ANALYSIS OF THE NUCLEOTIDE SEQUENCE OF *fmrT* ENCODING THE SELF-DEFENSE GENE OF THE ISTAMYCIN PRODUCER, *Streptomyces tenjimariensis* ATCC 31602; COMPARISON WITH THE SQUENCES OF *kamB* OF *Streptomyces tenebrarius* NCIB 11028 AND *kamC* OF *Saccharopolyspora hirsuta* CL102

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The pseudodisaccharide fortimicin (FTM)-group antibiotics are produced by seven strains of four genera in actinomycetes, *Micromonospora*, *Dactylosporangium*, *Streptomyces*, and *Saccharopolyspora*.[1~7] All the FTM-group antibiotics are thought to be synthesized by similar biosynthetic pathways.[8,9] Intergeneric distribution of antibiotic production genes is an intriguing aspect of the genetics of actinomycetes with the respect to the evolution and horizontal transfer of secondary metabolic biosynthetic systems.[10]

We have cloned previously a gene (*sms13*) encoding sannamycin B-glycyltransferase from the sannamycin producer *Streptomyces sannanensis* IFO 14239 and showed that related genes were conserved among all producers of the FTM-group antibiotics.[11] In contrast, the resistance genes (fortimicin-resistance genes; *fmr*s) could be classified into two families on the basis of the resistance profiles and DNA homologies.[12] The *fmrT* gene cloned from an istamycin producer *Streptomyces tenjimariensis* ATCC 31602 hybridized with *fmrS* of *S. sannanensis* and *fmrH* of a sporaricin producer *Saccharopolyspora hirsuta* ATCC 20501 in Southern-blot analysis, but not with resistance genes cloned from *Micromonospora* and *Dactylosporangium* strains. SKEGGS *et al.* described *kamA* of *S. tenjimariensis* which had a similar resistance profile to *fmrT*[13,14], however, it is not clear if the two are identical since there are differences in the physical maps of the two cloned DNA fragments.[12] In this study, we have determined the nucleotide (nt) sequence of *fmrT* and compared it to *kamB* of the nebramycin producer *Streptomyces tenebrarius* NCIB 11028 and *kamC* of another sporaricin producer *S. hirsuta* CL102[7], both of which were reported to be homologous to *kamA*.[14]

The nt sequence of the 2,783 bp *Bam*H I fragment containing *fmrT* (Fig. 1) was determined using the ABI Model 373A DNA sequencer (Applied Biosystems Inc.); protein coding frames were analyzed by the method of BIBB *et al.*[15] Two complete and one partial open reading frames (ORFs) were identified (Fig. 2). ORF-2 (nt 1553 to 2185) determines resistance expression, and is considered to be *fmrT*. The *fmrT* ORF encoded a protein of 211 amino acids (aa) with a predicted M_r 22,871 Da; *fmrT* is transcribed as a single gene. The two ORFs adjacent to *fmrT* are transcribed in the opposite direction. A palindromic sequence which might represent a transcriptional terminator was found in the 3'-flanking region of ORF-1.

Since resistance genes are often located in the biosynthetic gene clusters for antibiotics[16] ORF-1 and ORF-3 might be involved in istamycin biosynthesis. ORF-1 (nt 1278 to 280) showed 37~39% aa identities with the rat, rabbit and human microsomal epoxide hydrolases.[17~19] The biosynthesis of the FTM-group antibiotics[20] involves epimerization steps in which double bond formation and successive oxidative cleavage *via* epoxide formation are believed to occur. Minor components of FTM-intermediates contain carbon-carbon double bonds.[21] Thus an epoxide hydrolase may be

Fig. 1. The nucleotide (nt) sequence of the 2783 bp *Bam*H I fragment and the deduced amino acid sequence of *fmrT*.

