Extended Numerical Taxonomy Study of *Nocardia*

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Numerical classification of 80 strains received as *Nocardia* using six different sets of characters was carried out. A set of 57 "differentiating" characters gave six distinct clusters: *Nocardia farcinica*, *N. asteroides* A, *N. asteroides* B, *N. caviae*, *N. brasiliensis*, and *Gordona lentifragmenta* (formerly, *N. rubra*). Hence, the strains received as *N. asteroides* were divided into three subgroups, *N. farcinica*, *N. asteroides* A, and *N. asteroides* B. The cluster of *G. lentifragmenta* was placed apart from the others, and a previous classification of this taxon as a species of *Gordona* was supported by the present study. The problem of a minimum number of differentiating characters necessary for numerical classification, which was considered theoretically as above 40 or 50, was studied experimentally in this study. The results supported the theoretical considerations. Inclusion of linked characters reduced the number of clusters.

Previous studies on the taxonomy of *Nocardia* were made by Gordon and Mihm (6-9) by use of physiological and biochemical characters. Many species were merged into a few species. Gordon and Mihm (6, 8) included the strain ATCC 3318, labeled as *Nocardia farcinica*, in the species *Nocardia asteroides*. The first numerical classification of *Nocardia* was carried out by Cerbón (3). He suggested that the species *N. asteroides* be divided into several subgroups. Subsequently, Tsukamura (22) carried out a numerical classification of *Nocardia* using a large number of characters and reported that the strains received as *N. asteroides* were divided into two distinct taxa. In this study, the strain ATCC 3318 of *N. farcinica* was included in one cluster. Hence, this cluster was named *N. farcinica* and another cluster was designated *N. asteroides*. These two taxa could be differentiated by several characters. The former grew at 45°C, showed acetamidase and benzamidase activities, formed acid from rhamnose, and utilized 1,3-butylene glycol as sole carbon source and acetamide and monoethanolamine as simultaneous nitrogen and carbon sources; the latter taxon did not. This finding was supported by Berd (1), Holm (10), and Franklin and McClung (4) by use of the same phenetic analysis, and by Bradley (2) and Franklin and McClung (4) by use of a deoxyribonucleic acid hybridization technique. Magnusson and Mariat (14) suggested that, by using a delayed type skin reaction, *M. farcinica* be separated from *N. asteroides*.

At a later date, Goodfellow (5) and Kurup and Schmitt (11) reported that the species *N. asteroides* is divided into five and four subgroups, respectively. Goodfellow used none of the same strains as used by Tsukamura (22),

and Kurup and Schmitt stated that one of their subgroups was identical to Tsukamura's *N. farcinica*.

The present study was carried out for two reasons: (i) to reinvestigate the heterogeneity of *N. asteroides* by use of a new set of characters which included several characters not used previously, and (ii) to observe the relationship between the clustering and the characters used in the study. Furthermore, the study was carried out to observe how many characters are needed to obtain a suitable clustering. Based on theoretical considerations, Sokal and Sneath (18) and Sneath (16) recommended the use of at least 50 to 60 characters, and Lockhart (13) recommended a minimum of about 40 for numerical classifications. In the present study, these recommendations were tested experimentally.

MATERIALS AND METHODS

The 80 strains used are listed in Table 1. The names *N. asteroides* and *N. farcinica* were used according to the definition of the previous study (22). Lechevalier et al. (12) and Ridell and Norlin (15) questioned the authenticity of the strain ATCC 3318 of *N. farcinica* and claimed that the name *N. farcinica* was a nomen dubium.

The sets of characters used for numerical classifications are shown in Tables 2 and 3. The set of characters shown in Table 2 was used for analysis of the relationship among *Mycobacterium*, *Gordona*, and *Nocardia* (23). Only 57 of these characters were effective for differentiation between *Nocardia* strains (omitting strain 71, which is now identified as *Mycobacterium intracellulare*). The number of "differentiating" characters in other sets, too, were calculated omitting this strain.

