Numerical Taxonomy of the Genus *Nocardia*

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SUMMARY

By a numerical classification, strains received as *Nocardia asteroides* were divided into two clusters; these two groups were named as *N. asteroides* (Eppinger) Blanchard and *N. farcinica* Trevisan, respectively, recognizing the name of *N. farcinica* for the group which included strain ATCC 3318, received as *N. asteroides*.

The genus *Nocardia* was divided into 7 groups; (1) *N. asteroides* (in a new sense); (2) *N. brasiliensis*; (3) *N. caviae*; (4) *N. farcinica*; (5) a group provisionally named *N. rubra*; (6) *N. pelletieri*; (7) *N. madurae*.

The species provisionally named *N. rubra* was made up of strains which, when received from other workers, bore 8 species names: *N. rubra*, *N. erythropolis*, *N. globerula*, *N. convoluta*, *N. minima*, *N. lutea*, *N. rubropertincta* and *N. polychromogenes*.

Relationships between seven species of *Nocardia* are shown as a dendrogram, and descriptions of characteristics (here called Hypothetical Mean Organism) of the species are presented.

A method called numerical identification is presented by which identification can be made, without subjective judgement, by comparison of the characters of a test strain with the Hypothetical Mean Organism after permissible limits of S-values for the Hypothetical Mean Organism have been established.

INTRODUCTION

After the publication of *Bergey's Manual of Determinative Bacteriology* (1957), the taxonomy of the genus *Nocardia* was advanced by Gordon & Mihm (1957, 1959, 1962*a*, *b*) and Gordon (1966*a*, *b*), who characterized the species by biochemical tests. In other groups of organisms numerical taxonomy (Sneath, 1957*a*, *b*, 1964; Sneath & Cowan, 1958; Sokal & Sneath, 1963) has made a contribution to the elucidation of taxonomic problems, and since no numerical classification has been made on the genus *Nocardia* (see Appendix), it was decided to apply this method to the genus.

METHODS

One hundred and twelve strains received as nocardiae were used for the study. Thirty-four strains were received in 1967 from Dr Ruth E. Gordon, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, U.S.A., under the following species names: *N. asteroides* (10 strains); *N. brasiliensis* (7 strains); *N. caviae* (7 strains); *N. pelletieri* (5 strains); and *N. madurae* (5 strains). For these strains we retained the strain names. The other 78 strains were received in 1965 and 1967 from Dr I. Uesaka, Tuberculosis Research Institute, Kyoto University, Kyoto, Japan;

among these, 66 had been supplied to Dr Uesaka by Dr N. M. McClung, University of South Florida, Tampa, Florida, U.S.A., and these 66 strains were designated by the letter M. Eight strains had been supplied to Dr Uesaka by Dr McClung through Dr Arai, Chiba University, Chiba, Japan. These also were designated with the letter M. Four strains had been supplied to Dr Uesaka by Dr Gordon through Dr Arai; these were designated by the letter C. During investigations in our laboratory the strains were given a laboratory number so that their identities were concealed.

All strains were subcultured on glucose agar and kept at -20° . The composition of the glucose agar was as follows: glucose, 10 g.; sodium glutamate, 4.0 g.; KH_2PO_4 , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; agar, 20.0 g.; distilled water, 1,000 ml.; adjusted to pH 7.0 by addition of 10% (w/v) KOH.

One hundred characters were tested (see below); the methods employed were described previously (Tsukamura, 1967a); incubation was at 28° , unless specially noted.

Among the 100 characters 97 were set up originally for differentiating between the members of the genus *Mycobacterium*. Only 3 other characters were added; these were: (1) partial acid-fastness; (2) permanent mycelium; and (3) temporary mycelium.

Morphological characters were observed on smears from organisms growing on Ogawa egg medium or glucose agar, and on slide cultures of glucose agar after 1, 2, 3, and 7 days of incubation, by the Ziehl-Neelsen method. The term 'permanent' mycelium was used for well-developed mycelium showing fragmentation to rods or coccoids, mycelia being observed throughout the incubation period; 'temporary' mycelium for mycelia visible at an early stage of growth which soon became rods and coccoids. The term 'complete' acid-fastness was applied to organisms as acid-fast as growing mycobacteria.

Amidase tests were done according to the method of Bönicke (1962), the time of incubation being modified to 16 hr at 37° . To avoid contamination of the organisms with components of medium in the amidase tests, only glucose agar-grown cultures were used. Egg media seemed to be unsuitable for nocardiae, producing growth adherent to egg media.

All tests were done three times and the results obtained in at least two replications were used for preparation of charts of characters. The results were expressed by symbols (+, -), and the S-value was calculated as follows:

$$\text{S-value (\%)} = (n_s/n_s + n_d) \times 100 \%,$$

where n_s is the number of characters in which two strains showed similar code symbols (+ + or - -) and n_d is the number of characters in which two strains showed different symbols (+ -). The method used for calculation of S-values was described previously (Tsukamura, 1966). Clustering was made by single linkage (Sokal & Sneath, 1963).

Among the characters used 25 were ineffective for differentiation between the members of the genus *Nocardia*, giving either all positive or all negative results for the whole set of organisms. Nevertheless, these were retained in the test series. On the other hand, several characters reported as useful for differentiation between the members of the genus *Nocardia* (Gordon & Mihm, 1957, 1962a) were not included in this study because the same test series was applied to both mycobacteria and nocardias.

Hypothetical Mean Organism (HMO) is the term used to express a mean charac-

terization of a species, which is a slight modification of the 'Calculated Median Organism' of Liston, Wiebe & Colwell (1963).

For preparation of HMOs, the mean number of positive characters (mean of the characters showing a positive reaction in a strain) is calculated for every species or group. The number of positive characters in an HMO is equal to the mean number of positive characters per strain. The characters to be used for preparation of an HMO are chosen, first taking a character appearing at the highest frequency and then taking a character appearing at the next (lower) frequency, and the choice of such positive characters continues until the number of characters obtained equals the mean number of positive characters.

The number of characters used for the preparation of HMOs is not always equal to the mean number. If, after selection of one less than the mean number of characters, three characters showing the same frequency are found, these three are added, obtaining two characters more than the mean number. The HMOs thus prepared are similar to the Calculated Median Organisms of Liston *et al.* (1963). The average S-value between the HMO and the Calculated Median Organism in various mycobacterial species was 98.5 % (Tsukamura & Mizuno, 1968). Details of the method of preparation of the HMO and a few advantages in the use of the HMO (for example, it is possible to calculate the degree of deviation in the characters of an HMO) were described previously (Tsukamura & Mizuno, 1968).

