Jour. Microbiol. June 1996 p. 184-191

Vol. 34, No. 2

Lipid Analysis of Streptomycetes Isolated from Volcanic Soil

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(Received January 10, 1996/Accepted April 5, 1996)

The cellular fatty acids and quinones of streptomycetes isolated from volcanic soils were analysed. The strains contained fatty acids of 14 to 17 carbon chains, and 12-methyltetradecanoic acid and 14-methylpentadecanoic acid were dominant in most strains. The total profiles consisted of 74% branched fatty acid family, 16.8% linear family and 8.2% unsaturated family. The largest cluster of grey spore masses defined by numerical classification was separated from the remainders in the principal component analysis, but the other clusters were overlapped with one another. In the analysis of respiratory quinones, all of the strains contained either the menaquinone of 9 isoprene units with 6 hydrogenations or 8 hydrogenations as the major species. The distribution of menaquinones among the clusters could provide an important key in the chemotaxonomy of streptomycetes.

Key words: Fatty acids, streptomycetes, numerical classification, principal component analysis, menaquinone

Fatty acids are one of the most important building blocks of cellular materials, and in bacteria they occur in the cell membranes as the acyl constituents of the phospholipids (12). The straight-chain family is commonly found in bacteria, and constitutes a major proportion of the membrane in many bacterial groups. The branched-chain family, which includes *iso*-, *anteiso*-, and ω-alicyclic fatty acids with or without substitution (unsaturation and hydroxylation), is not so common as the straight-chain family, but is still very significant in bacterial taxonomy as well as their function in maintaining membrane fluidity (2, 12, 16, 20). In actinomycetes, the branched chain family occurs as a major family in many cases, and plays an important role as a chemical marker for discrimination of genera and species (12, 21). The third family, the unsaturated fatty acids, are found in minor amount, but are important in the bacterial groups having straight-chain fatty acids for the membrane fluidity (24).

Saddler and his colleagues (21) analyzed fatty acid profiles of streptomycete strains which had been previously studied in numerical ways, and compared them together their morphology and pigmentation properties.

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Isoprenoid quinones are a class of terpenoid lipids located in the plasma membranes of many bacteria, and play an important role in electron transport, oxidative phosphorylation, and possibly active transport (5, 10). The majority of the aerobic and facultatively anaerobic Gram-positive bacteria including actinomycetes produces menaquinones, and in streptomycetes menaquinones having 9 isoprenyl units with multiple saturation are mainly found. From the distribution of the quinone species among the prokaryotes, it appears that menaquinones have far greater discriminatory value than ubiquinones. In actinomycetes that contain menaquinones only, variations in isoprenyl side chains occur, which is of significant value in taxonomic view (4).

In the present study the streptomycetes which were isolated from soil and were numerically classified, were examined for their fatty acid profiles and menaquinone composition, together with their morphological characteristics to examine the significance of each tool in the classification of streptomycetes.

Materials and Methods

Isolation of streptomycetes

Soil samples were taken and treated as described by

Labeda (14). The samples were heat-dried and diluted to inoculate on starch casein agar plates (13) supplemented with cycloheximide and nystatin (50 g/ml each). Single colonies were picked and subcultured repeatedly on ISP 2 medium (23). And suspensions of spores and mycelial fragments were kept in glycerol (20%, v/v) at -4°C.

Morphology and pigmentation

Spore mass and substrate mycelium pigmentation as well as soluble pigment production were determined on ISP 3 agar plates whereas spore chain morphology was observed on the coverslips grown on ISP 4 medium. The morphological categories were described by Williams *et al.* (25).

Fatty acid analysis

Biomasses were obtained from liquid cultures in Sauton's broth medium (Difco) at 28°C for 2 days, freeze dried, and kept refrigerated for further analysis. Extraction of fatty acids as their methyl esters were performed by alkaline methanolysis (19). Fatty acid methyl esters were separated by HP-1 capillary column (530 mm I.D., 30 m length, 2.65 μm film) in HP 5809A gas chromatograph (Hewlett Packard) equiped with flame ionization detector. The temperature was programed to hold at 170°C for 1 min, then to rise by 5°C/min. Injector temperature was held at 250°C , and detector at 300°C. The peaks were identified by VG Quattro mass spectrometer (Fisons Instruments). The data was normalized, and multivariate analysis and cluster analysis were performed using MVSP 2.0 (Multivariate Statistical Package, Kovach Computing Services).