The matching coefficient (M-value) was calculated as described previously (24). Dendrograms were pre-

TABLE 1. *List of strains used in this study*

No. in this study	Laboratory no.	Identified as ^a :		Received as:	
			Name	Strain	Source ^b
1	23001	<i>Nocardia rubra</i>	<i>N. globerula</i>	M75	1
2	23002	<i>Gordona lentifragmenta</i>	<i>N. rubra</i>	M1	1
4	23004	<i>N. farcinica</i>	<i>N. asteroides</i>	M72	1
5	23005	<i>N. caviae</i>	<i>N. asteroides</i>	M73	1
6	23006	<i>N. asteroides</i>	<i>N. asteroides</i>	M93	1
7	23007	<i>N. asteroides</i>	<i>N. asteroides</i>	M94	1
9	23009	<i>N. asteroides</i>	<i>N. asteroides</i>	M124	1
10	23010	<i>N. farcinica</i>	<i>N. asteroides</i>	M126	1
11	23011	<i>N. farcinica</i>	<i>N. asteroides</i>	M128	1
12	23012	<i>N. asteroides</i>	<i>N. brasiliensis</i>	M145	1
13	23013	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M146	1
16	23016	<i>G. lentifragmenta</i>	<i>N. rubra</i>	M1	2
18	23018	<i>N. rubra</i>	<i>N. globerula</i>	M75	2
21	23021	<i>G. lentifragmenta</i>	<i>N. minima</i>	M103	2
22	23022	<i>G. lentifragmenta</i>	<i>N. coeliaca</i>	M122	2
23	23023	<i>N. rubra</i>	<i>N. coeliaca</i>	M191	2
24	23024	<i>G. lentifragmenta</i>	<i>N. lutea</i>	M192	2
25	23025	<i>N. farcinica</i>	<i>N. madurae</i>	C407	2
27	23027	<i>N. farcinica</i>	<i>N. madurae</i>	C743	2
30	23030	<i>N. rubra</i>	<i>N. polychromogenes</i>	M6	2
31	23031	?	<i>Proactinomyces restric-</i> <i>tus</i>	M9	2
32	23032	<i>N. asteroides</i>	<i>N. asteroides</i>	M10	2
33	23033	<i>N. caviae</i>	<i>N. caviae</i>	M54	2
34	23034	<i>N. farcinica</i>	<i>N. asteroides</i>	M72	2
35	23035	<i>N. caviae</i>	<i>N. asteroides</i>	M73	2
38	23038	<i>N. asteroides</i>	<i>N. asteroides</i>	M93	2
39	23039	<i>N. asteroides</i>	<i>N. asteroides</i>	M94	2
40	23040	<i>N. farcinica</i>	<i>N. asteroides</i>	M111	2
41	23041	<i>N. farcinica</i>	<i>N. eppingeri</i>	M112	2
44	23044	<i>N. asteroides</i>	<i>N. asteroides</i>	M124	2
45	23045	<i>N. asteroides</i>	<i>N. asteroides</i>	M128	2
46	23046	<i>N. asteroides</i>	<i>N. asteroides</i>	M129	2
48	23048	<i>N. farcinica</i>	<i>N. asteroides</i>	M131	2
49	23049	<i>N. asteroides</i>	<i>N. asteroides</i>	M132	2
51	23051	<i>N. farcinica</i>	<i>N. asteroides</i>	M138	2
53	23053	<i>N. farcinica</i>	<i>N. asteroides</i>	M144	2
54	23054	<i>N. asteroides</i>	<i>N. brasiliensis</i>	M145	2
55	23055	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M146	2
56	23056	<i>N. asteroides</i>	<i>N. asteroides</i>	M155	2
57	23057	<i>N. asteroides</i>	<i>N. asteroides</i>	M156	2
58	23058	<i>N. farcinica</i>	<i>N. asteroides</i>	M158	2
59	23059	<i>N. farcinica</i>	<i>N. asteroides</i>	M86	2
60	23060	<i>N. farcinica</i>	<i>N. asteroides</i>	M127	2
62	23062	<i>N. farcinica</i>	<i>N. asteroides</i>	M158A	2
65	23065	<i>N. farcinica</i>	<i>N. asteroides</i>	M162	2
66	23066	<i>N. asteroides</i>	<i>N. asteroides</i>	M163	2
67	23067	<i>N. farcinica</i>	<i>N. asteroides</i>	M164	2
69	23069	<i>N. farcinica</i>	<i>N. asteroides</i>	M167	2
70	23070	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M169	2
71	23071	<i>Mycobacterium intracel-</i> <i>lulare</i>	<i>N. intracellularis</i>	M170	2
73	23073	<i>N. farcinica</i>	<i>N. asteroides</i>	M175	2
74	23074	<i>N. farcinica</i>	<i>N. asteroides</i>	M177	2
75	23075	<i>N. farcinica</i>	<i>N. asteroides</i>	M179	2
77	23077	?	<i>N. asteroides</i>	M181	2
78	23078	<i>N. caviae</i>	<i>N. asteroides</i>	M185	2
81	23081	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M197	2
82	23082	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M198	2

