Reverse Transcriptase Sequencing of 16S Ribosomal RNA from *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta*, Three Wall Type IV Actinomycetes Which Lack Mycolic Acids

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The phylogenetic relationships of three mycolateless wall type IV actinomycetes, *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta*, were examined using reverse transcriptase sequencing of 16S ribosomal RNA. The sequences generated were aligned and the level of sequence homology calculated. The homology values were then used to produce a phylogenetic tree and to estimate S_{AB} values for the construction of a dendrogram. Both analyses show the three taxa to be closely related genera which form a distinct subdivision within the broader phylogenetic grouping defined by *Mycobacterium*, *Dactylosporangium* and their relatives.

INTRODUCTION

Actinomycetes which contain *meso*-diaminopimelic acid, arabinose and galactose in their cell envelope (wall type IV *sensu* Lechevalier & Lechevalier, 1970) can be divided into at least two groups based on the presence or absence of mycolic acids. Representatives of *Corynebacterium*, *Mycobacterium*, *Nocardia* and *Rhodococcus* all contain these compounds while the genera *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharopolyspora* and *Saccharomonospora* do not (Goodfellow & Cross, 1984). The latter mycolateless genera are morphologically and phenotypically diverse but can also be subdivided to some extent using chemotaxonomic characters (Lechevalier *et al.*, 1977, 1986; Kroppenstedt, 1985; Embley *et al.*, 1986, 1988).

Ribosomal RNA analyses are currently the most powerful methods available for determining phylogenetic relationships (Stackebrandt & Woese, 1981; Woese, 1987). rRNA cataloguing has been used to analyse the higher relationships among many actinomycetes and the distribution of chemotaxonomic markers has been shown to correlate with phylogeny to a high extent (Stackebrandt & Schleifer, 1984). The mycolateless wall type IV actinomycetes have not been included in cataloguing studies (Stackebrandt, 1986). Sequencing of 16S rRNA using reverse transcriptase (Qu *et al.*, 1983) and 'universal' primers (Lane *et al.*, 1985) has recently been introduced as an improved alternative to cataloguing. Using reverse transcriptase, approximately 75% of the 16S rRNA sequence can be determined routinely (Lane *et al.*, 1985), which compares favourably with the 40% obtained using the cataloguing procedure (Stackebrandt & Woese, 1981). This larger amount of sequence can also be analysed (Hori, 1975) to provide information on the relative speeds of RNA clocks for different organisms.

In the present study, 16S rRNA from three mycolateless wall type IV actinomycete strains was sequenced using the reverse transcriptase method. Since *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta* share many chemotaxonomic features (Lechevalier *et al.*, 1977; Kroppenstedt, 1985; Embley *et al.*, 1988) the possibility that they might form a distinct phylogenetic subgroup within the actinomycetes was therefore investigated.

METHODS

Strains and cultivation. The type strains of *Faenia rectivirgula* (ATCC 33515), *Pseudonocardia thermophila* (ATCC 19285) and *Saccharopolyspora hirsuta* (ATCC 27875) were grown to approximately early stationary phase in shake-flasks of nutrient broth (Difco) at 45 °C. Cells were harvested by centrifugation, washed with cold (4 °C) Tris/EDTA buffer (10 mM-Tris/HCl, 1 mM-EDTA, pH 7.2) and stored at -70 °C.

Extraction of cellular RNA. The method used was modified from that described by Johnson (1981). Approximately 5 g wet weight of cells was resuspended in 10 ml cold (4 °C) standard saline citrate (SSC, comprising 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0) and lysed by shaking with 45 g glass beads (0.10–0.11 mm, Braun) for 35 s in a cell homogenizer (Braun). The supernatant was extracted twice with SSC-saturated phenol, and total nucleic acids were precipitated with 2 vols absolute ethanol at -20 °C. The nucleic acid pellet was homogenized in 10 pellet volumes of cold (4 °C) 6 M-sodium acetate at pH 6.0 (Kirby, 1968) using an Ultra Turrax (Janke & Kunkel, Breisgau, FRG). The RNA was sedimented by centrifugation at 10000 g and the sodium acetate treatment repeated until the pellet was clear or only slightly turbid. The sodium acetate was decanted, the pellet redissolved in sterile distilled water and the RNA precipitated with ethanol at -20 °C. The precipitated RNA was then redissolved in sterile distilled water, the yield determined spectrophotometrically at 260 nm, and the solution diluted to give a final concentration of 3 µg µl⁻¹.

