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The Evaluation of Alkalitolerant-Mesophilic *Streptomyces* by Gas Chromatography and Pyrolysis Mass Spectrometry

Nevzat ŞAHİN, Kamil IŞIK, Ergin KARİPTAŞ
O.M.Ü. Fen Edebiyat Fakültesi, Biyoloji Bölümü, 55139 Kurupelit-Samsun - TURKEY
Michael GOODFELLOW
Newcastle University, Medical School, Microbiology Dept., Newcastle upon Tyne - U.K.

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Abstract: Thirty-eight alkalitolerant-mesophilic *Streptomyces*, defined by Saddler in his numerical taxonomic study, were evaluated by gas chromotography and pyrolysis mass spectrometry. Group I *Streptomyces* sp. AK409 and group II *Streptomyces* sp. AK029 selected duplicated strains were clustered in their own groups. It was observed that the results of gas chromatography and pyrolysis mass spectrometry was in good congruance with the numerical taxonomic results. Therefore, it can be concluded that the different chemotaxonomic methods provide rapid and reproducible information for classifying biotechnologically significant *Streptomyces* strains.

Key Words: *Streptomyces*, Gas chromotography, PyMS, Chemotaxonomy.

Pirolizis Kütle Spektrometri ve Gaz Kromatografisi ile Alkalitolerant-Mezofilik *Streptomyces*'lerin Değerlendirilmesi

Özet: Saddler tarafından nümerik taksonomisi yapılan alkalitolerant-mezofilik otuz sekiz *Streptomyces* suşu gaz kromatografi ve pirolizis kütle spektrometri metotları ile değerlendirildi. Grup I' den *Streptomyces* sp. AK409 ve grup II'den *Streptomyces* sp. AK029 nolu seçilen duplikat suşlar ilgili grupları ile kümelendiler. Gaz kromatografisi ve pirolizis kütle spektrometri sonuçlarının nümerik taksonomik sonuçlarla tam bir uyum içerisinde olduğu gözlendi. Farklı kemotaksonomik metotların biyoteknolojik önemi olan *Streptomyces* suşlarının sınıflandırılmasında hızlı ve üretken bilgiler sağladığı sonucuna varılmaktadır.

Anahtar Sözcükler: *Streptomyces*, Gaz kromatografisi, PyMS, Kemotaksonomi.

Introduction

The genus *Streptomyces* is now well circumscribed due to the extensive application of chemotaxonomic, molecular systematic and numerical phenetic methods (1-5). Many bacteria can be characterized and defined on the basis of cellular fatty acid composition (6- 8). It is,

however, vitally important that the preparation and analyses of fatty acid samples are carried out using highly standardized conditions to limit test error. Generally, all fatty acids are given equal weight in numerical analyses, even though some fatty acids are produced subsequent to essential fatty acid synthesis (9).

Fatty acids can be defined as carboxylic acid derivatives of long-chain aliphatic molecules (9). In bacteria, they range in chain length from two carbon molecules to over 90 carbon components, as found in mycolic acids. Taxonomically, fatty acids in the range C_{10} to C_{24} have provided the greatest information and are present across a diverse range of microorganisms. The diversity in fatty acid types makes these compounds valuable markers in bacterial systematics (8, 10-14).

The classification and identification of microorganisms are central themes in microbial systematics, but many of the methods currently used for these purposes are complex, expensive in terms of labour and materials and quite often give poor reproducibility. There is, therefore, a real need to develop rapid, reproducible and cost-effective methods for these purposes. It is well known that chemical and molecular techniques have been used to generate a lot of valuable data for detecting relationships between microorganisms isolated from natural habitats (3, 15-18). Many chemical and molecular techniques are relatively expensive in terms of both cost per sample and in time. This is not the case with some analytical chemical techniques, notably Curie-point pyrolysis mass spectrometry (19).

The primary aims of the present investigation were to evaluate intra-species relationships in numerically circumscribed two alkalitolerant-mesophilic clusters defined by Saddler (20) using gas chromatography and rapid technique pyrolysis mass spectrometry.

Materials and Methods

Pyrolysis mass spectrometry

Test strains: Thirty-eight members of two alkalitolerant, mesophilic clusters, defined by Saddler (20) in his numerical taxonomic study, were examined. The histories of the test strains are given in Table 1. All of the strains were maintained as frozen glycerol suspensions.