RESULTS

Numerical classification of Nocardia (strains provided by Dr Gordon)

Numerical classification of 34 strains of *Nocardia* received from Dr Gordon as reference strains showed clearly 6 clusters: (1, 2) *N. asteroides* (two clusters); (3) *N. brasiliensis*; (4) *N. caviae*; (5) *N. pelletieri*; (6) *N. madurae* (Fig. 1). The clusters of *N. asteroides* were labelled group A (from ATCC 9970 to R-553) and group B (from R-764 to ATCC 3318), respectively. Strain R-1351 received as *N. caviae* was not in the cluster of *N. caviae* and, therefore, was omitted from the group of *N. caviae*.

A dendrogram showing relationships between the species was prepared from the S-value table shown in Fig. 1 and is shown in Fig. 2.

Since a type strain of *Nocardia asteroides* seems to be non-existent, it was desired to consider which group contained, as nearly as possible, the characters attributed to *N. asteroides*. All strains showed the characters described by Gordon & Mihm (1957, 1959, 1962*a*), but three characters used by them were variable. Not all of the 7 strains of group A formed acid from rhamnose, or grew at 45°, and 5 of 7 showed reduction of nitrate to nitrite. On the other hand, all 3 strains of group B formed acid from rhamnose, grew at 45°, and failed to reduce nitrate. Gordon & Mihm (1957, 1959, 1962*a*) described these characters as follows: (a) 32 % of the strains tested formed acid from rhamnose; (b) 42 % of the strains grew at 45°; and (c) 88 % of the strains reduced nitrate to nitrite (Gordon & Mihm, 1962*a*). These characters resemble our group A rather than group B; accordingly, group A was named *N. asteroides*, but group B required another name. Group B included strain ATCC 3318, named *N. farcinica* Trevisan (Gordon & Mihm, 1957, 1962*a*), which name seemed to be suitable for group B.

Differentiation between groups A and B could be made not only by these characters

Fig. 1. Diagrammatic representation of the S-value table prepared by shading the square of a S-value between the strains.

The species names above are the names received.

Fig. 2. Dendrogram prepared from the S-value table shown in Fig. 1.

but more distinctly by many other characters (see below). It is now intended to demonstrate differences between these two species in tests of more strains and to make acceptable the proposal for the division of the species *N. asteroides* into two separate species.

Numerical classification of Nocardia (strains provided by Dr Uesaka and Dr McClung)

Results of the numerical classification of 78 strains received from Dr Uesaka, which were mainly from the collection of Dr McClung, are shown in Fig. 3. Two distinct clusters were present, but half of the strains remained unclustered. One of the clusters consisted of 34 strains (from C-407 to M-175); the other of 7 strains (from M-1 to

Fig. 3. Diagrammatic representation of the S-value table prepared by shading the square of a S-value between the strains.

Table 1. 'Hypothetical Mean Organisms' (HMOs) of the species of the genus *Nocardia* (comparison with Table 2)

	<i>N. asteroides</i> (group A)	<i>N. rubra</i> (Kyoto-II)	<i>N. farcinica</i> (Kyoto-I)	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>
1. Gram reaction	+	+	+	+	+	+	+
2. Acid-fastness, complete	-	-	-	-	-	-	-
3. Acid-fastness, partial	+	+	+	+	+	+	+
4. Mycelium, permanent	-	-	-	-	-	+	+
5. Mycelium, temporary	+	+	+	+	+	+	+
6. Rod shape (with or without coccoids)	+	+	+	+	+	+	+
7. Cord formation (compact grouping)	+	+	+	+	+	+	+
8. Colonial morphology, rough	+	+	+	+	+	-	-
9. Colonial pigmentation	-	+	-	-	-	+	-
10. Photochromogenicity	-	-	-	-	-	-	-
11. Growth rate, rapid	+	+	+	+	+	-	-
12. Catalase	+	+	+	+	+	+	+
13. Nitrate reduction	+	-	-	-	+	-	-
14. Three-day aerysulphatase	-	-	-	-	-	-	-
15. Two-week aerysulphatase	-	-	-	-	-	-	-
16. Salicylate degradation	-	-	-	-	-	-	-
17. PAS degradation	-	-	-	-	-	.	.
18. Growth on 0.2 % PAS medium	+	+	+	+	+	.	.
19. Growth on NH ₂ OH medium (62.5 µg./ml.)	+	+	+	+	+	.	.
20. Growth on NH ₂ OH medium (125 µg./ml.)	+	+	+	+	+	.	.
21. Growth on NH ₂ OH medium (250 µg./ml.)	-	+	+	-	-	.	.
22. Growth on NH ₂ OH medium (500 µg./ml.)	-	+	-	-	-	.	.
23. Tolerance to 0.1 % picric acid	+	+	+	+	+	+	+
24. Tolerance to 0.2 % picric acid	+	+	+	+	+	+	+
25. Growth at 28°	+	+	+	+	+	+	+
26. Growth at 37°	+	+	+	+	+	+	+
27. Growth at 45°	-	-	+	-	+	+	+
28. Growth at 52°	-	-	-	-	-	-	-
29. Acetamidase	-	+	+	-	-	-	-
30. Benzamidase	-	-	+	-	-	-	-
31. Urease	+	+	+	+	+	-	-
32. Isonicotinamidase	-	-	-	-	-	-	-
33. Nicotinamidase	+	-	+	-	+	+	-
34. Pyrazinamidase	-	-	+	-	+	+	-
35. Salicylamidase	-	-	-	-	-	-	-
36. Allantoinase	-	-	-	-	+	-	-
37. Succinamidase	-	-	-	-	-	-	-
38. Malonamidase	-	-	-	-	-	-	-
39. Acetate (as C source)	+	+	+	+	+	+	+
40. Citrate (as C source)	+	+	-	+	-	-	-
41. Succinate (as C source)	+	+	+	+	+	-	+
42. Malate (as C source)	+	+	+	+	+	-	+
43. Pyruvate (as C source)	+	+	+	+	+	+	+
44. Benzoate (as C source)	-	-	-	-	-	-	-
45. Malonate (as C source)	-	-	-	-	-	-	-
46. Fumarate (as C source)	+	+	+	+	+	-	+
47. Acid from glucose	+	+	+	+	+	+	+
48. Acid from mannose	-	-	-	-	-	-	-
49. Acid from galactose	-	-	-	+	-	-	-
50. Acid from arabinose	-	-	-	-	-	-	+

Table 1 (cont.)