Quinone analysis

Some 30~40 mg of dried cells was treated with chloroform/methanol (2 : 1, v/v) by shaking overnight. The extracts were concentrated *in vacuo* using electric aspirator, and the cell debris was removed by centrifugation. The solvents were evaporated completely, and the remnant was resuspended in hexane. High performance liquid chromatographic separation of the quinones was done with ODS Hypersil column (200×4.6 mm, particle size 5 μm, Hewlett Packard) and acetonitrile/tetrahydrofuran (70 : 30, v/v) as the mobile phase. The flow rate was 1 ml/min at 37°C, and the detector was a UV detector operated at 254 nm. The peak fractions were collected, evaporated, and then redissolved in hexane. Direct probe mass spectrometric analysis was performed to identify each quinone peak, in which the analysis was performed with electron impact ionization at 70 eV and probe temperature operated from 150 to 250°C by 10°C/min. Parental ion scanning was performed by tandem mass spectrometry where the select-

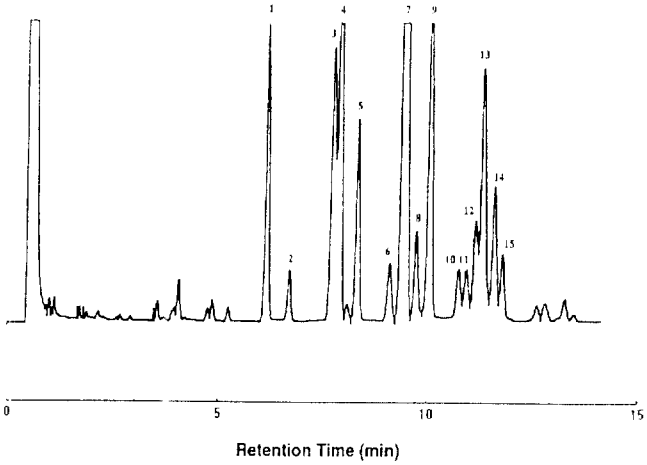


Fig. 1. Fatty acid profile of a test strain (SB201) as their methyl esters analysed by gas chromatography. The numbered peaks are *iso*-C14 : 0, *n*-C14 : 0, *iso*-C15 : 0, anteiso-C15 : 0, C15 : 0, C16 : 1, *iso*-C16 : 1, C16 : 1, n-C16 : 0, C17 : 1, C17 : 1, *iso*-C17 : 0, C17 : 1 and *n*-C17 : 0.

Table 1. List of clusters and their morphological properties

Cluster	#strains	Color of[1]				Diffusible pigment[1]		Spore chain[2]	
		Spore mass		Substrate mycelium					
1A	39	Grey	(90)[3]	Yellow-brown	(92)	Yellow-brown	(51)	*Retinaculiaperti*	(72)
1B	5	Blue	(60)	Yellow-brown	(100)	Yellow-brown	(100)	*Retinaculiaperti*	(80)
1C	8	White	(100)	Yellow-brown	(88)	Yellow-brown	(50)	*Retinaculiaperti*	(75)
3A	10	Ivory	(70)	Yellow-brown	(90)	Yellow-brown	(60)	*Rectiflexibiles*	(90)
3B	18	Grey	(56)	Yellow-brown	(100)	Yellow-brown	(89)	*Rectiflexibiles*	(100)
10	4	Grey/	white	Yellow-brown	(100)	Yellow-brown	(100)	*Retinaculiaperti*	(75)
20	4	Grey	(100)	Yellow-brown	(100)	Yellow-brown	(50)	*Rectiflexibiles*	(75)

[1] Determined on ISP 3 agar plate after growth at 28°C for 2 weeks.
[2] Determined on ISP 4 agar plate after growth at 28°C for 2 weeks.
[3] Numbers in parenthesis are the percentage of characters in the cluster.

