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Numerical Classification of *Actinomadura* and *Nocardiopsis*

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We studied 170 strains of *Actinomadura*, *Nocardiopsis*, and related taxa. The overall similarities of the test strains were determined by comparing 120 unit characters, using the simple matching and pattern coefficients. Clustering was achieved by using the unweighted pair group method with averages. Cluster composition was not markedly affected by the coefficient used or by test error (estimated at 4.9%). The numerical data, together with results from previous chemical and genetic studies, showed that the genus *Actinomadura* is markedly heterogeneous. Strains received as *Actinomadura* fell into two large aggregate groups. One, *Actinomadura* sensu stricto, contained *Actinomadura citrea*, *Actinomadura coerulea*, *Actinomadura coeruleoviolacea*, *Actinomadura cremea*, *Actinomadura livida*, *Actinomadura madurae*, *Actinomadura malachitica*, *Actinomadura pelletieri*, and *Actinomadura verrucosospora*. *Actinomadura kijaniata* was not studied, but other genetic data suggest that it should also be included in this group. The other aggregate group encompassed *Actinomadura ferruginea*, *Actinomadura pusilla*, *Actinomadura roseola*, *Actinomadura roseoviolacea*, *Actinomadura rubra*, and *Actinomadura salmonea*. The representatives of *Actinomadura helvata*, *Actinomadura libanotica*, *Actinomadura luteofluorescens*, *Actinomadura spadix*, and *Actinomadura spiralis* studied formed distinct clusters separate from both of the aggregate groups. *Nocardiopsis* strains formed a cluster clearly distinguished from the clusters containing the *Actinomadura* strains.

The genus *Actinomadura* (36) was introduced for sporoactinomycetes that form branched, usually stable substrate mycelia with walls containing *meso*-2,6-diaminopimelic acid but lacking arabinose and galactose (36, 38). This taxon was cited as a genus incertae sedis in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (40), but was included on the Approved Lists of Bacterial Names (52). The genus originally contained three species, *Actinomadura dassonvillei*, *Actinomadura madurae*, and *Actinomadura pelletieri*, and was classified in the family *Thermoactinomycetaceae* (36). It was subsequently transferred to the family *Thermomonosporaceae* because endospores were not produced (11), but it is now thought to be related to genera with chemotype III walls, notably *Microbispora* and *Microtetraspora* (3, 20, 24, 64). The size of the genus *Actinomadura* has increased so that 34 species are currently recognized or have been proposed (31, 32, 42, 46, 52, 60), primarily on the basis of morphology and wall chemotype. However, there is some evidence that both *A. madurae* and *A. pelletieri* are heterogeneous (4, 7, 19). The third original species, *A. dassonvillei*, formed the basis of the genus *Nocardiopsis* (41), the integrity of which is supported by an abundance of chemical, genetic, morphological, and numerical phenetic data (5, 12, 24, 35, 64).

The genus *Actinomadura* encompasses a range of morphological types (35, 65) and has also been shown to be heterogeneous on the basis of fatty acid (2, 12), menaquinone (5, 12, 67), polar lipid (37, 39), and numerical phenetic data (4, 17, 19, 24). Fischer et al. (12) provided the most compelling evidence of heterogeneity when they recovered representative actinomadurae in two aggregate groups based on deoxyribonucleic acid (DNA)-ribosomal ribonucleic acid (rRNA) and DNA homology values. The composition of the two aggregate taxa was supported by fatty acid and menaquinone data. The type strains of *Actinomadura spadix* and *Actinomadura spiralis* showed little DNA homology to members of either aggregate cluster or to each other.

Conventional numerical taxonomy has helped to clarify the intra- and intergeneric relationships of several actinomycete taxa, notably *Actinomyces* (49), *Mycobacterium* (26), *Nocardia* (21), and *Streptomyces* (64). In the present study, representatives of 20 *Actinomadura* species and marker cultures of *Microbispora*, *Microtetraspora*, *Nocardiopsis*, and *Streptomyces somaliensis* were compared by using numerical techniques in an attempt to further clarify the taxonomy of *Actinomadura* and related sporoactinomycetes.

MATERIALS AND METHODS

Strains. The 155 test strains were obtained from public and private culture collections (Table 1). When possible, type cultures were included, and duplicate cultures prepared from 15 of the strains were chosen to serve as internal checks on experimental test error. All cultures were maintained as suspensions in 20% (wt/vol) glycerol and were stored at −20°C (62). Glucose-yeast extract agar (GYEA) (30) was used as the basal medium.

Collection of data. Each strain was examined for 120 unit characters (Table 2). Unless otherwise stated, all test preparations were incubated at 30°C, and the results were recorded after 7, 14, 21, and 28 days. All tests were performed at least once with each strain and were repeated when ambiguous or unexpected results were obtained. When possible, tests were done in Replidishes (Table 2). With the exception of the antibiotic tolerance tests, in which 7-day-old cultures grown in glucose-yeast extract broth were used as inocula, Replidishes were inoculated from 10- to 14-day-old cultures grown on GYEA master Replidishes by using a multipoint inoculation procedure (57). Inocula for all other tests were taken from freshly thawed glycerol suspensions. Data for computation were taken from the final test readings.

Biochemical tests. Allantoinase and urease production were detected by the method of Gordon (28), nitrate reduction

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TABLE 1. Designations and sources of strains assigned to clusters based on the Dp-UPGMA analysis

