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

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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 Micromonospora olivasterospora ATCC 21819, fmrM from the antibiotic SF-2052 producer Micromonospora sp. SF-2098 (ATCC 31580) and fmrD from the dactimicin producer Dactylosporangium 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 Micromonospora olivasterospora ATCC 21819 (FTM-A) (Nara et al., 1977), Micromonospora 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 (istantycins) (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 (Odagakura 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...
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, Sac. hirsuta ATCC 20501, M. olivasterospora ATCC 21819, Micromonospora sp. ATCC 31580 and D. matsuzakiense 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 (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).

**Determination of the antibiotic resistance patterns of the strains.** About 10⁶ spores were spread on ATCC no. 5 medium containing 0, 25, 50, 125, 250 and 500 μg ml⁻¹ of the antibiotics to be tested: FTM-A hydrochloride, Km sulphate (Sigma), Gm sulphate (Sigma), Nm-B sulphate (Sigma) and streptomycin (Sm) sulphate (Sigma). Growth was monitored after incubation at 30 °C for 3–5 d.

**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 Sac. hirsuta ATCC 20501 was digested with BamHI and ligated to pEN101B cut by BamHI. Total DNA of M. olivasterospora ATCC 21819 was partially digested with Sau3AI and ligated to pEN101 cut by BglII. Total DNA of Micromonospora sp. SF-2098 ATCC 31580 was partially digested with Sau3AI and ligated to pEN101 cut by BglII. Total DNA of D. matsuzakiense ATCC 31570 was digested with SacI and ligated to pEN101 cut by BglII. All of these digested plasmid vectors were used after CIAP treatment. Protoplasts of S. lividans TK23 were transformed with these digested DNA preparations and regenerated on R5 medium (Hintermann et al., 1984).

**Thiopetin-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 thiopetin 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 BglII, 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: pFMRT1 with BamHI, pFMRS1 with BglII, pFMHR1 with BamHI and EcoRI, pFMRO1 with BglII, 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 cosmid 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

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<th>Table 1. Sensitivities to aminoglycoside antibiotics of test strains and S. lividans transformants carrying various fmr genes</th>
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<td><strong>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 ml⁻¹.</strong></td>
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<td>S. lividans (pFMRT01)*</td>
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* pFMRT01 is a pFMRO2-derived plasmid carrying both fmrT and fmrO, but it does not carry tsr.
Research Products) according to the method of Reed & Mann (1985). The 0.8 kb \textit{SalI} fragment within pFMRTB1 (Fig. 1a) and the 3.2 kb \textit{PvuII} fragment within pFMRS2 (Fig. 1d) were used as the \textit{‘fmrT} probe\text{"} and the \textit{‘fmrO} probe\text{"}, respectively. These DNA fragments were labelled with \textit{[32P]}dCTP (Amersham) by nick-translation. Hybridization was carried out at 65 °C for 16 h in 6 × SSC (1 × SSC is 0.15 M-NaCl plus 0.015 M-sodium citrate, pH 7.2), 5 × Denhardt’s solution (Maniatis\textit{ et al.}, 1982), 100 μg sonicated/denatured calf thymus DNA ml\textsuperscript{-1}, with the radioactive DNA probe. The filters were washed twice for 15 min at room temperature in 150 ml 0.5 × SSC, 0.2% (w/v) SDS, and four times for 30 min at 65 °C in 250 ml 0.3 × SSC, 0.2% (w/v) SDS. Autoradiograms were made by exposing the filters to Fuji X-ray films (type AIF RX) at -80 °C for 1–2 d.

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. \textit{S. tenjimariensis}, \textit{S. sannanensis} and \textit{Sac. hirsuta} were resistant to Nm-B, but sensitive to Gm. In contrast, \textit{M. olivasterospora}, \textit{Micromonospora} sp. SF-2098 and \textit{D. matsuzakiense} were resistant to Gm, but sensitive to Nm-B. The \textit{fmrT} gene isolated from \textit{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, \textit{M. olivasterospora}, \textit{Micromonospora} sp. SF-2098 and \textit{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 \textit{S. lividans} TK23 was sensitive to these antibiotics (Table 1), it was used as the host to clone the resistance genes.

Isolation of \textit{fmr} genes

Isolation of \textit{fmrT} from the istamycin producer \textit{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\textit{ et al.} (1987) cloned an aminoglycoside-resistance gene (\textit{kamA}; kanamycin-apramycin resistance methyltransferase) from the same strain of \textit{S. tenjimariensis}, its physical map was quite different from our clone (Skeggs\textit{ et al.}, 1985, 1987). In the present study, \textit{fmr} genes were cloned into \textit{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 \textit{S. sannanensis} IFO 14239, four FTM-A-resistant transformants were obtained in the shotgun cloning experiment. These plasmids, pFMR1, pFMR1, pFMR2, pFMR3 and pFMR4, contained 1.5, 2.3, 1.6 and 1.4 kb inserts, respectively. The resistance determinant, \textit{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 pFMR2 and the presumed location of \textit{fmrS}. From the sporaricin producer \textit{Sac. hirsuta} ATCC 20501, one resistant transformant, harbouring pFMRH1, was obtained. pFMRH1 contained a 5.9 kb \textit{BamHI} fragment (Fig.1c). During the preparation of this report, the isolation of \textit{kamC} (see above for designation of \textit{kam}) of

