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Fatty Acid Characterization of Rapidly Growing Pathogenic Aerobic Actinomycetes as a Means of Identification

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The fatty acid compositions of 39 type strains and 529 clinical or reference strains of pathogenic aerobic actinomycetes were analyzed after standardized culture by using the Microbial Identification System (MIS). Library entries for each type strain were created by using the MIS Library Generation Software, and the fatty acid profiles of clinical and reference strains were compared to these library entries. The bacteria separated into two large groups based upon major amounts of branched-chain or of saturated or monounsaturated straight-chain fatty acids. Identification of isolates was possible by using only the type strains for comparison, but fatty acid heterogeneity occurred within most species.

The genera *Gordona*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Rothia*, and *Streptomyces*, as well as strains of *Amycolatopsis orientalis*, *Dermatophilus congolensis*, *Nocardiopsis dassonvillei*, *Pseudonocardia autotrophica*, *Saccharothrix aerocolonigenes*, and *Tsukamurella paurometabola*, are aerobic actinomycetes that have been implicated in human infections (3, 29, 40). Prior attempts to improve the identification of these aerobic actinomycetes by cellular fatty acid analysis have used various culture media, growth conditions, incubation times, extraction methods, and chromatography techniques, all factors which affect the fatty acids expressed (9, 20, 22, 23, 26). The Microbial Identification System (MIS; Microbial Identification Inc., Newark, Del.) standardizes the chromatography variables associated with quantitative fatty acid analysis and facilitates fatty acid analysis as a means of identifying bacteria (53).

We analyzed the type strains of 39 pathogenic aerobic actinomycete species after standardized growth and incubation and created library entries for each species. The fatty acid profiles of 529 clinical and reference strains were then compared to these library entries. Fatty acid analysis is a limited, but practical method for the rapid identification of aerobic actinomycetes, and our results are consistent with some prior claims for the existence of subgroups within defined taxa (8, 33, 39, 42, 48).

MATERIALS AND METHODS

Bacteria. The sources of the type strains, reference strains, and other strains that were used to establish additional taxa defined by fatty acid profiles are listed in Table 1. Other isolates were obtained from commercial culture collections, the collections of other researchers, and clinical specimens. *Actinomadura madurae* and *Actinomadura pelletieri* were excluded from this study due to their fastidious growth requirements and need for prolonged incubation.

Culture conditions. All strains, with the exception of *Nocardia brevicatena*, were grown at 28°C for 96 h in ambient air on four TSBA plates composed of Trypticase soy broth (no. 11768; BBL) and 1.5% agar (no. 11849; BBL). Isolates of *N. brevicatena* were cultured on plates of TSBA with 1% Tween 80 (T164-500; Fisher Scientific), because we found a slight growth enhancement in the presence of Tween 80.

Identification by biochemical tests. Clinical isolates of *Rothia dentocariosa* were identified by using the scheme of Hollis and Weaver (17). *Nocardia* species were reidentified as needed by using Gordon characteristics supplemented by opacification of Middlebrook 7H10 agar; carbon source utilization of rhamnose, galactose, inositol, mannitol, propanol, butanol, butylene glycol, citrate, acetamide, and monoethanolamine; and 2-week arylsulfatase activity (6, 10, 31, 44, 49). The susceptibilities of *Nocardia* species to amikacin, ampicillin, carbenicillin, cefamandole, cefotaxime, erythromycin, and tobramycin, determined by disk diffusion, were also used to aid in identification, as described by Wallace and Steele (48).

Preparation of bacterial extracts. Bacterial growth sufficient to fill a disposable plastic loop (approximately 40 mg; no. 1906-95-9; Difco) was harvested from the last quadrant exhibiting confluent growth and was transferred to a 13-by-100-mm glass test tube fitted with a Teflon-lined cap. Saponification, esterification, extraction, and washing were performed according to MIS guidelines (30). Following this, a few drops of saturated aqueous sodium chloride solution were added, and the extract was spun at 600 to 650 × *g* for 2 min to maximize separation between the organic and aqueous phases. The organic phase was removed, flushed through a small volume of anhydrous sodium sulfate (no. 3898-01; J. T. Baker) in a sample vial to remove all traces of water, and transferred to a second vial for analysis.

Heuristic library development. Type strains of all species were analyzed on five separate occasions to provide adequate data for library entry generation. Comparison of data from multiple runs for the same isolate was done by using MIS dendrogram software to exclude runs that were greater than nine Euclidian distances (EDs) from the data from the other analyses. Such outliers were infrequent and were usually due to technical variables such as contamination of the culture or poor growth of the isolates. Repurification of the isolate or further subculture corrected this problem with all strains.

The type strain library entries were designated group A. The fatty acid profiles of the test strains were compared to the type strain entries by using the MIS software. The MIS software calculates a similarity index (SI), which is a numerical value that expresses how closely the fatty acid composition of an unknown isolate compares with the "average" composition profiles stored in a fatty acid library (30). A perfect match gives an SI of 1.000. In this study strains that had SIs of ≥0.300 with no second choice or a first-choice SI of ≥0.500 to a type strain library entry with a minimum separation of 0.100 between the first and second choices were considered acceptable identifications, in accordance with the manufacturer's recommendations (30).

After all strains had been analyzed, the type strain library entries were redeveloped by including the strains that had been identified. The redeveloped entries were placed in a new library, and the identification-redevelopment process was repeated until no further strains were identified by the latest library version. To allow for the inclusion of biochemically typical strains that did not meet our MIS identification criteria, well-characterized isolates were chosen at random from each species that had unidentified isolates and were analyzed a further four times to create group B library entries. Strains that met the identification criteria with the group B entries were used to redevelop the corresponding library entries. This process was carried out until no further strains were identified. Further fatty acid groups were created until most isolates had been included in a fatty acid group of their biochemically assigned species.