Reverse transcriptase sequencing of RNA. The method devised by Lane *et al.* (1985) was used to sequence 16S rRNA from the test strains. The nine primers used in the sequencing reactions included (in IUB nomenclature with *Escherichia coli* 3' position indicated): 1, CTGCTGCCTCCCGTA (357); 2, GTATTACCGCGGCTGCTG (536); 3, TCTACGCATTTACACGCTAC (704); 4, CTACCAGGGTATCTAAT (803); 5, CCGTCAATT-CATTTGAGTTT (926); 6, AGGGTTGCGCTCGTTG (1115); 7, CATTGTAGCACGTGTGTA (1241); 8, CGGTGTGTACAAGGCC (1401); 9, GGTTACCTTGTTACGACTT (1510). Primer sequences 1, 3 and 6 were originally devised by C. R. Woese (personal communication), 9 and 2 by Lane *et al.* (1985), 5 and 4 by G. E. Fox (personal communication), and 7 by J.S. and E.S. All of the primers used in the present investigation were synthesized by standard methods using an Applied Biosystems 3801A DNA synthesizer. In some sequencing reactions, masking bands were a problem and in order to resolve these ambiguities, terminal transferase was used to elongate prematurely terminated fragments (de Borde *et al.*, 1986). The products of all the sequencing reactions were separated using 55 cm wedge-shaped (0.2–0.6 mm) 6% (w/v) polyacrylamide denaturing (7 M-urea) gels (Chen & Seeburg, 1985). Two gels were routinely run for each reaction: a short run in which the bromophenol blue tracking dye was allowed to migrate 55 cm and a long run in which the xylene cyanol dye was allowed to migrate the same distance. All separations were performed using an LKB Macrophor 2010 sequencing apparatus operated at 50 W per gel.

Data analysis. The sequence data generated using the nine primers were aligned and homology values computed with the aid of the Beckman Microgenie program (Queen & Korn, 1984). Positions where a nucleotide could not be determined unambiguously (designated n) in two or three sequences were not included in these calculations. An ambiguous base in only one of the sequences reduced the length of comparable sequence for that strain by one base. Where homologous deletions occurred in two sequences, they were treated as a positive match. K_{nuc} values (nucleotide substitution rate or 'evolutionary distance values'; Hori, 1975) were calculated and a phylogenetic tree produced using the algorithm of Fitch & Margoliash (1967) contained in a program written by Felsenstein (1982) (PHYLIP version 2) for the IBM PC.

RESULTS AND DISCUSSION

The sequencing strategy used in the present investigation generated between 1263 and 1305 bases of 16S RNA sequence for *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta*, of which 1122 bases could be unambiguously aligned (Fig. 1). Table 1 shows the homology values calculated for these regions in the test strains and selected reference organisms (J.S. & E.S., unpublished results). K_{nuc} values are also shown in Table 1; these were used to construct a phylogenetic tree (Fig. 2). *Pseudonocardia thermophila*, *Saccharopolyspora hirsuta* and *Faenia rectivirgula* (for convenience hereafter referred to as the PSF group) are more closely related to each other than to the reference organisms (Fig. 2). The taxonomic rank of this relationship appears to be at the level of closely related genera as judged by the branching pattern of reference genera (Fig. 2). The phylogenetic coherence of the PSF group confirms the findings of previous taxonomic investigations which suggested that *Pseudonocardia thermophila*, *Saccharopolyspora hirsuta* and *Faenia rectivirgula* form a distinct centre of variation within the actinomycetes (Embley *et al.*, 1986, 1988). Thus, all three contain phosphatidylcholine (Lechevalier *et al.*, 1977; Kroppenstedt, 1985) and an unusual dimethyl-branched fatty acid