Growth conditions: Thawed glycerol suspensions of the test strains were used to inoculate inorganic salts-starch agar plates (ISP4, Difco) which were incubated for 14 days at 25°C. After incubation, inocula were taken from single colonies, using sterile loops, and transferred to 250 ml conical flasks containing 30 ml of modified Sauton's medium (pH 7.0). The inoculated flasks were shaken at 25°C for 3 days in an orbital incubator (1H-400; Gallenkamp and Co. Ltd.) at 180 rpm. Biomass was harvested at 10,000 rpm for 10 minutes and the resultant pellets were washed twice with distilled water and then stored at -80°C.

Table 1. Designation and source of mesophilic, alkalitolerant streptomycetes included in the Curie-point pyrolysis mass spectrometric and fatty acid analyses*.

Strain number	Name of strains	Source
Cluster 2: *Streptomyces* sp.		
AK046	*Streptomyces* sp.	G.S. Saddler, dune slack, Warkworth, England, U.K.
AK108	*Streptomyces* sp.	G.S. Saddler, embryo dune 1, Warkworth, England, U.K.
AK110	*Streptomyces* sp.	G.S. Saddler, embryo dune 1, Warkworth, England, U.K.
AK321	*Streptomyces* sp.	G.S. Saddler, embryo dune 2, Warkworth, England, U.K.
AK409	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK571	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK584	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK097	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK404	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK579	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK459	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK420	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK440	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK468	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK473	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
Cluster 5: *Streptomyces* sp.		
AK015	*Streptomyces* sp.	G.S. Saddler, dune slack, Warkworth, England, U.K.
AK029	*Streptomyces* sp.	G.S. Saddler, dune slack, Warkworth, England, U.K.
AK542	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK582	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK091	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK095	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK511	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK520	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK521	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK537	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK540	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK557	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK598	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK078	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK076	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK439	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK071	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK444	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK454	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK385	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK494	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK503	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.
AK479	*Streptomyces* sp.	G.S. Saddler, Consett, England, U.K.

* Numerically circumscribed clusters defined by Saddler (20) in an S_J, UPGMA analysis.

Preparation and Analysis of Samples: Pyrolysis foils and pyrolysis tubes (Horizon Instruments Ltd.) were washed in acetone and dried overnight at 27°C. The cleaned foils were inserted, using sterile forceps, into the pyrolysis tubes so that they protruded about 6 mm from the mouth. For each strain, small amounts of biomass (*ca.* 50 µg) were taken, using sterile disposable plastic loops, and smeared uniformly over the upper surface of the protruding foils; care was taken to use approximately the same amount of biomass in all instances. Prepared triplicate samples were dried at 80°C for 5 minutes and Curie-point pyrolysis mass spectrometry carried out in a RAPyD 400X instrument (Horizon Instruments, Heathfield, East Sussex, U.K.). Pyrolysis time was 2.4 seconds with a temperature rise time of 0.6 of a second. The inlet heater was set at 160°C. The pyrolysate was ionised by a low energy (20 eV) electron beam and separated in the quadrupole mass spectrometer (100 scans at 180 ms/scan). Integrated ion counts for each sample at unit mass intervals from 51 to 200, were recorded on a harddisk, together with the pyrolysis sequence number and the total ions count for each sample, without background removal.

Data Analysis: Multivariate statistical analyses were carried out using the Genstat V statistical package (21). The PYMENU software (Horizon Instruments Ltd.) was used to perform a series of data reduction steps, namely characteristicity and principal component analysis (PCA), on the raw data in preparation for discriminant analysis. The PYMENU program provided instructions to the GENSTAT program as to which analysis steps were to be carried out.

PyMS spectra were corrected by iterative re-normalisation and individual masses ranked according to their characteristicity values prior to principal components analysis (PCA). Principal components (PCs) accounting for less than 0.5% of the total variance were discarded. Canonical variate analysis (CVA) was then used to group the samples on the basis of the retained PCs taking into account the sets of triplicates. An ordination diagram of the PyMS data was produced by plotting the eigenvectors from the CVA (22, 23).

