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) (Obba 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 glycitrantransferase, 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. 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 10⁸ 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, 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, FTM-A-resistant transformants were selected.

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 Sau3A1 and ligated to pIJ702 cut by BamHI. Total DNA of Sac. hirsuta ATCC 20501 was digested with BamHI and ligated to pIJ702 cut by BamHI. Total DNA of M. olivasterospora ATCC 21819 was partially digested with Sau3A1 and ligated to pIJ702 cut by BamHI. Total DNA of Micromonospora sp. SF-2098 ATCC 31580 was partially digested with Sau3A1 and ligated to pIJ702 cut by BamHI. Total DNA of D. matsuzakiense 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). Thiopeptin-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 thiopeptin 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, pFMRSI with BclI, pFMRH1 with BamHI and EcoRI, 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 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

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

<table>
<thead>
<tr>
<th>Test Strain</th>
<th>FTM-A</th>
<th>Km</th>
<th>Gms</th>
<th>Nm-B</th>
<th>Sm</th>
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<tbody>
<tr>
<td>S. tenjimariensis</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
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<tr>
<td>S. sannanensis</td>
<td>&gt; 500</td>
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<tr>
<td>Sac. hirsuta</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<tr>
<td>M. olivasterospora</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<tr>
<td>Micromonospora sp. SF-2098</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<td>D. matsuzakiense</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>S. lividans TK23</td>
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<td>s</td>
<td>s</td>
<td>s</td>
<td></td>
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<tr>
<td>S. lividans (pFMRTB1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
<td>250</td>
<td>s</td>
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<tr>
<td>S. lividans (pFMRS2)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>s</td>
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<td>s</td>
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<tr>
<td>S. lividans (pFMRH1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<tr>
<td>S. lividans (pFMRO2)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<tr>
<td>S. lividans (pFMRM1)</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
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<tr>
<td>S. lividans (pFMRD1)</td>
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<td>&gt; 500</td>
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<tr>
<td>S. lividans (pFMRT01)*</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>250</td>
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* pFMRT01 is a pFMRO2-derived plasmid carrying both fmrT and fmrO, but it does not carry tsr.
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-apyramycin 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

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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. matsuzakienese*, 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* NCIB 11028 (kamB) (Skeggs *et al.*, 1987) and a sporaricin producer *Sac. hirsuta* CL102 (kamC) (Holmes *et al.*, 1991), in spite of the discrepancy.

![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 *Hind*III); 2, pFMRTB1 digested with *Bam*II (*fmrT* is contained in the 28 kb fragment); 3, pFMRS1 digested with *Bgl*II (*fmrS* is contained in the 30 kb fragment); 4, pFMRH1 digested with *Bam*II and *Eco*RI (*fmrH* is contained in the 5.9 kb fragment); 5, pFMRO1 digested with *Bgl*II (*fmrO* is contained in the 5.3 kb fragment); 6, pFMRM1 digested with *Bgl*II (*fmrM* is contained in the 3.4 kb fragment); 7, pFMRD1 digested with SacI (*fmrD* is contained in the 3.2 kb fragment).](image-url)
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) (Skogg 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 pFMRTBI (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. 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 producing, 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. 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. 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,
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 glycyltransferase 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-

**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.

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independently 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 dis-
semination 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 modific-
cation 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
Streptomycyes strains. This seems to be consistent with
the taxonomic distances among the genera Micro-
monospora, Dactylosporangium and Streptomyces. Re-
cent comparisons of 16S rRNA sequences showed that
the former two genera are very closely related, and are
distant from Streptomycyes and Saccharopolyspora
(Stackebrandt & Charfreltait, 1990).

Positive signals hybridizing to the fmrO probe were
commonly detected in Micromonospora strains which
produced other aminoglycoside antibiotics, i.e. genta-
micins, 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)
confers a ribosome modification. Ribosomal resistance is
a widespread mechanism among aminoglycoside-produc-
ing Micromonospora strains (Matkovic et al., 1984). No
FTM-A-inactivation activity was detected in M. oliva-
sterospa (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 (Beauclerk & Cundliffe, 1987).
The kamA of S. tenjimariensis, kamB of S. tenebrarius
NCIB 11028 and kamC of another sporaricin-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 conlers resistance to FTM-A, Km and Nm-
B 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., 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-
glycyltransferase gene as a probe (Ohba 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. echino-
spora subsp. pallida ATCC 15838 and M. echinospora
subsp. ferruginea ATCC 15836, and between M. saga-
iensis subsp. nonreducans ATCC 21803 and M. saga-
iensis 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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