp. SF-2098 ATCC 31580 was partially digested with *Sau3AI* and ligated to pEN101 cut by *BglII*. Total DNA of *D. matsuzakienense* ATCC 31570 was digested with *SacI* and ligated to pEN101 cut by *BamHI*. All of these digested plasmid vectors were used after CIAP treatment. Protoplasts of *S. lividans* TK23 were transformed with these ligated DNA preparations and regenerated on R5 medium (Hintermann et al., 1984). Thioppeptin-resistant transformants were obtained by overlaying with soft-agar medium consisting of Nutrient broth (Difco) 0.5% (w/v), Bacto agar (Difco) 0.5% (w/v) and 20 µg thioppeptin ml⁻¹ (Fujisawa Pharmaceutical). These transformants were replica-plated onto ATCC no. 5 medium containing FTM-A (50 µg ml⁻¹). After incubation at 30 °C for 3–5 d, FTM-A-resistant transformants were selected.

**Construction of a plasmid carrying both *fmrT* and *fmrO*.** The 1.5 kb *BamHI* fragment containing *fmrT* in pFMRT3 (Ohta & Hasegawa, 1989) was ligated to an *fmrO*-containing recombinant plasmid, pFMRO2, partially digested with *BclI*, and *S. lividans* TK23 was transformed with this DNA preparation. After regeneration on R5 medium without selection by antibiotics, the colonies were transferred to ATCC no. 5 medium containing 50 µg Nm-B ml⁻¹. They were then transferred to ATCC no. 5 containing 250 µg Gm ml⁻¹, and the transformants resistant to Nm and Gm were selected.

**Southern-blot hybridization.** For the analysis of the cloned *fmr* genes, each plasmid was digested as follows: pFMRTB1 with *BamHI*, pFMRS1 with *BclI*, pFMHR1 with *BglII*, and pFMRO1 with *BclI*, pFMRM1 with *BglII*, and pFMRD1 with *SacI*. In the experiment in which the total DNA from various actinomycetes was probed, all total DNA samples were digested with *BamHI*. To determine the location of *fmrO* within the gene cluster for FTM-A biosynthesis, a cosmide clone pGLM559 (Dairi et al., 1993) was digested with *MluI* or *XhoI*. These preparations were separated by 0.7% agarose gel electrophoresis in TAE buffer (40 mM-Tris/acetate and 2 mM-EDTA, pH 8.0), and blotted onto Genescreen plus (New England Nuclear

Table 1. **Sensitivities to aminoglycoside antibiotics of test strains and *S. lividans* transformants carrying various *fmr* genes**

<table>
<thead>
<tr>
<th></th>
<th>FTM-A</th>
<th>Km</th>
<th>Gms</th>
<th>Nm-B</th>
<th>Sm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. tenjamariensis</em></td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
</tr>
<tr>
<td><em>S. sannanensis</em></td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
</tr>
<tr>
<td><em>M. olivasterospora</em></td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>Micromonaspora</em> sp. SF-2098</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>D. matsuzakienense</em></td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> TK23</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRTB1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRS2)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMHR1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRO2)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRM1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRD1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td><em>S. lividans</em> (pFMRTO1)*</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>250</td>
<td>s</td>
</tr>
</tbody>
</table>

* pFMRT01 is a pFMRO2-derived plasmid carrying both *fmrT* and *fmrO*, but it does not carry *tsr*. 

---

Table 1. **Sensitivities to aminoglycoside antibiotics of test strains and *S. lividans* transformants carrying various *fmr* genes**

Bacterial growth was monitored after 3–5 d cultivation on ATCC no. 5 medium containing 0, 25, 50, 125, 250 and 500 µg ml⁻¹ of the antibiotics. Numbers represent the minimum concentrations (µg ml⁻¹) of the antibiotics which inhibit the growth of the bacteria. s, Sensitive to 25 µg of the antibiotic µg ml⁻¹.
Results