	<i>N. asteroides</i> (group A)	<i>N. rubra</i> (Kyoto-II)	<i>N. farcinica</i> (Kyoto-I)	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>
51. Acid from xylose	-	-	-	-	-	-	+
52. Acid from rhamnose	-	-	+	-	-	-	+
53. Acid from trehalose	-	-	-	+	-	-	+
54. Acid from raffinose	-	-	-	-	-	-	-
55. Acid from inositol	-	-	-	+	+	-	-
56. Acid from mannitol	-	+	-	-	+	-	+
57. Acid from sorbitol	-	+	-	-	+	-	-
58. Glycerol (as C source)	+	+	+	+	+	+	+
59. Glucose (as C source)	+	+	+	+	+	+	+
60. Mannose (as C source)	-	+	+	-	+	-	-
61. Galactose (as C source)	-	-	-	+	-	-	-
62. Arabinose (as C source)	-	-	-	-	-	-	+
63. Xylose (as C source)	-	-	-	-	-	-	+
64. Rhamnose (as C source)	-	-	+	-	-	-	+
65. Trehalose (as C source)	-	+	-	+	-	+	+
66. Raffinose (as C source)	-	-	-	-	-	-	-
67. Inositol (as C source)	-	-	-	+	+	-	+
68. Mannitol (as C source)	-	+	-	+	+	-	+
69. Sorbitol (as C source)	-	+	-	-	+	-	+
70. Fructose (as C source)	+	+	+	+	+	-	+
71. Sucrose (as C source)	-	+	-	-	-	-	-
72. Ethanol (as C source)	-	+	+	-	-	-	+
73. Propanol (as C source)	+	+	+	+	-	-	+
74. Propylene glycol (as C source)	-	+	+	-	-	-	-
75. 1,3-Butylene glycol (as C source)	-	-	+	-	-	-	-
76. 1,4-Butylene glycol (as C source)	-	-	-	-	-	-	-
77. 2,3-Butylene glycol (as C source)	-	-	+	-	-	-	-
78. L-Glutamate (as N and C sources)	+	+	+	+	+	+	+
79. L-Serine (as N and C sources)	-	-	-	+	+	-	-
80. Glucosamine (as N and C sources)	+	+	+	+	+	-	+
81. Acetamide (as N and C sources)	-	+	+	-	-	-	-
82. Benzamide (as N and C sources)	-	-	-	-	-	-	-
83. Monoethanolamine (as N and C sources)	-	+	+	-	+	-	-
84. Trimethylene diamine (as N and C sources)	-	-	-	-	-	-	-
85. L-Glutamate (as N source)	+	+	+	+	+	+	+
86. L-Serine (as N source)	+	+	-	+	+	-	+
87. L-Methionine (as N source)	+	+	-	+	+	-	+
88. Acetamide (as N source)	+	+	+	+	+	-	+
89. Benzamide (as N source)	-	-	-	-	-	-	-
90. Urea (as N source)	+	+	+	+	+	-	+
91. Pyrazinamide (as N source)	+	+	+	+	+	-	+
92. Isonicotinamide (as N source)	+	+	-	-	-	-	+
93. Nicotinamide (as N source)	+	+	+	+	+	-	+
94. Succinamide (as N source)	+	+	+	+	+	-	+
95. Nitrate (as N source)	+	+	+	+	+	-	+
96. Nitrite (as N source)	-	-	-	-	+	-	-
97. Nicotinic acid	-	-	-	-	-	-	-
98. Growth on TCH medium (10 µg./ml.)	+	+	+	+	+	.	.
99. Growth on salicylate medium (0.05 %)	+	+	+	+	+	.	.
100. Growth on salicylate medium (0.1 %)	+	+	+	+	+	.	.

NRRL 61531). As shown later, strains of the large group were identical with group B, that is, *N. farcinica*, and another group was shown to be a species distinguishable from the other species. In the following, the course of classification and identification will be described in detail.

Table 2. *Properties of 7 groups of Nocardia (mean no. of positive characters, intragroup-S-value for each group, and mean S-value to the HMO in each group)*

Group	No. of strains	Mean no. of positive characters	Intragroup-S-value (%)	Mean S-value to the HMO (%)
<i>N. asteroides</i> (group A of <i>N. asteroides</i>)	7	44.3 ± 4.03 (n = 7)	89.1 ± 3.38 (n = 21)	93.6 ± 2.58 (n = 7)
<i>N. rubra</i> (group Kyoto-II)	7	56.6 ± 3.05 (n = 7)	91.9 ± 2.74 (n = 21)	95.3 ± 1.98 (n = 7)
<i>N. farcinica</i> (group Kyoto-I)	30	52.7 ± 4.26 (n = 30)	91.0 ± 3.87 (n = 435)	94.6 ± 3.17 (n = 30)
<i>N. brasiliensis</i>	7	48.3 ± 3.15 (n = 7)	92.8 ± 2.25 (n = 21)	95.9 ± 2.55 (n = 7)
<i>N. caviae</i>	6	52.5 ± 2.43 (n = 6)	96.0 ± 1.46 (n = 15)	97.5 ± 1.38 (n = 6)
<i>N. pelletieri</i>	5	20.8 ± 1.48* (n = 5)	92.2 ± 3.84 (n = 10)	96.6 ± 1.34 (n = 5)
<i>N. madurae</i>	5	46.8 ± 3.11* (n = 5)	89.5 ± 2.22 (n = 10)	92.4 ± 2.07 (n = 5)

* Data on egg media were excluded. Accordingly, a total of 91 characters was tested. In other groups, a total of 100 characters was tested.

Table 3. *S-value table (%) of the 'Hypothetical Mean Organisms' (HMOs) of 7 groups of Nocardia*

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) <i>N. asteroides</i> (group A)	—	—	—	—	—	—	—
(2) <i>N. brasiliensis</i>	88	—	—	—	—	—	—
(3) <i>N. caviae</i>	84	84	—	—	—	—	—
(4) <i>N. rubra</i> (Kyoto-II)	81	79	77	—	—	—	—
(5) <i>N. farcinica</i> (Kyoto-I)	80	74	78	79	—	—	—
(6) <i>N. madurae</i>	76	78	79	78	70	—	—
(7) <i>N. pelletieri</i>	74	67	67	64	69	67	—

Intragroup-mean S-values to the HMO are as follows: (1) 93.6 ± 2.58 % (n = 7); (2) 95.9 ± 2.55 % (n = 7); (3) 97.5 ± 1.38 % (n = 6); (4) 95.3 ± 1.98 % (n = 7); (5) 94.6 ± 3.17 % (n = 30); (6) 92.4 ± 2.07 % (n = 5); (7) 96.6 ± 1.34 % (n = 5). (Refer to the right column in Table 2.)

Figure 3 was prepared by a rearrangement of the S-value table of 78 strains. When a diagrammatic representation of the S-value table of the strains was first prepared, two clusters were recognized, one large cluster consisting of 30 strains (from C-407 to M-200) and another small cluster consisting of 7 strains (from M-1 to NRRL 61531). These clusters were named provisionally Kyoto-I and Kyoto-II, respectively. In order to compare these two groups with the 6 groups obtained by a numerical classification of Dr Gordon's strains, HMOs were prepared for each of the following groups (Table 1): (1) Kyoto-I; (2) Kyoto-II; (3) *Nocardia asteroides* (group A); (4) *N. brasili-*

ensis; (5) *N. caviae*; (6) *N. pelletieri*; (7) *N. madurae* (Table 1). The strains used for preparation of the HMOs were as follows: (1) Kyoto-I (30 strains, C-407 (received as *N. madurae*) to M-200 (received as *N. brasiliensis*) in Fig. 3); (2) Kyoto-II (7 strains,

Fig. 4. Dendrogram prepared from the S-value table of HMOs of the species of *Nocardia*.