Fr	CUAAUACCGGAUAGGACAUU <u>U</u> GCCGAU
Sh	UGAGUGGCGAACGGGUGAGUAACACGUGGGUAAUCUGCCCGACACUCUGGGUAAAGCCUUGGAAACGGGGUCAAUACCGGAUAGACAGAAAGGCAU
Pt	CGCAU
Fr	ngUGGGGUGUUUGAAAGUUCGGCGGUAUGGGAAUn.GCUUGCGGCCUAUCAGCUnnUUGGUGGGGUAUAGGCCUACCAAGGGCAGCAGCGGUnGCCGGCCU
Sh	CUUUGUUUGGAAAGAUUCUGCGGGUACAGGAUnAGCCnCGCCGUAUCnGCUUnGUnUGGGGUGAUGGCCUACCAAGGGCAGCAGCGGUAUGCGGCCU
Pt	GUUCUGGUGUGGAAAGUUUUUUGCGGUGGGGAUG.GCCCGCGGCCUAUCAGCUnGUnUGGUGGGGUAUAGGCCUACCAAGGGCAGGUGAGGGUAGCGCGCCU
Fr	nAGAGGGUnACCGGnACACUGGGACUGAGACACGGCCUAGACUCCUACGGGAGnUAGCAGUGGGnAUUCnGCGCAUUGGGUGAAAGCCUnAUGCAGCA
Sh	GAGAGGGUnACCGGCAACACUGGGACUGAGACACGGCCUnGACUCCUACGGGAGCGCAGUGGGGAUUCUUGCGCAUUGGGCGAAAGCCUnACGCAGCA
Pt	GAGAGGGUGACCGGCGACACUGGGACUGAGACACGGCCUnGACUCCUACGGGAGCGCAGUGGGGAUUAUUGCGCAUUGGGCGAAAGCCUnACGCAGCG
Fr	ACGCGUGUGGGGnnAUGAAGGCUncGGGUnGUnAACnnUUU <u>1</u> ACCAUCCGACGAAG..CCUUUUG.GGGUnACGGUAGGUGGnnnAGAACGCCCGGCUAA
Sh	ACGCCGUGGGGGUAGACCGCUUCGGGUGUUnAACCUUUU <u>1</u> CGACUCCGACGAAGCCAUUUUGGUGGUGAGCGGUAGGUGUAGAACAGCACCGGCUAA
Pt	ACGChUGUGGGGGUAGAGCGUnnGGGUGUAAACnnUUU <u>1</u> CGACAGGACGAAG..CCUUGCG.GGGUACGGUAGCCUU <u>1</u> AGAAAGCACCGGCUAA
Fr	CUACGUGCCAGCACCGCCGGUAAUACGUAGGGUGCGAGCGUAGUCCGGAAUUUAUUGGGUGUAAAGAGCUGGUAGGCGGUGUUGCGGUGCGGCGUAAAA
Sh	UCACGUGCCAGCACCGCGGUAUAGUAGGUGGCGAGCGUUGUCCGGAAUUUAUUGGGCGUAAAGAGCUGGUAGGCGGUGUUGCGGUGUAGCGGUGUAAAA
Pt	CUACGUGCCAGCACCGCGGUAUAGCGUAGGGUGCGAGCGUUGUCCGGAAUUUAUUGGGCGUAAAGAGCUGGUAGGCGGUGUUGCGGUGCGGCGUAAAA
Fr	CCUACAGCUAAACUGGGGUGUGCGGUGCAUACGGGCA.GACUUGAGUUGCGGAGGGGAGACUGGAAUUCUGGUGUAGCGGUGAAUUGCGCAGAUUCA
Sh	CCGGGAGCUAAACUCCUnGCUUGCGGUGGUAUACGGGCA.GACUUGAGUUGCGGAGGGGAGACUGGAAUUCUGGUGUAGCGGUGAAUUGCGCAGAUUCA
Pt	CUUUCAGCUAAACUGGGGUGUGCGGUGCAUACGGGCAUACUAGAGUUGCGGAGGGGAGACUGGAAUUCAGGUGUAGCGGUGAAUUGCGCAGAUUCU
Fr	GGAGGAACACCGGUGGCGAAGGCGGGUCUCUGGGCGGAUACUGACGCGUGAGGAGCGAAAGCGUGGGUAGCGAAACAGGAUUAGAUACCCUnGUAGUCCAC
Sh	GGAGGAACnCCGGUGGCGAAGGCGGGUCUCUGGGCGGAUACUGACGCGUGAGGAGCGAAAGCGUGGGn.ACGCAACAGGAUUAGAUACCCUnGUAGUCCAC
Pt	UGGGAACnCCGGUGGCGAAGGCGGGUCUCUGGGCGGAUACUGACGCGUGAGGAGCGAAAGCGUGGGG.ACGCAACAGGAUUAGAUACCCUnGUAGUCCAC