Resistance profiles of strains to aminoglycoside antibiotics

Table 1 shows sensitivities of the FTM-group antibiotic producers to various aminoglycoside antibiotics. All strains were highly resistant to FTM-A and Km, and were sensitive to Sm. S. tenjimariensis, S. sannanensis and Sac. hirsuta were resistant to Nm-B, but sensitive to Gm. In contrast, M. olivasterospora, Micromonospora sp. SF-2098 and D. matsuzakiense were resistant to Gm, but sensitive to Nm-B. The fmrT gene isolated from S. tenjimariensis was considered to be a resistance gene for self-defence because of the structural similarity of FTM-A and istamycin A, its own product (Ohta & Hasegawa, 1989). The gene conferred resistance to Nm-B; however, M. olivasterospora, Micromonospora sp. SF-2098 and D. matsuzakiense were sensitive to this antibiotic. These results indicated the presence of two or more different types of resistance genes in these producers. As S. lividans TK23 was sensitive to these antibiotics (Table 1), it was used as the host to clone the resistance genes.

Isolation of fmr genes

Isolation of fmrT from the istamycin producer S. tenjimariensis was previously described (Ohta & Hasegawa, 1989). The physical map of the cloned DNA in pFMRTB1 is shown in Fig. 1(a). Although Skeggs et al. (1987) cloned an aminoglycoside-resistance gene (kamA; kanamycin-apramycin resistance methyltransferase) from the same strain of S. tenjimariensis, its physical map was quite different from our clone (Skeggs et al., 1985, 1987). In the present study, fmr genes were cloned into S. lividans TK23 from the other five strains. Plasmids pIJ702, pEN101 and pEN101B were used as vectors. Plasmid pEN101 is a multi-copy plasmid which is similar in length, copy number and stability to those of pIJ702 (unpublished data); pEN101B is a pEN101 derivative.

From the sannamycin producer S. sannanensis IFO 14239, four FTM-A-resistant transformants were obtained in the shotgun cloning experiment. These plasmids, pFMRS1, pFMRS1, pFMRS2, pFMRS3 and pFMRS4, contained 1.5, 2.3, 1.6 and 1.4 kb inserts, respectively. The resistance determinant, fmrS, should be located within the common 1.3 kb region in these cloned DNA fragments. Fig. 1(b) shows the physical map of the insert in pFMRS2 and the presumed location of fmrS. From the sporaricin producer Sac. hirsuta ATCC 20501, one resistant transformant, harbouring pFMRH1, was obtained. pFMRH1 contained a 5.9 kb BamHI fragment (Fig. 1c). During the preparation of this report, the isolation of kamC (see above for designation of kam) of

Fig. 1. Physical maps of the cloned DNA fragments containing fmr genes. The restriction maps of the cloned fragments of (a) pFMRTB1 from S. tenjimariensis (Ohta & Hasegawa, 1989), (b) pFMRS2 from S. sannanensis, (c) pFMRH1 from Sac. hirsuta (ATCC 20501), (d) pFMRO2 from M. olivasterospora, (e) pFMRM1 from Micromonospora sp. SF-2098, and (f) pFMRD1 from D. matsuzakiense are shown. The black regions represent the cloned fragments. The location of fmrT, fmrS and fmrO are indicated as open bars under the physical maps. Dotted lines represent the 0.8 kb SalI fragment of the fmrT probe (a) and the 3.2 kb PvuII fragment of the fmrO probe (d) used in Southern-blot hybridization analysis.
another sporaricin producer, Sac. hirsuta CL102, was reported by Holmes et al. (1991). However, the physical map of our clone was quite different from their kamC-containing DNA fragment; the taxonomic relationship of the two strains is unclear. As the structures of these two resistance genes were different and as the resistance mechanism of our gene has not been clarified, our resistance gene obtained from Sac. hirsuta ATCC 20501 was designated fmrH. From the FTM-A producer M. olivasterospora, three resistant transformants were obtained. The plasmids, pFMRO1, pFMRO2 and pFMRO3, carried 2.6, 3.6 and 3.0 kb inserts, respectively. The resistance determinant, fmrO, should be located within the common 1.9 kb region in the cloned DNA fragments of these three plasmids. Fig. 1(d) shows the physical map of the insert in pFMRO2 and the presumed location of fmrO. From the antibiotic SF-2052 producer Micromonospora sp. strain SF-2098, one resistant transformant, harbouring pFMRM1, was obtained. pFMRM1 contained a 3.4 kb insert (Fig. 1e). The resistance determinant was designated fmrM. From the dactimicin producer D. matsuzakiense, one resistant transformant, harbouring pFMRD1 was obtained. Although the shotgun cloning was performed by Sac1 digestion, pFMRD1 contained two sac1 fragments, of 3.1 and 0.7 kb (Fig. 1f).