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BamH I
GGATCCTGAGGCCGTGATCGCTGCCCTCGGTCGCGGTCTCAGTTGCGGTCTCACTTGACC    60
CCTAGGACTCCGGCACTAGCGACGGGAGCCAGCGCCAGAGTCAACGCCAGAGTGAACTGG

TCCGTTCCGGAAGTGTCCACCGCAGTGCGCCAGAGGCCGCCAAGCCGCGCGACCAGGATC   120
AGGCAAGGCCTTCACAGGTGGCGTCACGCGGTCTCCGGCGGTTCGGCGCGCTGGTCCTAG

GTAGCGGTCTGGACGGACGGGCGCGGACGGTCCCGGACCAAGATCGGACAGCTCGTAATG   180
CATCGCCAGACCTGCCTGCCCGCGCCTGCCAGGGCCTGGTTCTAGCCTGTCGAGCATTAC
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CGTAGGTCGCGGGTTCGAATCCCGCAGGTGGCTCAGCTCGAAAGCCCAGGTCAGTTTCGC   240
GCATCCAGCGCCCAAGCTTAGGGCGTCCACCGAGTCGAGCTTTCGGGTCCAGTCAAAGCG

TGACCTGGGCTTTTGTCGTTGGTGGATGCCGTGGCGTCAGAGGGTCGCGAAGAAGCGGGT   300
ACTGGACCCGAAAACAGCAACCACCTACGGCACCGCAGTCTCCCAGCGCTTCTTCGCCCA
                                  *  L  T  A  F  F  R  T

CAGGTCGGCGGCCAGGTGGTGGGGTTCCTCGGTGGGGGCGAAGTGGCCGCCTGAGGGCAT   360
GTCCAGCCGCCGGTCCACCACCCCAAGGAGCCACCCCCGCTTCACCGGCGGACTCCCGTA
 L  D  A  A  L  H  H  P  E  E  T  P  A  F  H  G  G  S  P  M

GACGGTCCAGTGGTGGACGGTGTAGAGGCGTTCCGCCCAGGAGCGGGGTGGGGTGCCGTC   420
CTGCCAGGTCACCACCTGCCACATCTCCGCAAGGCGGGTCCTCGCCCCACCCCACGGCAG
 V  T  W  H  H  V  T  Y  L  R  E  A  W  S  R  P  P  T  G  D

GTCGATGTACTCGTTGCCGAAGAGACCGATGGCGGTGGGGACCTGGACGTGGTCGGTGGC   480
CAGCTACATGAGCAACGGCTTCTCTGGCTACCGCCACCCCTGGACCTGCACCAGCCACCG
 D  I  Y  E  N  G  F  L  G  I  A  T  P  V  Q  V  H  D  T  A

GGTGAGGGAGTCGTACAGCTCGCGGTTGTCGTGGTAGTCGCGGAGCGAGGGGGCGATGCT   540
CCACTCCCTCAGCATGTCGAGCGCCAACAGCACCATCAGCGCCTCGCTCCCCCGCTACGA
 T  L  S  D  Y  L  E  R  N  D  H  Y  D  R  L  S  P  A  I  S
                                        Sma I
GTCGGTCACCCAGAGGAGGGTCAGGGTGGTGAGGAGGAAGTCCCGGGTGAAGCGGCTCTC   600
CAGCCAGTGGGTCTCCTCCCAGTCCCACCACTCCTCCTTCAGGGCCCACTTCGCCGAGAG
 D  T  V  W  L  L  T  L  T  T  L  L  F  D  R  T  F  R  S  E

CAGGTCGCCCCCGCTGTCGGACCAGGCGCGCCACTTCTCCAGGACCCAGGCGGCCAGGCC   660
GTCCAGCGGGGGCGACAGCCTGGTCCGCGCGGTGAAGAGGTCCTGGGTCCGCCGGTCCGG
 L  D  G  G  S  D  S  W  A  R  W  K  E  L  V  W  A  A  L  G

GGTGGGGGAGTCGGTCAGGGCGTAGGCGAGGGACTGGGGTTTCGTGGACTGGACGTGGTT   720
CCACCCCCTCAGCCAGTCCCGCATCCGCTCCCTGACCCCAAAGCACCTGACCTGCACCAA
 T  P  S  D  T  L  A  Y  A  L  S  Q  P  K  T  S  Q  V  H  N