TABLE 1. *Continued*

No. in this study	Laboratory no.	Identified as ^a :		Received as:	
			Name	Strain	Source ^b
83	23083	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	M199	2
84	23084	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M201	2
85	23085	?	<i>N. tenuis-convoluta</i>	M203	2
86	23086	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	M204	2
87	23087	<i>N. farcinica</i>	<i>N. asteroides</i>	M205	2
88	23088	<i>N. asteroides</i>	<i>N. asteroides</i>	M206	2
92	23092	<i>N. farcinica</i>	<i>N. asteroides</i>	M189	2
93	23093	<i>N. farcinica</i>	<i>N. brasiliensis</i>	M200	2
95	23095	<i>N. asteroides</i>	<i>N. asteroides</i>	R443(1)	3
97	23097	<i>N. asteroides</i>	<i>N. asteroides</i>	R553	3
98	23098	<i>N. farcinica</i>	<i>N. asteroides</i>	R784	3
101	23101	<i>N. farcinica</i>	<i>N. asteroides</i>	W3409B	3
102	23102	<i>N. farcinica</i>	<i>N. asteroides</i>	R3318 (ATCC3318)	3
103	23103	<i>N. asteroides</i>	<i>N. asteroides</i>	ATCC9970	3
104	23104	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	R405	3
105	23105	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	R432	3
106	23106	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	R887	3
108	23108	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	R1117A	3
109	23109	<i>N. brasiliensis</i>	<i>N. brasiliensis</i>	R1188	3
111	23111	<i>N. caviae</i>	<i>N. caviae</i>	R416	3
112	23112	<i>N. caviae</i>	<i>N. caviae</i>	R547	3
113	23113	<i>N. caviae</i>	<i>N. caviae</i>	R617	3
114	23114	<i>N. caviae</i>	<i>N. caviae</i>	R1291	3

^a All *Nocardia* strains, except for numbers 10, 12, 54, and 66, were identified in a previous study (22). *Gordona* strains were identified in another study (26). These were once identified as *N. rubra* (22).

^b 1, I. Uesaka, Kyoto University, Kyoto, Japan (received in 1965); 2, I. Uesaka, Kyoto University, Kyoto, Japan (received in 1967); 3, R. E. Gordon, Rutgers State University, New Brunswick, N.J. The strains received from I. Uesaka belonged to the collection of N. M. McClung, University of South Florida, Tampa, Fla., from whom I. Uesaka received the strains in 1960. The history of the strains, except for M126, M145 and M163, were described previously in detail (22). M126: I. Uesaka; N. M. McClung; R. E. Gordon; NCTC; S. T. Cowan; N. Conant. M145: I. Uesaka; N. M. McClung; McMillan (D-410); Gonzales-Ochoa. M163: I. Uesaka; N. M. McClung; J. D. Schneidau; University of Minnesota (Henrici's strain no. A10A, var. *gypsoides*).

pared by the single-linkage method (18). The computer used was IBM-S370 model 135, which is located in the Nagoya Factory, the Sumitomo Machinery Co., Obu, Aichi, Japan. The program was written in English (CÔBÔL) (no. G90002).

The data for the original "strain × characters" matrix have been deposited with the World Data Centre for Microorganisms, Brisbane, Australia. The data are available to anyone wishing to examine them by any other methods.

RESULTS

The data are shown in Fig. 1 to 6 as dendrograms.

The dendrogram obtained by set A1 (Fig. 1) shows six well-defined clusters, which correspond to *N. farcinica*, *Nocardia brasiliensis*, *Nocardia caviae*, *N. asteroides* A, *N. asteroides* B, and *Gordona lentifragmenta*. *N. asteroides* was divided into two clusters. Hence, the strains originally designated as *N. asteroides* when received were divided into three clusters, *N. farcinica*, *N. asteroides* A, and *N. asteroides* B. *G.*

lentifragmenta, formerly named *N. rubra*, was placed apart from the others, forming a distinct cluster.

The dendrogram obtained by set A2 (Fig. 2) showed similar clusters to those obtained by set A1. The dendrogram obtained by set A3 (Fig. 3), in which the same number of differentiating characters as used in set A2, but different characters, were included, showed five clusters, all *N. asteroides* strains being combined into one.

The dendrogram obtained by set A4 (Fig. 4) showed only three clusters, which closely corresponded to *N. farcinica*, *N. caviae*, and *G. lentifragmenta*.