Cluster	Laboratory no.[a]	Name	Source[b]
1	A3^T; A155	*A. pusilla*	ATCC 27296^T (= DSM 43357^T = KCC A-0144^T); HN A-115
	A2^T; A154	*A. roseoviolacea*	ATCC 27297^T(= DSM 43144^T = KCC A-0145^T); HN A-103
2	A234^T	*A. luteofluorescens*	ATCC 25469^T(= DSM 43098^T = ISP 5398^T)
	(A195^T, D3^T)	*A. spiralis*	IMET 9621^T (= DSM 43555^T)
3	(A230^T, D10^T)	*A. salmonea*	INA 2488^T
4	(A197, D4); A198; A227^T	*A. roseola*	IMET 9564; IMET 9565; INA 1671^T(= DSM 43551^T)
5	A196	*A. madurae*	IMET 9562
	A4^T; (A193, D2); A194	*A. verrucosospora*	ATCC 27299^T (= DSM 43558^T = KCC A-0147^T); IMET 9619; IMET 9620
6	A94; A124; A126	*A. madurae*	CDC N273; IMET 7144; IMET 7146
	A123; A128	*A. pelletieri*	IMET 7141; laboratory strain
7	A138^T	*A. citrea*	ATCC 27887^T(= DSM 43461^T = INA 1849^T)
	(A16^T, A113^T); A17; A22; A32; A38; A132; A166; A176; A177; A178	*A. madurae*	ATCC 19425^T (= DSM 43067^T = NCTC 5654^T); DSM 43236 (= NCTC 1070); IP 725; IP 364 (= DSM 43380); CBS 331.54; MAG; RNSH 202; RV 12875 (= IP 380); RV 27822 (= IP 83); RV 33888
8	(A224^T, D8^T)	*A. cremea*	INA 292^T
9	A11; A12; A165	*A. madurae*	LSHTM 393; LSHTM 373; RNSH 201
	A228^T	*A. livida*	INA 1678^T
	A139^T	*A. malachitica*	ATCC 27888^T(= DSM 43262^T = INA 1920^T)
10	A175; A181; A182; A237	*A. madurae*	RV 7051; IP 391; IP 767; NCPF 1180
11	A41; A43; A25; A30; A31	*A. madurae*	CBS 134.65; CBS 254.58; IP 393; IP 703; IP 363
	A18; (A116, A167); A122; A130	"*A. pelletieri*"	NCTC 3026; RNSH 203; IMET 9592; MAG 295
12	A26; (A27, A188); A28; A29; A34; A235; A236; A239; A240; A241; A242; A243; A244; A245	*Streptomyces somaliensis*	IP 702; IP 395; IP 313; IP 314; IP 384; NCPF 1069; NCPF 1070; NCPF 1220; NCPF 1221; NCPF 1222; NCPF 1223; NCPF 1224; NCPF 1225; NCPF 1226
13	A7; A8; A9; A10; A13; A19^T; A20; A21; (A23, A170); (A24, A117); A33; A35; A36; A39; A42; A97; A115; A153; A169; A171; A172; A173; A183; A184; A185; A186; A187; A246; A247; A248; A249; A250; A251	*A. pelletieri*	CP 377; CP 388S; CP 368; CP 1065; CP 388H; DSM 43117^T (= NCTC 4162^T); NCTC 9999; NCTC 10,000; IP 374; IP 385; IP 326; IP 308; (= DSM 43384); IP 381; CBS 436.57; CBS 294.64; CDC N86; laboratory strain; DSM 43118; RV 7053; RV 27823 (= IP 84); RV 28240; RV 39036 (= IP 89); IP 389; IP 390; IP 394; IP 388; IP 729; NCPF 1066; NCPF 1171; NCPF 1172; NCPF 1173; NCPF 1211; NCPF 1270
14	(A199^T, D5^T)	*A. ferruginea*	DSM 43553^T (= IMET 9567^T)
15	(A225^T, D9^T)	*A. rubra*	ATCC 27031^T (= INA 325^T)
16	N250	*A. madurae*	CUB 122
	A76; A82	"*Actinomadura* sp."	JL A440; JL A429
	(A14^T; A118^T); A15; A92; A93; A95; A115; A114; A119; A129; A144; A145; A146; A147; A148; A149; A150; A151; A201; A202; (A203, D6); A204; A205; A206; A207; A208; A209; A210; A211; A222; A223; A232; A233	*Nocardiopsis dassonvillei*	ATCC 23218^T(= DSM 4311^T = IMRU 509^T = NCTC 10488^T); NCTC 10489 (= IMRU 1250); CDC W2569; CDC N249; CDC N2491; CDC N291; laboratory strain: DSM 43378 (= IMRU 714); MAG 289G; JL A1438; JL A1439; JL 1440; JL 1441; JL 1442; JL A 1443; JL A1460; JL CW87; JL A1504; JL A1505; JL 1506; JL A1507; JL A1509; JL A1510; JL A1511; JL A1512; JL A1513; JL A1514; JL A1515; JL A1575; JL A1576; JL A1503; JL A1508
17	(A189, D1); A190^T; A191; A192; A200	*A libanotica*	IMET 9615; IMET 9616^T(= DSM 43556^T); IMET 9618; IMET 9617; IMET 9571

Continued on following page

TABLE 1—*Continued*

Cluster	Laboratory no.[a]	Name	Source[b]
Single-member clusters	A226^T	*A. coerulea*	INA 765^T
	A231^T	"*A. coeruleoviolacea*"	INA 3904^T
	A5^T	*A. helvata*	ATCC 27295^T(= DSM 43142^T = KCC A-0143^T)
	A133; A137; A179; A180; A253	*A. madurae*	MAG A291A; laboratory strain; IP 373; IP 384; NCPF1197
	A96	*A. pelletieri*	CDC N242
	A1^T	*A. spadix*	ATCC 27298^T(= DSM 43459^T = KCC A-0146^T)
	G28^T	*Microbispora rosea*	STW E6^T
	G27^T	*Microtetraspora glauca*	ATCC 23057^T
	G103^T	*Microtetraspora niveoalba*	ATCC 27301^T (= KCC A-0149^T)
	G101^T	*Microtetraspora viridis*	ATCC 27103^T (= DSM 43174^T = KCC A-0142^T)
	A98^T	"*Nocardia gardneri*"	RIA 634^T
	A102^T	"*Nocardia polychromogenes*"	RIA 675^T
	G45	"*Streptosporangium* sp."	CUB 662

[a] Parentheses indicate duplicate cultures.

[b] ATCC, American Type Culture Collection, Rockville, Md.; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; CDC, Centers for Disease Control, Atlanta, Ga.; CP, C. Philpot, London School of Hygiene and Tropical Medicine, London, United Kingdom; CUB, Collection of the University of Bradford, Bradford, United Kingdom; DSM, Deutsche Sammlung von Microorganismen, Gottingen, Federal Republic of Germany; HN, H. Nonomura, Yamanashi University, Kofu, Japan; IMET, Institute of Microbiology and Experimental Therapy, Jena, German Democratic Republic; IMRU, Institute of Microbiology, Rutgers University, New Brunswick, N.J.; INA, Institute of New Antibiotics, Moscow, U.S.S.R.; IP, Institut Pasteur, Paris, France; ISP, International Streptomyces Project, Ohio Wesleyan University, Delaware, Ohio; JL, J. Lacey, Rothamsted Experimental Station, Harpenden, United Kingdom; KCC, Kaken Chemical Company Ltd. Tokyo, Japan; LSHTM, London School of Hygiene and Tropical Medicine, London, United Kingdom; MAG, M. A. Gordon, State of New York Department of Health, Albany, N.Y.; NCTC, National Collection of Type Cultures, London, United Kingdom; NCPF, National Collection of Pathogenic Fungi, London School of Hygiene and Tropical Medicine, London, United Kingdom; RIA, Research Institute for Antibiotics, Moscow, U.S.S.R.; RNSH, Royal North Shore Hospital, Crows Nest, New South Wales, Australia; RV, R. Vanbreuseghem, Institute of Tropical Medicine, Antwerp, Belgium; STW, S.T. Williams, University of Liverpool, Liverpool, United Kingdom.

was detected by the method of Gordon and Mihm (30), and the production of prodiginine type pigments was detected by the method of Gerber and Lechevalier (16). Melanin production was determined in the basal medium of Gordon (28).

Degradation tests. Tests for degradation of gelatin, hypoxanthine, L-tyrosine, xanthine, and xylan (each at a concentration of 4%, wt/vol) were performed on GYEA by using the method of Gordon (28), and casein degradation was determined on GYEA supplemented with 0.1% (wt/vol) skim milk. The breakdown of DNA (0.2%, wt/vol) and the breakdown of ribonucleic acid (0.3%, wt/vol) were determined by using the methods of Goodfellow et al. (19) and Goodfellow and Schaal (25), respectively, and starch degradation was determined on GYEA supplemented with 1% (wt/vol) potato starch by flooding the plates with an iodine solution (10) after 10 days. Sierra medium (51) supplemented with 1% (vol/vol) Tween 20 and 1% (vol/vol) Tween 80 was examined for opacity, and hydrolysis of esculin (0.1%, wt/vol) and hydrolysis of arbutin (0.1%, wt/vol) were determined by the method of Williams et al. (64). In these latter tests the development of a rich brown pigment was scored as a positive result. In cases where organisms normally produced a brown pigment on GYEA (Table 2), positive results were scored only when the test media showed a deeper color than the negative control.

Colonial characteristics. Colonial morphology and pigmentation were determined with a binocular microscope (Olympus Metallurgical Services Ltd., Betchworth, England), using 10-day-old cultures grown on GYEA.