GTAGCCGCCCTCCTCCTCCAGCCAGCGCTCCTCCTGGGCCAGATAGGCGCGTTCGGCCTC   780
CATCGGCGGGAGGAGGAGGTCGGTCGCGAGGAGGACCCGGTCTATCCGCGCAAGCCGGAG
 Y  G  G  E  E  E  L  W  R  E  E  Q  A  L  Y  A  R  E  A  E
          Sma IApa I
GGTGAGCGGCGGGGCGCCCGGGCCCCAGGTACGGGGACAGCTCGATGTTGGACAGGTACAG   840
CCACTCGCCGCCCCGCGGGCCCGGGTCCATGCCCCTGTCGAGCTACAACCTGTCCATGTC
 T  L  P  P  A  G  P  G  L  Y  P  S  L  E  I  N  S  L  Y  L

GCCCCGTACCGCCGACGGCTGATCCAGCGCCAGGAACGTACCGATGCCCGATCCGAAGTC   900
CGGGGCATGGCGGCTGCCGACTAGGTCGCGGTCCTTGCATGGCTACGGGCTAGGCTTCAG
 G  R  V  A  S  P  Q  D  L  A  L  F  T  G  I  G  S  G  F  D

GCCGCCCTGGACGCCGTACCGCTCATAGCCGAGGCCGCGCATCAGGGTGTGCCAGAGGCG   960
CGGCGGGACCTGCGGCATGGCGAGTATCGGCTCCGGCGCGTAGTCCCACACGGTCTCCGC
 G  G  Q  V  G  Y  R  E  Y  G  L  G  R  M  L  T  H  W  L  R

GGCGGTGTCCCGCATCGTCAGCGCGCGGTCGGGGCGGGACGAGAAGCAGTAGCCGGGCAG  1020
CCGCCACAGGGCGTAGCAGTCGCGCGCCAGCCCCGCCCTGCTCTTCGTCATCGGCCCGTC
 A  T  D  R  M  T  L  A  R  D  P  R  S  S  F  C  Y  G  P  L
                    Apa I                 Sma I
CGACGGGATCACCACGTCGAAGGCGGGGCCCTCGATGCCGTGCGCCCCGGGGTCCGTGAG  1080
GCTGCCCTAGTGGTGCAGCTTCCGCCCCGGGAGCTACGGCACGCGGGGCCCCAGGCACTC
 S  P  I  V  V  D  F  A  P  G  E  I  G  H  A  G  P  D  T  L

CAGCGGGACCAGGGGGAGCAGCTCCGCGAACGTGCTCGGCCAGCCGTGGGTGAGGATCAG  1140
GTCGCCCTGGTCCCCCTCGTCGAGGCGCTTGCACGAGCCGGTCGGCACCCACTCCTAGTC
 L  P  V  L  P  L  L  E  A  F  T  S  P  W  G  H  T  L  I  L

GGGGATGCCCTCGCCGTGGGCCGACCGGTGGTGGACGAAGTGGATGCGGGTGTCCTCGAT  1200
CCCCTACGGGAGCGGCACCCGGCTGGCCACCACCTGCTTCACCTACGCCCACAGGAGCTA
 P  I  G  E  G  H  A  S  R  H  H  V  F  H  I  R  T  D  E  I

CTCGGTGCGGTGGTGGGCGAAGGTGTTGAGTCTGCGCTCCTGCGCCCGCCAGTCGAACTT  1260
GAGCCACGCCACCACCCGCTTCCACAACTCAGACGCGAGGACGCGGGCGGTCAGCTTGAA
 E  T  R  H  H  A  F  T  N  L  R  R  E  Q  A  R  W  D  F  K