Cluster analysis. Multivariate analysis to compare isolates was performed by using the dendrogram software supplied with the MIS. This program uses two-dimensional cluster analysis to calculate unweighted pair matchings based on named fatty acid composition. Relatedness is expressed as proximity in two-dimensional space as the ED. The results are presented graphically in a tree

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TABLE 1. Aerobic actinomycete type and reference strains[a]

Taxon	Strain designation
Amycolatopsis orientalis group A	ATCC 19795^T
Amycolatopsis orientalis group B	ATCC 35164
Amycolatopsis orientalis group C	ATCC 35166
Amycolatopsis orientalis group D	Yassin N355
Dermatophilus congolensis group A	ATCC 14637^T
Gordona aichiensis group A	ATCC 33611^T
Gordona bronchialis group A	ATCC 25592^T
Gordona bronchialis group B	UHL 6542
Gordona rubropertinctus group A	ATCC 14352^T
Gordona sputi group A	ATCC 29627^T
Gordona sputi group B	ATCC 33610
Gordona terrae group A	ATCC 25594^T
Mycobacterium abscessus group A	ATCC 19977^T
Mycobacterium chelonae group A	ATCC 35725^T
Mycobacterium fortuitum group A	ATCC 6841^T
Mycobacterium fortuitum group B	UAF 62169
Mycobacterium fortuitum group C	UAF 62517
Mycobacterium fortuitum group D	UAF 62201
Mycobacterium fortuitum group E	UAF 62171
Mycobacterium peregrinum group A	ATCC 14467^T
Nocardia asteroides group A	ATCC 19247^T
Nocardia asteroides group B	ATCC 14759
Nocardia asteroides group C	CSHWA 3167-A-93
Nocardia asteroides group D	CSHWA 2457-92
Nocardia asteroides group E	CSHWA 4635-91
Nocardia asteroides group F	CIP 94.0941
Nocardia asteroides group G	CIP 94.1519
Nocardia brasiliensis group A	ATCC 19296^T
Nocardia farcinica group A	ATCC 3318^T
Nocardia nova group A	ATCC 33726^T
Nocardia nova group B	ATCC 33727
Nocardia nova group C	CSHWA 170-93
Nocardia nova group D	CSHWA 7522-89
Nocardia otitidiscaviarum group A	ATCC 14629^T
Nocardia otitidiscaviarum group B	ATCC 14630
Nocardia otitidiscaviarum group C	ATCC 27941
Nocardia pseudobrasiliensis group A	TXN 249/649
Nocardia transvalensis group A	ATCC 6865^T
Nocardia transvalensis group B	ATCC 29982
Nocardiopsis dassonvillei group A	ATCC 23218^T
Pseudonocardia autotrophica group A	ATCC 19727^T
Pseudonocardia autotrophica group B	ATCC 33795
Pseudonocardia autotrophica group C	ATCC 33797
Pseudonocardia autotrophica group D	DSM 43088
Pseudonocardia autotrophica group E	DSM 43083
Pseudonocardia autotrophica group F	DSM 43084
Pseudonocardia autotrophica group G	DSM 43103
Pseudonocardia autotrophica group H	DSM 43091
Pseudonocardia autotrophica group I	DSM 43085
Rhodococcus coprophilus group A	ATCC 29080^T
Rhodococcus equi group A	ATCC 6939^T
Rhodococcus equi group B	UHL 6188
Rhodococcus erythropolis group A	ATCC 4277^T
Rhodococcus globerulus group A	ATCC 25714^T
Rhodococcus luteus group A	ATCC 35014
Rhodococcus rhodochrous group A	ATCC 13808^T
Rhodococcus rhodococcus group B	ATCC 184
Rhodococcus rhodococcus group C	ATCC 4001
Rhodococcus rhodococcus group D	ATCC 999
Rothia dentocariosa group A	ATCC 17931^T
Rothia dentocariosa group B	UHL 3893
Rothia dentocariosa group C	UHL 4934
Saccharothrix aerocolonigenes group A	ATCC 23870^T
Saccharothrix aerocolonigenes group B	ATCC 35162
Streptomyces albus group A	ATCC 3004^T
Streptomyces anulatus group A	ATCC 27416^T
Streptomyces coelicolor group A	ATCC 19894^T
Streptomyces griseus group A	ATCC 23345^T
Streptomyces griseus group B	ATCC 19347
Streptomyces rimosus group A	ATCC 10970^T
Streptomyces somaliensis group A	ATCC 33201^T
Streptomyces violaceoruber group A	ATCC 14980^T
Tsukamurella paurometabola group A	ATCC 8368^T
Tsukamurella paurometabola group B	ATCC 25938

[a] T, type strain; ATCC, American Type Culture Collection, Rockville, Md.; CIP, Institut Pasteur, Paris, France, CSHWA, California State Health and Welfare Agency, Berkeley, Calif.; DSM, Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH, Braunschweig, Germany; UAF, Mycobacteriology Section, Public Health Laboratories, Toronto, Ontario, Canada; TXN, The University of Texas Health Center at Tyler, Tyler, Tex.; UHL, University Hospital Campus, London Health Sciences Centre, London, Ontario, Canada; Yassin, Institut für Medizinische Mikrobiologie und Immunologie, Rheinische Friedrich-Wilhelms-Universitat, Bonn, Germany.

diagram (30). The manufacturer recommends that isolates less than 9 EDs apart are the same species and that those less than 25 EDs apart belong in the same genus.

RESULTS

Aerobic actinomycetes, like coryneforms, were separated into two fatty acid types, which we labeled type I and type II (2). Type I species exhibited a major percentage of branched-chain fatty acids (Table 2), while type II species exhibited a major percentage of saturated or monounsaturated straight-chain fatty acids (Table 3).

Type I species possessing a major percentage of fatty acids of the branched-chain type. (i) *Rothia dentocariosa*. *R. dentocariosa* isolates were unique because they possessed greater than 30% 15:0 anteiso fatty acids as their major fatty acid; all other species possessed 16:0 iso or a smaller percentage of 15:0 anteiso fatty acids as their major fatty acid.

(ii) *Amycolatopsis orientalis*. Three *A. orientalis* strains including DSM 43134 (*A. orientalis* subsp. *lurida*^{T}) were identified by the type strain library entry. Two *A. orientalis* strains including DSM 43388 were in group B, while ATCC 35166 was the sole member of group C. Group D strains contained the fatty acids 16:1 iso H, 16:1 ω7c, and iso 17:1 ω9c, which were not encountered in any other *A. orientalis* strains and lacked sum feature 4 (Table 2), which was common to all other *A. orientalis* strains. One isolate received as a putative *A. orientalis* strain had a fatty acid composition dissimilar to that of any *A. orientalis* strain that we have encountered and appeared to be related to *Streptomyces* species.