The dendrogram obtained by set A5 (Fig. 5) showed five clusters, which roughly corresponded to *N. farcinica*, *N. caviae*, *N. brasiliensis*, *N. asteroides*, and *G. lentifragmenta*. Several strains of *N. asteroides* were included in the cluster of *N. farcinica*.

The dendrogram obtained by set B (Fig. 6) showed five clusters, which approximately cor-

TABLE 2. *List of characters used in set A1^a*

1. Strong acid fastness	46. Growth at 52°C
2. Weak or partial acid fastness	47. Glucose as C source
3. Rod shape	48. Mannose as C source
4. Cord or compact grouping	49. Sucrose as C source
5. Fragmenting mycelium	50. <i>n</i> -Propanol as C source
6. Permanent mycelium	51. <i>n</i> -Butanol as C source
7. Rough colonies	52. Isobutanol as C source
8. Colony pigmentation in dark	53. Propylene glycol as C source
9. Photochromogenicity	54. Galactose as C source
10. Growth after 3 days	55. Arabinose as C source
11. Tolerance to 0.1% NaNO ₂	56. Xylose as C source
12. Tolerance to 0.2% picric acid	57. Rhamnose as C source
13. Growth on rifampin medium (25 µg/ml)	58. Trehalose as C source
14. Growth on <i>p</i> -aminosalicylic acid medium (2 mg/ml)	59. Inositol as C source
15. NH ₂ OH · HCl medium (0.125 mg/ml)	60. Mannitol as C source
16. NH ₂ OH · HCl medium (0.25 mg/ml)	61. Sorbitol as C source
17. NH ₂ OH · HCl medium (0.5 mg/ml)	62. Acetate as C source
18. Growth on TCH medium (10 µg/ml)	63. Citrate as C source
19. Salicylate medium (0.5 mg/ml)	64. Succinate as C source
20. <i>p</i> -Nitrobenzoic acid medium (0.5 mg/ml)	65. Malate as C source
21. Niacin	66. Pyruvate as C source
22. Tween hydrolysis (7 days)	67. Benzoate as C source
23. Tween hydrolysis (14 days)	68. Malonate as C source
24. α-Esterase	69. Fumarate as C source
25. β-Esterase	70. Acid from glucose
26. Acid phosphatase	71. Acid from mannose
27. Catalase (semiquantitative)	72. Glucose as C source (glutamate-N)
28. β-Galactosidase	73. Acetate as C source (glutamate-N)
29. Nitrate reduction (24 h)	74. Succinate as C source (glutamate-N)
30. Three-day arylsulfatase	75. Pyruvate as C source (glutamate-N)
31. Two-week arylsulfatase	76. Glycerol as C source (glutamate-N)
32. Salicylate degradation	77. Glutamate as N and C source
33. <i>p</i> -Aminosalicylic acid degradation	78. L-Serine as N and C source
34. Acetamidase	79. Glucosamine as N and C source
35. Benzamidase	80. Acetamide as N and C source
36. Urease	81. Benzamide as N and C source
37. Isonicotinamidase	82. Monoethanolamine as N and C source
38. Nicotinamidase	83. Trimethylene diamine as N and C source
39. Pyrazinamidase	84. Benzamide as N source
40. Salicylamidase	85. Nicotinamide as N source
41. Allantoinase	86. Nitrate as N source
42. Succinamidase	87. Nitrite as N source
43. Growth at 28°C	88. Ethambutol medium (5 µg/ml)
44. Growth at 37°C	89. 1,3-Butylene glycol as C source
45. Growth at 45°C	90. 2,3-Butylene glycol as C source

^a The references of the characters, except for characters 89 and 90, are described in a previous paper (24). Characters 89 and 90 were described previously (19, 20).

responded to *N. farcinica*, *N. asteroides*, *N. caviae*, *N. brasiliensis*, and *G. lentifragmenta*. In this dendrogram, the strains of *N. asteroides* B were included in the cluster of *N. farcinica* (compare Fig. 1 and Fig. 6).

"Distinguishing" characters useful for differentiation between the clusters obtained by set A1, which gave the most clusters, are shown in Table 4. In this table, characters of the strains that formed a compact cluster are shown. The clusters of *N. farcinica*, *N. caviae*, *N. brasiliensis*, *N. asteroides* A, *N. asteroides* B, and *G. lentifragmenta* were formed by 93, 94, 92, 91, 91, and 94% levels, respectively (Fig. 1).