The data from the PC-CVA analyses were presented as Mahalanobis distances (24). The Mahalanobis distance matrix was standardised by dividing the maximum intergroup distance and then treated as an ordinary Euclidean distance, which was mathematically converted to a similarity matrix using Gower's coefficient SG (23, 25). The similarity values were examined using the UPGMA algorithm (22) and the results presented as dendrograms. Ordination plots, which express the relative position of each set of replicate strains in a multidimensional space that accounted for the first three dimensions, were also generated. A 95% confidence limit represented by a circle was plotted around the samples to assess the variance and distinguish between the groups. It is important to analyse dendrograms with reference to ordination plots due to the tendency for pyrolysis data to be non-hierarchical (26).

Analaysis of Fatty Acids

Cultures: The designation, names and sources of the 38 test strains are shown in Table 1.

Growth of Test Strains: Thawed glycerol suspensions of the test strains were used to inoculate modified Bennett's agar plates (27) which were incubated for 3 weeks at 25°C. After incubation, inocula were taken from colonies and transferred to each of two 250 ml conical flasks containing 100 ml of modified Sauton's broth (pH 7.0). The inoculated flasks were shaken at 25°C at 200 rpm for 3 days. Aliquots (10 ml) from each of the 3-day old starter cultures were used to inoculate 2x1 litre flasks containing 200 ml of modified Sauton's broth. The inoculated flasks were incubated as before for 10 days. After incubation, the organisms were checked for purity, killed by shaking with formalin (1%, v/v; BDH Chemicals Ltd.), harvested by centrifugation at 10,000 rpm for 10 minutes and washed three times with distilled water. The harvested biomass was lyophilized and stored at room temperature.

Extraction of Fatty Acids: Fatty acids were extracted using a whole-organism alkaline methanolysis procedure (28). Freeze dried biomass (*ca.* 50 mg) was degraded at 80°C overnight with 3 ml of methanol, toluene and concentrated sulphuric acid (6:3:0.2, v/v) in a 10 ml test tube fitted with a polytetrafluoroethylene (PTFE)-lined screw cap. The reaction mixture was allowed to cool to room temperature prior to extraction with 2 ml of hexane. The tubes were shaken for 15 minutes in a Model TM1 Tumbler (Luckham Ltd.) and then centrifuged at low speed for 5 minutes to facilitate the separation of the two layers. The upper layer, which contained the fatty acid esters, was transferred to a clean tube containing 4 ml of 0.3 M phosphate buffer, the contents thoroughly mixed and then centrifuged at low speed as before. The upper layer was collected and evaporated under a stream of nitrogen at a temperature below 40°C to give a crude fatty acid methyl ester (FAME) preparation. All samples were stored at 4°C.

Analytical one-dimensional thin-layer chromatography of fatty acid methyl esters: The stored dried extracts (FAME) were dissolved in 0.2 ml of petroleum ether (b.p. 60-80°C) and 3 to 4 drops applied to a 10x10 cm aluminium backed silica gel plate (Merck 5554; Darmstadt, FRG) using a capillary pipette. The plates were developed in a solvent system consisting of petroleum ether (b.p. 60-80°C)-acetone (95:5, v/v) and dried in air prior to spraying with a solution of molybdophosphoric acid in ethanol (50 g l^{-1}) and heating at 120°C for 5 minutes. Fatty acid methyl esters and other long chain components appeared as blue/black spots on a yellow background. The remaining sample was purified by preparative thin-layer chromatography.

Preparative thin-layer chromatography of fatty acid methyl esters: The long-chain components were isolated by applying the remainder of the petroleum ether extracts as 3 cm bands onto 10x10 cm plastic backed silica gel plates (Merck 5735). Two samples were applied per plate approximately 1cm from the base. The plates were developed as before, air-dried and

sprayed with rhodamine 6G (0.01%, w/v in ethanol). Separated FAME were detected by examining the plates under ultra-violet light. Separated components were ringed lightly in pencil and the marked bands were then cut from the plates, placed in test tubes, and extracted using 1ml of anhydrous diethyl ether. The tube contents were mixed for 15 minutes on a tube rotator. The extracted FAME were passed down a 0.5 cm column of anhydrous diethyl ether-washed neutral aluminium oxide (BDH) in a Pasteur pipette to remove any residual rhodamine 6G. The extraction was repeated and the combined eluates transferred to a small glass vial and evaporated to dryness under a stream of nitrogen at a temperature below 40°C. The preparations were stored at 4°C.