Table 4. Comparison between *Nocardia asteroides* (group A) and *N. brasiliensis*

	<i>N. asteroides</i> (group A) (%)	<i>N. brasiliensis</i> (%)
Mean S-value to own HMO	93.6 ± 2.58 (n = 7)	95.7 ± 2.55 (n = 7)
Mean S-value to other HMO	83.7 ± 2.01 (n = 7)	86.4 ± 2.51 (n = 7)

(Mean) ± (standard deviation) %. (n = number of strains.)

Table 5. Intra- and inter-groups mean S-values, *Nocardia asteroides* and *N. brasiliensis*

	(1) (%)	(2) (%)
(1) <i>N. asteroides</i> (group A) (7 strains)	89.1 ± 3.38 (n = 21)	—
(2) <i>N. brasiliensis</i> (7 strains)	82.9 ± 3.37 (n = 49)	92.8 ± 2.25 (n = 21)

Intra-group mean S-values are significantly larger than inter-groups mean S-value (*P* less than 5 %).

M-1 (received as *N. rubra*) to NRRL 61531 (received as *N. polychromogenes*) in Fig. 3); (3) *N. asteroides* (7 strains, ATCC 9970 to R-553 in Fig. 1); (4) *N. brasiliensis* (7 strains, R-405 to R-1336 in Fig. 1); (5) *N. caviae* (6 strains, R-416 to R-1316 in Fig. 1); (6) *N. pelletieri* (5 strains in Fig. 1); (7) *N. madurae* (5 strains in Fig. 1). Group B, *N. farcinica*, was omitted from the above groups, as it consisted of only 3 strains.

Table 6. *Intra- and inter-groups mean S-values, Nocardia brasiliensis and N. caviae*

	(1) (%)	(2) (%)
(1) <i>N. brasiliensis</i> (7 strains)	92.8 ± 2.25 (n = 21)	—
(2) <i>N. caviae</i> (6 strains)	84.6 ± 2.62 (n = 42)	96.0 ± 1.46 (n = 15)

Intra-group S-values are significantly larger than inter-groups mean S-value (*P* less than 5 %).

Table 7. *Intra- and inter-groups mean S-values, Kyoto-I and Kyoto-II groups*

	(1) (%)	(2) (%)
(1) Kyoto-I (30 strains)	91.0 ± 3.87 (n = 435)	—
(2) Kyoto-II (7 strains)	77.9 ± 3.87 (n = 210)	91.9 ± 2.74 (n = 21)

Intra-group mean S-values are significantly larger than inter-groups mean S-value (*P* less than 5 %).

Table 8. *Identification of strains ATCC 3318, W-3409B and R-784 (group B of Nocardia asteroides)*

Hypothetical Mean Organism (HMO)	S-value to HMO (%)			Permissible low limit of S-value for species* (%)
	ATCC 3318	W-3409B	R-784	
<i>N. farcinica</i> (Kyoto-I)	93	93	89	88.3
<i>N. rubra</i> (Kyoto-II)	78	75	78	91.3
<i>N. asteroides</i> (group A)	77	74	83	88.4
<i>N. brasiliensis</i>	74	68	77	90.8
<i>N. caviae</i>	71	70	79	94.7
<i>N. pelletieri</i>	70	76	74	93.9
<i>N. madurae</i>	73	67	69	88.3

* The permissible low limit of the S-value to the HMO for members of a species is at (mean) - 2 × (standard deviation) %.

Table 9. *Strains identified, provisionally, as Nocardia rubra (Kruse) Chalmers & Christopherson*

Strain*	History
<i>N. rubra</i> M-1	N. M. McClung; CBS, Holland (= R-562)
<i>N. erythropolis</i> M-8	N. M. McClung; Waksman (3407)
<i>N. globerula</i> M-75	N. M. McClung; NRRL no. B1306 (= ATCC 9356 = R-417)
<i>N. convoluta</i> M-79	N. M. McClung; ATCC 4275
<i>N. minima</i> M-103	N. M. McClung; ATCC 8674
<i>N. lutea</i> M-192	N. M. McClung; D. Schneidau (365); R. E. Gordon (R-680) E. N. Azarowicz (147); Inst. Pasteur, Paris
<i>N. polychromogenes</i> NRRL 61531	N. M. McClung; NRRL 61531
<i>N. polychromogenes</i> M-6	N. M. McClung; <i>N. polychromogenes</i> (Vallée) Jensen; CBS; (= R-565)
<i>N. rubropertincta</i> M-191†	N. M. McClung; J. D. Schneidau

* Species name is the name when received.

† Suggested to be a member of this species (see Table 10).

Table 10. Identification by comparison with Hypothetical Mean Organisms (HMOs) of strains not forming a cluster in preliminary sorting

