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Characterization of Methyltransferase and Hydroxylase Genes Involved in the Biosynthesis of the Immunosuppressants FK506 and FK520

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FK506 and FK520 are 23-membered macrocyclic polyketides with potent immunosuppressive and antifungal activities. The gene encoding 31-*O*-demethyl-FK506 methyltransferase, *fkbM*, was isolated from *Streptomyces* sp. strains MA6858 and MA6548, two FK506 producers, and *Streptomyces hygroscopicus* subsp. *ascomyceticus*, an FK520 producer. The nucleotide sequence of the *fkbM* gene revealed an open reading frame encoding a polypeptide of 260 amino acids. Disruption of *fkbM* in *Streptomyces* sp. strain MA6548 yielded a mutant that produced 31-*O*-demethyl-FK506, confirming the involvement of the isolated genes in the biosynthesis of FK506 and FK520. Heterologous expression of *fkbM* in *Streptomyces lividans* established that *fkbM* encodes an *O*-methyltransferase catalyzing the methylation of the C-31 hydroxyl group of 31-*O*-demethyl-FK506 and FK520. A second open reading frame, *fkbD*, was found upstream of *fkbM* in all three aforementioned species and was predicted to encode a protein of 388 residues that showed a strong resemblance to cytochrome P-450 hydroxylases. Disruption of *fkbD* had a polar effect on the synthesis of the downstream *fkbM* gene product and resulted in the formation of 9-deoxo-31-*O*-demethyl-FK506. This established the product of *fkbD* as the cytochrome P-450 9-deoxo-FK506 hydroxylase, which is responsible for hydroxylation at position C-9 of the FK506 and FK520 macrolactone ring.

The polyketide, immunosuppressant compound FK506 (Fig. 1) (13) is a 23-membered macrolide with potent antifungal activity produced by several *Streptomyces* species. FK506 is approximately 100-fold more potent than the structurally unrelated immunosuppressive compound cyclosporin A. Both drugs are important therapeutic agents for the prevention of graft rejection following organ and bone marrow transplantations and for the treatment of autoimmune diseases (22). FK520 (also known as immunomycin and ascomycin) is another immunosuppressive compound similar to FK506 (Fig. 1) in which the allyl group is replaced by an ethyl group at position C-21 of the macrolactone ring (9). Both the antifungal and the immunosuppressive activities of FK520 are approximately one-half of those exhibited by FK506 (9).

Through precursor incorporation experiments, Byrne et al. (3) demonstrated that the polyketide portion of FK506 and FK520 is derived, for the most part, from acetate and propionate. Those authors also established the origin of the pipecolate and the cyclohexyl rings to be lysine and shikimic acid, respectively, and demonstrated that the source of the methyl portion of the methoxyl groups at C-13, C-15, and C-31 of FK520 (Fig. 1) is L-methionine.

The enzymology of FK506 biosynthesis has also been explored to some extent. The pipecolate-activating enzyme which presumably incorporates pipecolate into the completed polyketide chain has been characterized previously (19). Both 31-*O*-demethyl-FK520 methyltransferase and 31-*O*-demethyl-FK506 methyltransferase (FKMT) have been isolated from the producing strains (3, 27). These two enzymes can use each other's substrate interchangeably and methylate the C-31 OH and not the C-13 or C-15 OH group (27).

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Here, we report the isolation and molecular characterization of two genes involved in the biosynthesis of FK506. One gene, *fkbM*, encodes FKMT, and the other, *fkbD*, encodes a cytochrome P-450 9-deoxo-FK506 hydroxylase that catalyzes hydroxylation at C-9.

MATERIALS AND METHODS

Standard recombinant DNA techniques were performed as described by Sambrook et al. (24).

Probe design. N-terminal amino acid sequencing of FKMT from *Streptomyces* sp. strain MA6858 (27) gave a 39-mer with the sequence SDVVETLRLPNGA TVAHVNAGEAQFLYREIFTDRCYLRH. This peptide sequence was then used to design two nonoverlapping degenerate oligonucleotide probes, P1 and P2, in which inosine was incorporated at the third position of highly degenerate codons (2). P1 corresponded to amino acid residues 2 through 15, with the sequence 5′-GGCGC(CG)GGCGGCGC(CG)TCIGTIGAGGAGGTICTICT IGA GAC(CG)G, and P2 corresponded to amino acid residues 17 to 35, with the sequence 5′-GGIGCIGGIGGIGCITCIGTIGA(GA)GA(GA)GTICTICTIGA (GA)AC.