Table 2. Fatty acid profiles of test strains

Strains	4.1	4.9	6.2[1]	6.8[1]	7.8[1]	8[1]	8.2	8.4[1]	9.2[1]	9.6[1]
1A										
SB180	0.5	1.1	12.3	1.6	9.3	26.5	0.3	5.6	0.8	25.1
SB182	0.5	0.9	12.4	1.9	9.7	24.1	0.3	6.2	0.8	25.1
SB223	0.9	0.0	2.4	0.9	12.8	17.4	2.3	13.9	1.1	9.4
SB234	0.5	1.3	7.3	1.4	4.8	29.8	0.5	8.3	0.9	20.0
SB244	0.7	2.2	10.4	1.3	1.6	34.0	0.4	9.2	1.0	21.6
SB271	3.0	0.0	4.8	0.0	15.8	22.7	0.0	7.5	0.0	24.2
SB305	0.2	0.2	8.2	1.3	6.8	9.8	0.1	10.6	0.9	31.4
SB675	0.4	0.3	7.0	1.6	9.2	19.1	0.3	6.7	0.7	24.0
S. parvulus	0.4	0.7	7.0	0.4	0.0	40.5	0.5	4.4	2.2	19.3
1B										
SB217	0.4	0.2	6.9	0.7	12.3	13.3	0.2	3.1	1.6	30.6
SB237	0.3	0.2	3.2	0.9	5.0	13.4	0.0	2.1	1.4	24.2
SB264	0.8	2.4	9.8	1.3	0.0	33.8	0.3	10.7	0.3	19.9
SB266	0.3	0.3	2.9	0.6	11.4	17.2	0.5	5.9	1.7	24.4
SB303	0.4	0.0	5.3	0.4	15.7	22.4	0.3	2.6	2.6	20.7
1C										
SB201	4.0	0.0	4.6	0.0	9.3	29.1	0.0	0.0	0.0	15.8
SB246	1.7	0.1	3.9	0.3	13.6	19.9	0.2	6.7	0.9	24.3
SB274	0.9	1.3	3.9	0.7	11.4	34.0	0.8	1.6	1.2	10.7
SB654	1.2	0.0	4.0	1.3	6.8	40.4	0.0	5.9	0.0	12.7
S. ptilosus	0.0	0.0	9.0	1.2	17.6	29.1	1.2	5.8	1.8	16.3
3A										
ASB186	2.1	0.0	7.6	0.0	11.6	25.3	0.0	8.1	0.0	27.1
SB222	0.9	0.0	5.3	0.9	6.9	14.6	0.0	3.4	2.1	31.7
SB230	1.6	0.0	5.1	1.5	6.5	17.6	0.0	2.4	0.0	23.4
SB278	0.1	0.1	6.0	1.7	0.0	21.1	0.1	6.6	0.5	20.5
SB294	0.7	0.6	7.0	1.0	4.5	25.0	0.8	4.2	1.0	19.5
SB296	0.8	0.4	2.1	0.6	1.6	22.6	0.0	0.8	1.1	19.8
SB306	0.2	0.2	8.0	1.3	5.9	10.8	0.1	10.7	0.9	30.9
S. vanaceus	0.9	0.0	4.4	0.6	11.5	21.6	0.6	4.4	2.2	18.8
3B										
BSB198	2.0	0.0	4.9	0.9	10.2	27.4	0.0	2.1	0.0	16.4
SB277	0.0	0.0	2.1	0.0	5.5	13.4	0.0	1.6	2.1	29.7
SB290	0.5	0.6	5.0	1.1	0.0	30.8	0.6	1.8	4.9	17.4
SB659	0.0	0.0	13.0	0.0	5.2	17.3	0.0	6.9	2.2	32.7
SB672	1.0	0.0	4.5	0.0	2.7	25.6	1.0	1.7	1.2	21.3
S. atroolivaceus	0.6	1.1	8.2	0.9	15.3	23.7	0.6	5.0	1.7	15.7
S. kanamyceticus	1.2	0.3	6.8	0.3	7.7	23.9	0.3	2.3	1.8	19.6
10										
SB284-1	0.8	0.5	6.2	0.8	4.6	26.7	0.8	3.7	1.1	18.7
SB284-2	0.6	1.1	3.6	0.7	11.0	33.2	0.6	1.5	1.2	10.9
SB307	0.3	0.6	8.6	1.0	16.5	26.4	0.9	5.4	1.7	16.0
S. janthinus	0.9	0.0	2.2	1.3	5.1	52.4	0.0	3.7	0.0	10.1
20										
SB190	2.2	0.0	4.2	0.0	6.7	22.8	0.0	4.1	0.0	23.3
SB673-1	0.7	0.0	6.2	2.3	8.6	26.0	0.0	2.0	0.3	19.4
SB673-2	1.2	0.5	2.7	0.7	3.3	22.4	0.0	0.9	1.3	20.7
S. exfoliatus	1.6	0.5	11.1	1.3	8.8	41.7	0.1	3.7	4.5	52.7
Single-member cluster										
SB203-1	0.0	0.0	5.3	1.7	11.1	25.7	0.0	4.2	0.0	16.9
SB203-2	1.4	0.0	10.0	1.6	9.1	15.3	0.0	5.7	1.6	29.8
SB254-1	0.6	2.0	10.9	1.3	3.1	34.2	0.5	7.7	1.8	23.3