Species name and strain name as received	S-value (%) to the HMO of							Identification*
	<i>N. asteroides</i> (group A)	<i>N. rubra</i> (Kyoto-II)	<i>N. farcinica</i> (Kyoto-I)	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>	
Permissible low limit (%)†	88.4	91.3	88.3	90.8	94.7	93.9	88.3	
<i>N. coeliaca</i> M-122	69	84	71	75	71	50	72	.
<i>N. madurae</i> C-743	84	83	92	80	76	68	70	F
<i>N. blackwellii</i> M-81	83	84	95	77	77	65	76	F
<i>N. asteroides</i> M-130	83	80	97	77	77	69	74	F
<i>N. asteroides</i> M-163	80	77	84	72	74	72	68	F‡
<i>N. corrallina</i> M-78	86	75	74	82	76	80	67	A‡
<i>N. brasiliensis</i> C-851	85	82	73	95	83	63	78	B
<i>N. asteroides</i> M-94	91	82	81	85	81	76	76	A
<i>N. asteroides</i> M-124	92	81	80	84	82	72	78	A
<i>N. asteroides</i> M-128	90	83	80	84	80	74	78	A
<i>N. asteroides</i> M-132	93	80	85	87	87	77	72	A
<i>N. brasiliensis</i> M-145	84	76	73	89	79	76	77	B‡
<i>N. asteroides</i> M-155	91	80	79	87	81	76	76	A
<i>N. asteroides</i> M-161	90	75	78	84	84	77	75	A
<i>N. transvalensis</i> M-193	79	77	73	81	81	60	71	.
<i>N. brasiliensis</i> M-199	83	78	75	91	85	68	72	B
<i>N. brasiliensis</i> M-204	86	79	76	96	82	68	77	B
<i>N. asteroides</i> M-206	91	82	83	85	81	68	75	A
<i>N. brasiliensis</i> M-168	87	78	75	93	85	67	76	B
<i>Nocardia</i> sp. M-54	80	77	80	82	92	75	75	C‡
<i>N. asteroides</i> M-73	83	80	81	83	93	71	76	C‡
<i>N. asteroides</i> M-180	75	70	75	77	89	77	68	C‡
<i>N. tenuis convoluta</i> M-203	78	80	75	83	81	55	72	.
<i>N. restrictus</i> M-9	72	61	64	66	62	83	57	.
<i>N. asteroides</i> M-10	94	79	84	82	82	72	75	A
<i>N. asteroides</i> M-93	88	79	78	80	76	79	72	A
<i>N. asteroides</i> M-129	97	80	81	89	85	77	75	A
<i>N. asteroides</i> M-156	94	75	76	82	82	71	69	A
<i>N. bostroemi</i> M-159	84	71	78	76	80	85	67	.
<i>N. asteroides</i> M-166	85	76	79	83	91	77	75	C‡
<i>N. intracellularis</i> M-170	80	67	74	72	72	83	64	.
<i>N. pretoriana</i> M-194	90	77	74	86	80	75	75	A
<i>N. rubropertincta</i> M-191	82	89	74	80	76	64	75	R‡
<i>N. polychromogenes</i> M-6	82	93	76	80	76	61	75	R
<i>N. madurae</i> M-172	77	72	73	73	73	76	80	.
<i>N. asteroides</i> M-175	83	80	97	75	77	69	74	F
<i>N. asteroides</i> M-185	84	75	80	84	92	74	74	C‡
<i>N. madurae</i> C-637	60	75	68	65	63	52	74	.
<i>N. leishmanii</i> M-82	69	72	61	78	67	58	76	.
<i>N. alba</i> (Schoen) M-113	75	72	67	79	73	60	80	.
<i>N. asteroides</i> M-123	80	64	68	71	67	85	63	.

* A = *N. asteroides* (group A); R = *N. rubra* (Kyoto-II); F = *N. farcinica* (Kyoto-I); B = *N. brasiliensis*; C = *N. caviae*; Pe = *N. pelletieri*; M = *N. madurae*.

† As to the permissible limit of S-value, see Table 5.

‡ If an S-value was outside the permissible limit of 7 species but remained within a 5 % range from the permissible limit of a species, this species name was indicated with the double dagger. If an S-value was within the permissible low limit of a species, this species name was indicated as the species identified. Spaces remaining blank show that no species was identified.

Mean number of positive features for each group or species, intragroup-S-value for each group, and mean S-value to the HMO for the members of each group are shown in Table 2.

An S-value table of the HMOs is shown in Table 3, and a dendrogram prepared from it is shown in Fig. 4. Comparing the mean S-values to the HMO, which are shown in Table 2, and the S-values between the HMOs, which are shown in Table 3, it is conceivable that there are statistically significant differences between an intra-group-S-value to the HMO, which is shown on the right column in Table 2, and an intergroup-S-value (Table 3), and these 7 groups were considered to be distinct species.

Table 11. *Strains identified as Nocardia asteroides* (Eppinger)
Blanchard (Blanchard, 1896)

Strain*	History
<i>N. asteroides</i> ATCC 9970	R. E. Gordon; C. W. Emmons (NIH) (9956); C. A. Perry
<i>N. asteroides</i> R-399	R. E. Gordon; C. W. Emmons (NIH) (9966)
<i>N. asteroides</i> R-860	R. E. Gordon; W. D. Jones, Jun.
<i>N. asteroides</i> R-443 (1)	R. E. Gordon; I. B. Christison; N. F. Conant (2338)
<i>N. asteroides</i> R-443 (2)	R. E. Gordon; I. B. Christison; N. F. Conant (2338)
<i>N. asteroides</i> W-3409	R. E. Gordon; <i>N. polychromogenes</i> ; S. A. Waksman; H. L. Jensen
<i>N. asteroides</i> R-553	R. E. Gordon
<i>N. caviae</i> R-1351	R. E. Gordon
<i>N. asteroides</i> M-94	N. M. McClung; C. W. Emmons (9976)
<i>N. asteroides</i> M-124	N. M. McClung; R. E. Gordon (R-409); J. Caffey
<i>N. asteroides</i> M-128	N. M. McClung; R. E. Gordon (R-420 (1)); N. F. Conant (1004)
<i>N. asteroides</i> M-132	N. M. McClung; R. E. Gordon (W-3599); S. V. Keating
<i>N. asteroides</i> M-155	N. M. McClung; W. Jones (6M*)
<i>N. asteroides</i> M-161	N. M. McClung; D. Schneidau (306); pulmonary nocardiosis
<i>N. asteroides</i> M-206	N. M. McClung; S. McMillan; R. E. Gordon (R-421); N. F. Conant (1017)
<i>N. asteroides</i> M-10	N. M. McClung; var. <i>crateriforme</i> Bald. CBS
<i>N. asteroides</i> M-93	N. M. McClung; C. W. Emmons (NIH) (9935)
<i>N. asteroides</i> M-129	N. M. McClung; R. E. Gordon (W-3663); E. Haynes
<i>N. asteroides</i> M-156	N. M. McClung; W. Jones (8N*)
<i>N. pretoriana</i> M-194	N. M. McClung; D. Schneidau (368); S. T. Cowan (15)
<i>N. corallina</i> M-78†	N. M. McClung; ATCC 4273

* Species name is the name as received.

† Suggested to be a member of *N. asteroides* (see Table 10).

When two groups, *Nocardia asteroides* (group A) and *N. brasiliensis*, were compared, the HMO of these showed an S-value of 88 %, the highest among the intergroup-S-values (Table 3). Mean S-value for members of *N. asteroides* to its own HMO was significantly greater than mean S-value to the HMO of another species, *N. brasiliensis* (Table 4). On the other hand, mean S-value for the members of *N. brasiliensis* to its own HMO was significantly larger than that to the HMO of *N. asteroides* (Table 4). These results indicate that *N. asteroides* and *N. brasiliensis* are distinct from each other.