Southern analysis. DNAs from various sources were digested with the desired restriction enzymes and electrophoretically separated on a 0.8% agarose gel. The DNA was transferred to a Nytran membrane (Schleicher & Schuell, Keene, N.H.) and hybridized with ^{32}P-labeled probe employing the random priming DNA labeling system (Bethesda Research Laboratories, Gaithersburg, Md.). Conditions for hybridization and washes were as described previously (18).

Construction of the genomic libraries. Size-fractionated (approximately 35-kb) partial *Sau*3A-digested chromosomal DNAs from *Streptomyces* sp. strain MA6858, *Streptomyces hygroscopicus* subsp. *ascomyceticus*, and *Streptomyces* sp. strain MA6548 were cloned into the *Bam*HI site of the *Escherichia coli* cosmid vector pHC79 (Bethesda Research Laboratories) in three separate experiments. Ligation products were packaged into λ particles and then transduced into *E. coli* NM554 (Bethesda Research Laboratories). Colonies (2,000) from each library were picked onto Luria broth (24) supplemented with ampicillin (100 μg/ml) in 96-well microtiter dishes and used for screening.

Screening of the cosmid libraries. The DNA from individual clones was transferred to Nytran nylon membranes (24), and the filters were prehybridized at 65°C for 2 h in 5× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate)–5× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–100 μg of denatured calf thymus DNA per ml. Following prehybridization, the filters were incubated with probe P1, which had been end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase, for 16 h under the same conditions. The filters were washed three times (10 min each wash) with 2× SSC–0.2% SDS at room tem-

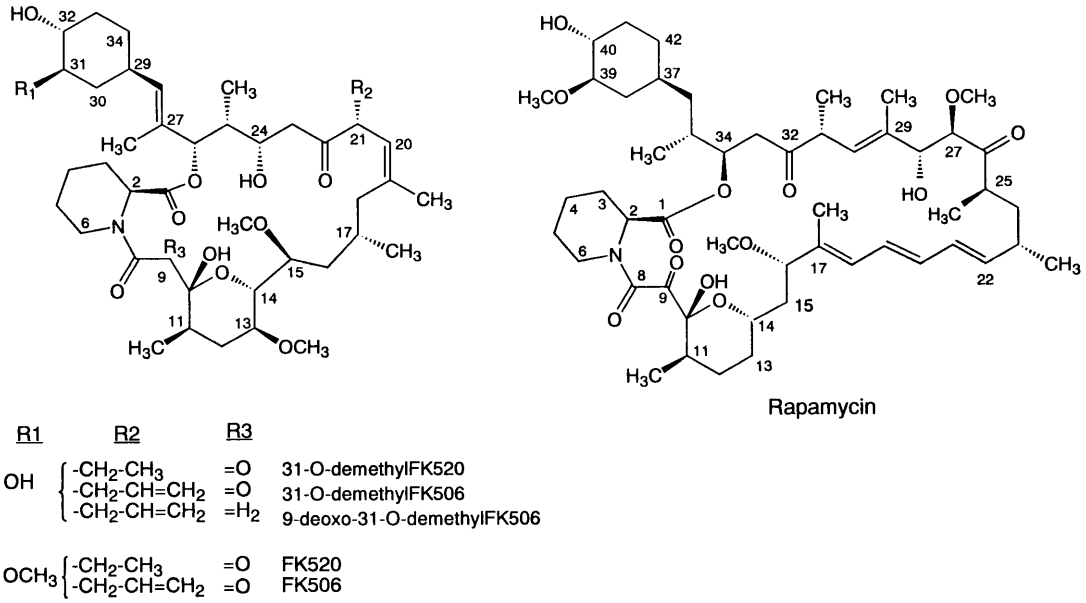

FIG. 1. Structures of FK506 and FK520 and their derivatives and structure of rapamycin.

perature and subsequently three times (10 min each wash) with 1× SSC–0.2% SDS at 65°C and finally once (30 min) with 0.1× SSC–0.1% SDS at 65°C.

Nucleotide sequence analysis. Nucleotide sequencing was carried out by the dideoxy method of Sanger et al. (25) on the denatured plasmid templates using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) and α-^{35}S-dATP as the radiolabeled nucleotide. Both strands were sequenced without ambiguities. Comparison of the deduced amino acid sequences with the GenBank database was done by using the Genetics Computer Group program package, version 7.2 (5). Sequence alignments were carried out with GAP and PILEUP programs (5).