Organic compounds as sole sources of carbon. The ability of test strains to use 28 carbon compounds as sole sources of carbon and energy was examined by using the basal medium of Stevenson (59).

Physiological tolerance tests. Resistance to various inhibitors and temperature regimes (Table 2) was determined on GYEA. The ability of strains to grow at 10 and 45°C was determined after 42 and 21 days, respectively. Resistance of strains to 11 antibacterial agents, each at two different concentrations, was determined as described below. Molten GYEA, appropriately supplemented with each of the antibiotics, was dispensed into Replidishes, allowed to set, and then inoculated with 0.02-ml portions of 7-day-old glucose-yeast extract broth cultures. Replidishes containing GYEA alone served as positive controls, and a culture of *Staphylococcus aureus* NCTC 6575 was used as the susceptible control. Inoculated Replidishes were left at room temperature for 2 h to allow the inoculum to soak into the medium. They were then inverted, incubated at 30°C, and examined after 1, 3, 5, 7, and 14 days. When growth on the antibiotic-containing media was considered to be similar to or greater than that on GYEA alone, the organisms were recorded as being resistant. Poor or no growth on antibiotic-containing media was recorded as susceptible.

Coding of data. Most of the characters existed in one of two mutually exclusive states and were scored as plus (1) or minus (0). Qualitative multistate characters, such as pigmentation and colony elevation, were coded as several independent characters, and each was scored plus (1) for the character state shown and minus (0) for the alternatives. Data for coding were taken from results recorded on the final day, except for the antibacterial agent resistance tests. Most of the latter tests were read after 3 to 5 days (that is, when growth on the positive control was good).

Computer analysis. Data were analyzed by using the CLASP package of programs (written by G. J. S. Ross, F. B. Laukner, and D. Hawkins, Rothamsted Experimental Sta-

TABLE 2. Frequency of positive characters in the major and minor clusters found by using the Dp and the UPGMA algorithm[a]

Test or characteristic	No. of strains positive																
	A. pusilla-A. roseoviolacea (cluster 1; 4 strains)	*A. luteofluorescens-A. spiralis* (cluster 2; 3 strains)	*A. salmonea* (cluster 3; 2 strains)	*A. roseola* (cluster 4; 4 strains)	*A. verrucosospora* (cluster 5; 5 strains)	*A. madurae* (cluster 6; 5 strains)	*A. citrea-A. madurae* (cluster 7; 12 strains)	*A. cremea* (cluster 8; 2 strains)	*A. madurae-A. malachitica* (cluster 9; 5 strains)	*A. madurae* (cluster 10; 4 strains)	"*A. pelletieri*" (cluster 11; 10 strains)	*Streptomyces somaliensis* (cluster 12; 15 strains)	*A. pelletieri* (cluster 13; 35 strains)	*A. ferruginea* (cluster 14; 2 strains)	*A. rubra* (cluster 15; 2 strains)	*Nocardiopsis dassonvillei* (cluster 16; 37 strains)	*A. libanotica* (cluster 17; 6 strains)
Biochemical tests																	
Allantoinase production	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Melanin production	3	1	1	0	0	4	12	2	4	4	9	6	22	0	2	15	0
Nitrate reduction	4	3	2	3	1	4	12	2	5	4	10	0[b]	29	2	2	25	6
Prodiginin production	0	0	0	0	0	0	0	0	0	0	4	0	34[b]	0	0	0	0
Urease production	0	0	0	0	0	0	0	2[b]	0	0	0	0	0	0	0	8	0
Colonial characters																	
Aerial mycelium sparse	2	1	1	0	1	0	0	0	0	0	0	2	3	2	2	2	0
Aerial mycelium moderate	0	2	1	0	0	0	0	2	0	0	0	0	0	0	0	10	2
Aerial mycelium abundant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	4
Colonies blue or green	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0	0	0
Colonies brown	2	2	2	0	0	0	0	0	0	0	0	2	0	0	0	18	0
Colonies pink or orange	1	0	0	3	1	1	3	0	0	0	4	0	3	2	0	0	0
Colonies red or maroon	1	0	0	0	0	0	0	0	0	0	4	0	31[b]	0	2	0	0
Colonies white, cream, or yellow	0	1	0	1	4	4	9	2	5	4	1	10	0	0	0	19	6
Diffusible pigment brown	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
Diffusible pigment red or reddish brown	1	0	0	0	0	0	1	0	0	0	2	0	15	0	2	0	0
Diffusible pigment yellow or orange	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	3	0
Colony elevation crateriform	4	3	2	2	0	4	1	1	3	2	1	8	14	0	0	5	6
Colony elevation convex	0	0	0	0	5	1	10	0	2	1	7	1	13	2	0	31	0
Colony elevation irregular	0	0	0	2	0	0	2	1	0	1	2	6	8	0	2	1	0
Colony margin entire	0	0	0	0	0	1	1	0	1	4	4	0	17	2	2	0	1
Colony margin filamentous	4	3	1	1	5	3	10	1	2	0	5	3	17	0	0	4	2
Colony margin highly filamentous	0	0	1	3	0	1	1	1	3	0	1	12	1	0	0	33	3
Degradation of:																	
Esculin	4	3	2	4	5	5	12	2	5	4	10	9	5	2	2	37	6
Arbutin	4	2	1	4	5	4	12	2	4	4	9	1	1	2	2	37	6
Casein	3	3	1	4	5	5	12	2	4	4	10	15	35	2	2	37	6
DNA	4	3	2	4	5	4	10	1	1	2	8	15	5	2	1	33	2
Elastin	0	0	2	0	5	5	12	2	5	3	9	15	31	2	2	36	0
Gelatin	4	3	2	4	5	5	12	2	5	0[b]	9	15	30	2	2	37	1
Guanine	2	2	1	4	4	1	8	0	0	4	0	1	6	2	0	35[b]	0
Hypoxanthine	4	2	2	4	4	5	12	0	4	2	9	0	26	2	2	34	0
Ribonucleic acid	2	1	0	4	4	3	7	1	3	1	6	9	3	1	2	35	6
Starch	2	3	1	3	0	3	6	0	3	3	5	8	2	0	2	36	6
Testosterone	2	3	1	4	5	4	12	2	3	4	8	11	0[b]	2	2	2	6
Tween 80	2	1	2	3	5	5	12	2	5	4	10	15	35	2	2	37	6
L-Tyrosine	3	3	0[b]	4	5	5	12	1	5	4	10	15	33	2	2	37	0[b]
Xanthine	0	0	0	0	0	0	0	0	0	0	0	7	6	0	0	34[b]	0
Xylan	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	21	0
Growth in the presence of:[c]																	
Adenine (0.4%, wt/vol)	0	0	1	3	1	1	0	0	0	1	0	0	2	0	1	34[b]	1
Bismuth citrate																	
0.0001% (wt/vol)	4	3	2	4	5	4	12	2	4	3	10	15	35	2	2	24	6
0.001% (wt/vol)	4	3	2	4	5	4	12	2	4	3	10	14	34	2	2	21	1
0.01% (wt/vol)	3	3	2	4	5	3	8	0	1	2	7	9	29	0	2	7	0