Similarity was observed between the physical maps of fmrO and fmrM, cloned from two Micromonospora strains; however, no structural similarities were observed among the other genes.

Resistance profiles of fmr genes
Resistance profiles of S. lividans TK23 transformants harbouring each fmr gene were examined. Exact comparison of the resistance levels conferred by resistance genes present in taxonomically different micro-organisms is difficult because of the difference in their optimum growth conditions and the difficulty of controlling their growth rates. We therefore used the homogeneous background of the host S. lividans carrying the fmr genes to compare the characteristics of these resistance genes. The results summarized in Table 1 clearly indicate that the resistance patterns of these transformants are similar to those of the corresponding producer strains. These results also show that fmr genes could undoubtedly be classified into two groups with respect to their resistance profiles, namely the Nm-resistant group (designated ‘fmrT-type’, consisting of fmrT, fmrS and fmrH) and the Gm-resistant group (designated ‘fmrO-type’, consisting of fmrO, fmrM and fmrD). The resistance pattern of the fmrT-type was similar to the kam genes isolated from S. tenjimariensis (kamA) (Skeggs et al., 1985), a nebramycin producer S. tenebrarius NC1B 11028 (kamB) (Skeggs et al., 1987) and a sporaricin producer Sac. hirsuta CL102 (kamC) (Holmes et al., 1991), in spite of the discrepancy
of the physical maps between kamA and our fmrT, and between kamC and our fmrH. On the other hand, the resistance pattern of the fmrO-type was similar to Km-Gm, resistance genes isolated from the Km producer Streptomyces kanamyceticus ISP 5500 (Nakano et al., 1984), S. tenebrarius NCIB 11028 (kgmB) (Skeggs et al., 1987), and the Gm producer M. purpurea (grm) (Kelemen et al., 1991).

An 8-7 kb plasmid, pFMRTO1, which carried both fmrT and fmrO, was constructed by introducing fmrT into pFMRO2, although the plasmid lost a portion of pFMRO2 including tsr during the construction. An S. lividans TK23 transformant harbouring pFMRTO1 was resistant to both Nm-B and Gm (Table 1), indicating that these resistance mechanisms worked independently and simultaneously in S. lividans.

DNA homologies of the cloned fmr genes

Southern-blot hybridization analysis was performed to examine the DNA homologies among the fmr genes. The 0.8 kb SalI fragment in pFMRTB1 (fmrT; Fig. 1a) and the 3.2 kb PvuII fragment in pFMRO2 (fmrO; Fig. 1d) were used as the hybridization probes. The plasmids carrying the fmr genes described above were digested with appropriate restriction enzymes in order to distinguish the cloned DNA fragments from the vector sequences. When the fmrT probe was employed, significant homologies were found in fmrS and fmrH, but not in fmrO, fmrM and fmrD (Fig. 2b). The opposite result was obtained when the fmrO probe was employed (Fig. 2c).