Heterologous expression. Cosmid clone 11F5, which contained the entire coding sequence of *fkbM* from *S. hygroscopicus* subsp. *ascomyceticus*, was partially digested with *Bam*HI, and a fragment larger than 25 kb was isolated and cloned into the *Bgl*II site of the low-copy-number *Streptomyces* plasmid pIJ943 (10), generating plasmid pHM15. Plasmid pHM15 and the parent plasmid pIJ943 were then transformed into protoplasts of *Streptomyces lividans*, and transformants were selected with thiostrepton (Sigma) as described previously (16). One transformant from each kind was grown in R5 broth (10), and their protein extracts were subjected to a partial enzyme purification as described by Shafiee et al. (27). The partially purified fractions were examined for methyltransferase activity by radio-thin-layer chromatography and high-performance liquid chromatography (HPLC)-based assays in which 31-*O*-demethyl-FK520 was used as a substrate according to procedures described previously (18).

Disruption of *fkbM*. Of the three FK506 or FK520 producers described in this communication, we were able to transform only *Streptomyces* sp. strain MA6548, which was used as the parental strain in our mutant construction experiments. The integration vector pIV23 was constructed by insertion of the 1.7-kb *Sma*I-*Bam*HI fragment (see Fig. 4B, lower map) into pVE1053 (8). Protoplasts of *Streptomyces* sp. strain MA6548 were transformed (16) with pIV23, and the integrant obtained was designated strain M23. M23 and a wild-type representative were grown in RSPB medium (17), and the products of their fermentation were extracted with ethyl acetate and then subjected to HPLC fractionation (27). The antifungal activity of each fraction was examined by employing a bioassay with *Aspergillus niger* ATCC 6275 as the indicator strain (17).

Disruption of *fkbD*. A 3.2-kb *Bam*HI-*Sma*I fragment (see Fig. 4B, lower map) was cloned into pVE1053 (8), generating integrative plasmid pIV38. Plasmid pIV38 was introduced into protoplasts of *Streptomyces* sp. strain MA6548, yielding the integrant M38. M38 was grown and treated as described for M23.

Nucleotide sequence accession number. The sequence data reported in this article have been submitted to GenBank and assigned accession no. U65940.

RESULTS

Isolation and sequence analysis of *fkbM* gene from *Streptomyces* sp. strain MA6548. The N-terminal amino acid sequence of the purified FKMT (27) provided information for the design of two nonoverlapping oligonucleotide probes, P1 and P2 (see Materials and Methods), one of which (P1) was used to screen a cosmid library of *Streptomyces* sp. strain MA6858; seven positive clones were obtained. Southern blot analysis of DNA from these clones digested with *Sma*I, *Sph*I, *Bgl*II, and *Bam*HI gave hybridizing fragments identical in size to those observed in genomic digests (Fig. 2A). Three of the smaller hybridizing DNA fragments were subcloned, and their restriction maps showed that they were overlapping (Fig. 2B); this helped to localize the relative position of the *fkbM* gene within a *Bgl*II-*Bam*HI fragment (Fig. 2B, sites 6 through 8). The nucleotide sequence of a 2.4-kb DNA segment including *fkbM* was determined. Analysis of this sequence revealed an open reading frame (ORF) coding for 260 amino acids (Fig. 3) beginning with GTG as the translation start codon (position 1359) and ending with TGA (position 2139). The GTG start codon is preceded by a probable ribosome-binding site (RBS), GGG GTG (nucleotide positions 1349 to 1353), with complementarity to the 3′ end of *S. lividans* 16S rRNA (1). At the 3′ end, we found a pair of inverted repeats of 21 nucleotides each, which can form a stem-and-loop structure (nucleotide positions 2144 to 2189; Fig. 3) typical of transcription terminator regions found in prokaryotes (23). The calculated molecular mass for the product of the *fkbM* gene is 28,180 Da, which is in good agreement with the apparent molecular mass of 30,000 Da reported for the purified FKMT (27).

Sequence analysis of the upstream region of the *fkbM* gene. Immediately upstream of the *fkbM* coding region we found another ORF, *fkbD*, from ATG, as the putative start site, at position 196 to TGA at position 1360 (Fig. 3). This ORF is preceded by the sequence GAAGGA, which may serve as an RBS for the *fkbD* gene. Translation of the *fkbD* gene would result in a polypeptide of 388 amino acids with a calculated molecular mass of 42,308 Da. The translation termination codon for *fkbD* overlaps by 2 nucleotides with the translation initiation codon for *fkbM*, and the putative RBS for *fkbM* is within the coding region of the *fkbD* gene. These are the properties of translationally coupled genes found in prokaryotes (30). The predicted amino acid sequence of *fkbD* revealed strong homology to cytochrome P-450 hydroxylases, especially to those from streptomycetes (12, 14, 21, 29). Di-