CTCCGTCCAGTACGTCACGATCTCGCGCAGGTATCCGAGATCCGCGCCCTGGCTCCAGGG  1320
GAGGCAGGTCATGCAGTGCTAGAGCGCGTCCATAGGCTCTAGGCGCGGGACCGAGGTCCC
 E  T  W  Y  T  V << ORF-1
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                .         .         .         .         .         .
GGCGCCCGGTGCCGGGGAGGGCCAGCGGGTGCGCTCCAGGCGGGTGGTCAGATCGGACAG  1380
CCGCGGGCCACGGCCCCTCCCGGTCGCCCACGCGAGGTCCGCCCACCACTCTAGCCTGTC

          .Sal I    .         .         .         .         .
CACGGCGTCGTCGACCGCGATCGTGAACGGCCGCGGGGAATGGGTCGCTGTGTCGGAGGT  1440
GTGCCGCAGCAGCTGGCGCTAGCACTTGCCGGCGCCCCTTACCCAGCGACACAGCCTCCA

                  .         .         .  Sal I.         .         .
CACTGGGGCAGGCTGCCATGCGCGCGGCCGGGGGTCGACCTTCTCGCGTACGGGAGCCG  1500
GTGACCCCGTCCGACGGTACGCGCGCCGGCCCCCAGCTGGGAAGAGCGCATGCCCTCGGC

                  .         .         .              ORF-2 (fmrT).>> V  E  L
GGGGCCGAACCGGTACGCTGGGCGTATGCGTCGTGTCAGCGGGAAGCGGGTCGTGGAGCT  1560
CCCCGGCTTGGCCATGCGACCCGCATACGCAGCACAGTCGCCCTTCGCCCAGCACCTCGA

  G  R  S  E  F  E  E  L  L  S  R  H  K  K  V  V  L  D  V  G
GGGCCGGAGCGAGTTCGAGGAACTGCTCTCGCGGCACAAGAAGGTGGTGCTGGACGTCGG  1620
CCCGGCCTCGCTCAAGCTCCTTGACGAGAGCGCCGTGTTCTTCCACCACGACCTGCAGCC

  T  G  D  G  K  H  A  F  Q  L  A  R  R  E  P  D  T  L  V  I
CACCGGCGACGGCAAGCACGCCTTCCAGCTCGCCCGGCGCGAGCCCGACACCCTGGTCAT  1680
GTGGCCGCTGCCGTTCGTGCGGAAGGTCGAGCGGGCCGCGCTCGGGCTGTGGGACCAGTA
                                                  Stu I
  G  L  D  A  A  K  D  N  M  R  K  V  A  T  K  A  S  A  S  P
CGGGCTGGACGCGGCCAAGGACAACATGCGCAAGGTCGCGACCAAGGCCTCGGCCTCACC  1740
GCCCGACCTGCGCCGGTTCCTGTTGTACGCGTTCCAGCGCTGGTTCCGGAGCCGGAGTGG

  N  K  G  G  L  P  N  L  L  Y  V  W  A  S  A  E  R  L  P  E
CAACAAGGGCGGGCTGCCGAACCTGCTGTACGTGTGGGCCTCGGCCGAGCGGCTGCCCGA  1800
GTTGTTCCCGCCCGACGGCTTGGACGACATGCACACCCGGAGCCGGCTCGCCGACGGGCT

  E  L  H  G  V  T  E  I  H  V  L  M  P  W  G  S  L  L  R  G
GGAACTGCACGGGGTCACCGAGATCCATGTGCTGATGCCCTGGGGGAGCCTGCTGCGCGG  1860
CCTTGACGTGCCCCAGTGGCTCTAGGTACACGACTACGGGACCCCCTCGGACGACGCGCC
Sph I
  M  L  G  S  D  P  K  M  L  R  D  L  A  G  V  C  V  P  E  A
CATGCTCGGGTCCGATCCGAAGATGCTGCGGGACCTCGCGGGCGTCTGTGTTCCCGAGGC  1920
GTACGAGCCCAGGCTAGGCTTCTACGACGCCCTGGAGCGCCCGCAGACACAAGGGCTCCG
          Bcl I
  S  F  L  I  T  L  N  L  H  A  W  R  P  A  V  P  E  V  G  D
CAGTTTCCTGATCACTCTGAACCTGCACGCCTGGCGGCCTGCCGTGCCCGAGGTCGGGGA  1980
GTCAAAGGACTAGTGAGACTTGGACGTGCGGACCGCCGGACGGCACGGGCTCCAGCCCCT
                                                      Sma I
  H  P  E  P  T  P  E  S  A  M  R  D  L  V  P  A  L  A  P  G
CCACCCGGAGCCGACGCCCGAGTCCGCCATGCGGGACCTGGTGCCCGCGCTCGCGCCCGG  2040
GGTGGGCCTCGGCTGCGGGCTCAGGCGGTACGCCCTGGACCACGGGCGCGAGCGCGGGCC