Table 2. Continued

Strains	4.1	4.9	6.2[1]	6.8[1]	7.8[1]	8[1]	8.2	8.4[1]	9.2[1]	9.6[1]
SB287	0.2	0.2	8.1	1.3	6.4	10.3	0.1	10.6	0.9	31.2
SB293	0.4	0.0	13.6	0.4	5.4	17.8	0.0	7.1	2.2	33.2
1A										
SB180	0.1	6.8	0.1	1.5	1.5	4.6	1.2	0.5	0.1	0.4
SB182	0.2	8.0	0.2	1.5	1.6	4.2	1.3	0.6	0.3	0.3
SB223	5.3	7.7	3.6	2.4	4.4	12.2	0.0	3.6	0.0	0.0
SB234	0.6	8.8	0.7	1.8	1.5	7.0	2.4	0.9	0.5	1.0
SB244	0.2	6.5	0.2	1.2	0.8	5.5	1.6	0.9	0.2	0.6
SB271	0.0	9.0	0.0	0.0	4.6	8.4	0.0	0.0	0.0	0.0
SB305	1.7	14.6	0.8	0.7	2.6	5.3	0.8	3.4	0.3	0.4
SB675	1.1	14.0	0.7	0.8	2.8	7.1	1.8	1.9	0.2	0.5
S. parvulus	0.7	3.7	1.9	2.5	2.9	10.0	0.8	0.9	0.4	0.8
1B										
SB217	0.9	10.1	1.5	1.2	5.8	7.4	1.1	1.5	0.4	0.6
SB237	3.6	12.5	3.8	3.2	4.6	14.3	2.9	1.8	1.1	1.5
SB264	0.3	7.5	0.3	1.1	1.1	6.4	2.0	0.9	0.4	0.7
SB266	1.0	9.7	2.0	1.9	5.3	10.1	1.3	2.5	0.4	0.7
SB303	0.8	5.3	1.6	2.1	6.2	10.8	0.9	1.2	0.3	0.4
1C										
SB201	4.2	13.1	0.0	3.7	0.0	9.9	6.3	0.0	0.0	0.0
SB246	0.5	9.3	1.0	0.9	5.0	9.2	0.7	1.3	0.2	0.3
SB274	0.7	5.9	1.0	1.4	7.8	13.3	1.8	0.5	0.0	1.0
SB654	0.0	7.5	0.0	0.6	1.4	16.0	0.8	1.3	0.0	0.0
S. pilosus	0.0	5.5	0.0	1.5	2.6	8.3	0.0	0.0	0.0	0.0
3A										
SB186	0.0	7.2	0.0	0.0	3.2	7.8	0.0	0.0	0.0	0.0
SB222	1.7	14.4	1.3	1.6	3.1	8.3	1.6	1.6	0.0	0.7
SB230	3.1	13.0	3.1	2.4	3.8	11.1	2.6	1.5	0.0	1.1
SB278	0.9	22.6	0.5	0.8	2.7	8.0	0.7	6.1	0.0	0.9
SB294	0.0	11.4	0.0	3.7	0.9	9.6	5.2	0.8	1.0	3.1
SB296	3.8	9.7	2.3	3.8	1.0	20.1	4.5	0.5	1.6	2.5
SB306	1.7	14.7	0.9	0.7	2.6	5.5	0.8	3.5	0.3	0.4
S. vanaceus	0.9	8.7	2.1	2.1	5.6	10.3	2.0	2.2	0.0	1.0
3B										
SB198	2.1	13.0	1.0	3.0	1.5	10.0	5.5	0.0	0.0	0.0
SB277	2.9	7.4	3.0	2.8	6.4	21.6	0.0	1.3	0.0	0.0
SB290	9.2	9.6	2.2	4.0	1.0	6.3	3.7	0.4	0.6	0.4
SB659	0.0	3.5	0.0	9.5	0.0	6.9	2.9	0.0	0.0	0.0
SB672	0.0	13.1	0.0	2.4	1.4	16.5	4.2	1.0	0.0	2.4
S. atroolivaceus	0.9	6.3	1.3	2.1	3.2	10.0	1.2	1.4	0.3	0.6
S. kanamyceticus	0.7	10.1	0.7	3.6	2.0	10.0	5.8	0.4	0.7	2.1
10										
SB284-1	0.0	9.7	0.1	4.3	1.1	10.9	4.8	0.7	1.1	3.3
SB284-2	0.8	6.0	1.1	1.6	8.3	14.1	2.0	0.6	0.0	1.1
SB307	0.5	5.9	0.6	1.8	2.9	9.2	0.6	0.7	0.1	0.3
S. janthinus	0.0	5.6	0.0	0.5	1.2	15.7	0.5	0.7	0.0	0.0
22										
SB190	1.7	8.3	2.4	2.3	4.9	14.8	0.0	2.3	0.0	0.0
SB673-1	1.9	12.6	2.2	2.3	2.9	7.2	4.5	0.0	0.4	0.4
SB673-2	4.2	8.7	2.1	3.9	2.1	16.7	4.4	0.5	1.5	2.4
S. exfoliatus	7.0	19.6	3.2	5.3	5.2	27.8	1.3	1.8	1.8	0.7
Single-member cluster										
SB203-1	0.0	13.0	2.1	2.3	2.9	10.1	4.7	0.0	0.0	0.0