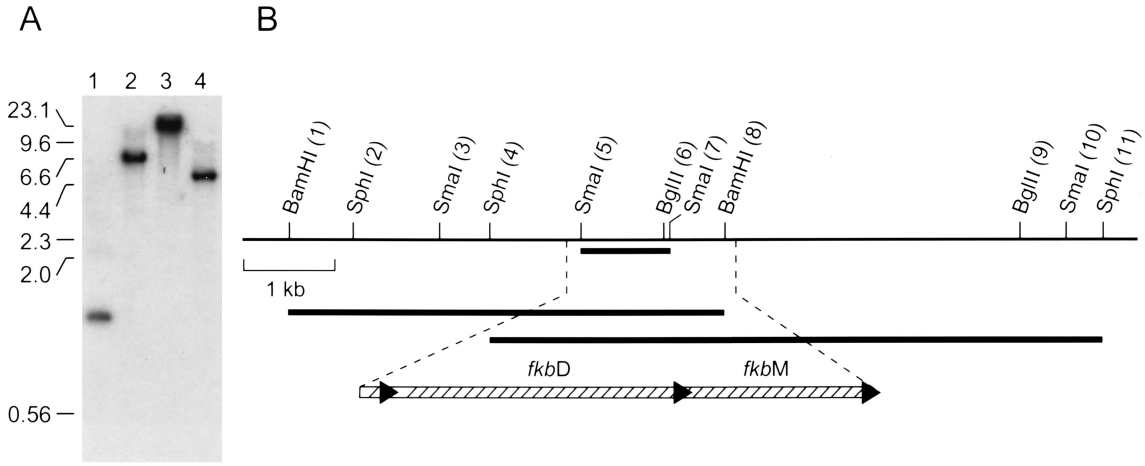

FIG. 2. Southern analysis and restriction map of the *fkbD* and *fkbM* regions from *Streptomyces* sp. strain MA6858. (A) Southern analysis of the chromosomal DNA from *Streptomyces* sp. strain MA6858 digested with *Sma*I (lane 1), *Sph*I (lane 2), *Bgl*II (lane 3), or *Bam*HI (lane 4) and probed with P1 primer. The sizes (in kilobases) of the λ-*Hin*dIII DNA markers are provided on the left. (B) Restriction map and gene organization of *fkbD* and *fkbM* regions. The fragments that were subcloned and used for restriction mapping and as templates for sequencing (black bars) and the direction and extent of each gene (arrowheads) are indicated. Probe P2 gave a pattern of hybridization similar to that of P1.

rectly upstream of *fkbD* and possibly translationally coupled to it lies the 3′ end of another ORF that is involved in the biosynthesis of the FK506 macrolactone ring (15).

Isolation and sequence analysis of *fkbM* and *fkbD* homologs. Since attempts to introduce plasmid DNA into *Streptomyces* sp. strain MA6858 were not successful, we directed our efforts to the isolation of the *fkbM* homologs from other *Streptomyces* species that produce FK506 and FK520 with the hope of finding a transformable species. Consequently, a 0.7-kb *Bam*HI-*Bgl*II fragment that contains most of the *fkbM* gene from *Streptomyces* sp. strain MA6858 was used as a hybridization probe to screen genomic digests of *S. hygroscopicus* subsp. *ascomyceticus*, an FK520 producer, and *Streptomyces* sp. strain MA6548, an FK506 producer. The results of the Southern blot are shown in Fig. 4A. The probe hybridized to single *Bam*HI and *Sma*I fragments of *S. hygroscopicus* subsp. *ascomyceticus* (Fig. 4A, lanes 1 and 2) and *Streptomyces* sp. strain MA6548 (lanes 3 and 4) DNAs. The *fkbM* and *fkbD* homologs were then isolated from the cosmid libraries of the two aformentioned species (Fig. 4B) and sequenced.

Heterologous expression of *fkbM* in *S. lividans*. Plasmid pHM15, which carries a DNA fragment encompassing the entire *fkbM* coding region from *S. hygroscopicus* subsp. *ascomyceticus* (Fig. 4B, upper map), was introduced into *S. lividans*. A cell extract from a resulting transformant was subjected to partial enzyme purification (3). A transformant containing pIJ943 (10), the parent vector of pHM15, was used as a control. Conversion of 31-*O*-demethyl-FK520 to FK520 was observed only with the enzyme from *S. lividans*(pHM15). Similar results were obtained when 31-*O*-demethyl-FK506 was used as a substrate, i.e., FK506 was produced, implying that the expressed enzyme does not discriminate between the two substrates.