It was noticed that an S-value between two HMOs may differ from an intergroup-mean-S-value actually calculated on two groups. As shown in Table 3, S-value between the HMO of *Nocardia asteroides* (group A) and the HMO of *N. brasiliensis* was 88 %, and S-value between the HMO of *N. brasiliensis* and the HMO of *N. caviae* was 84 %. In contrast, when calculated with these groups directly, intergroup-mean S-value between *N. asteroides* (group A) and *N. brasiliensis* was 82.9 ± 3.37 % ($n = 49$)

(Table 5) and intergroup-mean S-value between *N. brasiliensis* and *N. caviae* was $84.6 \pm 2.62\%$ ($n = 42$) (Table 6). The former was significantly ($P = 5\%$) smaller than the latter. It is considered that this discrepancy is due to HMOs being a sym-

Table 12. *Strains identified as Nocardia farcinica* Trevisan (Trevisan, 1889)
(= group Kyoto-I = group B of *N. asteroides*)

Strain*	History
<i>N. madurae</i> C-407	I. Uesaka; Arai; R. E. Gordon (R-407)
<i>N. asteroides</i> M-72	N. M. McClung; C. H. Drake; pulmonary nocardiosis
<i>N. asteroides</i> M-111	N. M. McClung; R. E. Gordon (R-504)
<i>N. eppingeri</i> M-112	N. M. McClung; R. E. Gordon (R-508)
<i>N. asteroides</i> M-131	N. M. McClung; R. E. Gordon (R-429); N. F. Conant (2123)
<i>N. asteroides</i> M-137	N. M. McClung; S. McMillan (N-31); brain abscess
<i>N. asteroides</i> M-138	N. M. McClung; S. McMillan (N-34)
<i>N. asteroides</i> M-139	N. M. McClung; S. McMillan (N-35)
<i>N. asteroides</i> M-144	N. M. McClung; S. McMillan (O-652)
<i>N. brasiliensis</i> M-146	N. M. McClung; S. McMillan (O-416)
<i>N. asteroides</i> M-158	N. M. McClung; Pinkerton, Texas (patient)
<i>N. asteroides</i> M-86	N. M. McClung; <i>N. farcinica</i> ATCC 3318
<i>N. asteroides</i> M-127	N. M. McClung; R. E. Gordon; <i>N. graminis</i> W-3601; G. J. Friou
<i>N. asteroides</i> M-133	N. M. McClung; S. McMillan (N-4)
<i>N. asteroides</i> M-158 A	N. M. McClung; Pinkerton, Texas
<i>N. asteroides</i> M-162	N. M. McClung; D. Schneidau (308)
<i>N. asteroides</i> M-164	N. M. McClung; D. Schneidau (*312); ATCC 9504
<i>N. asteroides</i> M-167	N. M. McClung; D. Schneidau (320)
<i>N. brasiliensis</i> M-169	N. M. McClung; D. Schneidau (327); A. Batista (586)
<i>N. asteroides</i> M-177	N. M. McClung; D. Schneidau (343); leg and lung lesions
<i>N. asteroides</i> M-179	N. M. McClung; D. Schneidau (345)
<i>N. asteroides</i> M-181	N. M. McClung; D. Schneidau (347)
<i>N. brasiliensis</i> M-197	N. M. McClung; D. Schneidau (387)
<i>N. brasiliensis</i> M-198	N. M. McClung; D. Schneidau (390)
<i>N. brasiliensis</i> M-201	N. M. McClung; D. Schneidau (501); C. Lacaz (299)
<i>N. asteroides</i> M-205	N. M. McClung; S. McMillan (N-12)
<i>N. asteroides</i> M-186	N. M. McClung; D. Schneidau (353); A. Batista (702)
<i>N. asteroides</i> M-187	N. M. McClung; D. Schneidau (354); Salvin (2001)
<i>N. asteroides</i> M-189	N. M. McClung; D. Schneidau (356); (dog)
<i>N. brasiliensis</i> M-200	N. M. McClung; D. Schneidau (397); C. Lacaz (130)
<i>N. madurae</i> C-743	I. Uesaka; Arai; R. E. Gordon (R-743)
<i>N. blackwellii</i> M-81	N. M. McClung; ATCC 6846
<i>N. asteroides</i> M-130	N. M. McClung; R. E. Gordon (R-436); N. F. Conant (2249)
<i>N. asteroides</i> M-175	N. M. McClung; D. Schneidau (341)
<i>N. asteroides</i> ATCC 3318	R. E. Gordon: originally, <i>N. farcinica</i> ATCC 3318
<i>N. asteroides</i> W-3409 B	R. E. Gordon; variant of W-3409 (<i>N. polychromogenes</i> ; S. A. Waksman; H. L. Jensen)
<i>N. asteroides</i> R-784	R. E. Gordon; B. W. Lacey; A 6 (NCTC)
<i>N. asteroides</i> M-163†	N. M. McClung; D. Schneidau; Henrici's strain A10A, var. <i>gypsoides</i>

* Species name is the name as received.

† Suggested to be a member of this species, *N. farcinica*. Remark: the first 30 strains, strain C-407 to strain M-200, are the strains first identified as the group Kyoto-I. Strains, ATCC 3318, W-3409 B and R-784, belonged to the group B of *N. asteroides*.

bolized feature of a species, prepared by omitting characters appearing at low frequencies. Accordingly, an S-value between two HMOs is not a direct reflexion of an intergroup-mean S-value.

Three examples of comparison between groups are shown in Tables 5, 6 and 7. The

Table 13. *Strains identified as Nocardia brasiliensis (Lindenberg) Pinoy (Pinoy, 1913)*

Strain*	History
<i>N. brasiliensis</i> R-405	R. E. Gordon; Julia M. Coffey; J. B. Fischer; N. F. Conant (<i>N. mexicana</i>)
<i>N. brasiliensis</i> R-432	R. E. Gordon; <i>N. mexicana</i> ; I. Christison; N. F. Conant
<i>N. brasiliensis</i> R-887	R. E. Gordon; J. L. Miranda (229)
<i>N. brasiliensis</i> R-1115	R. E. Gordon; L. F. Bojalil (N-30); Mycetoma
<i>N. brasiliensis</i> R-1117A	R. E. Gordon; L. F. Bojalil (variant of N-114)
<i>N. brasiliensis</i> R-1188	R. E. Gordon
<i>N. brasiliensis</i> R-1336	R. E. Gordon
<i>N. brasiliensis</i> C-851	I. Uesaka; Arai; R. E. Gordon (R-851)
<i>N. brasiliensis</i> M-199	N. M. McClung; D. Schneidau (393); C. Lacaz (602)
<i>N. brasiliensis</i> M-204	N. M. McClung; S. McMillan (N-2)
<i>N. brasiliensis</i> M-168	N. M. McClung; D. Schneidau (322); N. F. Conant (24); Waksman (3485); G. Ochoa (409)
<i>N. brasiliensis</i> M-145†	N. M. McClung; S. McMillan (O-410)

* Species name is the name as received.