  G  W  R  L  D  S  A  E  Y  L  D  S  A  A  I  E  A  L  A  T
GGGGTGGCGGCTGGATTCCGCCGAGTACCTCGACAGCGCCGCCATCGAGGCGCTGGCCAC  2100
CCCCACCGCCGACCTAAGGCGGCTCATGGAGCTGTCGCGGCGGTAGCTCCGCGACCGGTG

  S  W  T  R  R  L  N  S  S  R  D  Q  L  D  V  L  G  L  T  G
CTCCTGGACCCGGCGGCTCAACTCCTCCCGCGATCAGCTCGATGTGCTGGGGCTGACCGG  2160
GAGGACCTGGGCCGCCGAGTTGAGGAGGGCGCTAGTCGAGCTACACGACCCCGACTGGCC
   Bcl I
  V  I  N  P  G  E  S  D  *  .         .         .         .
GGTGATCAATCCGGGGGAGTCGGACTGAGCCGCGGGGCCAGGCTCTTGCGGGGGTCAGCT  2220
CCACTAGTTAGGCCCCCTCAGCCTGACTCGGCGCCCCGGTCCGAGAACGCCCCCAGTCGA
                                                      *  S
                .         .         .         .         .         .
CTTCTGACCGCCGGTCGCCTTCAGCACGAAGTCGTACGCGGTTCTGCGTGACCAGCCCGG  2280
GAAGACTGGCGGCCAGCGGAAGTCGTGCTTCAGCATGCGCCAAGACGCACTGGTCGGGCC
 K  Q  G  G  T  A  K  L  V  F  D  Y  A. T  R  R  S  W  G  P
                .         .         . Sal I   .         .         .
CTGCGCCGCCAGCGCCATCGCGATCGTCCTCGTCGACACCGACTGCTCCAGCAGCGCCGC  2340
GACGCGGCGGTCGCGGTAGCGCTAGCAGGAGCAGCTGTGGCTGACGAGGTCGTCGCGGCG
 Q  A  A  L  A  M  A  I  T  R  T  S  V  S  Q  E  L  L  A  A
                .         .         .         .         .         .
CACCAGCCCCGCCGGGTCCACCGCGGTCACCCCGGCGCCCGCCGGGGTCTCCACCGGGTC  2400
GTGGTCGGGGCGGCCCAGGTGGCGCCAGTGGGGCCGCGGGCGGCCCCAGAGGTGGCCCAG
 V  L  G  A  P  D  V  A  T  V  G  A  G  A  P  T  E  V  P  D
                .         .         .         .        .Apa I    .
CACCTGGCACACCACCTCGCCCCGGCCGCTCCAGTCCAGCTCGGCCACCGGGCCCCACCA  2460
GTGGACCGTGTGGTGGAGCGGGGCCGGCGAGGTCAGGTCGAGCCGGTGGCCCGGGGTGGT
 V  Q  C  V  V  E  G  R  G  S  W  D  L  E  A  V  P  G  W  W
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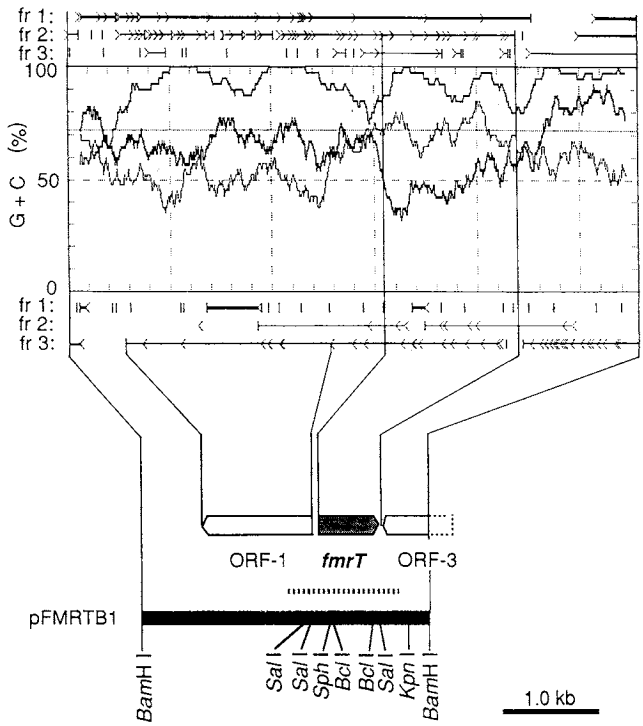
                .         .         .         .         .         .
GGGGCGTTCCGCGCCGGGGAGGGACGCCTCCGCCACGCCGACCGTGCGGCTGCCGGCCGG  2520
CCCCGCAAGGCGCGGCCCCTCCCTGCGGAGGCGGTGCGGCTGGCACGCCGACGGCCGGCC
 P  R  E  A  G  P  L  S  A  E  A  V  G  V  T  R  S  G  A  P
                . Sma I   .         .         .         .
GCGGTCCACCTCGGCCAGCACCCGGGGCAGTTCCTCGGCCGCGCAGCGGAACGTCACGGC  2580