Analytical gas chromatography of purified fatty acid methyl esters: The dried FAME were dissolved in petroleum ether (b.p. 60-80°C) and 2.5µl amounts injected into a GC-MINI-3 gas chromatograph equipped with a 30m SP-2330 Fused Silica Capillary Column using a 10µl microsyringe. Nitrogen was used as the carrier gas and the injector and detector temperatures were set at 270°C. The column oven was programmed to rise from 150°C, at 4°C minute^{-1}, to the final oven temperature of 250°C.

Retention times and the relative proportions of each FAME, expressed as a proportion of the total peak area, were measured using a TRIO Chromatography Computing Integrator. Relative retention times were calculated for each peak using hexadecanoic acid (16:0) as the internal standard. The identity of individual esters were established by comparison with the relative retention times of standard FAME mixtures. Straight chain standards (C_{12}-C_{20}) were obtained commercially whereas branched chain standards (C_{14}-C_{18}) were extracted from *Bacillus subtilis* NCIB 3610.

The results were transferred to the format suitable for NTSYS-pc software. The raw data were normalised and pairwise Euclidean distances calculated by using NTSYS-pc software. The information in the resultant distance matrix was sorted using the UPGMA algorithm in the NTSYS-pc software package.

Results and Discussion

The 38 alkalitolerant, mesophilic strains representing clusters 2 and 5 (Table 1) defined in the numerical classification of *Streptomyces* (20) were analysed by Curie-point pyrolysis mass spectrometry. The strains were assigned to two fairly well defined groups when biomass grown in the broth medium was used (Figures 1 and 2). Excellent agreement was found between the results of the triplicate analyses of each strain. It was also reassuring that the various duplicated cultures clustered together. Unlike the other phenotypic methods, PyMS is rapid (approximately 2 minutes per sample), automated, highly reproducible, requires very small samples (50-100 µg), little preparation and can be used to examine up to 300 samples per day. The speed and reproducibility of Curie-point pyrolysis mass spectrometry and its applicability to a wide range