Fr	GCCGUAACCGGUGGGCGUAGGUGUGGGnACUnGUUCCAGGCUUUCUGGCCUUAAGCUnACGCAUUAAGCGCCCGCCUnGGGAGUACGGCGCGAAGGCU
Sh	GCCGUAACCGUUGGGCGUAGGUGUGGGUUCACUGUUUUCUGGCCUUAAGCUnACGCAUUAAGCGCCCGCCUnGGGAGUACGGCGCGAAGGCU
Pt	GCCGUAACCGGUGGGCGUAGGUGUGGGGCUA.UUCCACGGGUCUCUGGGCGGACGUAACGCAUUAAGCGCCCGCCUnGGGAGUACGGCGCGAAGGCU
Fr	AAAAUCUAAAGGAUUGACGGGnCGnGCACAAAGCGGGGAGCAUGUGGAUUAUUCGAUGnAACCGGAAGAACCUUACCGUGGUGUAGCAU <u>2</u> ACUnGA
Sh	AAAAUCUAAAGGAUUGACGGGnCGnGCACAAAGCGGGGAGCAUGUGGAUUAUUCGAUGnAACCGGAAGAACCUUACCGUGGUGUAGCAU <u>2</u> ACUAGCA
Pt	AAAAUCUAAAGGAUUGACGGGnCGnGCACAAAGCGGGGAGCAUGUGGAUUAUUCGAUGnnCGCGAAGAACCUUACCGUGGUGUAGCAU <u>2</u> ACCGGA
Fr	UCCGCCUAGACAUAGCGGnCCCUngUnGCUngGACAGGUnGCUngGCUngAGCUnAGCUnGUGUGUGUAGCAUGUUGGGUAAAG
Sh	CAGCCCUAGACAGGCGGnCCCUngUnGCUngGUGUAGAGUngGCUngGCUngAGCUnAGCUnGUGUGUGUAGCAUGUUGGGUAAAG
Pt	CGCGUAGACAGUAGCGGnCCCUngUnGCUngGUGUAGAGUngGCUngGCUngAGCUnAGCUnGUGUGUGUAGCAUGUUGGGUAAAGACCCGCAUAGAGC
Fr	GACUUGCGGGGUnAACUUGGAGGAAGGUGGGGAGACGCUAAGUCAUCAUG
Sh	UGGGGAGUUGCGGGGUnAACUUGGAGGAAGGUGGGGAGACGCUAAGUCAUCAUG
Pt	GCAACCCUUGUCCAUUGUUGCCAGCAGCUUAGGUGGGGACUCUUGGGAGACUUGCGGGGUnAACUUGGAGGAAGGUGGGGAGACGCUAAGUCAUUAUG
Fr	CCCCUUAUGCCUAGGGCUGUACACAUUGCUACAAGUGCCGUGUACAGAGGGUGGCGAGACCGUGAGGUGGAGCGGAUUCGCCAGAAAGCCGGGnUCAGUUCGGA
Sh	CCCCUUAUGCCUAGGGCUGUACACAUUGCUACAAGUGCCGUGUACAGAGGGUGGCGAGAUUCGUGAGGUGUAGCGGAUUCGCCAGAAAGCCGGnUnAGUUCGGA
Pt	CCCCUUAUGCCUAGGGCUGUACACAUUGCUACAAGUGCCGAGUACnAGGGGUGCGAGACCGUGAGGUGGAGCGGAUUCGCCUnAAAGCUnGnnUCAGUUCGGA
Fr	UCGGGGUUCGACCCUnGCCCnGUGAAGUGCGAGUUGCGUAGUAAUCCGAGAUACAGAAUCCUGCGGUGAAUACGUUCCCGGGCCUUGUACACACCGCnCG
Sh	UCGGGGUUCGACCCUnGCCCnGUGAAGUGCGAGUUGCGUAGUAAUCCGAGAUACAGAAUCCUGCGGUGAAUACGUUCCCGGGU
Pt	UCGGGGUUCGACnUnGCCCCGUGAAGUUGGAGUUGCGUAGUAAUCCGAGAUACAGAAUCCUGCGGUG
Fr	UCACGUAUGAAAGUCGGUAAACACCCGAACCCAUUGGCCCAACCCCGUGUGGGGGA.GGGAGUnGUGCAAGGUGGGGACUnGCGGAUC
Sh	GUCUAUGAAAGCGUAAACACCCGAACCCAUUGGCCCAACC.CUUGUGGG...GGGAGUnGUGCAAGGUGGGGACUnGCGGAUC
Pt	GUCUAUGAAAGUUGGUAAACACCCGAACCCAUUGGCCCAACCCUCUUGGGGGA.GGGAGUGUGCAAGGUGGGGACCGGCGAUC