CGCCAGGTGGAGCCGGTCGTGGGCCCCGTCAAGGAGCCGGCGCGTCGCCTTGCAGTGCCG
 R  D  V  E  A  L  V  R  P  L  E  E  A  A  C  R  F  T  V  A
     Sma I         .         .         .      Kpn I.         .
GGCGCCCGGGTGGGCCGCCACGAGGCGCGGGACCTCGCGGGGCGGTACCTCCGTCGCCAG  2640
CCGCGGGCCCACCCGGCGGTGCTCCGCGCCCTGGAGCGCCCCGCCATGGAGGCAGCGGTC
 A  G  P  H  A  A  V  L  R  P  V  E  R  P  P  V  E  T  A  L
                .         .         .         .         .
GAGGCGCTCGGCCCGCACCGGGGACACCACGGAGAGCGCCAGCTCCGGCGGGAGGTTCAG  2700
CTCCGCGAGCCGGGCGTGGCCCCTGTGGTGCCTCTCGCGGTCGAGGCCGCCCTCCAAGTC
 L  R  E  A  R  V  P  S  V  V  S  L  A  L  E  P  P  L  N  L
                .         .         .      Apa I      .
GGCCTCGACCGTCGGGCGGGCGGTCTTCTCGAAGAGCGGCGGCAGCGGGCCCGGTACGGC  2760
CCGGAGCTGGCAGCCCGCCCGCCAGAAGAGCTTCTCGCCGCCGTCGCCCGGGCCATGCCG
 A  E  V  T  P  R  A  T  K  E  F  L  P  P  L  P  G  P  V  A
   Not I .       BamH I
CGCGGCCGCGACCGTCAGGATCC                                       2783
GCGCCGGCGCTGGCAGTCCTAGG
 A  A  A  V  T  L  I --- (<< ORF-3)
```

The stop codons are indicated by asterisks. Restriction sites are underlined. Last digits of numerals are aligned with corresponding nt. A palindromic sequence observed in the 3'-flanking region of open reading frame (ORF)-1 is underlined. The nt sequence data will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases under accession No. D13170.

Fig. 2. Analysis of open reading frames (ORFs) in the 2,783 bp *Bam*H I fragment.



Protein coding regions are predicted by the base compositions of the amino acid (aa) codons according to the method of BIBB *et al.*[15] using the "Frame Plot" program. Window size was set at 40 aa codons. The physical map of the *Bam*H I fragment of pFMRTB1[12] and the positions and directions of the predicted protein coding frames are indicated. Dotted line presents the minimum region limited by the subcloning of *fmrT* in *Streptomyces lividans*.[12]

Fig. 3. Alignment of the deduced amino acid (aa) sequences of the gene products of *fmrT* of *Streptomyces tenjimariensis* (middle line), and *kamB* of *Streptomyces tenebrarius*[7] (upper line) and *kamC* of *Saccharopolyspora hirsuta* CL102[7] (bottom line).