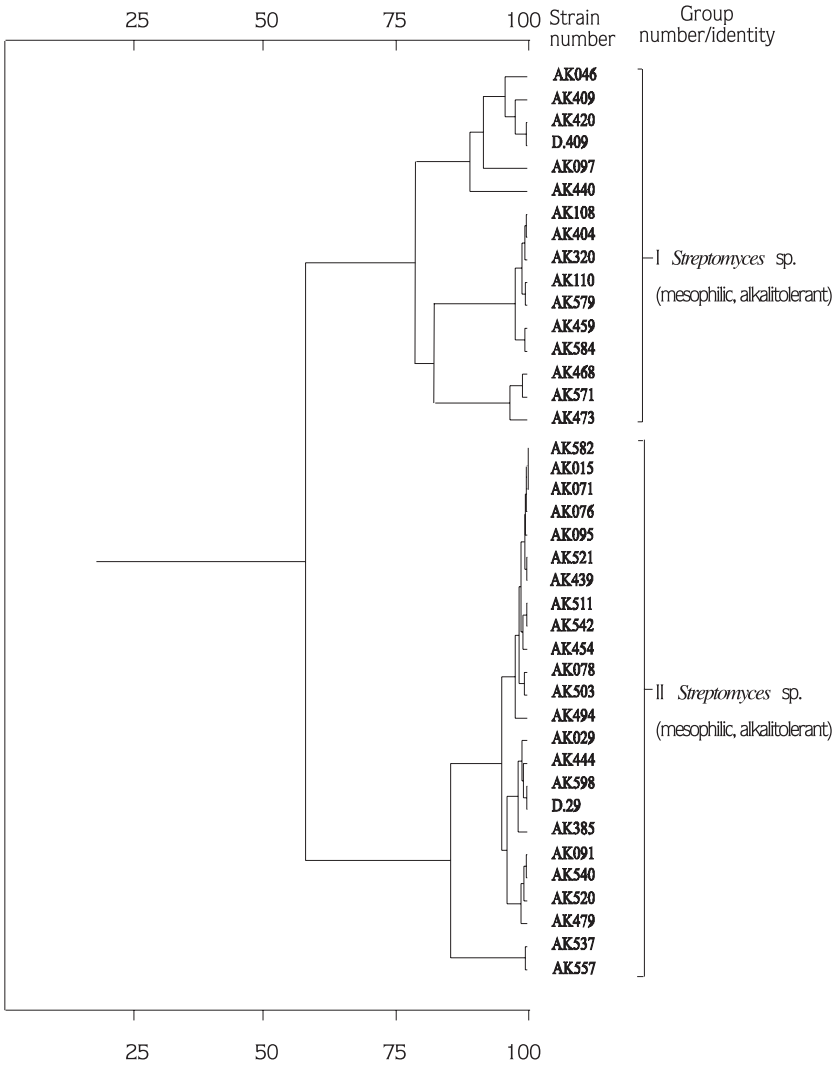

Figure 1. Dendrogram representing the relationships found between members of groups I (*Streptomyces* sp.) and II (*Streptomyces* sp.) based on pyrolysis mass spectral data. The dendrogram is based on similarity values derived from Mahalanobis distances with clustering achieved using the UPGMA algorithm.

of bacteria make it a particularly attractive method for evaluating the integrity of taxa circumscribed using coventional taxonomic criteria (29-31).

The cellular fatty acid composition of the 37 alkalitolerant, mesophilic streptomycetes (Table 1), which represented clusters 2 (*Streptomyces* sp.) and 5 (*Streptomyces* sp.) defined in

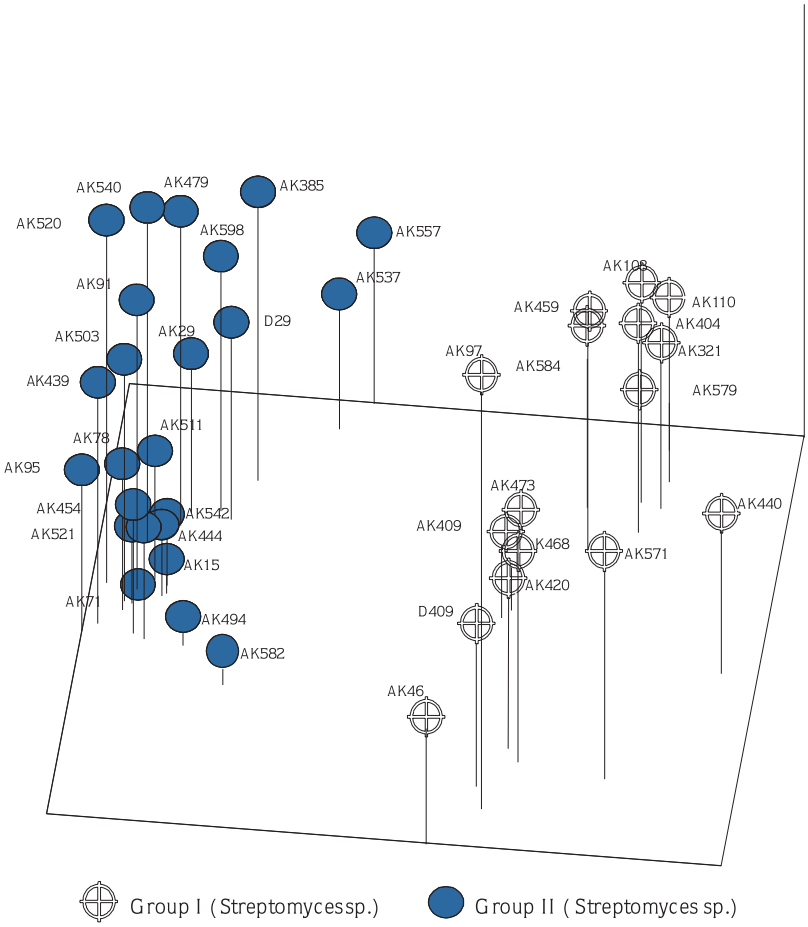

Figure 2. Ordination plot along the first three canonical variate axes showing the mean position of groups 1 (*Streptomyces* sp.) and 2 (*Streptomyces* sp.) based on pyrolysis mass spectral data. The first three canonical variate axes account for 96.82% of the total variation found between the strains.

a numerical taxonomic analysis based on the S_J coefficient and the UPGMA algorithm (20), were examined. Whole-organism methanolysates of the test strains showed the presence of predominant spots (R_f 0.8) corresponding to non-hydroxylated FAME (28); neither hydroxylated fatty acids nor mycolic acids were detected. All of the alkalitolerant, mesophilic strains produced qualitatively similar profiles that were dominated by methyl branched chain fatty acids. The chain lengths of the major fatty acid components were between 14 and 17 carbon atoms with 12-methyltetradecanoic (*anteiso*-C15:0), 14-methylpentadecanoic (*iso*-C16:0), hexadecanoic (C16:0) and 14-methylhexadecanoic (*anteiso*-C17:0) fatty acids as the predominant components (Table 2).