Table 2. Continued

Strains	4.1	4.9	6.2[1]	6.8[1]	7.8[1]	8[1]	8.2	8.4[1]	9.2[1]	9.6[1]
SB203-2	2.3	8.9	1.1	1.1	2.1	5.8	2.9	1.3	0.0	0.0
SB254	0.0	5.6	0.0	1.4	0.5	4.5	1.3	0.8	0.0	0.5
SB287	1.7	14.6	0.8	0.7	2.6	5.4	0.8	3.5	0.3	0.4
SB293	0.0	3.5	0.4	7.9	0.0	5.6	2.1	0.0	0.0	0.4

[1]Numbers designate the retention times of *i*-C14 : 0, C14 : 0, *i*-C15 : 0, *ai*-C15 : 0, C15 : 0, C16 : 1, *i*-C16 : 0, C16 : 1, C16 : 0, C17 : 1, C 17 : 1, *i*-C17 : 0, *ai*-C17 : 0, C17 : 1, and C17 : 0, respectively.

ed ions were monitored.

Results

Morphological features

Eighty eight strains of 7 representative clusters defined by numerical classification were used in the analysis. They included 7 reference strains of streptomycetes: *S. parvulus* (cluster 1A), *S. pilosus* (cluster 1C), *S. vanaceus* (cluster 3A), *S. atroolivaceus* and *S. kanamyceticus* (cluster 3B), *S. janthinus* (cluster 10), and *S. exfoliatus* (cluster 20). Most of the test strains produced grey spores, yellow to brown substrate mycelia and yellowish brown diffusible pigment. The colors of spore masses provided high discriminatory value while the other pigmentation properties did little. The strains in the cluster 1A, 1B and 1C mostly produced *Retinaculiaperti* (RA) chains of spores, and the strains in cluster 3A and 3B produced *Rectiflexibiles* (RF) chains of spores.

Fatty acid profile

Whole organism methanolyzates of the test strains contained the fatty acids having 14 to 17 carbon chains, which are commonly found in prokaryotes (12), and a GC chromatogram is given in Fig. 1. Most of the strains had 12-methyltetradecanoic acid (*anteiso*-C15) and 14-methylpentadecanoic acid (iso-C16) as the major species (Table 2). Branched fatty acids were dominant in all cases, constituting 74% of the total fatty acids in average, and the linear ones 16.8%. The unsaturated fatty acids, mostly hexadecenoic acid isomers (C16 : 1) and heptadecenoic acid isomers (C17 : 1), accounted for 8.2% of the total.

Principal component analysis (8) was performed with the quantitative profiles of the test strains, and the scatter plot with the first two principal component axes showed complex relationship among test strains and clusters as shown in Fig. 2. The strains of grey spores (cluster 1A) were scattered in the upper side, and those of light colored spores (cluster 3A and 3B) were clustered in the lower side. The strains in the cluster 1B were scattered in a wide range, and so were the strains in the cluster

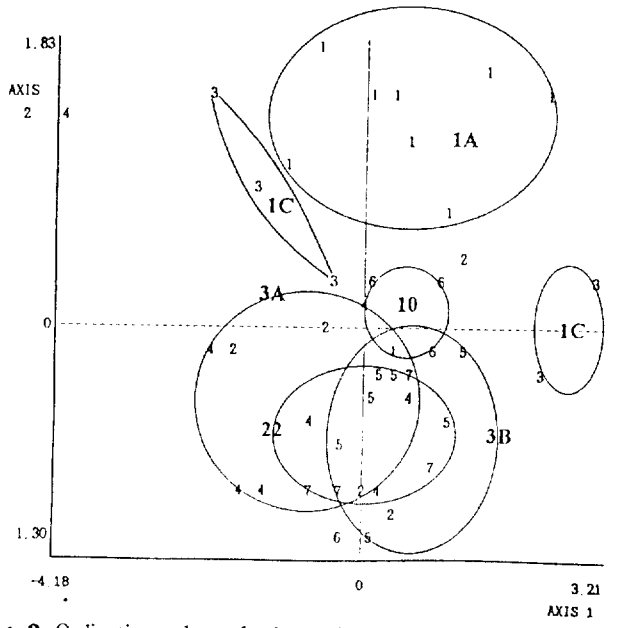


Fig. 2. Ordination plot of the principal components analysis results showing the relationship among the clusters (MVSP 2.0). The frist principal component axis explained 39.0% of the total variance, and the second axis 16.3%.

1C, thus showing that these clusters were not homogeneous. The members in the clusters 10 and 22 were recovered as separate groups each, and were close to cluster 3A and 3B. *S. vanaceus*, which belonged to cluster 3A, was far from all other strains. The first principal component axis accounted for 39.0% of total variance among the test strains, and the second axis explained 16.3%.