```
      1        10        20        30        40        50        60
kamB                                                  MEKISAKAA   [9]
                                                      * *  **
fmrT VELGRSEFEELLSRHKKVVLDVGTGDGKHAFQLARREPDTLVIGLDAAKDNMRKVATKAS  [60]
                                                      * * : **
kamC                                                  MQKIAGKAA   [9]

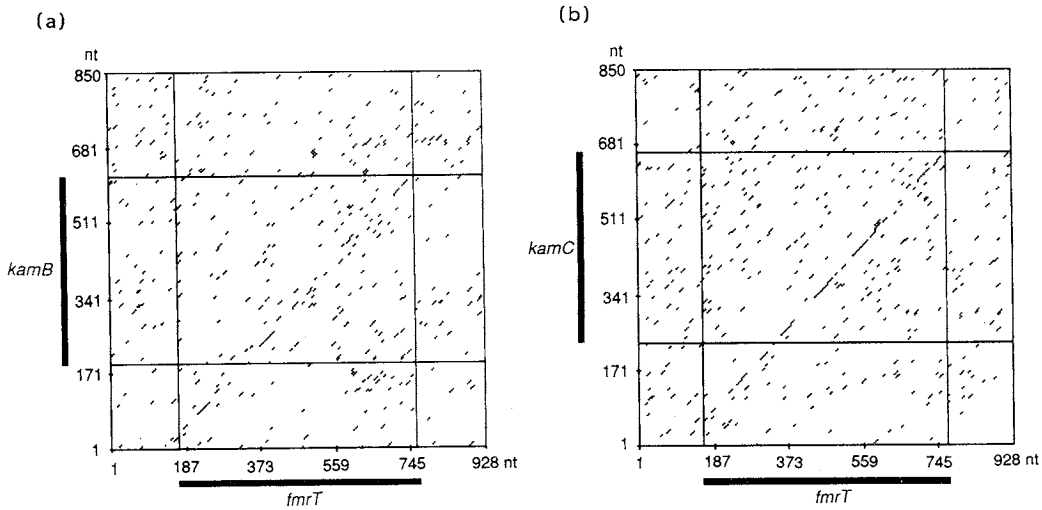
              70        80        90       100       110       120
AKPAK-ALPNLLYLWATAERLP-PLSGVGELHVLMPWGSLLRGVLGSSPEMLRGMAAVCR  [67]
* * *  :****: ** **::*  * :* * *:********** *** : *::  * :*
ASPNKGGLPNLLYVWASAERLPEELHGVTEIHVLMPWGSLLRGMLGSDPKMLRDLAGVCV [120]
*:* *:: **** :**:**  * :*  *:* * **********:***:  *   :*  *:
ASPKKGGAPNLLCVWASAEKPPPELARVTELHSLMPWGSLLRGMLGSDTEMRPGLAELCV  [69]