Continued on following page

TABLE 2—*Continued*

Test or characteristic	No. of strains positive																
	A. pusilla-A. roseoviolacea (cluster 1; 4 strains)	*A. luteofluorescens-A. spiralis* (cluster 2; 3 strains)	*A. salmonea* (cluster 3; 2 strains)	*A. roseola* (cluster 4; 4 strains)	*A. verrucosospora* (cluster 5; 5 strains)	*A. madurae* (cluster 6; 5 strains)	*A. citrea-A. madurae* (cluster 7; 12 strains)	*A. cremea* (cluster 8; 2 strains)	*A. madurae-A. malachitica* (cluster 9; 5 strains)	*A. madurae* (cluster 10; 4 strains)	"*A. pelletieri*" (cluster 11; 10 strains)	*Streptomyces somaliensis* (cluster 12; 15 strains)	*A. pelletieri* (cluster 13; 35 strains)	*A. ferruginea* (cluster 14; 2 strains)	*A. rubra* (cluster 15; 2 strains)	*Nocardiopsis dassonvillei* (cluster 16; 37 strains)	*A. libanotica* (cluster 17; 6 strains)
Crystal violet																	
0.0001% (wt/vol)	4	3	2	4	5	4	10	1	4	3	10	15	34	2	2	37	6
0.001% (wt/vol)	3	2	2	4	5	4	3	0	4	2	8	13	32	1	2	23	6
Phenol																	
0.01% (wt/vol)	4	3	2	4	5	4	11	2	4	3	9	13	32	2	2	36	5
0.1% (wt/vol)	1	2	2	2	0	2	3	0	1	1	0	1	11	2	2	8	0
Phenyl ethanol																	
0.1% (vol/vol)	4	3	2	4	5	4	10	2	4	2	10	12	31	2	2	36	4
0.2% (vol/vol)	4	1	0	4	4	4	9	0	3	0	6	2	17	1	0	11	1
0.3% (vol/vol)	1	0	0	0	0	3	1	0	2	0	3	2	7	0	0	10	0
0.4% (vol/vol)	0	0	0	0	0	2	1	0	1	0	1	0	1	0	0	0	0
Potassium tellurite																	
0.001% (wt/vol)	4	3	2	4	5	4	12	2	4	4	10	10	35	2	2	36	6
0.005% (wt/vol)	1	3	2	4	5	3	7	1	3	4	7	5	31	2	2	24	6
0.01% (wt/vol)	0	2	3	4	1	2	0	0	1	1	1	0	12	2	0	14	1
Sodium azide																	
0.001% (wt/vol)	3	2	1	4	5	4	12	2	4	2	10	10	34	2	2	23	6
0.01% (wt/vol)	0	2	0	0	5	3	4	0	1	0	4	6	17	0	0	2	4
0.02% (wt/vol)	0	1	0	0	4	1	4	0	1	0	1	2	4	0	0	0	4
Sodium chloride																	
3% (wt/vol)	0[b]	3	2	0[b]	3	5	12	2	5	4	9	11	21	2	2	37	1
5% (wt/vol)	0	1	2	0	0	5	12	1	5	4	7	5	10	0	0	37	0
7% (wt/vol)	0	0	1	0	0	5	6	0	4	3	1	0	3	0	0	37	0
10% (wt/vol)	0	0	0	0	0	4	6	0	4	2	0	0	2	0	0	36[b]	0
13% (wt/vol)	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	30	0
Teepol (0.01%, vol/vol)	4	3	2	4	5	4	11	2	4	4	10	14	34	2	2	37	6
Tetrazolium																	
0.01% (wt/vol)	4	5	2	4	5	4	12	2	4	4	10	15	34	2	2	33	6
0.1% (wt/vol)	0	2	2	1	2	4	10	0	2	4	8	11	31	1	2	6	1
Thallous acetate																	
0.0001% (wt/vol)	4	3	2	4	5	4	10	0[b]	4	4	7	14	31	2	2	32	5
0.001% (wt/vol)	3	3	1	4	0	4	6	0	3	4	7	6	17	0	2	16	5
0.01% (wt/vol)	1	2	0	1	0	1	1	0	1	2	1	0	4	0	0	2	5
Growth on sole carbon sources (1%, wt/vol)[c]																	
Adonitol	3	3	2	4	3	4	12	2	3	1	3	0[b]	8	2	2	5	6
L-(+)-Arabinose	4	3	1	4	5	5	11	2	5	2	8	2	3	1	2	26	6
Cellobiose	4	3	2	4	4	5	12	0[b]	5	3	8	0[b]	7	2	1	34	6
Dextrin	4	3	2	4	5	4	12	2	3	2	9	13	4	2	2	28	6
D-(−)-Fructose	4	3	2	3	5	4	12	2	2	2	9	7	7	2	2	35	6
D-(+)-Galactose	4	3	2	3	4	4	3	0[b]	2	2	8	2	6	2	2	30	6
D-(+)-Glucosamine	4	1	2	0	1	2	12[b]	0	2	0	5	3	1	0	2	1	0
Glycerol	3	3	2	4	5	5	9	2	5	4	8	6	0[b]	0[b]	2	30	0[b]
Glycogen	3	3	2	4	5	5	10	0[b]	4	3	8	1	7	2	2	6	6
Lactose	3	3	2	0	3	3	8	0	3	3	4	5	3	2	2	3	4
Maltose	4	3	2	4	5	4	12	2	4	4	8	10	1	0[b]	2	26	6
Mannitol	2	3	1	4	5	4	12	2	3	3	8	0[b]	4	1	2	33	0[b]
D-(+)-Mannose	4	3	2	3	3	2	12	0	2	1	8	6	5	1	2	22	6
L-(+)-Rhamnose	4	3	2	4	5	5	11	2	5	4	7	1	3	1	2	29	5
Starch	3	3	0	4	5	5	12	0	5	4	8	1	0	2	2	0	6
Sucrose	4	3	2	4	4	3	10	2	3	4	9	8	3	2	2	29	6