Menaquinone composition

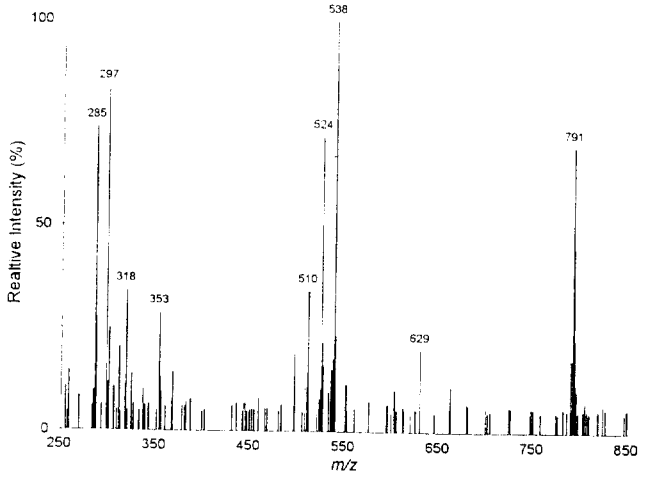
The menaquinone profiles were investigated in 17 representative strains from each cluster (Table 3). The mass spectra of the menaquinones always contained *m/z* 225 as the predominant peak. And from the molecular ion peaks the numbers of isoprene units and the degree of hydrogenation were calculated (Figure 3 and 4 and Table 3). In the parental ion scans, mass to charge ratio of 510, 524, 538, and 552 were always present in the spectra. These masses were possibly from the frag-

Table 3. Major menaquinone composition of some strains of the representitive clusters

Strains	Cluster	Molecular Masses[1]	Menaquinone species[2]
SB182	1A	793, 791	MK-9(H_8), MK-9(H_6)
SB249	1A	793, 791, 787	MK-9(H_8), MK-9(H_6), MK-9(H_2)
SB234	1A	793, 791	MK-9(H_8), MK-9(H_6)
SB180	1A	793, 791	MK-9(H_8), MK-9(H_6)
SB303	1B	793, 791, 789	MK-9(H_8), MK-9(H_6), MK-9(H_4)
SB266	1B	793, 791, 789	MK-9(H_8), MK-9(H_6), MK-9(H_4)
SB237	1B	793, 791	MK-9(H_8), MK-9(H_6)
SB310	1C	793	MK-9(H_8),
SB197	1C	791	MK-9(H_6)
SB201	1C	793, 791	MK-9(H_8), MK-9(H_6)
SB294	3A	791, 793, 789	MK-9(H_6), MK-9(H_8), MK-9(H_4)
SB222	3A	791, 793, 789	MK-9(H_6), MK-9(H_8), MK-9(H_4)
SB672	3B	791, 793, 789	MK-9(H_6), MK-9(H_8), MK-9(H_4)
SB659	3B	791, 793, 789,	MK-9(H_8), MK-9(H_6)
S. atrovaceus	3B	723	MK-9(H_6), MK-9(H_8), MK-9(H_4) MK-8(H_6)
SB284	10	793, 791, 789	MK-9(H_8), MK-9(H_6), MK-9(H_4)
SB190	22	793, 791, 789	MK-9(H_8), MK-9(H_6), MK-9(H_4)
		785, 723	MK-9, MK-8(H_6)

[1] Tandem mass spectrometric analysis of molecular ions was performed by parental ion selection of *m/z* 225.

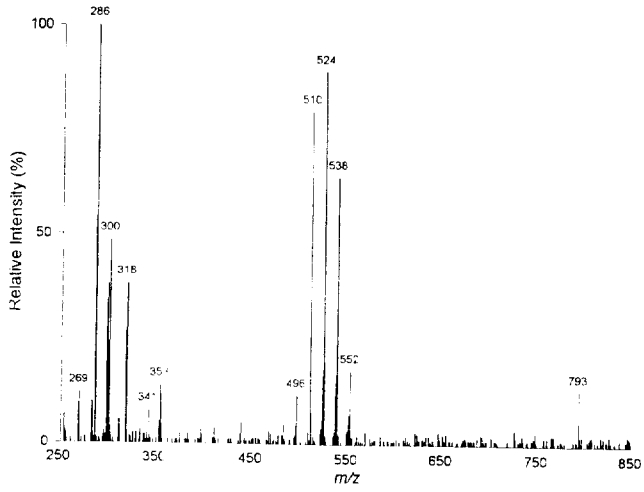
[2] Major species were put in order.