             130       140       150       160       170       180
PGASFLVECNRSCRGPPVPEVGEHPEPTPDSADEWLAPRYAEAGWKLADCRYLEPEEVAG [127]
* *:**   *     * ***** ****** **   * *  *  ** *    *:
PEASFLITLNLHAWRPAVPEVGDHPEPTPESAMRDLVPALAPGGWRLDSAEYLDSAAIEA [180]
* * **:::*:::::* *****:******:**:  * *  *  ** *  : *       *
PGAEFLITLNLHAWRPPVPEVGDHPEPTPESAMELLAPYYAAAGWTLEEARYFGAEELDA [129]

             190       200
LETSWTRRLHSSRDRFDVLALTGTISP       [154 aa]
* ******* *:**  *** :** * :
LATSWTRRLNSSRDQLDVLGLTGVINPGESD   [211 aa]
*:******* * ** :***: ****
LATSWTRRLGSTRDELDVLGITGVIGK       [156 aa]
```

Identical residues among three sequences are indicated by asterisks, and conserved residues in *fmrT* and *kamB* or *kamC* are indicated by colons. Numerals above the sequences indicate the amino acid numbers from the predicted NH_2-terminus of the *fmrT* gene product.

Fig. 4. Dot plot comparisons of the nucleotide (nt) sequences of the regions carrying *fmrT* and *kamB* (a) and *fmrT* and *kamC* (b).



The nt sequence of the 928 bp *Sal*I fragments containing *fmrT* is analyzed. Stretches up to 6 bp identical between the two sequences are plotted.

involved in these steps. No sequence similar to ORF-3 could be detected in screening the GenBank and NBRF data bases.

The *fmrT* showed strong sequence similarities with those of *kamB* of *S. tenebrarius* and *kamC* of *S. hirsuta* CL102[7] (66.3% and 72.8% identities, respectively). Southern-blot analysis indicated that *kamB* is similar to *kamA* of *S. tenjimariensis*.[14] In

our studies, *fmrT* was the only FTM-A, kanamycin and neomycin-B resistance gene obtained by shotgun cloning,[12] and only one DNA region similar to *fmrT* was detected in *S. tenjimariensis* DNA by Southern-blot analysis at low hybridization stringency (data not shown). Thus, *fmrT* appears to be identical to *kamA* encoding a kanamycin-apramycin resistance methyltransferase capable of modifying 16S rRNA.[22] The difference in physical maps of *fmrT*[12] and *kamA*[14] needs investigation.

Alignment of the deduced aa sequences revealed that *fmrT* is closely similar to *kamB* and *kamC* (60.3% and 70.1% identities, respectively) (Fig. 3). The protein sequences from aa 92 to 107 and from 136 to 152 of *fmrT* are highly conserved among the three genes. *fmrT* and *kamC* were identical between aa 127 and 135, where no similarity was present in *kamB*. *kamB* and *kamC* are reported to encode apparent proteins of 154 and 156 aa, respectively, which are smaller than that of 211 aa encoded by *fmrT* (Fig. 3). FmrT has a 51 aa addition at its NH_2-terminus. Interestingly, this NH_2-terminal sequence was highly conserved in the 5'-flanking regions of *kamB* and *kamC*, whereas little similarity was observed in the 3'-flanking regions (Fig. 4). Further work is required to determine the size and the NH_2-terminal aa sequences of the proteins encoded by these three genes, and to analyze the function of the NH_2-terminal 51 aa region of the *fmrT* gene product.

S. hirsuta CL102 produces sporaricins, FTM-group antibiotics.[7] On the other hand, *S. tenebrarius* produces the nebramycin complex.[23] The similarity (72.8%) in sequence between *fmrT* and *kamC* of the producers of the FTM-group antibiotics (*Streptomyces* and *Saccharopolyspora*) is higher than that between *fmrT* and *kamB* of the *Streptomyces* strains (66.3%). This appears to be variance with the taxonomic relationships of these microorganisms. One interpretation of our results is that the *kam* genes were distributed among producers of the FTM-group antibiotics together with FTM-production genes at a time independently from the separation of the genera *Streptomyces* and *Saccharopolyspora* in the evolution of actinomycetes. This implies that horizontal gene transfer may have occurred.

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References

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