(iii) *Nocardiopsis dassonvillei*. *N. dassonvillei* was the only member of this group to contain the fatty acids 18:0 and 18:0 10-methyl(tuberculostearic acid). Three putative isolates possessed major branched-chain fatty acids, but lacked tuberculostearic acid and were excluded from being *N. dassonvillei* (16, 54).

(iv) *Pseudonocardia autotrophica*. None of the *P. autotrophica* isolates were identified by the type strain library entry. Strains ATCC 19727^T and ATCC 33795 were related, although they did not meet the identification criteria, as were strains DSM 43083, DSM 43084, DSM 43085, DSM 43091, and DSM 43106. Strains NRRL B-16063 (DSM 43088) and ATCC 33797 were distinct. All strains were placed into separate groups.

(v) *Saccharothrix aerocolonigenes*. *S. aerocolonigenes* ATCC 35162 was not identified by the library entry for ATCC 23870^T. Multivariate analysis of the isolates showed them to be separated by approximately 29 EDs, which suggests that *S. aero-*

TABLE 2. Species with a majority of fatty acids of the branched-chain type

Organism (no. of isolates)	% of total fatty acids present																											
	13:0 anteiso	14:0 iso	14:0	15:0 iso	15:0 anteiso	15:1 ω6c	15:0	16:1 iso H	16:0 iso	16:1 ω7c	16:0	Iso 17:1 ω9c	16:0 10 methyl	Anteiso 17:1 ω9c	17:0 iso	17:0 anteiso	17:1 ω8c	17:1 ω6c	17:0 cyclo	17:0	17:0 10 methyl	18:0 iso	18:1 ω9c	18:0	TBSA 10Me18:0	Feature 2[a]	Feature 4
Amycolatopsis orientalis group A (4)	—[b]	9.2	—	13	2.5	—	4.8	—	23	—	3.7	—	—	—	1.1	1.3	10	—	—	6.3	—	—	—	—	—	—	13.4
Amycolatopsis orientalis group B (3)	—	16.7	1.0	4.7	0.8	—	5.0	—	41.5	—	3.8	—	—	—	—	—	0.7	—	—	2.5	—	—	—	—	—	—	3.2
Amycolatopsis orientalis group C (1)	—	19.0	—	8.7	1.5	—	5.7	—	34.7	—	1.6	—	—	—	0.6	0.7	5.2	—	—	5.1	—	—	—	—	—	—	5.6
Amycolatopsis orientalis group D (3)	—	7.0	—	3.5	3.0	0.6	0.8	7.0	31.4	8.0	3.6	1.0	—	—	4.3	3.2	3.5	—	—	0.9	—	0.4	2.2	—	—	—	—
Nocardiopsis dassonvillei (4)	—	1.9	—	0.7	3.2	—	—	—	34	—	2.0	—	—	—	2.4	13.8	7.7	—	—	2.3	3.6	2.8	15.1	3.5	4.9	—	1.7
Pseudonocardia autrotrophica group A (1)	—	—	—	5.0	—	1.4	1.1	14.0	44.2	5.2	1.4	7.2	—	—	5.4	1.7	7.9	—	—	—	5.0	—	—	—	—	—	—
Pseudonocardia autrotrophica group B (1)	—	—	—	6.5	—	1.9	1.4	11.4	39.0	5.3	1.1	7.3	—	—	6.5	1.4	11.9	—	—	0.8	5.5	—	—	—	—	—	—
Pseudonocardia autrotrophica group C (1)	—	4.8	—	4.9	—	0.6	—	30.0	43.0	4.8	0.6	3.9	—	—	2.0	3.1	0.8	—	—	—	—	—	—	—	—	1.0	—
Pseudonocardia autrotrophica group D (1)	—	1.7	0.7	11.5	0.9	1.2	1.5	5.1	36.8	—	3.9	—	—	—	8.0	5.8	6.2	—	—	1.3	0.4	0.4	1.0	—	—	0.3	12.4
Pseudonocardia autrotrophica group E (1)	—	1.2	—	5.2	—	—	—	19.7	42.9	—	1.2	—	6.5	—	4.8	1.6	1.3	—	—	—	1.2	0.4	0.8	—	—	0.6	12.4
Pseudonocardia autrotrophica group F (1)	—	2.2	—	7.9	0.4	0.8	—	22.6	41.0	—	1.1	—	6.3	—	4.2	2.1	1.5	—	—	—	1.0	—	—	—	—	1.2	7.1
Pseudonocardia autrotrophica group G (1)	—	2.0	0.5	9.3	0.5	1.5	0.9	12.5	41.1	—	1.8	—	4.4	—	4.9	2.6	4.5	—	—	0.5	1.0	0.4	0.5	—	—	0.7	9.7
Pseudonocardia autrotrophica group H (1)	—	1.2	—	9.8	0.4	1.2	—	20.4	36.5	—	1.0	—	8.4	—	5.7	3.0	2.1	—	—	—	1.3	—	—	—	—	1.6	7.3
Pseudonocardia autrotrophica group I (1)	—	1.5	0.4	9.5	0.5	1.1	0.5	17.3	36.5	—	1.8	—	3.3	—	6.5	3.8	3.8	—	—	—	1.2	0.5	0.7	0.8	—	1.2	9.7
Rothia dentocariosa group A (1)	—	2.2	3.5	7.4	34.6	—	—	—	11.9	—	24.1	—	—	0.6	—	12.4	—	—	—	—	—	—	—	—	—	—	—
Rothia dentocariosa group B (10)	0.6	2.7	1.2	4.0	51.9	—	—	—	18.6	—	4.9	—	—	—	0.4	15.6	—	—	—	—	—	—	—	—	—	—	—
Rothia dentocariosa group C (5)	1.6	4.6	1.7	4.3	58.5	—	—	—	15.4	—	5.1	—	—	—	—	8.5	—	—	—	—	—	—	—	—	—	—	—
Saccharothrix aerocolonigenes group A (1)	—	4.2	—	3.2	2.3	—	—	23.6	55.5	—	1.5	1.1	—	0.8	0.6	2.0	1.0	—	—	—	0.5	—	—	—	—	—	1.2
Saccharothrix aerocolonigenes group B (1)	—	14.1	2.0	4.4	3.7	1.6	1.2	4.2	40.1	—	3.7	—	2.6	—	0.4	1.0	1.8	1.4	—	0.3	—	—	0.4	—	—	—	15.5
Streptomyces albus (1)	0.4	18.1	—	10.1	19.8	0.9	1.6	5.0	33.1	—	1.4	—	—	—	1.5	3.9	—	—	0.4	0.3	—	0.4	—	—	—	—	0.3
Streptomyces anulatus (1)	—	13.2	0.4	3.5	26.5	—	1.0	2.5	28.1	—	3.3	1.0	—	—	1.2	7.5	0.4	—	3.0	—	—	—	—	—	—	—	1.4
Streptomyces coelicolor (2)	0.4	5.9	0.7	6.9	25.7	0.4	4.7	3.6	22.3	—	9.6	—	—	—	2.2	9.0	0.8	—	1.4	0.9	—	—	—	—	—	—	2.5
Streptomyces griseus group A (1)	—	9.5	0.4	8.2	24.8	—	2.4	3.3	27.4	—	5.3	—	—	—	2.0	5.7	1.1	—	1.0	0.4	—	—	—	—	—	—	4.6
Streptomyces griseus group B	—	9.0	—	8.0	22.3	1.0	3.6	7.1	24.3	—	4.8	1.2	—	—	1.9	7.5	0.9	—	0.9	1.1	—	—	—	—	—	—	2.4
Streptomyces lavendulae (1)	0.2	3.6	0.6	7.9	34	—	1.0	1.9	17.4	—	6.1	2.2	—	—	2.4	13.5	0.2	—	2.9	0.3	—	—	—	—	—	—	1.7
Streptomyces rimosus (1)	0.3	4.8	0.3	18.0	26.7	—	1.1	3.9	19.5	—	2.4	2.4	—	—	3.0	8.8	—	0.8	—	—	—	—	—	—	—	—	2.6
Streptomyces somaliensis (1)	—	1.8	0.7	13.4	10.6	1.2	5.1	3.2	15.0	—	8.0	6.0	—	—	3.0	5.8	5.8	0.5	—	0.9	0.3	—	0.4	—	—	—	14.3
Streptomyces violaceoruber (1)	—	3.4	4.0	7.8	25.3	—	2.2	4.1	21.4	—	7.7	2.1	—	—	3.3	13.1	0.5	—	1.4	0.6	—	—	—	—	—	—	2.0