Continued on following page

TABLE 2—*Continued*

Test or characteristic	No. of strains positive																
	A. pusilla-A. roseoviolacea (cluster 1; 4 strains)	*A. luteofluorescens-A. spiralis* (cluster 2; 3 strains)	*A. salmonea* (cluster 3; 2 strains)	*A. roseola* (cluster 4; 4 strains)	*A. verrucosospora* (cluster 5; 5 strains)	*A. madurae* (cluster 6; 5 strains)	*A. citrea-A. maduraе* (cluster 7; 12 strains)	*A. cremea* (cluster 8; 2 strains)	*A. madurae-A. malachitica* (cluster 9; 5 strains)	*A. madurae* (cluster 10; 4 strains)	"*A. pelletieri*" (cluster 11; 10 strains)	*Streptomyces somaliensis* (cluster 12; 15 strains)	*A. pelletieri* (cluster 13; 35 strains)	*A. ferruginea* (cluster 14; 2 strains)	*A. rubra* (cluster 15; 2 strains)	*Nocardiopsis dassonvillei* (cluster 16; 37 strains)	*A. libanotica* (cluster 17; 6 strains)
Trehalose	4	3	2	2	5	3	10	0	3	3	8	5	3	1	2	8	5
Xylose	4	3	2	0	5	5	12	1	5	2	8	7	1	1	2	30	6
Growth on sole carbon sources (0.01%, wt /vol)																	
D-(−)-Alanine	4	3	0	2	4	3	6	1	1	2	7	3	1	1	0	6	4
L-(−)-Alanine	4	3	0	4	5	3	11	0	2	2	8	9	2	1	0	8	6
Arbutin	1	3	0	4	1	4	11	2	2	1	2	0	0	0	0	1	6
Sodium butyrate	2	3	0	4	5	3	6	0	1	4	7	5	3	0	0	1	4
L-(−)-Proline	3	3	2	3	5	4	12	2	4	1	10	13	5	2	2	16	4
Sodium propionate	1	1	0	0	3	2	10	2	2	3	5	8	0	1	1	14	6
Protocatechuic acid	2	2	2	2	4	2	12	2	2	2	5	3	8	0	2	2	6
Sodium pyruvate	4	3	0	0	2	5	12	0	5	3	6	11	1	2	0	14	0
L-Serine	3	3	0	1	5	2	8	0	1	2	10	14	6	2	1	14	4
L-Tyrosine	3	3	0	2	5	4	9	2	4	2	10	10	2	2	1	13	1
Growth at:[c]																	
10°C	0	0	0	4	0	2	7	0	2	2	8	6	5	0	0	14	0
20°C	4	3	2	4	5	5	12	2	5	4	10	15	35	2	2	37	6
45°C	3	0	0	3	1	2	6	0	3	0	5	10	22	0	0	1	0
Resistance to antibiotics[c]																	
Cephaloridine hydrochloride																	
2 μg/ml	4	3	2	2	5	5	12	2	5	4	4	1	6	2	2	33	0
10 μg/ml	3	0	1	1	2	5	7	1	5	0	2	0	0	2	1	1	0
Demeclocyclin																	
2 μg/ml	4	3	2	2	5	3	6	0	2	4	5	4	10	2	2	31	6
8 μg/ml	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gentamicin sulfate																	
4 μg/ml	4	3	2	0	5	4	9	0	4	4	5	0	1	2	2	0	0
16 μg/ml	4[b]	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0
Lincomycin hydrochloride																	
10 μg/ml	4	3	2	2	5	5	11	2	4	4	7	0	10	2	2	37	6
20 μg/ml	4	2	2	0	4	5	11	1	4	3	5	0	6	1	2	30	0
Neomycin sulfate																	
3 μg/ml	4	3	0	2	3	3	1	0	3	0	0	1	3	0	0	0	0
10 μg/ml	4[b]	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Oleandomycin phosphate																	
2 μg/ml	4	3	2	2	5	5	12	2	5	4	9	0	11	2	2	37	6
16 μg/ml	4	3	1	0	5	5	5	0	2	2	0	0	0	2	1	22	0
Benzylpenicillin																	
10 μg/ml	4	3	2	0	5	5	12	2	5	4	4	1	0	2	2	35	2
20 μg/ml	4	3	2	0	4	5	12	0	5	0	2	0	0	2	2	23	0
Rifampin																	
2 μg/ml	4	3	2	2	5	4	12	2	4	3	8	3	19	2	2	1	0
6 μg/ml	4	3	2	2	5	3	10	2	3	2	2	3	17	2	2	0	0
Streptomycin sulfate																	
4 μg/ml	4	3	0	2	5	3	0	2	3	0	9	0	13	2	0	0	0
16 μg/ml	4	3	0	0	3	3	0	0	2	0	2	0	6	0	0	0	0
Tobramycin sulfate																	
1 μg/ml	4	3	0	1	5	4	3	2	3	3	0	2	10	0	0	0	0
8 μg/ml	1	0	0	0	0	3	0	0	2	0	1	0	0	0	0	0	0
Vancomycin hydrochloride (0.25 μg/ml)	4	3	2	2	5	4	5	0	4	0	3	3	15	2	2	20	0

[a] All strains degraded Tween 20 and were susceptible to vancomycin hydrochloride at a concentration of 2 μg/ml.
[b] Character states which gave consistant separation from most other taxa and may form the basis of a diagnostic table.
[c] Tests performed in Replidishes.

tion, Harpenden, United Kingdom), an ICL model 470/472 computer, and both the simple matching coefficient (S_{SM}) (58) and the pattern difference coefficient (Dp) (53). In each case, clustering was achieved by using the unweighted pair group method with averages (UPGMA) algorithm (56). Abbreviated dendrograms were produced for the results of both Dp-UPGMA and S_{SM}-UPGMA analyses (see Fig. 1 and 2).

RESULTS

Clustering of the strains by using the Dp coefficient with the UPGMA algorithm. The percentage of dissimilarity between strains can be separated into pattern and vigor components (53). The vigor component expresses differences in total metabolic activity between strains, whereas the pattern component (Dp) takes into account differences in growth rates, periods of incubation, and similar factors that distort similarity values. Dp is a measure of difference, so with this coefficient, strains are distributed from a value of 0 for identical organisms to a value of 1 for organisms which differ in all measured characters. Since the test strains differed widely in rates of growth, numerical classification based on the Dp-UPGMA analysis is described in detail below.

Nearly 90% of the 170 strains were assigned to nine major clusters (five or more strains) and eight minor clusters (between two and four strains) defined at or about a Dp level of 0.21; the remaining organisms formed single-member clusters (Fig. 1). Most of the clusters were distinct and homogeneous and were named after the type or authentic strains which they contained (Table 1). The defined clusters fell into six aggregate groups at a Dp level of 0.32.

The 20 strains in the first aggregate group formed four minor clusters and seven single-member clusters. The first cluster contained four strains, including type cultures *Actinomadura pusilla* (A3) and *Actinomadura roseoviolacea* (A2), and was closely related to the second cluster, which encompassed duplicate cultures of the *A. spiralis* type strain (A195) together with *Actinomadura luteofluorescens* A234. Cluster 3 contained the duplicate cultures of *Actinomadura salmonea* (cultures A230^T and D10^T), whereas the fourth cluster contained the three strains of *Actinomadura roseola* together with the duplicate culture of strain A227^T (T = type strain). Strains forming single-member clusters included "*Actinomadura coeruleoviolacea*" A231^T, *A. spadix* A1^T, *Microbiospora rosea* G28^T, *Microtetraspora glauca* G27^T, and *Microtetraspora niveoalba* G103^T.

The second and largest of the aggregate groups contained 96 strains assigned to seven major clusters, two minor clusters, and four single-member clusters. Strains labeled *A. madurae* were recovered in six of the seven clusters, the largest of which, cluster 7, included the type strains of *A. madurae* (A16) and *Actinomadura citrea* (A138) and 10 other strains received as *A. madurae*. Similarly, cluster 9 contained *Actinomadura malachitica* type strain A139 and three strains of *A. madurae*; *Actinomadura livida* type strain A228 was recovered on the periphery of this taxon. Clusters 6 and 10 were composed mainly of *A. madurae* strains, although the former also contained *A. pelletieri* A123. Cluster 13, the largest phenon in this aggregate group, encompassed 35 strains of *A. pelletieri*, including the type strain of the species, strain A19, but five strains bearing the same specific epithet were found in cluster 11 together with five cultures received as *A. madurae*. The 15 cultures of *Streptomyces somaliensis* formed cluster 12. Cluster 5 consisted of three strains (one in duplicate culture) of *Actinomadura verrucosospora*, including type strain A4, and *A. madurae* A196. *Actinomadura cremea* type strain A224 and its duplicate formed cluster 8, and *A. madurae* A133, A179, and A253 and *Actinomadura coerulea* type strain A226 each formed single-member clusters.

The third aggregate group included two minor clusters, clusters 14 and 15, which incorporated the duplicate cultures of the type strains of *Actinomadura ferruginea* and *Actinomadura rubra*, respectively, and four single-member clusters. Two of the latter consisted of *Actinomadura helvata* A5^T and *Microtetraspora viridis* G101^T. The largest phenon, cluster 16, contained 32 strains of *Nocardiopsis dassonvillei*, including type strain A14, and duplicates of five different cultures. This phenon made up the fourth aggregate group, whereas the fifth aggregate taxon accommodated the six *Actinomadura libanotica* cultures, including type strain A190. "*Nocardia polychromogenes*" A102^T and "*Nocardia gardneri*" A98^T formed single-member aggregate groups.