Fig. 1. Alignment of 16S rRNA partial sequences from *Faenia rectivirgula* (Fr), *Saccharopolyspora hirsuta* (Sh) and *Pseudonocardia thermophila* (Pt). The first nucleotide in the *S. hirsuta* sequence and the last nucleotide in the sequences of all three strains are analogous to positions 98 and 1485 (IUB numbering) of the *E. coli* 16S rRNA sequence (Brosius *et al.*, 1978), respectively. Boxed sequences indicate highly variable regions that may serve as suitable target sites for oligonucleotide probes. The 5' nucleotides of the regions are analogous to positions 440 (box 1) and 997 (box 2) of the *E. coli* sequence.

(Embley *et al.*, 1987, 1988), and they form a tight cluster when their fatty acid composition is compared by principal components analysis (Embley *et al.*, 1988). Differences in menaquinone composition (Collins *et al.*, 1977; Kroppenstedt, 1985; Embley *et al.*, 1986), morphology (Dorokhova *et al.*, 1969; Henssen & Schäfer, 1971; Lacey & Goodfellow, 1975) and many phenotypic features (Lacey & Goodfellow, 1975; Goodfellow & Pirouz, 1982) support their classification as distinct genera. The separation of the PSF group from wall type IV taxa which contain mycolic acids and from *Dactyloporangium aurantiacum* is also strongly supported by available chemotaxonomic data (Minnikin & Goodfellow, 1981; Collins *et al.*, 1982; Kroppenstedt, 1985). Clustering of the wall-type IV actinomycete *Amycolata autotrophica* with *Dactyloporangium aurantiacum*, which has a wall chemotype II (Fig. 2) may be clarified as more actinomycete rRNA sequences become available for comparison. *Amycolata autotrophica* shares a number of chemotaxonomic characters with *Pseudonocardia thermophila* and relatives but can be distinguished by its fatty acid composition and polar lipid profile (Lechevalier *et al.*, 1986; T.M.E., unpublished data).

The limited number of complete or nearly complete 16S RNA sequences determined in actinomycetes makes it impossible to directly ascertain the position of the PSF group relative to

Fig. 2. Unrooted phylogenetic tree showing the relationships between the test and reference strains, based upon K_{nuc} values contained in Table 1, lower left section.

Table 1. Comparison of phylogenetic parameters derived from 16S rRNA partial sequences and 16S rRNA catalogues of test and reference strains

The lower left section shows (upper rows) homology values based on the alignment of 1122 nucleotides and (lower rows) K_{nuc} values derived therefrom; the upper right section shows (upper rows) S_{AB} values, calculated from complete 16S rRNA catalogues and (lower rows) derived S_{AB} values (see text). The sequences of organisms 4–9 are derived from unpublished data of J. S. and E. S. The *B. subtilis* sequence was published by Green *et al.* (1985). ND, Not determined.