Distribution of DNA fragments homologous to fmrT and fmrO

Southern hybridization analyses of the total DNA from various actinomycetes were performed to examine the distribution of DNA fragments homologous to fmrT and fmrO. When the fmrT probe was employed, a positive signal of 2.8 kb was detected in S. tenjimaireshis, which agreed with the cloned fragment by BamHI digestion (Fig. 3, lane 2; Fig. 1a). Positive signals were clearly detected in S. sananensis and Sac. hisuta ATCC 20501 as expected, with sizes of 7.0 and 5.9 kb, respectively (Fig. 3, lanes 3 and 11). The size of the signal detected in the sporaricin producer Sac. hisuta ATCC 20501 agreed with the structure of the cloned fragment by BamHI digestion (Fig. 1c); however, it differed in size from the 2.6 kb BamHI fragment containing kamC in another sporaricin producer, Sac. hisuta CL102 (Holmes et al., 1991). Sac. hisuta ATCC 27875 also gave a signal of 2.4 kb (Fig. 3, lane 13). This strain has not been reported to produce FTM-group antibiotics; however, it showed a similar resistance profile to those of fmrT-type resistance genes – resistant to FTM-A, Km and Nm-B, but sensitive to Gm and Sm. These results suggest that Sac. hisuta ATCC 27875 has an fmrT-type resistance gene. On the other hand, no signal was detected in M. olivasterospora, Micromonospora sp. SF-2098, D. matsuakiense and other actinomycetes including producers of the other aminoglycoside antibiotics (see legend to Fig. 3).

When the fmrO probe was employed, positive signals of approximately 15, 15 and 25 kb were detected in M. olivasterospora, Micromonospora sp. SF2098 and D. matsuakiense, respectively (Fig. 4, lanes 1, 2 and 11). No positive signal was detected in S. tenjimaireshis, S. sananensis and sporaricin-producing Sac. hisuta (not shown). Hybridization signals were also commonly detected in Micromonospora strains which produced the other aminoglycoside antibiotics: 6-1 kb signals in M. purpurea ATCC 15835 and three M. echinospora strains,
Fig. 4. Distribution of DNA homologous to fmrO. The result of Southern-blot hybridization analysis using the 3.2 kb PstI fragment in pFMR02 (Fig. 1) as a probe is shown. Lanes: 1, Micromonospora olivasterospora ATCC 21819 (FTM-A); 2, Micromonospora sp. SF-2098 ATCC 31580 (SF-2052); 3, Micromonospora purpurea ATCC 15835 (identical to strain DSM 43036; Gms); 4, Micromonospora sagamiensis subsp. nonreducans ATCC 21803 (sagamicins and Gm-A1); 5, Micromonospora sagamiensis ATCC 21826 (sagamicins and Gm-A1); 6, Micromonospora echinospora subsp. echinospora ATCC 15837 (Gms); 7, Micromonospora echinospora subsp. pallida ATCC 15838 (Gms); 8, Micromonospora inyonensis ATCC 27600 (sisomicins); 9, Micromonospora zionensis IFO 14116 (sisomicins); 10, Micromonospora echinospora subsp. ferruginea ATCC 15836 (Gms); 11, Dactylosporangium matsuzakiense ATCC 31570 (dactimicin); 12, Dactylosporangium aurantiacum ATCC 23491; 13, Dactylosporangium varioporum (capreomycin). DNA preparations from the strains in Fig. 3 (lanes 1–13) were also probed, but gave no hybridization signals with fmrO (not shown). Other details as for Fig. 3.

ATCC 15837, ATCC 15838 and ATCC 15836 (Gm producers; lanes 3, 6, 7 and 10), 4.1 kb in two M. sagamiensis strains, ATCC 21803 and ATCC 21826 (sagamicins and Gm-A1; lanes 4 and 5), an approximately 13 kb signal and a weak 4.7 kb signal in M. inyonensis ATCC 27600 (sisomicins; lane 8), and a 7.3 kb signal and a weak 13 kb signal in M. zionensis IFO 14116 (sisomicins; lane 9). All these strains were highly resistant to FTM-A (more than 500 μg ml⁻¹); however, they are not known to produce any FTM-group antibiotics.