DISCUSSION

By comparing the dendrograms obtained by six different sets of characteristics, it is obvious that sets A1 and A2 provided the most clusters. Only these sets could divide *N. asteroides* strains (according to Tsukamura's definition [22]) into two subgroups. From the theoretical basis, Sokal and Sneath (18) stated that the number of differentiating characters for numerical classifications should be not less than 60, Sneath (16) suggested at least 50, and Lockhart (13) specified above a minimum of about 40. As sets A1 and A2 contained 57 and 40 differentiating characters, respectively, the theoretical considerations were shown experimentally to be correct.

Although set B contained 79 differentiating characters, it failed to give the most clusters. Sneath (17) and Tsukamura (19, 25) stated that the characters for numerical classifications should not contain linked characters. Linked characters which are controlled by the same cistron should be ignored. Set B contained the characters of the utilization of carbohydrates and those of acid formation from the same carbohydrates. The utilization of carbohydrates and the acid formation from them usually showed the same results. Probably based on the use of the linked characters, set B confused the *N. asteroides* B group with the *N. farcinica* group.

Sets A2 and A3 contained the same number of differentiating characters, but set A3 failed to differentiate between *N. asteroides* A and B. It is suggested that, when the minimum number according to Lockhart (13) is used, the kind of characters may influence the clustering. Set A3 consisted of the characters concerned only with amidases and nutritional requirements, whereas set A2 consisted of various characters. Our results show that the use of various characters is better. Set A1, which contains 57 differentiating characters, is an improved set in which the num-

TABLE 3. Sets of characters used in numerical classification

Set	Characters	No. of characters	No. of differentiating characters ^a
A1	1-90 ^b	90	57 ^d
A2	1-64 ^b	64	40
A3	35-90 ^b	56	40
A4	1-53 ^b	53	30
A5	51-90 ^b	40	30
B	1-105 ^c	105	79 ^e

^a The number of differentiating characters was counted omitting strain 71, which was identified as *Mycobacterium intracellulare*.

^b See Table 2.

^c From the 90 characters shown in Table 2, the following 7 characters were removed: no. 22 (Tween hydrolysis after 7 days); no. 72 (glucose as C source, glutamate-N); no. 73 (acetate as C source, glutamate-N); no. 74 (succinate as C source, glutamate-N); no. 75 (pyruvate as C source, glutamate-N); no. 76 (glycerol as C source, glutamate-N); and no. 88 (resistance to ethambutol). To the remaining 83 characters, the following 22 characters were added (19-21): (1) Gram positiveness; (2) growth on $\text{NH}_4\text{OH} \cdot \text{HCl}$ (62.5 $\mu\text{g}/\text{ml}$); (3) growth on salicylate medium (1.0 mg/ml); (4) tolerance to 0.1% picric acid; (5) tolerance to 0.2% NaNO_2 ; (6) growth on 1% Tween-Sauton agar; (7) malonamidase; (8) fructose as C source; (9) glycerol as C source; (10) ethanol as C source; (11) 1,4-butylene glycol as C source; (12) raffinose as C source; (13) acid from raffinose; (14) acid from galactose; (15) acid from arabinose; (16) acid from xylose; (17) acid from rhamnose; (18) acid from trehalose; (19) acid from inositol; (20) acid from mannitol; (21) acid from sorbitol; (22) nicotinamide as N and C sources.

^d The characters that showed a positive match were 2, 3, 5, 10, 12, 14, 18, 19, 20, 43, 44, 62, 66, 72, 73, 74, 75, 76, and 77 (19 characters). The characters that showed a negative match were 1, 4, 9, 21, 22, 30, 32, 33, 37, 40, 42, 46, 81, and 83 (14 characters). These numbers were counted omitting strain 71 (*M. intracellulare*).

^e The characters that showed a positive match were 3, 10, 14, 18, 19, 47, and additional characters 1, 2, 3, and 9 (see footnote c). The characters that showed a negative match were 1, 4, 9, 21, 30, 32, 33, 37, 40, 42, 46, 81, and additional characters 7, 12, 13, and 22 (see footnote c).

ber of linked characters is minimal. This set gave the most clusters.

Previously, Tsukamura (22) divided the strains received as *N. asteroides* into two taxa, *N. farcinica* and *N. asteroides* (a strict sense). In the present study, the taxon *N. asteroides* could be divided into two subgroups, A and B. After Tsukamura's previous study (22), Goodfellow (5) divided strains designated as *N. asteroides* into five subgroups, and Kurup and Schmitt (11) divided them into four subgroups. The identity of the clusters shown in the present

FIG. 1. Dendrogram obtained by the set A1. Abbreviations: F, *N. farcinica*; C, *N. caviae*; B, *N. brasiliensis*; AA, *N. asteroides* A; AB, *N. asteroides* B; G, *G. lentifragmenta*.