(a) MK-9(H_8), SB222

Fig. 3. A mass spectrum of the menaquinone SB222 by tandem mass spectrometry. Parental ions of *m/z* 225 were selectively recored, and the molecular ion peaks appeared as the dominant masses.

mentation of 6th isoprenyl unit, giving off the series of masses by the difference of 14-the mass of one carbon plus two hydrogens. Thus the point of saturation would be identical throughout all the tested strains, but the location of the point could not be determined by the mass spectra alone. All of the test strains had menaquinones



(b) MK-9(H_8), SB310

Fig. 4. A mass spectrum of the menaquinone from SB310 by tandem mass spectrometry.

of 9 isoprene units, designated as MK-9, with 1 to 4 double bonds saturated, MK-9(H_2)~MK-(H_8), of which the molecular masses were 787 to 793. MK-9(H_6) or MK-9(H_8) was dominant in all cases. The strains in cluster 1 had MK-9(H_8) as the major component in most cases whereas the strains in cluster 2 had MK-9(H_6) as the major component in most cases. The strains in subcluster 1A had MK-9(H_8) and MK-9(H_6) as the two major components, the strains in subcluster 1B had MK-9(H_4) as minor component in addition to MK-9(H_8) and MK-9(H_6), and the strains in subcluster 1C had either MK-9(H_8) or MK-9(H_6) as the major component. Most strains in subcluster 3A and 3B had MK-9(H_8) and MK-9(H_6), and an additional MK-9(H_4). The distribution of menaquinone species among the clusters implicated the possible potential of menaquinones as the marker for discrimination of clusters.

Discussion

The genus *Streptomyces* is a morphologically diverse group, forming characteristic structures and producing a variety of pigments, most of which are highly diagnostic (17, 18, 25). The numerical and chemical taxonomic studies with streptomycetes provided some criteria for their taxa with morphology and pigmentation, thus, the visual observation was important in the classification of streptomycetes (7, 11, 21, 22, 25). The morphology of spore chains, colors of spore masses, mycelia and diffusible pigments, are important keys in the characterization of the clusters defined by both numerical and chemical methods despite their possible variations according to the culture conditions or by mutations (15).

Fatty acid profiles in bacterial taxonomy were already proved useful with a wide range of bacterial groups (1, 3, 6, 21). The branched fatty acids were dominant in all cases, which was in good agreement with the previous studies on streptomycetes (17, 21). There was no characteristic fatty acid species for a specific group of test strains, rather quantitative differences and the presence or absence of a few fatty acids among strains were found. The principal component analysis showed the relative distances both among test strains and among groups which were defined by numerical classification, and the strains of common morphological features were gathered within a group in agreement with the numerical classification. The strains in the clusters 3A, 3B and 20, producing rectiflexibile spore chains, were close with each other, thus showing the importance of spore chain morphology as one of the key characters.

The quinone composition of some representative strains from major and minor clusters implied the taxonomic potential of menaquinones in streptomycetes. And the determination of double bond saturation in isoprenyl side chains would give a better discriminatory value. In general the distribution of MK-9 species with the saturation of 1 to 4 isoprene units gave as much valuable information as the relative composition of MK-9(H_6) and MK-9(H_8), in which altogether discrimination between the clusters defined by numerical classification could be made.

The tandem mass spectrometry proved to be a good tool for identifying the quinones by conveying informations about the point of double bond saturation. The degree of saturation probably affected the fragmentation of the molecules, so that the molecular ion peaks became relatively smaller as the degree of saturation became higher, while the fragmentation began to increase. The points of saturation were, as was in the previous reports, the second, third and eighth position in MK-9(H6), and the ninth in addition to the above positions in MK-9(H8). The location, however, can not be a decisive one if determined only by the mass spectra, and in order to better support the result, more data need to be collected, and some other additional information such as nuclear magnetic resonance spectroscopic analysis may be required.

The comparison between morphology and pigmentation properties and chemical data gave a good general correlation for each view. Molecular taxonomy, together with these, have a strong potential due to the fact that the genetic materials to be studied do not change in accordance with environmental or conditional variations, which has become one of the focuses for actinomycete taxonomy, providing new views with a number of methods (9). And performing a polyphasic taxonomy including numerical, chemical and molecular based works together will be a significant work.

Acknowledgement

This work was supported by a research grant for SRC (Research Center for Molecular Microbiology, Seoul National University) from the Korea Science and Engineering Foundation.