Clustering of strains by using the S_{SM} coefficient and the UPGMA algorithm. Of the 17 clusters found in the Dp-UPGMA analysis, 10 were recovered in their entirety in the S_{SM}-UPGMA analysis, which took into account both positive and negative matches. The clusters, which were defined at the 78% similarity level, were arranged differently, although it was still possible to recognize aggregate groups 1, 4, and 6 (Fig. 1 and 2). Changes in clustering behavior were restricted mainly to a small number of *A. madurae* and *A. pelletieri* strains. The most significant change affected clusters 6 (*A. madurae*) and 9 (*A. madurae*, *A. malachitica*), which merged to form a single cluster. *A. livida* A228^T was recovered on the periphery of this taxon as a single-member cluster. Similarly, *A. madurae* A25, A30, and A31 from cluster 11 ("*A. pelletieri*") and *A. madurae* A175 from cluster 10 (*A. madurae*) joined cluster 7 (*A. citrea*, *A. madurae*). Conversely, cluster 11 gained *A. pelletieri* A10 from cluster 13 (*A. pelletieri*) and *A. pelletieri* A128 from cluster 6 (*A. madurae*) but lost one strain, *A. pelletieri* A18, to cluster 12 (*Streptomyces somaliensis*).

Reproducibility of results. Inclusion of 15 duplicate strains (Table 1) in the analysis enabled experimental test error to be estimated. The probability (p) of an erroneous test result averaged 4.9%, which is equal to an observed S_{SM} value of about 90% between duplicate cultures.

Characterization of major and minor clusters. The properties of both the major and minor clusters and the characters considered to be diagnostically important are shown in Table 2. Results from two tests are not included in the data matrix because they have no diagnostic value since all strains were tolerant of vancomycin hydrochloride (2 μg/ml) and all degraded Tween 20.

DISCUSSION

The robustness and distinctness of clusters based on different statistics may be determined from an estimate of test error (6, 54, 55) supported by data from independent chemical, genetic, and serological investigations (26, 33, 45). The numerical classification scheme resulting from the present study needed careful examination because some similarity values were influenced by (i) difficulties in choosing a balanced set of tests, (ii) difficulties in growing some fastidious organisms, and (iii) difficulties resulting from the wide range of growth rates shown by the test strains. However, it is encouraging that neither the number nor the composition of clusters was markedly influenced by the coefficients used or by the test error (p) of 4.9%. An

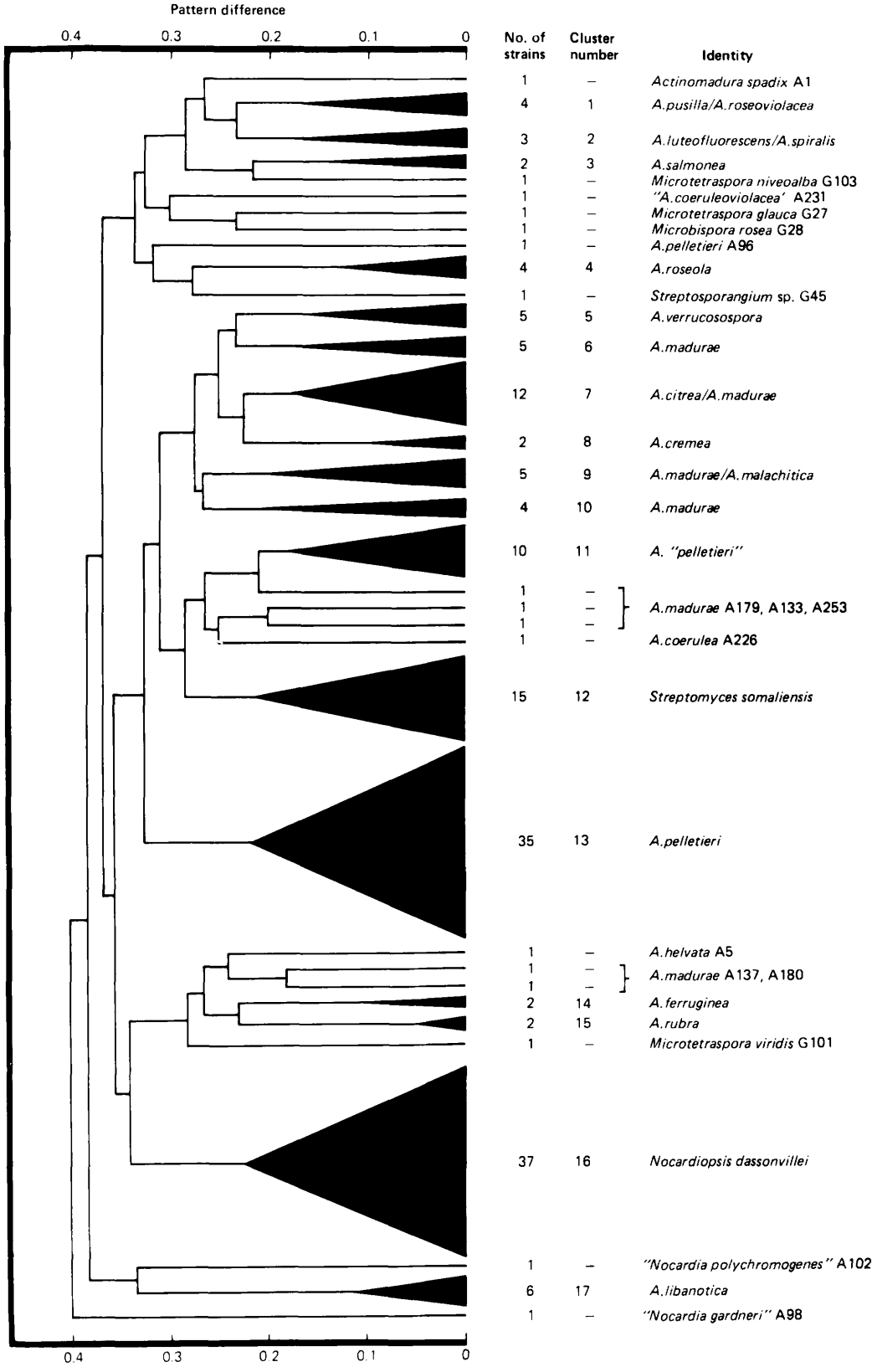

FIG. 1. Simplified dendrogram showing the relationships among clusters based on the Dp-UPGMA analysis.