[a] Summed feature 2 (Feature 2) is a combination of 15:1 iso H and 13:0 3OH fatty acids, and summed feature 4 (Feature 4) is a combination of 15:0 iso 2OH and 16:1 ω7t fatty acids.
[b] —, fatty acid not detected.

TABLE 3. Species with a majority of fatty acids of the saturated or monounsaturated type

Organism (no. of isolates)[a]	% of total fatty acids present																							
	14:0	15:1 ω5c	15:0	16:0 iso	16:1 ω11c	16:1 ω9c	16:0 ω7c	16:0	16:0 10 methyl	17:1 ω8c	17:0	17.0:10 methyl	18:1 ω9c	18:0	TBSA 10me 18:0	17:0 3OH	19:1 ω12t	19:0	20:4 ω6,9, 12,15c	20:1 ω9c	20:2 ω6,9c	20:0	Feature 4[c]	Feature 7	Feature 8
Dematophilus congolensis (2)	38.7	—[b]	2.8	—	—	9.5	5.8	25.2	—	2.7	—	—	6.9	1.1	—	—	—	—	—	—	—	—	—	—	—
Gordona aichiensis (2)	2.6	—	—	—	—	0.5	—	29.7	—	—	0.7	—	14.2	4.8	27.0	—	—	—	—	—	—	—	16.1	—	—
Gordona bronchialis group A (2)	1.4	1.5	6.6	—	—	—	—	17.8	—	12.9	14.1	5.4	14.0	3.2	12.0	—	—	—	—	—	—	—	8.9	—	1.2
Gordona bronchialis group B (1)	2.6	0.4	4.4	—	—	0.5	—	26.0	—	7.9	13.2	1.3	19.6	6.0	6.8	—	—	0.3	—	—	—	—	9.2	—	0.9
Gordona rubropertinctus (1)	2.9	1.0	1.6	—	—	—	—	26.2	—	1.9	1.8	0.5	17.5	—	14.0	—	—	—	—	—	0.7	—	22.5	—	3.1
Gordona sputi group A (1)	3.3	—	2.2	—	—	—	—	47.8	—	0.7	1.3	—	29.0	2.4	12.0	—	—	—	—	—	—	—	—	—	—
Gordona sputi group B (2)	3.4	0.6	2.1	—	—	—	—	30.1	—	1.0	1.7	0.6	7.8	1.2	28.7	—	—	—	—	—	—	—	17.0	—	—
Gordona terrae (2)	2.9	—	0.5	—	—	—	—	34.9	—	0.9	0.9	—	20.4	1.7	19.0	—	—	—	—	—	2.0	—	16.1	—	0.7
Mycobacterium abscessus (4)	5.0	—	—	—	—	2.0	3.1	34.4	—	1.0	0.6	—	35.3	1.2	7.3	—	—	—	—	—	—	—	9.6	—	—
Mycobacterium chelonae (8)	11.3	—	0.5	—	—	2.0	2.2	30.0	—	1.0	0.7	—	37.0	1.3	5.6	—	—	—	—	—	—	—	9.0	—	—
Mycobacterium fortuitum group A (9)	8.8	—	0.9	—	—	1.8	—	33.3	—	0.6	0.7	—	24.0	3.0	11.0	0.5	—	—	—	—	—	—	14.3	—	0.7
Mycobacterium fortuitum group B (8)	6.3	—	0.4	—	—	2.2	—	34.0	—	0.4	0.3	—	35.7	1.3	7.5	—	—	—	—	—	—	—	11.0	—	—
Mycobacterium fortuitum group C (4)	6.0	—	0.4	—	—	2.0	—	34.0	—	0.7	0.5	—	38.0	1.6	5.7	—	—	—	—	0.5	—	—	10.4	—	—
Mycobacterium fortuitum group D (4)	6.7	—	0.6	—	—	2.4	—	33.4	—	0.9	—	—	33.4	0.9	9.8	—	—	—	—	—	—	—	11.6	—	—
Mycobacterium fortuitum group E (2)	5.4	—	0.4	—	—	2.3	—	34.0	—	0.7	0.5	—	41.3	1.6	3.9	—	—	—	—	0.7	—	—	9.1	—	—
Mycobacterium peregrinum (10)	8.2	—	0.7	—	—	1.5	—	34.4	—	1.0	0.6	—	31.4	1.1	7.5	—	—	—	—	—	—	—	12.6	—	—
Nocardia asteroides group A (1)	—	—	1.1	—	—	—	—	30.9	—	1.5	3.8	1.3	7.5	2.8	23.0	—	—	—	—	—	3.3	—	24.5	—	—
Nocardia asteroides group B (36)	1.5	—	1.4	—	—	—	—	29.6	—	1.2	3.0	0.7	10.1	12.8	15.0	4.7	—	—	—	—	—	—	19.1	—	—
Nocardia asteroides group C (2)	2.0	—	—	—	—	—	1.4	34.5	—	—	3.2	—	20.7	19.7	10.6	—	—	—	—	—	1.0	—	6.5	—	—
Nocardia asteroides group D (2)	2.8	—	—	0.9	—	—	5.2	42.2	—	—	—	—	29.1	2.9	6.5	—	—	—	—	—	—	—	10.2	—	—
Nocardia asteroides group E (1)	2.5	—	3.7	—	—	—	—	31.3	—	4.2	3.5	1.4	15.9	4.7	12.3	1.9	—	—	—	—	—	—	17.5	—	—
Nocardia asteroides group F (1)	0.8	—	—	3.0	0.9	—	—	48.5	—	—	—	—	20.8	0.8	22.7	—	—	—	—	—	—	—	1.0	—	—
Nocardia asteroides group G (1)	2.0	0.6	4.7	—	—	—	—	30.0	—	4.9	3.8	3.0	12.5	5.4	12.9	—	—	—	—	—	—	—	18.1	—	0.8