Table 2. Percentage fatty acid composition of alkalitolerant, mesophilic streptomycetes assigned to clusters 2 and 5 in a numerical taxonomic study based on S_J, UPGMA algorithm (20).

	Percentage of total fatty acids*																	
Strain number	12:0	i-14:0	14:0	A	i-15:0	ai-15:0	15:0	14:0-3OH	i-16:0	16:1	16:0	B	i-17:0	ai-17:0	17:0	18:1	18:0	S24	20:0
Cluster 2: *Streptomyces* sp.																			
AK046	0	4.3	0.4	1.5	2.7	29.6	1.4	0.5	23.9	1.3	6.5	4.2	1.8	10.6	3.5	1.0	0	0	0
AK097	1.9	4.0	1.3	1.9	2.0	32.2	2.2	0.6	22.6	1.8	4.3	2.0	1.4	15.8	0.8	0	0	0	0
AK108	2.0	4.0	1.5	2.0	3.0	30.7	1.8	0	22.0	1.6	5.2	2.0	2.3	13.2	0.6	0	0	0	0
AK321	2.2	3.9	0.5	2.3	2.9	30.8	1.8	0	21.0	0	6.6	1.1	1.8	12.9	0.7	0	0	0.8	0
AK404	1.3	4.3	0.9	1.3	1.9	34.0	1.9	0.4	25.0	0.6	5.9	1.8	0	14.2	0.6	0	0	0	0
AK409	0	4.7	0.6	3.0	4.5	30.5	2.1	0	23.8	0	5.3	4.1	0.6	10.8	4.5	0	0	0.7	1.2
AK420	0	4.0	0.5	2.4	2.3	30.0	2.8	0	24.8	0	4.5	1.0	1.2	14.4	0.7	0	0	0.3	0
AK440	0	2.4	0.5	1.7	3.8	29.7	2.5	0	26.8	0.5	5.2	3.0	1.1	13.7	3.3	0.8	0	0	0.6
AK459	0	4.1	1.0	2.5	3.4	32.4	2.3	0	25.0	0	5.2	2.4	0	13.6	2.6	1.2	0	0	1.1
AK468	0	4.8	1.0	2.5	4.6	29.0	2.0	0	26.2	1.0	4.5	4.4	0.6	11.0	2.6	0.6	0	0.6	1.0
AK473	1.6	4.6	1.3	1.8	2.1	29.7	1.2	0	26.4	1.0	3.7	2.9	1.0	14.4	1.7	0.7	0	0	0
AK571	0	4.8	1.0	2.5	3.7	31.5	2.5	0	26.0	1.0	4.9	1.0	0	9.8	4.3	0.8	0	0.6	1.5
AK110	0	4.4	1.5	2.2	4.1	30.6	1.7	0	22.3	0	5.7	1.1	2.4	12.9	0.8	0	0	0	0
AK579	0	4.5	1.0	2.0	4.6	31.0	2.0	0	24.2	0	4.9	4.8	1.0	10.9	3.8	1.4	0	0.6	2.0
AK584	1.9	4.3	1.2	1.7	2.0	31.4	2.0	0	26.0	0	5.0	0	1.8	14.6	0.9	0	0	0	0
Cluster 5: *Streptomyces* sp.																			
AK015	1.5	5.7	0.5	1.7	3.2	25.7	3.2	0.9	24.2	3.1	6.6	1.7	1.5	8.6	0.8	0	0	0.7	0