References

1. **Bousfield, I.J., G.L. Smith, T.R. Dando, and G. Hobbs,** 1983. Numerical analysis of total fatty acid profiles in the identification of coryneform, nocardioform and some other bacteria. *J. Gen. Microbiol.* **129**, 375-394.
2. **Brennan, P.J.,** 1988. *Mycobacterium* and other actinomycetes. p. 203-298. *In* Ratledge, C. and S.G. Wilkinson (eds.), Microbial Lipids. Vol. 1. Academic Press; New York.
3. **Brondz, J., I. Olsen, M. Haapasalo and A.J.V. Winkelhoff,** 1991. Multivariate analysis of fatty acid data from whole-cell methanolysates of *Prevotella, Bacteroides* and *Porphyromonas spp. J. Gen. Microbiol.* **137**, 1445-1452.
4. **Collins, M.D.,** 1994. Isoprenoid quinones. p.266-309. *In* Goodfellow, M. and A. G. O'Donnell (eds.), Chemical Methods in Prokaryotic Systematics. John Wiley and Sons; Chichester.
5. **Collins, M.D. and D. Jones,** 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Mircobiol. Rev.* **45**, 316-354.
6. **Couderc, F., D.D. Briel, N. Demont, V. Gilard and J.C. Prom,** 1991. Mass spectrometry as a tool for identifying group D2 corynebacteria by their fatty acid profiles. *J. Gen. Microbiol.* **137**, 1903-1909.
7. **Goodfellow, M., J. Lacey and C. Todd,** 1987. Numerical classification of thermophilic streptomycetes. *J. Gen. Microbiol.* **133,** 3135-3149.
8. **Gower, J.C.** 1966. Some distance properties of latent root and vector methods used in multivatiate analysis. *Biometrica.* **53**, 325-338.
9. **Johnson, J.,** 1989. Nucleic acids in bacterial classification. p.2306-2309. *In* Williams, S.T. , M.E. Sharpe and J.G. Holt (eds.), Bergey's Manual of Systematic Bacteriology. Vol. 4. Williams and Wilkins; Baltimore.
10. **Jones, D. and N. Krieg,** 1989. Serology and chemotaxonomy. p.2340-2343. *In* Williams, S.T. , M.E. Sharpe and J.G. Holt (eds.), Bergey's Manual of Systematic Bacteriology. Vol. 4. Williams and Wilkins; Baltimore.
11. **Kämpfer, P., R.M. Kroppenstedt and W. Dott.** 1991. Numerical classification of the genera *Streptomyces* and *Streptoverticillium using* miniaturized physiological tests. *J. Gen. Microbiol.* **137**, 1831-1891.

12. **Kaneda, T.,** 1991. Iso- and anteiso-fatty acids in bacteria; biosynthesis, function, and taxonomic significance. *Microbiol Rev.* **55**, 288-302.
13. **Küster, E. and S.T. Williams,** 1964. Production of hydrogen sulphide by streptomycetes and methods for its detection. *Appl. Microbiol.* **12**, 46-52.
14. **Labeda, D.P.,** 1987. Isolation of actinomycetes for biotechnological applications. p.1-20. *In* Labeda, D.P. (ed.), Isolation of Biotechnological Organisms from Nature. McGraw-Hill.
15. **Leblond, P. & B. Decaris,** 1994. New insights into the genetic instability of streptomycetes. *FEMS Microbiol. Lett.* **123**, 225-232.
16. **Lechevalier, M.P.,** 1977. Lipids in bacterial taxonomy- a taxonomist's view. *Crit. Rev. Microbiol.* **5**, 109-210.
17. **Locci, R.,** 1989. Streptomycetes and related genera. p.2451-2508. *In* Williams, S.T., M.E. Sharpe and J.G. Holt (eds.), Bergey's Manual of Systematic Bacteriology. Vol. 4. Williams and Wilkins; Baltimore.
18. **Locci, R. and G.P. Sharples,** 1983. Morphology. In Biology of Actinomycetes. pp.165-199. Edited by Goodfellow, M., M. Modarski and S. T. Williams. Academic Press; London.
19. **Minnikin, D.E., A.G. O'Donnell, M. Goodfellow, G. Alderson, M. Athalye, A. Schaal and J.H. Parlett,** 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods.* **2**, 233-241.
20. **O'Leary, W.M. and S. G. Wilkinson,** 1988. Gram-positive bacteria. p. 117-201. In Ratledge, C. and S. G. Wilkinson (eds.), Microbial Lipids. Vol. 1. Academic Press; New York.
21. **Saddler, G.S., A.G. O'Donnell, M. Goodfellow and D.E. Minnikin,** 1987. SIMCA pattern recognition in the analysis of streptomycete fatty acids. *J. Gen. Microbiol.* **133**, 1137-1147.
22. **Seong, C.N.,** 1992. Numerical taxonomy of actinomycetes isolated from acid soil in Korea. Ph.D. Thesis. Seoul National University.
23. **Shirling, E.B. and D. Gottlieb,** 1966. Methods for characterization of Streptomyces species. *Int. J. Syst. Bacteriol.* **16**, 313-340.
24. **Silbert, D.F., R.C. Ladenson and J.L. Honegger,** 1973. The unsaturated fatty acid requirement in *Escherichia coli*. Temperature dependence and total replacement by branched-chain fatty acids. *Biochim. Biophys. Acta.* **311**, 349-361.
25. **Williams, S.T., M. Goodfellow, G. Alderson, E.M.H. Wellington, P.H.A. Sneath, and M.J. Sackin,** 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* **129**, 1743-1813.