Fig. 1. Physical maps of the cloned DNA fragments containing \textit{fmr} genes. The restriction maps of the cloned fragments of (a) pFMRTB1 from \textit{S. tenjimariensis} (Ohta & Hasegawa, 1989), (b) pFMR2 from \textit{S. sannanensis}, (c) pFMRH1 from \textit{Sac. hirsuta} (ATCC 20501), (d) pFMR2 from \textit{M. olivasterospora}, (e) pFMR1 from \textit{Micromonospora} sp. SF-2098, and (f) pFMRD1 from \textit{D. matsuzakiense} are shown. The black regions represent the cloned fragments. The location of \textit{fmrT}, \textit{fmrS} and \textit{fmrO} are indicated as open bars under the physical maps. Dotted lines represent the 0.8 kb \textit{SalI} fragment of the \textit{fmrT} probe (a) and the 3.2 kb \textit{PvuII} fragment of the \textit{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 *Sac* digestion, pFMRD1 contained two *Sac* 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

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**Fig. 2. DNA homologies among the cloned *fmr* genes.** Ethidium bromide-stained gel (a) and the results of Southern-blot hybridization analysis using the *fmrT* probe (b) and the *fmrO* probe (c) are shown. The 0.8 kb *Sac* fragment in pFMRTB1 (Fig. 1a) and the 3.2 kb *Pvu*II fragment in pFMRO2 (Fig. 1d) as the *fmrT* and *fmrO* probes, respectively. Lanes: 1, size markers (*λ* phage DNA digested with *HindIII*); 2, pFMRTB1 digested with *BamHI* (*fmrT* is contained in the 2.8 kb fragment); 3, pFMRS1 digested with *BclI* (*fmrS* is contained in the 3.0 kb fragment); 4, pFMRH1 digested with *BamHI* and *EcoRI* (*fmrH* is contained in the 5.9 kb fragment); 5, pFMRO1 digested with *BclI* (*fmrO* is contained in the 5.3 kb fragment); 6, pFMRM1 digested with *BglII* (*fmrM* is contained in the 3.2 kb fragment); 7, pFMRD1 digested with *SacI* (*fmrD* is contained in the 3.2 kb fragment).
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. tendrarius NCIB 11028 (kgmB) (Skeggs et al., 1987), and the Gm producer M. purpurea (grm) (Kelemen et al., 1991).

An 8.7 kb plasmid, pFMR01, which carried both fmrT and fmrO, was constructed by introducing fmrT into pFMR02, although the plasmid lost a portion of pFMR02 including tsr during the construction. An S. lividans TK23 transformant harbouring pFMR01 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 pFMR02 (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. tenjimariensis, which agreed with the cloned fragment by BamHI digestion (Fig. 3, lane 2; Fig. 1a). Positive signals were clearly detected in S. sannanensis and Sac. hirsuta 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. hirsuta 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. hirsuta CL102 (Holmes et al., 1991). Sac. hirsuta 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. hirsuta ATCC 27875 has an fmrT-type resistance gene. On the other hand, no signal was detected in M. olivasterospora, Micromonospora sp. SF-2098, D. matsuzakiense 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. matsuzakiense, respectively (Fig. 4, lanes 1, 2 and 11). No positive signal was detected in S. tenjimariensis, S. sannanensis and sporaricin-producing Sac. hirsuta (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 PvuII fragment in pFMR02 (Fig. 1d) 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. pallida ATCC 15838 (Gms); 7, *Micromonospora inyonensis* ATCC 27600 (sisomicins); 9, *Micromonospora zionensis* IFO 14116 (sisomicins); 10, *Micromonospora echinospora* subsp. ferruginea ATCC 15836 (Gms); 11, *Dactylosporangium matsuzakiense* ATCC 31570 (dactimicins); 12, *Dactylosporangium aurantiacum* ATCC 22491; 13, *Dactylosporangium varisporum* (capreomycin). DNA preparations from the strains in Fig. 3 (lanes 1–13) were also probed, but gave no hybridization signals with fmrO. 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 kb *MfuI* 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 sporaricin-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-
FmTm resistance genes, or that the similarity in
ATCC probe in their strain
modification. It was reported that grrn of
Piendl et al.,
sterospora (data not shown). Thus, one can suppose that
the fmrO-type resistance genes confer such a ribosome
3) patterns similar to fmr0 isolated from
ing Micromonospora strains (Matkovic et al.,
RNA (Kelemen et al.,
produced other aminoglycoside antibiotics, i.e. genta-
recent comparisons of
micromonospora, Dactylosporangium and Streptomyces. Re-
the taxonomic distances between the genera Micro-
methyltransferase gene (kgmB) of
S. tenebrarius ATCC
11028 and karnC of another sporaricin-producing
Sac. hirsuta strain, CL102, have been reported to have
DNA similarity (Skeggs et al.,
Cundliffe, 1989; Holmes et al.,
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.,
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 com-
parative 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.,
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-
glycyltransferase gene as a probe (Ohta et al.,
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.

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References
ribosomal RNA methylases responsible for resistance to aminogly-
Burniam, M. K. R., Earl, A. J., Bull, J. H., Smith, D. J. & Turner,


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