Disruption of the *fkbM* gene. Insertion of the integration vector pIV23 into the chromosome of *Streptomyces* sp. strain MA6548 resulted in generation of strain M23, in which *fkbM* had been disrupted at the *Bam*HI site (Fig. 4B, lower map), 90 bp before the end of the gene. To investigate the phenotype of the integrant M23, ethyl acetate-extracted material from the cultures of both the wild type and strain M23 was subjected to HPLC fractionation, and the fractions were evaluated for antifungal activities. Fractions that inhibited the growth of *A. niger* ATCC 6275, an FK506-sensitive strain, were purified and characterized by nuclear magnetic resonance and mass spectroscopy (17). The results identified the products as 31-*O*-demethyl-FK506 and FK506 (Fig. 1) for M23 and the wild-type *Streptomyces* sp. strain MA6548, respectively (17).

Disruption of the *fkbD* gene. Sequence analysis established that the junction regions between *fkbM* and *fkbD* possessed properties of translationally coupled cistrons. To test whether the two genes are within a single transcript as well as to determine the role of *fkbD* in the biosynthesis of FK506 and FK520, we inactivated *fkbD* through targeted gene disruption. The integrative vector pIV38 was inserted into the chromosome of *Streptomyces* sp. strain MA6548 via homologous recombination to produce strain M38. From this integration event, a disruption within the *fkbD* coding sequence at the *Sma*I site would be predicted (Fig. 4B). To elucidate the phenotype of the *fkbD* integrant, an extract of a culture of M38 was treated as described for M23. An active fraction which was shown by mass and nuclear magnetic resonance spectroscopy (17) to contain 9-deoxo-31-*O*-demethyl-FK506 (Fig. 1), suggesting that *fkbD* encodes the cytochrome P-450 9-deoxo-FK506 hydroxylase, was found.

DISCUSSION

Data reported here establish the presence of two genes, *fkbM* and *fkbD*, in two FK506 producers and one FK520 producer. Heterologous expression and gene disruption demonstrated that *fkbM* encodes 31-*O*-demethyl-FK506 and/or FK520-*O*-methyltransferase, catalyzing O methylation at position 31 of the cyclohexyl ring of FK506 and FK520. On the basis of our hybridization data, *fkbM* DNA did not show sequence similarity to two other methyltransferase genes encoding the 13- and 15-*O*-methyltransferases involved in the biosynthesis of FK506 and FK520 (27). However, it exhibited good homology to the DNA of a rapamycin producer, *S. hygroscopicus* MA6434 (Fig. 4A, lanes 5 and 6). Rapamycin biosynthesis requires a methyltransferase to carry out O methylation at C-39 of the cyclohexyl ring, which is the equivalent of C-31 in FK506 (Fig. 1). Garrity et al. (7) used the same probe