Nocardia asteroides drug type VI (9)	1.4	—	1.6	—	—	—	—	33.1	—	1.1	2.5	1.0	12.3	10.2	17.8	—	—	—	—	—	—	—	16.5	—	0.4
Nocardia brasiliensis (19)	2.0	0.3	3.8	—	—	0.5	—	37.1	—	4.9	3.8	0.9	19.8	2.9	7.2	1.8	—	—	—	0.3	—	—	11.7	—	0.7
Nocardia brevicatena (2)	2.1	—	1.3	3.0	1.9	1.3	8.1	16.7	—	1.9	2.0	—	41.5	2.1	10.8	—	—	—	—	—	—	—	—	5.3	—
Nocardia carnea group A (1)	1.3	—	—	0.4	—	—	6.9	25.9	—	—	—	—	30.2	6.9	12.5	—	—	—	—	4.7	—	—	9.9	—	—
Nocardia carnea group B (1)	0.5	—	0.9	—	—	—	—	32.3	—	0.4	2.1	—	19.2	1.7	13.9	—	—	—	—	—	2.6	—	24.5	1.0	—
Nocardia farcinica (93)	1.4	—	—	—	—	—	—	29.5	—	0.6	1.2	—	19.7	6.4	15.2	—	—	—	—	2.1	—	—	22.2	—	0.5
Nocardia nova group A (7)	1.1	—	—	—	—	—	—	37.7	—	—	1.6	—	21.4	7.4	13.8	—	—	—	—	0.8	—	—	14.1	—	—
Nocardia nova group B (32)	—	—	—	—	—	—	—	30.0	—	2.1	5.4	—	24.1	12.8	13.5	—	—	—	—	—	—	—	9.9	—	—
Nocardia nova group C (3)	—	—	—	—	—	—	—	27.9	—	—	2.4	—	16.1	25.7	17.1	—	—	—	—	—	—	—	10.0	—	—
Nocardia nova group D (1)	2.5	0.4	1.4	—	—	0.8	—	33.4	—	3.3	3.0	—	27.1	1.0	7.4	1.0	—	—	1.6	—	—	—	15.2	—	0.6
Nocardia otitidiscaviarum group A (3)	1.4	0.6	3.2	—	—	—	—	35.9	—	2.6	1.6	1.7	10.4	—	17.3	—	—	—	—	—	—	—	23.4	—	1.3
Nocardia otitidiscaviarum group B (1)	0.9	0.4	3.2	—	—	—	—	28.0	—	3.8	2.8	2.3	20.8	5.6	13.7	0.6	—	—	—	—	—	—	14.5	0.9	1.7
Nocardia otitidiscaviarum group C (2)	0.9	0.5	2.6	—	—	—	—	31.3	—	1.9	2.2	1.2	14.6	3.8	21.7	—	—	—	—	0.8	—	—	16.0	—	2.2
Nocardia pseudobrasiliensis (21)	1.1	—	2.3	—	—	2.4	—	38.7	—	2.7	1.4	0.6	20.1	1.2	9.7	—	—	—	—	—	—	—	18.3	—	—
Nocardia transvalensis group A (1)	3.6	—	3.3	1.5	—	0.7	—	33.1	—	4.2	3.8	3.4	11.9	1.4	17.6	—	—	—	—	0.4	—	—	10.9	—	0.7
Nocardia transvalensis group B (1)	2.2	0.6	3.2	1.8	—	—	—	32.1	—	3.2	4.8	1.4	14.0	3.4	15.2	—	—	—	—	1.0	—	—	14.9	—	0.7
Rhodococcus coprophilus (1)	3.9	—	2.0	—	—	—	18.0	19.4	25.3	1.6	0.4	1.7	1.6	—	5.7	—	—	—	—	—	—	—	16.1	—	—
Rhodococcus equi group A (10)	3.3	—	1.3	—	—	—	—	26.4	2.6	—	9.8	1.5	3.6	1.1	20.1	—	3.4	—	2.7	1.9	—	—	10.6	1.4	1.8
Rhodococcus equi group B (96)	9.9	—	1.6	—	—	—	6.9	30.0	10.9	1.2	—	1.7	5.1	0.9	12.7	—	2.4	1.9	0.8	—	—	—	10.7	—	1.7
Rhodococcus erythropolis (9)	6.9	0.7	3.5	—	—	—	—	25.2	2.9	1.7	1.8	2.8	4.5	0.8	16.0	—	—	7.4	—	—	—	3.6	15.4	—	1.3
Rhodococcus fascians (1)	6.5	0.8	13.7	—	—	—	—	21.2	0.6	4.8	—	2.0	15.0	2.4	9.5	—	—	1.6	—	0.9	—	—	12.6	—	6.9
Rhodococcus globerulus (1)	18.7	—	2.7	—	—	—	—	17.2	1.1	0.8	0.9	—	12.0	0.9	12.7	—	—	8.7	—	—	5.7	1.2	10.1	—	3.0
Rhodococcus rhodochrous group A (1)	2.1	—	1.6	—	—	—	—	26.3	7.2	1.8	2.5	3.1	4.6	1.4	20.6	—	—	2.6	0.4	—	—	3.9	22.1	—	—
Rhodococcus rhodochrous group B (1)	0.9	—	5.5	—	—	—	—	22.4	0.3	13.3	13.7	1.9	18.2	12.4	3.9	—	—	2.7	0.3	—	—	—	4.6	—	—
Rhodococcus rhodochrous group C (1)	3.8	0.5	3.2	—	—	—	—	28.6	18.1	2.2	1.2	2.8	3.0	1.3	6.5	—	—	2.1	—	—	—	1.6	23.2	—	—
Rhodococcus rhodochrous group D (1)	2.5	1.6	4.4	—	—	—	—	30.4	20.6	3.0	2.9	6.9	1.8	0.6	9.6	—	—	—	—	—	—	—	14.5	—	—
Tsukamurella paurometabola group A (1)	4.5	—	0.5	—	—	0.4	—	28.0	—	—	1.0	—	22.0	3.7	12.4	—	—	—	0.3	5.4	—	—	19.7	—	1.9
Tsukamurella paurometabola group B (9)	4.6	—	0.9	—	—	0.8	—	31.2	—	0.5	1.6	—	31.1	2.2	10.1	—	—	—	—	2.1	—	—	14.0	—	0.6