	1	2	3	4	5	6	7	8	9	10
1. <i>Faenia rectivirgula</i> ATCC 33515		ND 0.72	ND 0.65	ND 0.56	ND 0.54	ND 0.56	ND 0.53	ND 0.58	ND 0.49	ND 0.26
2. <i>Saccharopolyspora hirsuta</i> ATCC 27875	93.4 0.063		ND 0.60	ND 0.56	ND 0.51	ND 0.53	ND 0.55	ND 0.58	ND 0.47	ND 0.26
3. <i>Pseudonocardia thermophila</i> ATCC 19285	91.7 0.088	90.3 0.103		ND 0.60	ND 0.55	ND 0.55	ND 0.53	ND 0.58	ND 0.48	ND 0.28
4. <i>Amycolata autotrophica</i> DSM 535	88.6 0.124	88.9 0.121	90.1 0.106		ND 0.60	ND 0.60	ND 0.58	ND 0.64	ND 0.50	ND 0.25
5. <i>Dactylosporangium aurantiacum</i> DSM 43157	88.2 0.128	87.0 0.143	88.4 0.125	90.0 0.108		0.62 0.57	0.62 0.55	0.60 0.60	0.50 0.49	0.30 0.26
6. <i>Mycobacterium phlei</i> TMC 1516	88.6 0.124	87.7 0.134	88.4 0.126	90.6 0.100	89.1 0.118		0.70 0.60	0.65 0.68	0.55 0.53	0.30 0.26
7. <i>Nocardia asteroides</i> DSM 43005	87.7 0.134	88.4 0.125	87.7 0.134	89.4 0.115	88.4 0.126	90.1 0.106		0.69 0.72	0.56 0.49	0.33 0.25
8. <i>Rhodococcus erythropolis</i> DSM 43188	89.6 0.112	89.5 0.114	89.3 0.115	91.2 0.094	90.2 0.105	92.2 0.082	93.4 0.068		0.56 0.58	0.31 0.28
9. <i>Corynebacterium glutamicum</i> DSM 20300	86.4 0.150	85.8 0.157	86.1 0.154	86.7 0.147	86.4 0.150	87.8 0.132	86.6 0.148	89.4 0.114		0.31 0.25
10. <i>Bacillus subtilis</i>	76.4 0.283	76.7 0.280	77.8 0.264	75.9 0.291	76.7 0.280	76.5 0.282	75.9 0.290	77.8 0.264	76.1 0.288	

the major actinomycete groupings as defined by S_{AB} (binary association coefficient; Fox *et al.*, 1977) values. The fact that the sequences generated by reverse transcriptase are seldom complete means that RNAse T1 resistant catalogues, upon which the published dendrograms are based, cannot be extracted. Woese (1987) has published a calibration plot which expresses the correlation between homology values obtained for full 16S rRNA sequences and S_{AB} values.

Fig. 3. Dendrogram showing the relationships between the test and reference strains, based upon the derived S_{AB} values shown in Table 1, upper right section.

Table 1 contains S_{AB} values which were derived from this calibration plot and our partial (73% of the length of the *E. coli* sequence) sequence data. S_{AB} values produced from cataloguing data for the same strain are included where available and, as possibly expected, there is incomplete agreement between the values (Table 1). In most cases, the derived values are lower by up to 0.1 S than the S_{AB} values obtained by experiment. Better congruence might be obtained if the calibration curve were based upon more data and included more actinomycete sequences. A dendrogram constructed from the derived S_{AB} values using average linkage clustering is presented in Fig. 3. It is encouraging that, despite the discrepancies mentioned above, the branching order (but not necessarily the branch length) obtained for the reference organisms, i.e. members of *Dactylosporangium*, *Nocardia*, *Rhodococcus*, *Mycobacterium* and *Corynebacterium*, is the same as that obtained using published S_{AB} values (Stackebrandt & Woese, 1981; Stackebrandt, 1986). This confirms that *Faenia rectivirgula*, *Pseudonocardia thermophila* and *Saccharopolyspora hirsuta* form a distinct subline within the broader grouping which comprises *Mycobacterium*, *Dactylosporangium* and their relatives (Stackebrandt, 1986).

An advantage of using reverse transcriptase is that it provides sequences from the variable regions of the 16S RNA molecule. These domains may show considerable sequence diversity between closely related species (Woese, 1987) and can therefore be used as target sites for taxon-specific probes which can be used for rapid identification, as already demonstrated for *Mycoplasma* species (Göbel *et al.*, 1987). The biotechnological importance of certain members of the wall type IV actinomycetes as producers of antibiotics suggests that the development of probes for use in industrial screening programs is desirable. From our data we suggest that the most appropriate target sites for probe hybridization are those indicated in Fig. 1.

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