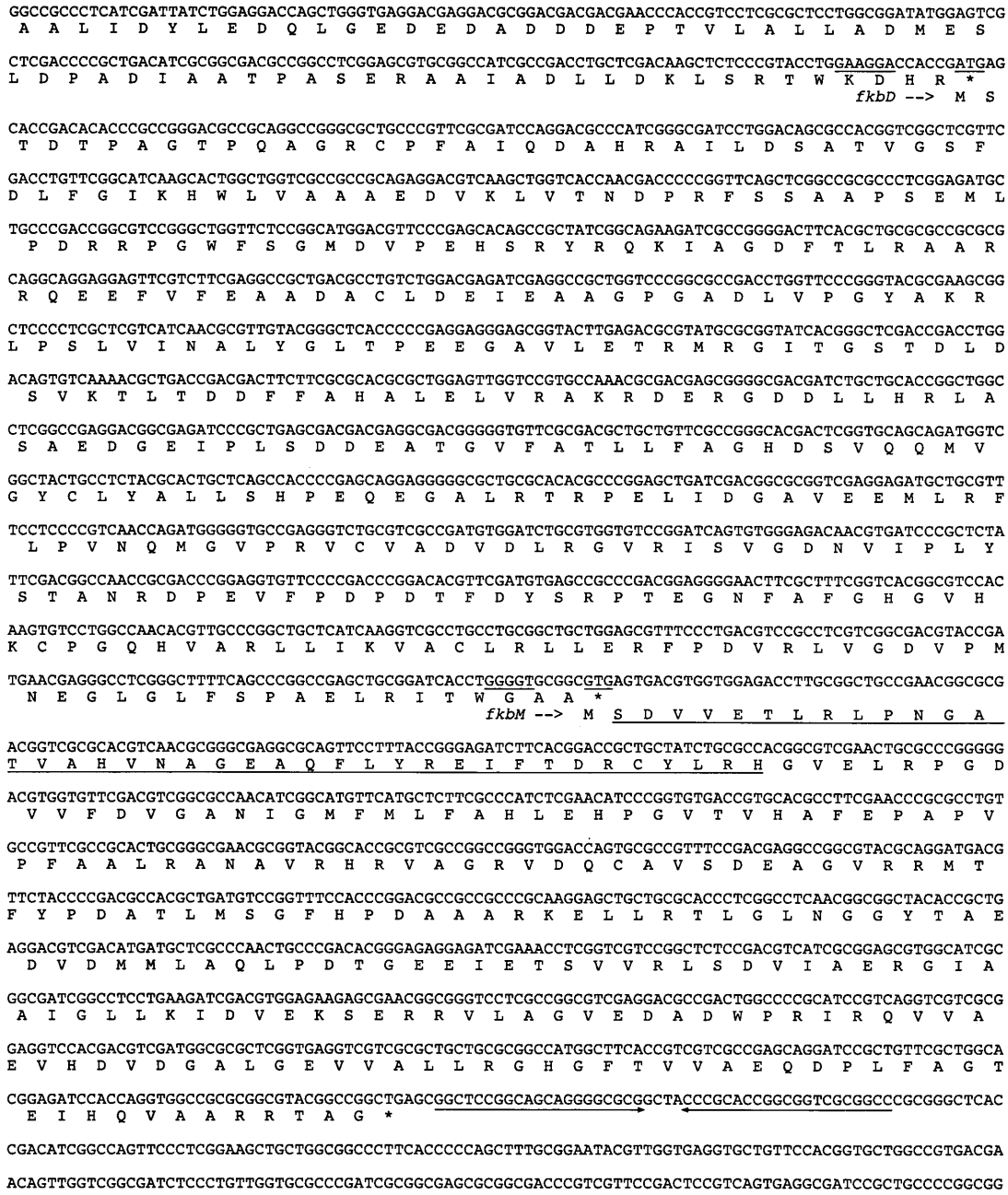

FIG. 3. Nucleotide and corresponding amino acid sequences of the *fkbD* and *fkbM* regions of *Streptomyces* sp. strain MA6858. Nucleotides are numbered beginning with the first sequenced nucleotide. Amino acids are numbered beginning with the N-terminal residue of each complete ORF. The amino acid sequence of the upstream incomplete ORF (negative numbers), the peptide sequence (39-mer) derived from the N-terminal amino acid sequence of the purified FKMT protein (27) (underlined amino acids), the putative RBSs and translation initiation codons (underlined), the positions of a pair of inverted repeats (convergently pointing arrows at the end of the *fkbM* gene), and the stop codons (asterisks) are indicated.

to study the genetic and phenotypic relationships among several actinomycetes that produce FK506 and its analogs as well as rapamycin. Their results indicated that all the species that produced these immunosuppressive agents hybridized to the *fkbM* probe.

Recently, a biosynthetic gene cluster for the immunosuppressant rapamycin, which is structurally related to FK506 (Fig. 1), has been reported (14, 26). Comparison of the *fkbM* sequence with the presumed rapamycin biosynthetic genes revealed 72% identity between the deduced amino acid sequences of *fkbM* and *rapI*, suggesting that *rapI* encodes 39-*O*-demethyl-rapamycin methyltransferase (Fig. 5). The role of *rapI* in the biosynthesis of rapamycin has not been determined previously (14, 26). Apart from the *rapI* gene product, FkbM protein did not show any significant matches to any other known proteins in the available databases. Likewise, *fkbM* did not exhibit any significant resemblance to nucleotide binding motifs described as the putative *S*-adenosylmethionine (SAM)-binding sites in other methyltransferases (11). This was not completely unexpected, since one of the three SAM-dependent