AK029	1.8	7.0	1.3	1.8	3.8	26.5	1.7	0.5	26.8	1.7	5.4	1.5	2.0	8.2	2.1	0	0	0.5	0
AK076	0	5.1	0	0	4.5	28.4	1.0	2.0	28.9	5.0	5.7	2.5	1.5	14.0	0	0	0	0	0
AK071	1.5	6.4	0.9	1.0	3.4	27.5	1.4	1.4	25.6	5.0	5.4	2.6	1.5	12.3	1.4	0	0	0	0
AK095	0	6.1	0.7	0.4	4.8	27.9	2.4	1.2	28.4	4.2	9.0	1.5	0.9	7.9	1.8	0	0	0	0
AK385	0	9.5	0.4	1.0	3.8	28.7	2.4	0.6	28.1	3.3	5.9	2.2	0.5	9.0	2.0	0	0	0	0
AK439	0	6.1	0.6	0.7	3.5	26.5	1.3	1.2	30.3	4.7	8.5	2.3	1.3	11.0	0.9	0	0	0	0
AK454	0	6.0	0.7	0.4	3.6	27.0	2.4	1.2	28.5	3.6	8.8	2.3	0.8	10.3	1.8	0	0	0	0
AK494	0	5.3	0.6	0.4	2.7	26.9	0.7	2.6	30.5	6.2	8.5	2.3	1.5	11.0	1.8	0	0	0	0
AK503	0	6.8	0.8	0	2.9	26.4	1.5	0.7	30.3	2.0	8.1	2.5	1.1	12.5	2.4	0	0	0	0
AK520	0	9.0	0.3	0	2.8	28.9	1.0	1.8	29.3	3.5	4.2	4.2	0.7	8.9	2.9	0	0	0	0
AK521	0	6.0	0.5	0	2.2	29.5	2.0	2.0	29.4	5.8	6.6	2.4	0	12.4	0.6	0	0	0	0
AK540	0	7.7	1.2	0	3.0	26.3	1.5	1.3	27.5	3.5	5.0	2.0	1.7	8.2	0.9	0	0	0	0
AK598	0	4.7	0.5	1.0	2.9	29.7	1.0	1.7	28.6	5.3	6.7	3.1	0.7	13.0	0.8	0	0	0	0
AK078	0	4.4	0.7	1.9	2.8	27.2	1.8	1.3	27.3	3.9	9.7	1.7	0.4	11.4	2.3	0	0	0	0
AK091	0	6.8	0	0.8	3.5	28.9	0.8	1.7	28.3	2.7	5.9	3.0	1.0	10.6	2.0	0	0	0	0
AK444	0	8.8	0.8	1.9	3.0	28.8	3.1	0.9	28.6	3.6	5.6	1.2	0.8	9.3	4.4	0	0	0	0
AK479	0	7.0	1.0	0.7	2.2	29.9	1.9	1.0	28.7	3.5	8.2	3.5	0.5	11.8	1.2	0	0	0	0
AK511	0	4.5	0.7	0	4.3	26.9	1.4	1.0	27.0	5.4	9.5	2.2	1.3	11.6	0.9	0	0	0	0
AK537	0	6.0	0.6	0	2.4	27.5	3.5	1.7	28.0	4.8	7.7	2.4	0.5	10.4	0.9	0	0	0	0
AK542	0	6.8	0.8	0	3.5	26.7	2.6	1.2	28.8	5.2	9.0	2.4	0.6	8.0	4.0	0	0	0	0
AK557	0	6.0	0.6	0	2.5	15.9	3.5	1.6	29.4	4.9	7.6	2.1	0	10.5	1.0	0	0	0	0
AK582	0	2.9	0.4	0	3.0	27.5	1.8	1.1	29.8	4.9	6.6	3.5	0	11.3	2.9	0	0	0	0