**Location of fmrO in the gene cluster of FTM-A biosynthesis**

The genes for FTM-A biosynthesis are clustered within an approximately 25 kb region (Dairi et al., 1993). The fmrO probe hybridized to the 6.4 kb MluI and the 6.0 kb XhoI fragments of pGLM559 carrying the FTM-A biosynthesis gene cluster (Dairi et al., 1993). This indicated that fmrO is adjacent to one end of the gene cluster for FTM-A biosynthesis in the *M. olivasterospora* genome. The size of the signal detected in the Southern analysis of the total DNA (Fig. 4, lane 1) is also consistent with the structure of the gene cluster.

**Discussion**

Self-defence against their own products is essential for the growth of antibiotic producers (Hotta et al., 1983). Antibiotic biosynthesis genes are generally clustered in limited regions in the genome, and the genes conferring resistance to the organism's own products are usually located in or near the gene clusters (Rhodes et al., 1984; Ohnuki et al., 1985; Chater & Bruton, 1985; Malpartida & Hopwood, 1986; Murakami et al., 1986; Stanzak et al., 1986; Fishman et al., 1987). These facts seem to suggest a tight genetic linkage between antibiotic biosynthesis genes and the resistance genes during the evolution and the horizontal distribution of antibiotic biosynthesis systems.

The FTM-group antibiotics, which are produced by six actinomycetes of four genera, are believed to be synthesized via similar biosynthetic pathways (Dairi & Hasegawa, 1989; Hotta et al., 1989), and at least parts of these antibiotic biosynthetic genes are conserved among these producers (Ohta et al., 1992). We had previously postulated that the resistance genes for self-defence would also be conserved, like the biosynthesis genes (Ohta & Hasegawa, 1989). However, the present study showed that the resistance genes of these strains could be clearly classified into two different families with respect to their resistance profiles and DNA homologies. The results suggest that the differences in the resistance patterns are caused by differences in the nucleotide sequences and hence differences in the enzyme structures. Thus there may exist at least two functionally different families of resistance genes among the FTM-group antibiotic producers.

Importantly, fmrO was found to be located adjacent to the gene cluster for FTM-A biosynthesis. It is very curious that the fmrO-type resistance genes could not be found in *S. tenjimariensis*, *S. sannanensis* and sporarin-producing *Sac. hirsuta*, which carry the homologous glycyrltransferase gene that is located in this gene cluster. This fact suggests that the biosynthetic genes and the resistance genes have evolved separately in these producers. It can be considered that the genes for antibiotic biosynthesis and resistance are horizontally distributed by genetic transfer, such as conjugation or transduction via phages, in addition to the vertical succession accompanied by bacterial evolution. It is possible that part of the gene cluster for the biosynthesis of FTM-group antibiotics has been transferred inde-
pendently of its corresponding resistance gene to one of the FTM-group antibiotic producers which had another class of resistance genes. Our results suggest a looser genetic linkage between the antibiotic biosynthesis genes and the self-defence genes during their presumed dissemination among the FTM-group antibiotic producers.

The resistance pattern conferred by the fmrO-type resistance genes was similar to those conferred by the Km-Gm resistance gene conferring the ribosome modification isolated from S. kanamyceticus ISP 5500 (Nakano et al., 1984) and by the kanamycin-gentamicin resistance methyltransferase gene (kgmB) of S. tenebrarius NCIB 11028 (Skeggs et al., 1987). However, no hybridization signals were detected in these strains using the fmrO probe under the conditions employed in this study. This suggests that the resistance determinant found in S. kanamyceticus and S. tenebrarius is different from the fmrO-type resistance genes, or that the similarity in nucleotide sequences is too small to be detected by this hybridization experiment. The fmrO-type resistance genes of Micromonospora and Dactylosporangium are more similar to each other than to those of the Streptomycetes strains. This seems to be consistent with the taxonomic distances between the genera Micromonospora, Dactylosporangium and Streptomycetes. Recent comparisons of 16S rRNA sequences showed that the former two genera are very closely related, and are distant from Streptomycetes and Saccharopolyspora (Stackebrandt & Charfrezig, 1990).