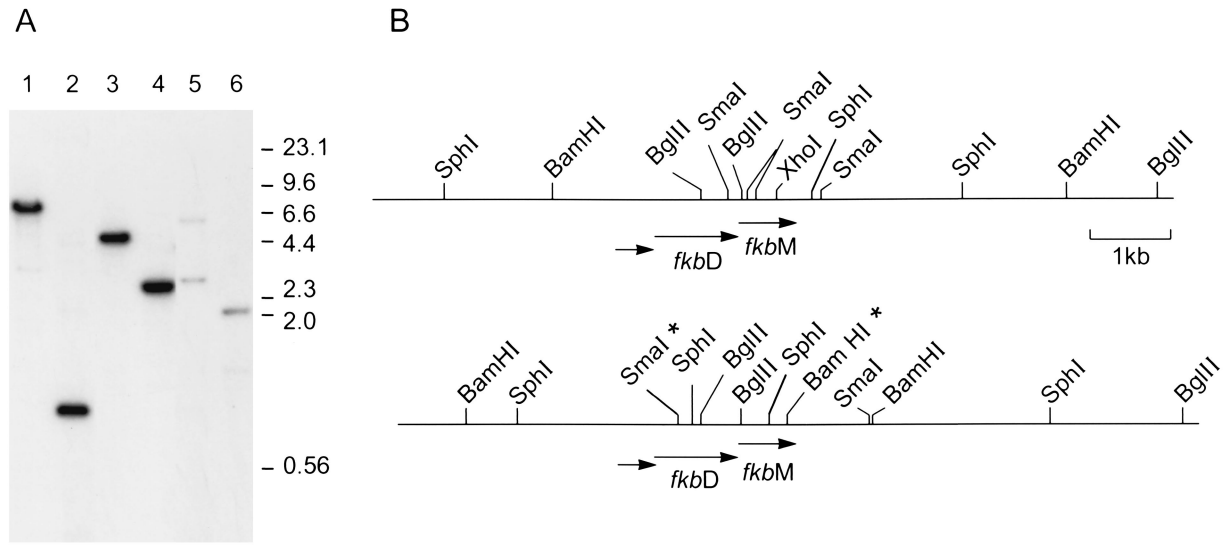

FIG. 4. Southern analysis and restriction map of *fkbD* and *fkbM* regions of *S. hygroscopicus* subsp. *ascomyceticus* and *Streptomyces* sp. strain MA6548 and Southern blot of *S. hygroscopicus* MA6434. (A) Genomic DNAs from *S. hygroscopicus* subsp. *ascomyceticus* (lanes 1 and 2), *Streptomyces* sp. strain MA6548 (lanes 3 and 4), and *S. hygroscopicus* MA6434 (lanes 5 and 6) were digested with *Bam*HI (lanes 1, 3, and 5) or *Sma*I (lanes 2, 4, and 6) and subjected to Southern analysis using the 0.7-kb *fkbM* probe. Fragment sizes (in kilobases) are indicated on the right. (B) Restriction map and gene organizations within FK520 (top) and FK506 (bottom) gene clusters of *S. hygroscopicus* MA6434 and *Streptomyces* sp. strain MA6548, respectively. The direction and extent of each gene (arrows) and the restriction sites at which mutations were introduced in *fkbD* (*Sma*I) and *fkbM* (*Bam*HI) (asterisks) are indicated.

methyltransferases involved in the biosynthesis of tetracenomycin C, encoded by *tcmP*, also lacks this motif (4, 28).

Targeted gene disruption established the function of *fkbD*, the second gene described in this paper, as a cytochrome P-450-9-deoxo-FK506 hydroxylase involved in hydroxylation at C-9 of the FK506 macrolactone ring. It is possible that the same hydroxylase catalyzes the subsequent oxidation of C-9 OH to generate the C-9-oxo derivative. A P-450 hydroxylase with dual function has been reported to be involved in cholesterol biosynthesis (6). However, the possibility of the involvement of an oxidase for oxidation of C-9 OH cannot be ruled out at this point. The presence of a gene, *rapK*, encoding a pteridin-dependent dioxygenase has been reported in the rapamycin biosynthetic pathway (14). Rapamycin, like FK506, has a keto group at position C-9 (Fig. 1). It is tempting to speculate that *rapK* carries out oxidation of the C-9 OH group.

9-Deoxo-31-*O*-demethyl-FK506, rather than 9-deoxo-FK506, is produced upon disruption of *fkbD*, indicating a polar effect on the expression of the downstream *fkbM* gene leading to the loss of methyltransferase activity. This result implies that the *fkbD* and *fkbM* genes are cotranscribed. The immunosuppressive activity of 31-*O*-demethyl-FK506 is about 10-fold less than that of FK506, and 9-deoxo-31-*O*-demethyl-FK506 is somewhat less active than 31-*O*-demethyl-FK506 (17). In a related study, Nishida et al. (20) reported that addition of metyrapone, a cytochrome P-450 inhibitor, to cultures of the rapamycin producer *Actinoplanes* sp. strain N902109 allowed them to isolate four rapamycin analogs, among which were 9-deoxo- and 9-deoxo-27-demethoxy-rapamycin. Their results suggest that enzymes responsible for hydroxylation at C-9 and C-27 of the rapamycin macrolactone ring are cytochrome P-450 hydroxylases (Fig. 1).