[a] *D. congolensis* contains 5% 12:0, *G. aichiensis* contains 2% 20:3 ω6,9,12c, *G. rubropertinctus* contains 3% 17:1 ω9c, *G. sputi* group B contains 4% 20:3 ω6,9,12c, *R. coprophilus* contains 5% 17:0 cyclo, *R. equi* group B contains 4% 17:0 ω7c and 2% feature 9, *R. erythropolis* contains 5% 17:1 ω5c, *R. globerulus* contains 2% 17:1 ω5c, and *R. rhodochrous* group C contains 7% 16:1 ω5c and 3% 17:0 ω7c.

[b] —, fatty acid not detected.

[c] Summed feature 4 (Feature 4) is a combination of 15:0 iso 2OH and 16:1 ω7t fatty acids, summed feature 7 (Feature 7) is a combination of 18:1 ω9c, ω12t, ω9t, and ω7c fatty acids, summed feature 8 (Feature 8) is a combination of the unknown fatty acid with the equivalent chain length of 18.856 and 19:1 ω11c fatty acids, and summed feature 9 (Feature 9) is a combination of the unknown fatty acids with the equivalent chain length of 18.846, and 18.858 and 19:0 cyclo ω10c.

colonigenes ATCC 35162 is improperly classified to the species level and possibly to the genus level (30).

(vi) *Streptomyces* species. All type strains were differentiated as separate species. The *Streptomyces somaliensis* type strain had a different fatty acid content than the other *Streptomyces* type strains, as did *S. somaliensis* ATCC 19437. The proposed neotype strain of *S. somaliensis* ATCC 19437 has been reidentified as a strain of *S. griseus* (14). Strain ATCC 19437 was not identified by the type strain library entry and was assigned to *S. griseus* group B. Eleven strains received as *Streptomyces* species as well as three strains received as *N. dassonvillei* were identified by the *S. griseus* group B library entry. Another isolate identified by the *S. coelicolor* library entry and nine isolates were not identified. All strains with the exception of *Streptomyces rimosus* and *S. somaliensis* contained a small percentage of a 17:0 cyclo fatty acid, which may be a useful marker in the identification of *Streptomyces* species.

To identify a greater percentage of isolates a single-genus-level library entry was constructed from the *Streptomyces* type strains with the exception of *S. somaliensis* ATCC 33201^T. This library entry identified 20 isolates submitted as *Streptomyces* species as well as three misidentified strains. This level of identification could prove to be satisfactory for clinical laboratories because it would rule out the more significant pathogens.

Type II species possessing a major percentage of fatty acids of the straight-chain saturated or monounsaturated type. The type II species possessing a major percentage of fatty acids of the straight-chain saturated or monounsaturated type are members of closely related genera and include the most commonly isolated aerobic actinomycetes causing human infections.

(i) *Dermatophilus congolensis.* *D. congolensis* differed from the other type II members because it possessed 14:0 as its major fatty acid and lacked tuberculostearic acid. All other genera possessed 16:0, 18:1 ω9c, and tuberculostearic acid fatty acids as their major fatty acids.

(ii) *Gordona* species. The type strains of the *Gordona* species *G. aichiensis*, *G. bronchialis*, *G. rubropertinctus*, *G. sputi*, and *G. terrae* could be differentiated as separate species. *Gordona sputi* ATCC 33610 was not identified by the type strain library entry, and it was assigned to group B, which identified ATCC 33609. One strain was identified by the type strain entry for *G. aichiensis*, and another isolate met the identification criteria with *G. bronchialis* ATCC 25592^T.