Positive signals hybridizing to the fmrO probe were commonly detected in Micromonospora strains which produced other aminoglycoside antibiotics, i.e. gentamicins, sagamicins or sisomicins. The signals detected in Micromonospora strains should be resistance genes similar to fmrO considering the high resistance of these strains to FTM-A. The resistance genes with resistance patterns similar to fmrO isolated from S. kanamyceticus (Nakano et al., 1984), S. tenebrarius (kgmB) (Skeggs et al., 1987) and M. purpurea (grm) (Kelemen et al., 1991) confer ribosome modification. Ribosomal resistance is a widespread mechanism among aminoglycoside-producing Micromonospora strains (Matkovic et al., 1984). No FTM-A-inactivation activity was detected in M. olivasterospora (data not shown). Thus, one can suppose that the fmrO-type resistance genes confer such a ribosome modification. It was reported that grm of M. purpurea encoded a specific methyltransferase of 16S ribosomal RNA (Kelemen et al., 1991). However, the size of the band that hybridized to the fmrO probe in M. purpurea ATCC 15835 (identical to strain DSM 43036 used by Piendl et al., 1984) (6-1 kb BamHI fragment; Fig. 4, lane 3) was not the same as that which hybridized to the grm probe in their strain (1-9 kb BamHI fragment).

It has been shown that the self-defence mechanism of S. tenjimariensis is ribosomal resistance (Yamamoto et al., 1981, 1986; Piendl et al., 1984; Skeggs et al., 1985), and that its kamA encodes a specific methyltransferase of 16S ribosomal RNA (Beaclerk & Cundliffe, 1987). The kamA of S. tenjimariensis, kamB of S. tenebrarius NCIB 11028 and kamC of another sporarin-producing Sac. hirsuta strain, CL102, have been reported to have DNA similarity (Skeggs et al., 1987; Cundliffe, 1989; Holmes et al., 1991). The fmrT-type resistance genes obtained in this study gave a similar resistance profile to that of the kam type resistance genes. However, the physical map of fmrT disagreed with that of kamA cloned from the same strain of S. tenjimariensis (Skeggs et al., 1987), and no signal hybridizing to the fmrT probe was detected in S. tenebrarius ATCC 17920 under the conditions used in this study. fmrT seems to be the only gene which confers resistance to FTM-A, Km and Nmb in our strain. At this stage we cannot explain why fmrT does not appear to be related to kam. Further comparative studies, including sequence analysis, between the fmrT and kam genes are needed.

Although the two Micromonospora strains which produce the FTM-group antibiotics were independently isolated by different research groups (Nara et al., 1977; Ohba et al., 1981), the patterns of hybridization signals obtained with the fmrO probe agreed well. Such agreement was also observed using the sannamycin B-glycyrrhizinase gene as a probe (Ohta et al., 1992). These observations suggest that the biosynthetic gene clusters for FTM-A and SF-2052 compounds are well conserved. Similarities in the hybridization signals to the fmrO probe were observed among the producers of the Gm-group antibiotics, M. purpurea ATCC 15835, M. echinospora subsp. echinospora ATCC 15837, M. echinospora subsp. pallida ATCC 15838 and M. echinospora subsp. ferruginea ATCC 15836, and between M. saga-miensis subsp. nonreducans ATCC 21803 and M. saga-miensis ATCC 21826. The difference between the former and the latter groups is only in the level and composition of their Gm-group antibiotics. The properties of these antibiotics seem to be correlated with the degree of conservation of the gene organizations around the resistance genes, probably including the biosynthetic genes for these antibiotics.

We are grateful to Dr I. Kawamoto for his critical discussion on the taxonomic field in this study. We also thank Ms K. Fujiwara and Ms K. Naito for their excellent technical assistance.

References


Burnham, M. K. R., Earl, A. J., Bull, J. H., Smith, D. J. & Turner,


Fortimicin resistance genes

erythromycin biosynthesis genes from *Streptomyces erythreus*. *Bio/Technology* 4, 229–232.