The deduced product of *fkbD* is very similar to the known cytochrome P-450 hydroxylases. The strongest similarity was to *rapJ* (74% identity) from the rapamycin gene cluster (14, 26), which, at 386 residues, is 2 amino acids shorter than its FK506 counterpart (Fig. 6). Therefore, by inference, the *rapJ* protein functions as the cytochrome P-450-9-deoxo-rapamycin hydroxylase. Like *rapI*, the function of *rapJ* in the biosynthesis of rapamycin has not been clarified (14). Besides the *rapJ* protein, sequences most similar to the *fkbD* gene product (with an overall identity of 30 to 34%) are those of SuI and SuII, the sulfonyl urea herbicide-induced P-450 hydroxylases from *Streptomyces griseolus* (21); ChoP, the P-450 hydroxylase from a *Streptomyces* sp. involved in cholesterol metabolism (12); and SoyB from *Streptomyces griseus*, involved in xenobiotic trans-

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fkbM VSDVVETLRLPNGATVAHVNAGEAQFLYREIFTDRCYLRHGVELRPGDVVFDVGANIGMFMLFAHLEHPGVTVHAFEPAPVPFAALRANAVRHRVAGRVD 100
     ||: |:|::|| | : ||||:|||||||:|||::|||||:|:|||:|||||||||||||| |||||| |:||||||||||||:|||||||  |: :||| :
rapI VSASVQTIKLPYGRPSAHVNPGEAQFLYQEIFAERCYLRRGLELRAGDVVFDVGANIGMFSLFAHLECPDVTVHAFEPAPVPYAALRANAERYAIAGRFE 100

fkbM QCAVSDEAGVRRMTFYPDATLMSGFHPDAAARKELLRTLGLNGGYTAEDVDMMLAQLPDTGEEIETSVVRLSDVIAERGIAAIGLLKIDVEKSERRVLAG 200
     |||||| ||  :||||:|:|:|||||||:|:| |||| |::|||||:||::|:|||:||||:: |||||||||||||||||::|||||||||||:|||:|:||
rapI QCAVSDVAGRGKMTFYTDTTMMSGFHPDPATRAELLRRLAINGGYSAEAADRMLAELPDTSQVIETSVVRLSDVIAERGITSIGLLKIDVEKNERHVMAG 200

fkbM VEDADWPRIRQVVAEVHDVDGALGEVVALLRGHGFTVVAEQDPLFAGTEIHQVAARRTAG*                                          260
     :::::||||||||||:|||||:|| |:||::|||||:|||||::||:|||||||:|:||:|||:::
rapI IDAGDWPRIRQVVTEVHDIDGRLDEVLTLLRGQGFTVLSEQEPLFAGTDIYQVVARRGDA*                                          260
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FIG. 5. Comparison of the predicted amino acid sequences of *fkbM* (from *Streptomyces* sp. strain MA6548) and *rapI* (26). Identity (bars) and similarity (double and single dots) are indicated according to the GAP program default settings (5).

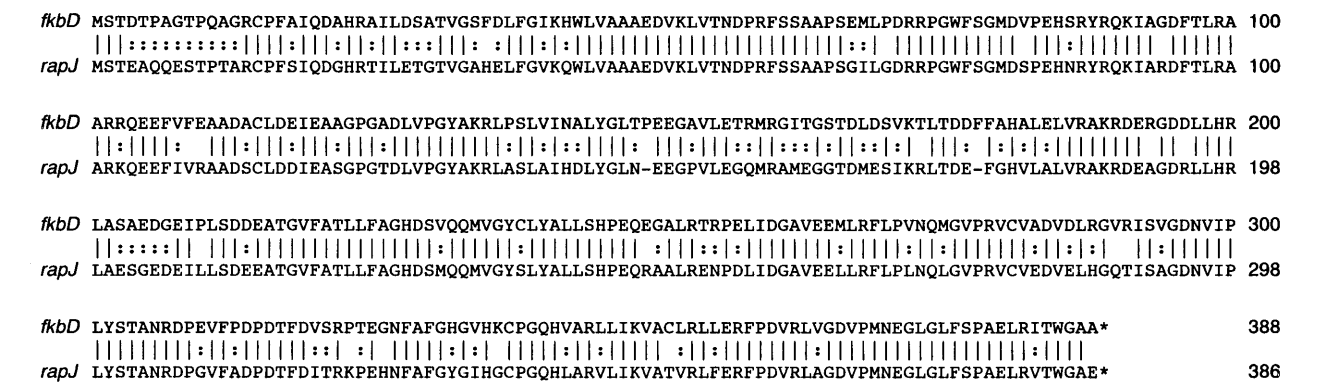

FIG. 6. Comparison of the deduced amino acid sequences of *fkbD* (from *Streptomyces* sp. strain MA6548) and *rapJ* (26) using the GAP program (5). Identity and similarity are indicated as in the legend to Fig. 5.

formation (29). In these cases, the homology is most distinct surrounding a cysteine residue near the C terminus which is located inside the heme-binding pocket of cytochrome P-450 hydroxylases.

The work presented here demonstrates that specific alteration of the FK506 biosynthetic genes results in the formation of analogs with predicted structures. This allows chemical derivatization of the generated metabolites, which could lead to the discovery of important antifungal agents and immunosuppressants with improved properties.

ACKNOWLEDGMENT

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