(iii) *Mycobacterium* species. The type strains of the *Mycobacterium* species *M. abscessus*, *M. chelonae*, *M. fortuitum*, and *M. peregrinum* could be differentiated by fatty acid composition. All isolates of *M. abscessus*, *M. chelonae*, and *M. peregrinum*, but only 8 of 28 strains of *M. fortuitum*, were correctly identified by the type strain library entries. The remaining *M. fortuitum* strains were incorrectly identified by our library as *M. peregrinum* or were not identified, leaving the differentiation of these two species in doubt. One *M. fortuitum* isolate, biochemically identified as a member of the third biovar, was not identified by any library entry. Another isolate previously identified biochemically as a member of the *M. fortuitum* group was identified by the *M. abscessus* type strain. Subsequent biochemical testing supported the fatty acid identification.

(iv) *Nocardia asteroides.* Twenty-two *N. asteroides* isolates were identified as *Nocardia farcinica*, and six isolates were identified as *Nocardia nova*. These findings support the conclusions of other workers for ATCC 3308 (21, 50), ATCC 6864 (44, 50), ATCC 9504 (44), ATCC 10904 (49), and ATCC 23825 (50). *Nocardia asteroides* ATCC 13781 had a fatty acid composition closely related to that of rapidly growing *Mycobacterium* species and has been reidentified as *Mycobacterium senegalense* (7, 33). Ten cultures were found to be contaminated, containing mixtures of *N. asteroides* and *N. farcinica* or *N. nova*. Ten isolates of *N. asteroides* grew too slowly to produce sufficient biomass for analysis. The type strain library entry did not identify any of the 23 remaining isolates. Sixteen isolates were identified by the *N. asteroides* group B library entry, and the remaining isolates were assigned to a further five groups. An isolate received as an aberrant strain of *N. asteroides* was found to possess type I fatty acids and lacked tuberculostearic acid, excluding it from the genus *Nocardia*. Multivariate analysis of all type I isolates linked this strain to *Pseudonocardia autotrophica* ATCC 19727 and ATCC 33795. Eleven strains of *N. asteroides* drug type VI proposed by Wallace et al. (51) were analyzed. Nine strains were identified as *N. asteroides* group B, one strain was identified as *N. nova* group A, and one strain was not identified but had low SIs with *N. asteroides* groups B and E.

(v) *Nocardia brasiliensis* and *Nocardia pseudobrasiliensis.* The four *N. brasiliensis* isolates in our culture collection partitioned into three groups, two of which were related. When 23 additional *N. brasiliensis* and 12 *N. pseudobrasiliensis* isolates were analyzed, 18 *N. brasiliensis* isolates were identified as *N. brasiliensis* group A and 5 isolates were identified as *N. brasiliensis* group B. The 12 *N. pseudobrasiliensis* isolates were identified as *N. brasiliensis* group B or C. The type strain of *N. pseudobrasiliensis* was not commercially available. Instead, two of the strains that had been used in the original description of the new species were used to create a single library entry. All *N. pseudobrasiliensis* strains with one exception were identified by this entry.

(vi) *Nocardia brevicatena.* Multivariate analysis of the two *N. brevicatena* isolates indicated they were related, but *N. brevicatena* ATCC 15725 was not identified by the type strain library entry.

(vii) *Nocardia carnea.* *N. carnea* ATCC 35163 was not identified by the library entry for ATCC 6847^T. Twelve isolates of *N. carnea* from clinical sources had insufficient biomass production, which precluded analysis.

(viii) *Nocardia farcinica.* Strains of *N. farcinica* formed a homogeneous group with all but 4 of the 73 isolates identified by the type strain library entry. Of these four isolates, two were unrelated to any *Nocardia* type strain, one was identified as *N. nova* group A, and one was identified as *N. asteroides* group B. The last isolate had the biochemical pattern of *N. farcinica* but the susceptibility pattern of *N. asteroides* drug type VI of Wallace et al. (51). Another isolate possessed an orange-red pigment which is unlike that of any other strain of *N. farcinica* that we have examined.

(ix) *Nocardia nova.* The type strain library entry identified three strains received as *N. nova* and two clinical strains received as *N. asteroides* as well as ATCC 10904. The group B library entry identified 27 isolates as *N. nova* and identified as *N. nova* 4 strains received as *N. asteroides*. Two strains received as *N. nova* were identified as *N. farcinica*, two strains were unrelated to any *Nocardia* library entry, and a single strain contained a mixture of two *N. nova* fatty acid groups. Two strains were not identified by either of the library entries and formed the nuclei for groups C and D.

(x) *Nocardia otitidiscaviarum.* The 20 *N. otitidiscaviarum* strains examined were heterogeneous, and ATCC 14629^T, ATCC 14630, and ATCC 27941 were used as nuclei for three groups. ATCC 27943 was identified by the type strain library entry. Multivariate analysis of the remaining strains indicated that they were most likely separate groups.

(xi) *Nocardia transvalensis*. None of the 13 *N. transvalensis* strains was identified by the type strain library entry, and with the exception of 2 isolates, the 13 strains were dissimilar.

(xii) *Rhodococcus* species. The *Rhodococcus* type strains could be separated by their fatty acid compositions. *Rhodococcus erythropolis* ATCC 11048 was identified by the type strain library entry, as were eight other strains. Some of these strains hydrolyzed tyrosine and were negative for acid production from glucose, glycerol, mannose, trehalose, and xylose, and their identification could not be confirmed. All of the *Rhodococcus equi* strains from human sources were identified with the group B library entry. Six other strains possessed unrelated fatty acid profiles and were not allocated to groups. The four American Type Culture Collection (ATCC) strains of *R. rhodochrous* were sufficiently different to be placed in separate groups.

(xiii) *Tsukamurella paurometabola*. None of the *T. paurometabola* isolates was identified by the type strain library entry. All other isolates were identified by the group B library entry. Multivariate analysis of *T. paurometabola* ATCC 8368^T (formerly *Corynebacterium paurometabolum*) and ATCC 25938 (formerly *Gordona aurantiaca*) showed them to be separated by greater than 10 EDs, indicating separate species.

DISCUSSION

Correct and timely identification of aerobic actinomycetes is important, because their pathogenic potentials vary and some species are more likely to cause disseminated disease (29). Identification has traditionally been accomplished with biochemical testing and the determination of growth characteristics (31, 44). These methods may have insufficient discriminatory power, as evidenced by the emergence of several new species and subgroups and the reassignment of taxa to different species or even genera (18, 19, 35, 36, 38, 42, 50–52). Molecular biology-based methods involving DNA probes, 16S rRNA sequencing, DNA amplification, or restriction fragment length polymorphisms have been shown to be useful in the identification of *Mycobacterium* and *Nocardia* species (4, 8, 42, 52).