† Suggested to be a member of *N. brasiliensis* (see Table 10).Table 14. *Strains identified as Nocardia caviae (Erikson) Erikson (Erikson, 1935)*

Strain*	History
<i>N. caviae</i> R-416	R. E. Gordon; <i>N. asteroides</i> , W. C. Haynes (B-970)
<i>N. caviae</i> R-547	R. E. Gordon; <i>N. asteroides</i> , E. N. Azarowicz
<i>N. caviae</i> R-617	R. E. Gordon; E. N. Azarowicz
<i>N. caviae</i> R-1291	R. E. Gordon
<i>N. caviae</i> R-1315	R. E. Gordon
<i>N. caviae</i> R-1316	R. E. Gordon
<i>Nocardia</i> sp. M-54	N. M. McClung; E. Munch-Peterson
<i>N. asteroides</i> M-73	N. M. McClung; NRRL, Peoria
<i>N. asteroides</i> M-180	N. M. McClung; D. Schneidau (346); CDC, Atlanta (A-343)
<i>N. asteroides</i> M-166	N. M. McClung; D. Schneidau (318); CDC, Atlanta (A-343)
<i>N. asteroides</i> M-185	N. M. McClung; D. Schneidau (351); B. H. Webster (17292A)

* Species name is the name as received.

Table 15. *Strains identified as Nocardia pelletieri (Laveran) Pinoy (Pinoy, 1912)*

Strain*	History
<i>N. pelletieri</i> R-408	R. E. Gordon; <i>N. pelletieri</i> (Laveran) Pinoy; Julia M. Coffey (47293)
<i>N. pelletieri</i> R-755	R. E. Gordon; <i>N. pelletieri</i> ; J. E. Mackinnon (IHM 614); Lab. de Parasit. Fac. Med. Paris
<i>N. pelletieri</i> R-755 var.	R. E. Gordon
<i>N. pelletieri</i> R-1352	R. E. Gordon
<i>N. pelletieri</i> R-1354	R. E. Gordon

* Species name is the name as received.

Table 16. *Strains identified as Nocardia madurae (Vincent) Blanchard (Blanchard, 1896)*

Strain*	History
<i>N. madurae</i> SAL I	R. E. Gordon
<i>N. madurae</i> R-431	R. E. Gordon; <i>N. madurae</i> ; Isabel Christison; N. F. Conant (2177); A. González Ochoa (415)
<i>N. madurae</i> R-706	R. E. Gordon; <i>N. madurae</i> ; C. W. Emmons (NIH) (9979)
<i>N. madurae</i> R-1091	R. E. Gordon; <i>Streptomyces albus</i> ; H. D. Tresner (AO-928)
<i>N. madurae</i> R-1260	R. E. Gordon; <i>Streptomyces madurae</i> ; F. Mariat, Paris (812)

* Species name is the name as received.

Table 17. Frequency of positive characters appearing in each species of *Nocardia*

	<i>N. asteroides</i>	<i>N. rubra</i>	<i>N. farcinica</i>	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>
No. of strains tested . . .	21	9	38	12	11	5	5
1. Gram reaction	21	9	38	12	11	5	5
2. Acid-fastness, complete	0	0	0	0	0	0	0
3. Acid-fastness, partial	21	8	35	12	10	5	5
4. Mycelium, permanent	2	0	9	0	1	5	5
5. Mycelium, temporary	21	9	38	12	11	5	5
6. Rod shape (with or without coccoids)	21	9	38	12	11	5	5
7. Cord formation (compact grouping)	21	9	38	12	11	5	5
8. Colonial morphology, rough	19	9	36	10	10	1	0
9. Colonial pigmentation	4	8	1	0	0	3	0
10. Photochromogenicity	0	0	0	0	0	0	0
11. Growth rate, rapid	20	9	38	12	11	0	0
12. Catalase	21	9	38	12	11	5	5
13. Nitrate reduction	8	0	0	0	6	2	2
14. Three-day arylsulphatase	0	0	0	0	0	0	0
15. Two-week arylsulphatase	8	0	0	0	2	0	0
16. Salicylate degradation	0	0	0	0	0	0	0
17. PAS degradation	0	0	0	0	0	—	—
18. Growth on 0.2 % PAS medium	21	9	38	12	11	—	—
19. Growth on NH ₂ OH medium (62.5 µg./ml.)	21	9	38	12	11	—	—
20. Growth on NH ₂ OH medium (125 µg./ml.)	17	9	35	9	11	—	—
21. Growth on NH ₂ OH medium (250 µg./ml.)	9	9	26	3	4	—	—
22. Growth on NH ₂ OH medium (500 µg./ml.)	3	4	5	2	1	—	—
23. Tolerance to 0.1 % picric acid	21	9	38	12	11	4	5
24. Tolerance to 0.2 % picric acid	20	9	38	12	11	3	5
25. Growth at 28°	21	9	38	12	11	5	5
26. Growth at 37°	18	8	38	11	11	5	5
27. Growth at 45°	0	2	35	0	11	3	4
28. Growth at 52°	0	0	6	0	0	0	0
29. Acetamidase	2	8	38	1	0	0	0
30. Benzamidase	0	0	27	0	0	0	0
31. Urease	16	5	37	12	11	0	0
32. Isonicotinamidase	0	0	0	0	0	0	0
33. Nicotinamidase	10	0	36	2	11	3	1
34. Pyrazinamidase	4	0	33	0	11	3	1
35. Salicylamidase	0	0	0	0	0	0	0
36. Allantoinase	1	0	0	0	7	0	0
37. Succinamidase	0	0	0	0	0	0	0
38. Malonamidase	0	0	0	0	0	0	0
39. Acetate (as C source)	21	9	38	12	11	5	5
40. Citrate (as C source)	12	9	7	12	0	0	3
41. Succinate (as C source)	20	9	35	12	11	0	4
42. Malate (as C source)	20	9	35	12	11	0	4
43. Pyruvate (as C source)	21	9	38	12	11	4	5
44. Benzoate (as C source)	1	1	0	0	0	0	0
45. Malonate (as C source)	1	1	0	0	0	0	1
46. Fumarate (as C source)	19	8	34	12	11	0	3
47. Acid from glucose	16	9	38	12	11	5	5
48. Acid from mannose	0	5	1	1	0	0	1
49. Acid from galactose	0	0	0	10	0	0	0
50. Acid from arabinose	0	0	0	0	0	0	5
51. Acid from xylose	0	0	0	0	0	0	4
52. Acid from rhamnose	0	0	34	1	0	0	5

Table 17 (*cont.*)