FIG. 2. Dendrogram obtained by the set A2. Abbreviations: F, *N. farcinica*; C, *N. caviae*; AA, *N. asteroides* A; AB, *N. asteroides* B; B, *N. brasiliensis*; G, *G. lentifragmenta*.

FIG. 3. Dendrogram obtained by the set A3. Abbreviations: F, *N. farcinica*; C, *N. caviae*; B, *N. brasiliensis*; A, *N. asteroides* (subgroups A and B in Fig. 1 are combined into one); G, *G. lentifragmenta*.

FIG. 4. Dendrogram obtained by the set A4. Abbreviations: F, *N. farcinica*; C, *N. caviae*; G, *G. lentifrag-menta*.

FIG. 5. Dendrogram obtained by the set A5. Abbreviations: F, *N. farcinica*; C, *N. caviae*; B, *N. brasiliensis*; A, *N. asteroides*; G, *G. lentifragmenta*.

FIG. 6. Dendrogram obtained by the set B. Abbreviations: F, *N. farcinica*; A, *N. asteroides* (this cluster almost equals the cluster *N. asteroides* A in Fig. 1); C, *N. caviae*; G, *G. lentifragmenta*; B, *N. brasiliensis*. Note: The cluster *N. asteroides* B in Fig. 1 is included into the cluster *N. farcinica*.

TABLE 4. *Characters useful for distinguishing Nocardia species^a*

Character	<i>N. farcin- ica</i>	<i>N. aster- oides A</i>	<i>N. aster- oides B</i>	<i>N. cav- iae</i>	<i>N. bras- iliensis</i>	<i>G. lentifrag- menta</i> (for- merly, <i>N.</i> <i>rubra</i>)
	31 ^b	7 ^b	10 ^b	7 ^b	4 ^b	5 ^b
1. Colony pigmentation in dark	6	14	80	0	0	100
2. Growth at 45°C	100	0	0	29	0	0
3. Tween hydrolysis (14 days)	68	43	30	71	0	0
4. α-Esterase	0	0	100	0	100	0
5. β-Esterase	77	14	100	100	100	0
6. Acid phosphatase	0	43	0	0	75	0
7. β-Galactosidase	100	100	100	100	100	0
8. Nitrate reduction	3	14	70	0	0	100
9. Catalase (semiquantitative)	29	0	50	71	75	60
10. Acetamidase	97	0	40	0	25	100
11. Benzamidase	68	0	0	0	0	0
12. Urease	100	100	70	100	100	0
13. Allantoinase	19	57	10	100	75	0
14. Growth on rifampin medium (25 µg/ml)	97	86	90	100	100	0
15. Tolerance to ethambutol (5 µg/ml)	100	86	100	100	100	0
16. Sucrose as C source	26	0	0	0	0	100
17. <i>n</i> -Propanol as C source	100	29	20	29	0	100
18. Propylene glycol as C source	55	0	10	0	0	100
19. 1,3-Butyleneglycol as C source	77	0	0	0	0	20
20. Mannose as C source	100	29	10	100	75	100
21. Galactose as C source	0	0	0	0	100	0
22. Rhamnose as C source	81	0	0	0	0	0
23. Trehalose as C source	26	0	0	14	100	0
24. Inositol as C source	0	0	0	86	100	0
25. Mannitol as C source	0	0	0	100	100	100
26. Sorbitol as C source	0	0	0	29	0	100
27. Citrate as C source	6	86	0	0	100	100
28. Benzoate as C source	0	0	0	0	0	80
29. Malonate as C source	0	0	0	0	0	60
30. L-Serine as N and C sources	3	0	0	100	0	20
31. Glucosamine as N and C sources	94	57	0	100	75	80
32. Acetamide as N and C source	100	0	0	0	0	100
33. Monoethanolamide as N and C source	100	0	0	100	0	80
34. Benzamide as N source	61	0	70	0	0	0
35. Nitrite as N source	45	43	40	0	0	0

^a Values indicate the percentage of strains that showed a positive reaction.^b Number of strains tested (the strains that showed a compact cluster in Fig. 1).

study with those obtained by these authors will be a problem in further investigations. The relationship between the clustering and the kind of characters used in numerical classifications should also be studied in detail in the future.

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REPRINT REQUESTS

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