*Abbreviations: Iso-14:0, 12-methyltridecanoic acid; 14:0, tetradecanoic acid; iso-15:0, 13-methyltetradecanoic acid; *anteiso*-15:0, 12-methyltetradecanoic acid; 15:0, pentadecanoic acid; iso-16:0, 14-methylpentadecanoic acid; 16:0, hexadecanoic acid; *iso*-17:0, 15-methylhexadecanoic acid; *anteiso*-17:0, 14-methylhexadecanoic acid and 17:0, methylheptadecanoic acid;

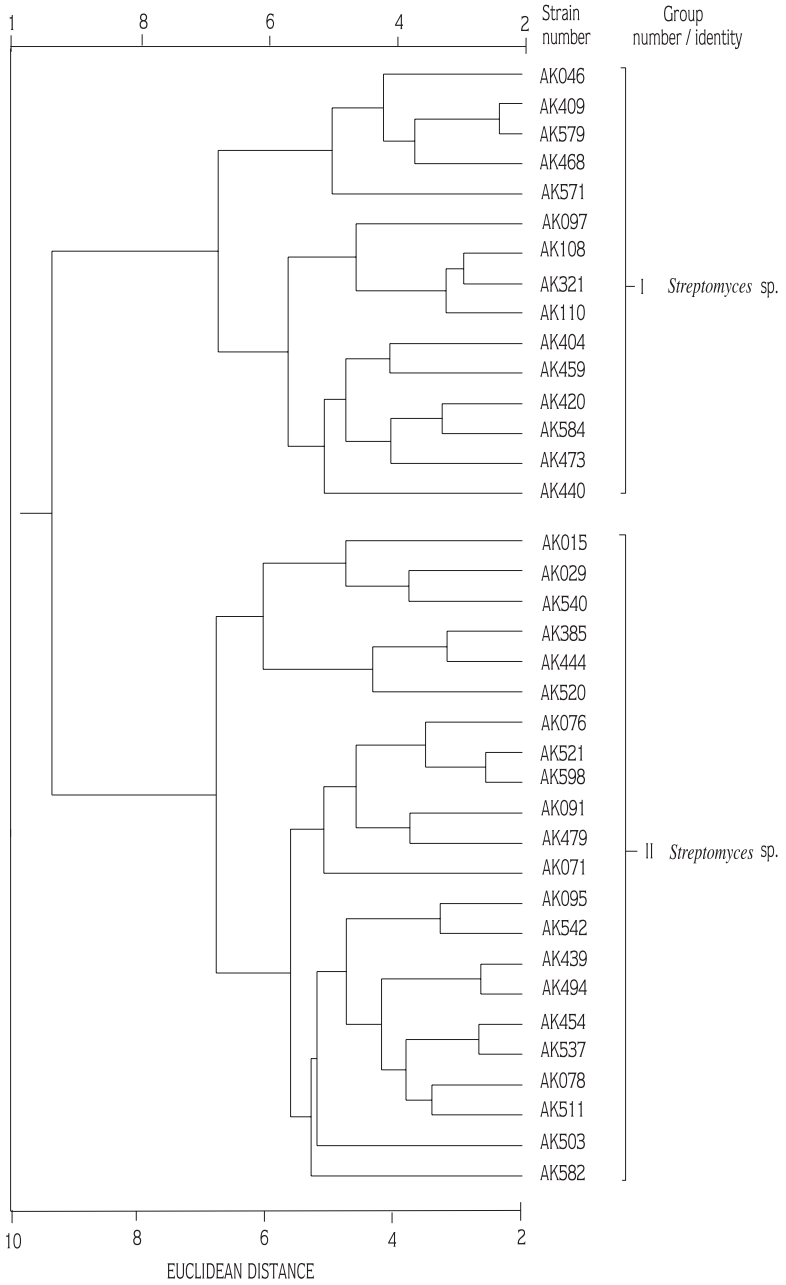

Figure 3. Dendrogram showing relationships between members of *Streptomyces* clusters 2 and 5 (20) based on the analysis of fatty acid methyl ester composition using pairwise Euclidean distances and the UPGMA algorithm.

The cluster 2 strains (*Streptomyces* sp.) contained major proportions of 12-methyltetradecanoic (range 29-34%; *anteiso*-C15:0), 14-methylpentadecanoic (range 21-26.8%; *iso*-C16:0) and 14-methylhexadecanoic (range 9.8-15.8%; *anteiso*-C17:0) fatty acids. In contrast, the members of cluster 5 (*Streptomyces* sp.) contained major proportions of 14-methylpentadecanoic (range 24.2-30.5%; *iso*-C16:0), 12-methyltetradecanoic (range 25.7-29.9%; *anteiso*-C15:0) and 14-methyl-hexadecanoic acid (range 7.9-14%; *anteiso*-C17:0). The strains assigned to clusters 2 (*Streptomyces* sp.) and 5 (*Streptomyces* sp.) were recovered in two well separated groups when the fatty acid data were analysed quantitatively (Figure 3).

The detection of major amounts of saturated iso- and anteiso- branched fatty acids of between 14 and 17 carbons in all of the strains was in good agrement with previous results (32, 33). It is difficult to evaluate the significance of fatty acid profiles in streptomycete systematics as most studied are based on poorly described strains examined using a variety of experimental methods. Moreover, the overall similarities found in streptomycete fatty acid profiles make it necesary to use numerical analyses in order to exploit data for taxonomic purposes. In the present study it was most encouraging that good agreement was found between the results of the numerical phenetic and quantitative fatty acid studies.

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