Analysis of fatty acids is a reliable means of bacterial identification (47, 53). However, application as a means of identifying aerobic actinomycetes has remained limited, even though the determination of fatty acids has long been used as an aid in species determination (22). Our attempt at characterizing pathogenic aerobic actinomycetes using fatty acid profiles met with limited success, because only 177 of 529 (33.5%) isolates were identified as valid strains. The remaining isolates were identified by library entries based on nonvalid species strains or were not able to be analyzed due to insufficient growth. In our study the only uncontrolled factors were intrinsic characteristics of the isolate. Therefore, our fatty acid groups, developed by using a heuristic or self-educating approach, reflect either variables which affect fatty acid composition or limitations of current taxonomy.

The heterogeneity of species has been recognized by a variety of techniques for many of the aerobic actinomycetes. *A. orientalis* isolates exhibit variability in lysozyme and phage susceptibility as well as their fatty acid makeup (13, 24). Our finding of fatty acid diversity within *R. dentocariosa* was supported by the findings of Lesher et al. (25), who recognized four biotypes and three serotypes, but was not in accord with the findings of Bernard et al. (2), who did not report fatty acid heterogeneity within this species.

The genus *Gordona* currently consists of six validly recognized species (19, 38, 41, 55). Infections with *G. aichiensis*, *G. rubropertinctus*, *G. sputi*, *G. terrae*, and *G. bronchialis* have been reported, with infections with *G. bronchialis* being the most frequent (29, 45). We examined a small number of *Gordona* isolates and found diversity within recognized species. This supports the work of Tsukamura (45), who in his original description noted variants of *G. bronchialis* as well as other isolates that he thought were *Gordona* species but that he did not assign to taxons. We were not able to differentiate *M. fortuitum* from *M. peregrinum*. Similarly, Thibert and LaPierre (43) were unable to identify the three former biovars of *M. fortuitum* using analysis of high-molecular-weight mycolic acids.

Based on antibiotic susceptibility, phenotypic testing, molecular evidence, and our own work, it is apparent that *N. asteroides* new sense still contains a number of distinct groups or taxa (4, 31, 46, 51). Ridell (37), using immunodiffusion studies, concluded that ATCC 19247^T was divergent from most strains of the species, and Wallace et al. (49) found the isolate to have a rare drug resistance pattern. This supports our findings because none of the 23 isolates that we examined was identified by the type strain library entry. However, we were able to identify 95% of *N. farcinica* isolates submitted to us. Wallace et al. (50) noted that some variation existed within the species because two of the strains used in the original description were susceptible to lysozyme.

The final identification of isolates as *N. asteroides* new sense, *N. farcinica*, or *N. nova* could often be predicted from their colonial morphologies. Most *N. asteroides* new sense isolates produced good growth that was adherent to the TSBA, abundant white aerial mycelia, and no colonial pigmentation. *N. farcinica* isolates usually produced good growth that had a nonadherent creamy or waxy consistency, without aerial hyphae, and a distinct orange pigment. *N. nova* isolates usually produced fine adherent growth, abundant white aerial mycelia, and an orange pigment.

Multivariate analysis of our four original strains of *N. brasiliensis* corroborated the conclusions of Wallace et al. (52) and Ruimy et al. (39) that two species existed within this taxon and demonstrates the differentiating power of fatty acid analysis.

Heterogeneity within *N. otitidiscaviarum* and *N. transvalensis* has been described previously (34, 37, 42) and is consistent with our findings. Recently, Steingrube et al. (42), using PCR to amplify a 439-bp segment of the 65-kDa heat shock protein gene from *Nocardia* species, detected three groups in *N. otitidiscaviarum* and two groups in *N. transvalensis.*

Taxonomic revisions within the genus *Rhodococcus* have been extensive, and it currently consists of 12 species, 6 of which have been associated with human infections (18, 19, 29, 36, 38). The six species that we studied possessed the fatty acid 16:0 10-methyl. This fatty acid was absent from *Rhodococcus* species reassigned to other genera but was present in species reassigned to *Rhodococcus* from other genera (18, 19, 36) and might be a useful marker for *Rhodococcus* species. Diversity within *R. equi* was expected because two groups have been detected by using biochemical testing, three groups have been detected by using mycolic acid pattern analysis, and fatty acid heterogeneity has been reported (5, 12, 15, 28, 32). Fatty acid differences in the strains that we examined suggest that two groups exist within the current taxon. Group B, based on a strain cultured from a prostatic abscess, is the most commonly isolated group and identified all strains of human origin (27). We encountered several clinical isolates that, on the basis of phenotypic testing, were initially thought to be *Rhodococcus* species but that were not identified by our library entries. Goodfellow et al. (12) described 26 clusters of suspect *Rhodococcus* isolates that could not be assigned to a valid taxon.

Our findings agree with those of Auerbach et al. (1) who

analyzed 12 outbreak-related strains of *T. paurometabola* plus two strains isolated after the outbreak and found them to be similar to ATCC 25938, but our findings are in contrast to those of Goodfellow et al. (11), who found environmental strains of *T. paurometabola* to be distinct from ATCC 25938.

Many species of aerobic actinomycetes were described prior to the availability of or without DNA hybridization techniques. Some have been described by DNA hybridization techniques, but with the biochemical data based only on the type strain or a limited number of reference strains. Since the majority of clinical laboratories identify isolates solely by biochemical testing, this may lead to incorrect identifications. Fatty acid analysis certainly offers improved identification, but its discriminatory power leads to the detection of much heterogeneity within recognized species. This can obscure identification for those who are inexperienced.

Fatty acid analysis appears to be a useful technique for the rapid identification of some aerobic actinomycetes. The fatty acid heterogeneity within most species necessitates the creation of fatty acid groups of presently unknown significance. These groups likely represent phenotypically similar species or subspecies, or they could possibly be artifacts caused by growth variables. Analysis of more isolates of all species plus data from DNA methods is required to clarify the validity and significance of fatty acid groups.

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