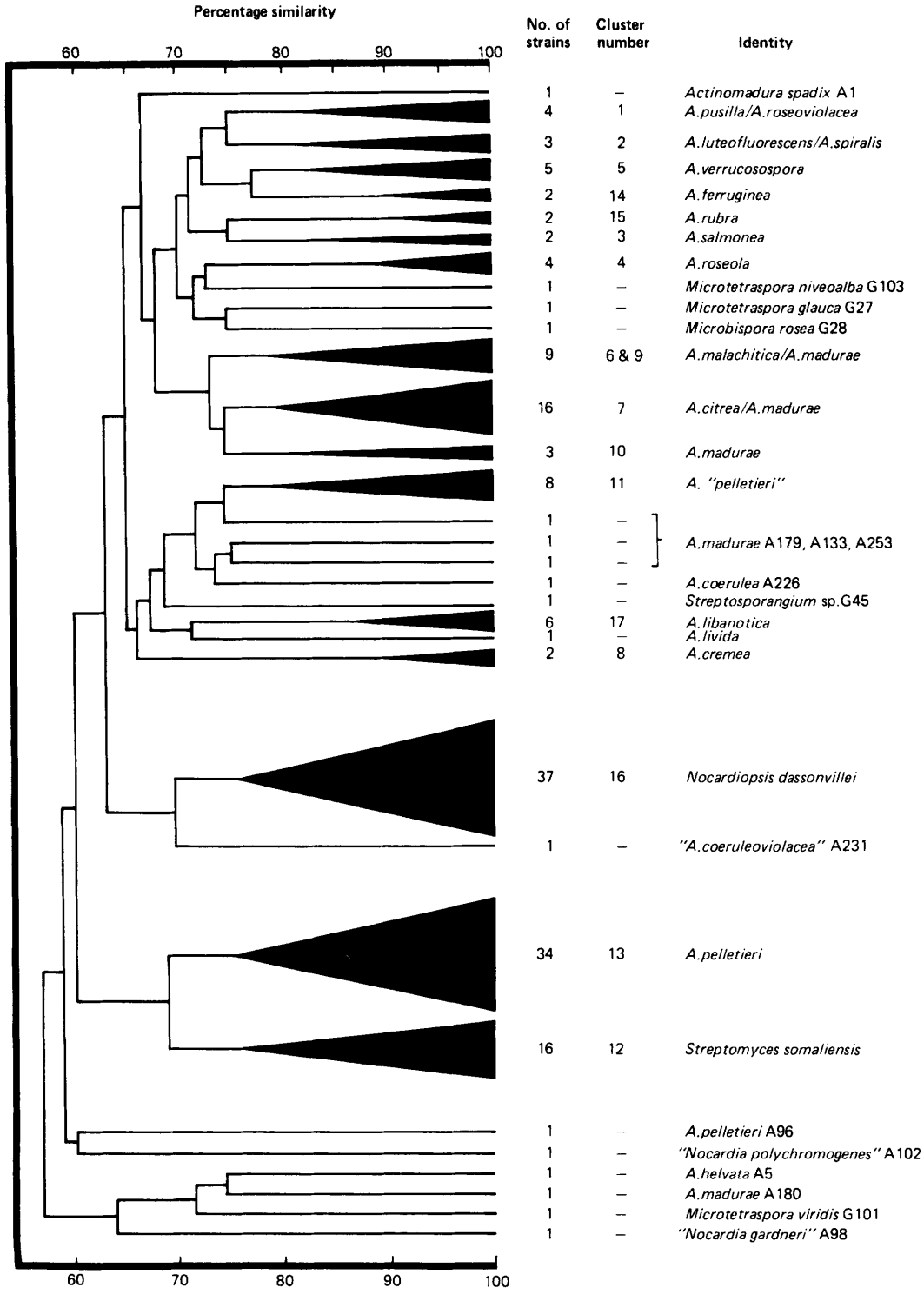

FIG. 2. Simplified dendrogram showing the relationships among clusters based on the S_{SM}-UPGMA analysis.

experimental test error of this order is comparable to the errors from earlier studies of actinomadurae (p = 4.5%) (4, 19), facultative anaerobic actinomycetes (p = 1.6%) (49), actinomycetes with wall chemotype III (p = 2.2%) (24), "*Gordona aurantiaca*" strains (p = 1.1%) (23), nocardiae (p = 1.5%) (22), rhodococci (p = 3.7%) (18), and streptomycetes (p = 3.4%) (64). It is also well within the 10% limit of Sneath and Johnson (55). The compositions of the aggregate groups were sensitive to the coefficients used, but the three largest aggregate clusters recovered in the Dp-UPGMA analysis have also been shown to differ inchemical and nucleic acid pairing studies (5, 12).

The numerical taxonomy results based upon the S_{SM} and Dp were derived mainly from tests in which we used media

and methods that were found to be useful in previous studies of actinomadurae (19, 24). Nevertheless, even with this improved data base, the relationships between some taxa were distorted by negative matches. Thus, although *A. pelletieri* and *Streptomyces somaliensis* strains were recovered as distinct clusters, the higher intercluster similarity resulted largely from a lack of activity in many of the tests. However, *Streptomyces somaliensis* has a chemotype I wall and lipids like other streptomycetes and can thus be distinguished from *A. pelletieri*, which has a chemotype III wall and different polar lipids (7, 36–38, 47). Representatives of these taxa have also been distinguished by menaquinone structure (5), by serological investigations (48), and in taxonomic studies using the Jaccard coefficient, which excludes negative matches (19, 64). These results provide good grounds for continuing to classify *A. pelletieri* and *Streptomyces somaliensis* in separate genera.

The clear separation of the *Actinomadura* and *Nocardiopsis* strains agrees well with the recent reclassification of these organisms in separate genera and with the results of other studies (5, 12, 41, 66; E. Stackebrandt and K. H. Schleifer, *in* L. Bojalil and L. Ortiz-Ortiz, ed., *Actinomycete Biology*, in press), including less extensive numerical phenetic studies of representatives of these taxa (4, 17, 19, 24, 34, 61, 64). Meyer (41) defined *Nocardiopsis dassonvillei* primarily on the basis of biochemical and morphological criteria, but recent evidence suggests that this taxon is heterogeneous (5, 19, 67). Thus, representative strains of *Nocardiopsis dassonvillei* fall into two distinct groups based on DNA homology and DNA base pair studies (12) and menaquinone composition (5). Comparative studies will be required to determine the taxonomic structure of the genus *Nocardiopsis* and its suprageneric relationship, especially to *Streptomyces*, with which it shares many morphological and physiological properties (29, 64). Such studies should include representatives of the three recently described species, *Nocardiopsis syringae* (13), *Nocardiopsis mutabilis* (50), and *Nocardiopsis antarcticus* (1). Preliminary phylogenetic data suggest that *Nocardiopsis* is related to *Planobispora*, *Planomonospora*, and *Streptosporangium* (Stackebrandt and Schleifer, in press) rather than to *Thermonospora*, even though strains of the latter genus also contain major amounts of menaquinones with 10 isoprene units (8).

In the Dp-UPGMA analysis most actinomadurae were assigned to one of two aggregate groups, the first of which was also recovered in the corresponding S_{SM} analysis. This aggregate taxon contained the type strains of *A. luteofluorescens*, *A. pusilla*, *A. roseola*, *A. roseoviolacea*, *A. salmonea*, *A. spadix*, *A. spiralis*, *Microbispora rosea*, *Microtetraspora glauca*, and *Microtetraspopa niveoalba*. The second aggregate cluster contained the type strains of *A. citrea*, *A. coerulea*, *A. cremea*, *A. livida*, *A. madurae*, *A. malachitica*, *A. pelletieri*, and *A. verrucosospora*. These two aggregate clusters corresponded well with the genetically and chemically defined taxa recognized by Fischer et al. (12). Nucleic acid and chemical data (5, 12) and the results of the S_{SM}-UPGMA analysis support the inclusion of *A. ferruginea* (cluster 14) and *A. rubra* (cluster 15) in the first aggregate taxon and *A. libanotica* (cluster 17) in the second aggregate taxon. Conversely, *A. spadix*, *A. spiralis*, and perhaps *A. luteofluorescens* should be excluded from the first aggregate taxon (5, 12). The aggregate clusters also differ in menaquinone composition (5), with the first cluster characterized by tetrahydrogenated menaquinones with nine isoprene units [Mk-9(H_4)] and the second cluster characterized by Mk-9(H_6) or, in *A. pelletieri*, by Mk-9(H_8) as the predominant isoprenologs.

Poor or slow growth, particularly of *A. helvata* A5^T, may have contributed to erratic clustering of some strains, but it is also well known that single marker strains are not always good representatives of species in numerical phenetic surveys (27, 63). The predominance of MK-9(H_6) in *A. helvata* A5^T (5) and its close affinity with *A. madurae* (19) suggest a similarity to the second aggregate cluster. However, Fischer et al. (12) have suggested that this taxon may belong to the *A. pusilla* aggregate group.

Aggregate cluster 2 can be considered to represent *Actinomadura* sensu stricto, as it includes the type strain of the type species of the genus, *A. madurae*. It is reassuring that phena corresponding to the well-established taxa *A. madurae* (cluster 7) and *A. pelletieri* (cluster 13) were recovered in the same aggregate taxon. Members of these species contain characteristic prodigiosin-like pigments (14, 15), have similar fatty acid (2), menaquinone (5, 9, 67), and polar lipid profiles (37, 44), and have been shown to be moderately related in DNA pairing studies (12). The relatively small overall similarities found between *A. madurae* and *A. pelletieri* in earlier numerical taxonomic studies (19, 24, 61, 64) can be attributed to the tests and statistics used in these investigations. The sharp separation of clusters 7 and 9 (*A. madurae*) and cluster 13 (*A. pelletieri*) in the S_{SM}-UPGMA analysis underlines the importance of using the Dp when comparing actinomycetes that have different growth rates.

The recovery of strains received as *A. madurae* and *A. pelletieri* in more than one phenon in the second aggregate cluster is in line with previous reports that these taxa are heterogeneous (4, 17, 19). In both the Dp-UPGMA and S_{SM}-UPGMA analyses, the two clusters containing *A. madurae* strains (clusters 7 and 9) had very high intercluster similarity. However, cluster 12, containing the metabolically active strains of *A. pelletieri*, had a greater affinity for the two *A. madurae* clusters than for *A. pelletieri* sensu stricto (cluster 13), a finding confirmed by DNA-DNA reassociation studies (12). Thus, further comparative studies will be required to unravel the detailed relationships of *A. madurae* and the metabolically active strains of *A. pelletieri* (cluster 12). These studies should include representatives of *A. citrea* and *A. malachitica*, as the type strains of these taxa were recovered in clusters 7 and 9, respectively, although Fischer et al. (12) concluded that both were distinct species in their *A. madurae* rRNA cluster. The classification of *A. livida* A228^T together with three *A. madurae* strains in cluster 4 in the Dp-UPGMA analysis is not supported by the results of the corresponding S_{SM}-UPGMA analysis. This apparent contradiction is probably a function of the relatively small number of positive characters shown by *A. livida* A228^T. It has been demonstrated (22, 23, 27) that when organisms showing many positive responses are compared with much less vigorous strains, then the pattern difference between them can be artificially small.

A. ferruginea A199^T, *A. pusilla* A3^T, *A. roseola* A227^T, and *A. roseoviolacea* A2^T, members of the first aggregate cluster, comprised the core of an rRNA cluster (12). The position of the type strain of *A. libanotica*, the fifth member of this rRNA cluster, is equivocal as it was recovered in the penultimate aggregate cluster in the Dp-UPGMA analysis and has been variously reported as containing Mk-9(H_4) (12) and Mk-9 (H_6) as the predominant isoprenolog (5). Menaquinone data support the retention of "*A. coeruleoviolacea*" and inclusion of *A. rubra*, *Microbispora rosea* G28^T, *Microtetraspora glauca* G27^T, and *Microtetraspora niveoalba*

G103^T in the first aggregate taxon. Although *A. spiralis* A195^T also contains Mk-9(H_4) as its predominant isoprenolog, it nevertheless forms the nucleus of a distinct rRNA cluster (12). The recovery of *A. spadix* A1^T in the first aggregate cluster is also probably anomalous for it is genetically distinct from both the *A. pusilla* and *A. madurae* rRNA clusters (12), whereas opinions on menaquinone composition differ (5, 12). Although this and previous studies show that *A. pusilla* and *A. roseoviolacea* are relatively closely related (2, 59), the type strains of these taxa have little DNA in common (12), and they were recovered as single-member clusters in earlier numerical phenetic surveys (19, 24). The recovery of *A. luteofluorescens* A234^T and *A. spiralis* A195^T in one cluster is also probably anomalous, as these strains have different menaquinone profiles (5).

Conventional numerical taxonomy has been used to greatest effect in the definition of species (18, 20, 26, 64), but chemical and genetic techniques are increasingly necessary for circumscribing and separating actinomycete genera (20, 37, 43; Stackebrandt and Schleifer, in press). Chemical, genetic, and numerical phenetic studies all show that the genus *Actinomadura* is in need of taxonomic revision, although they support the integrity of most of the recently described species. There are good grounds for restricting *Actinomadura* sensu stricto to *A. citrea*, *A. coerulea*, "*A. coeruleoviolacea*," *A. cremea*, *A. livida*, *A. madurae*, *A. malachitica*, *A. pelletieri*, and *A. verrucosospora*, although further work will be required to unscramble the taxonomy of the heterogeneous taxon *A. madurae* and to locate *Actinomadura* species not included in the present survey, such as *Actinomadura kijaniata*.

Based on our study and previous numerical phenetic and chemotaxonomic studies, we propose that *Actinomadura* sensu stricto should be defined as follows: substrate mycelium well developed, branched, septate, usually stable, giving rise to sparse aerial mycelium bearing chains of spores up to 15 spores long; spore chains straight, hooked, or in spirals; colonies often slow growing, usually convex or crateriform with filamentous or entire margins; aerobic; gram positive; non-acid-fast; nonmotile; susceptible to lysozyme; wall peptidoglycan containing *meso*-diaminopimelic acid (wall chemotype III); cell extracts contain madurose (3-*O*-methyl-D-galactose) with Mk-9(H_6) or Mk-9(H_8) as the major menaquinones; cells rich in iso-branched fatty acids and tuberculostearic acid, with few polar lipids, phosphatidylinositol, and phosphatidylinositol mannosides predominating; produces prodigiosin-like pigments; guanine-plus-cytosine content, 70 to 78 mol%. Additional biochemical and physiological characteristics are shown in Table 2.

The taxa assigned to cluster group 1 (*A. ferruginea*, *A. pusilla*, *A. roseola*, *A. roseoviolacea*, *A. rubra*, and *A. salmonea*) are very similar in morphology and physiology to *Actinomadura* sensu stricto but can be distinguished by the predominance of Mk-9(H_4) menaquinones and the greater range of polar lipids produced, with phosphatidylinositol, phosphatidylethanolamine, and diphosphatidylglycerol predominant but with several uncharacterized glycolipids and phospholipids also present. These taxa should be the subject of additional studies to determine whether they form the nucleus of one or more genera. These studies should also include representatives of understudied taxa, such as *Microbispora* and *Microtetraspora*, since representatives of these genera have properties in common with *Actinomadura* species (20, 24, 37, 39). Comparative studies of additional strains of *A. helvata*, *A. libanotica*, *A. luteofluorescens*, *A. spadix*, and *A. spiralis* are needed to determine their relationships to one another and to other *Actinomadura* species not included in the present investigation.

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