	<i>N. asteroides</i>	<i>N. rubra</i>	<i>N. farcinica</i>	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pellerieri</i>	<i>N. madurae</i>
No. of strains tested ...	21	9	38	12	11	5	5
53. Acid from trehalose	0	5	7	9	0	0	4
54. Acid from raffinose	0	0	0	0	0	0	0
55. Acid from inositol	1	1	0	11	10	0	0
56. Acid from mannitol	0	9	0	1	11	0	5
57. Acid from sorbitol	0	9	0	0	0	0	0
58. Glycerol (as C source)	21	9	37	12	11	5	5
59. Glucose (as C source)	21	9	37	12	11	5	5
60. Mannose (as C source)	4	8	27	4	7	0	1
61. Galactose (as C source)	0	0	0	11	0	0	0
62. Arabinose (as C source)	0	0	0	2	0	0	5
63. Xylose (as C source)	0	0	0	1	0	0	5
64. Rhamnose (as C source)	0	0	34	1	0	0	5
65. Trehalose (as C source)	0	6	10	10	0	3	5
66. Raffinose (as C source)	0	0	0	0	0	0	0
67. Inositol (as C source)	2	1	0	12	11	0	4
68. Mannitol (as C source)	0	9	0	11	11	0	5
69. Sorbitol (as C source)	0	9	0	7	10	0	4
70. Fructose (as C source)	21	9	38	12	11	0	4
71. Sucrose (as C source)	0	6	1	0	0	0	3
72. Ethanol (as C source)	3	9	37	0	0	0	4
73. Propanol (as C source)	8	9	38	8	0	0	4
74. Propylene glycol (as C source)	1	5	30	0	0	0	0
75. 1,3-Butylene glycol (as C source)	0	1	36	1	0	0	0
76. 1,4-Butylene glycol (as C source)	0	0	3	0	0	0	0
77. 2,3-Butylene glycol (as C source)	4	4	37	0	0	0	0
78. L-Glutamate (as N-C sources)	19	9	38	12	11	5	5
79. L-Serine (as N-C sources)	0	0	0	7	9	0	0
80. Glucosamine (as N-C sources)	12	7	38	12	10	0	4
81. Acetamide (as N-C sources)	0	8	37	0	0	0	2
82. Benzamide (as N-C sources)	0	0	0	0	0	0	0
83. Monoethanolamine (as N-C sources)	1	9	37	0	10	0	0
84. Trimethylene diamine (as N-C sources)	0	2	4	0	0	0	0
85. L-Glutamate (as N source)	21	9	38	12	11	5	5
86. L-Serine (as N source)	16	9	7	11	10	0	5
87. L-Methionine (as N source)	18	9	9	8	6	0	5
88. Acetamide (as N source)	19	8	37	8	8	0	5
89. Benzamide (as N source)	3	1	6	1	0	0	1
90. Urea (as N source)	20	9	35	12	10	0	5
91. Pyrazinamide (as N source)	20	9	31	12	10	0	5
92. Isonicotinamide (as N source)	15	9	12	2	2	0	5
93. Nicotinamide (as N source)	20	9	31	12	10	0	5
94. Succinamide (as N source)	20	9	24	12	7	0	4
95. Nitrate (as N source)	20	9	32	12	10	0	5
96. Nitrite (as N source)	8	0	3	2	4	0	1
97. Nicotinic acid	0	0	0	0	0	0	0
98. Growth on TCH medium (10 µg./ml.)	21	9	38	12	11	—	—
99. Growth on salicylate medium (0.05 %)	21	9	38	12	11	—	—
100. Growth on salicylate medium (0.1 %)	21	9	37	12	11	—	—
Mean no. of positive characters per strain	42.0	56.4	52.1	48.8	49.9	20.8	47.6

comparisons, made by the method described by Tsukamura (1967*b*) showed that the groups tested were two different clusters.

To return to the problem of *Nocardia farcinica*, that is, group B of *N. asteroides*. Group Kyoto-I, consisting of 30 strains received from Dr Uesaka, formed one large

Table 18. *Strains not identified as any of 7 species*

Strain	History
<i>N. coeliaca</i> M-122	N. M. McClung; C. W. Emmons (NIH)
<i>N. transvalensis</i> M-193	N. M. McClung; D. Schneidau (366); R. E. Gordon (366); N.C.T.C.
<i>N. tenuis convoluta</i> M-203	N. M. McClung; D. Schneidau (551); A. Batista (189)
<i>N. restrictus</i> M-9	N. M. McClung; CBS; (= R-566)
<i>N. bostroemi</i> M-159	N. M. McClung; D. Schneidau (303); A. Batista (630); Inst. Pasteur, Paris (321); (= R-1011)
<i>N. intracellularis</i> M-170	N. M. McClung; D. Schneidau (328); M. L. Littman; Wiley
<i>N. madurae</i> M-172	N. M. McClung; D. Schneidau (333); N. F. Conant (2177); G. Ochoa
<i>N. madurae</i> C-637	I. Uesaka; Arai; R. E. Gordon (R-637); A. G. Ochoa (486)
<i>N. leishmani</i> M-82	N. M. McClung; ATCC 6855; lung disease and pericarditis
<i>N. alba</i> (schoen) M-113	N. M. McClung; R. E. Gordon (R-506); Inst. Pasteur, Paris
<i>N. asteroides</i> M-123	N. M. McClung; R. E. Gordon (W-3661); C. A. Payne; brain abscess

Table 19. *Typical strains for the species of Nocardia*

Species	Typical strain (species name as received is shown)	S-value to the HMO of its species (%)
<i>N. farcinica</i> (group Kyoto-I; group B of ' <i>N. asteroides</i> ')	<i>N. asteroides</i> M-133 (ATCC 23825)	99
	<i>N. asteroides</i> M-205 (ATCC 23826)	99
	<i>N. asteroides</i> M-175	97
<i>N. rubra</i> (group Kyoto-II)	<i>N. rubra</i> M-1	97
	<i>N. globerula</i> M-75	97
	<i>N. lutea</i> M-192	97
<i>N. asteroides</i> (group A of <i>N. asteroides</i>)	<i>N. asteroides</i> R-399* (ATCC 23824)	99
	<i>N. asteroides</i> M-129	97
	<i>N. asteroides</i> ATCC 9970 (Gordon)	95
<i>N. brasiliensis</i>	<i>N. brasiliensis</i> R-432	100
	<i>N. brasiliensis</i> R-887	98
<i>N. caviae</i>	<i>N. caviae</i> R-1315	99
	<i>N. caviae</i> R-1316	99
	<i>N. caviae</i> R-416	98
<i>N. pelletieri</i>	<i>N. pelletieri</i> R-1352	98
	<i>N. pelletieri</i> R-408	98
<i>N. madurae</i>	<i>N. madurae</i> SAL I	95
	<i>N. madurae</i> R-706	94

* Proposed neotype strain deposited in the American Type Culture Collection, Rockville, Maryland, 20852, U.S.A.

cluster in numerical classification; characters of this group seemed to be similar to those of the group B of *N. asteroides* (*N. farcinica*). Twenty-two of the 30 strains had been received as *N. asteroides*, and the group Kyoto-I included strain M-86 of *N. asteroides*, which is the same as strain ATCC 3318 of *N. farcinica*.

Identification of the group B of *Nocardia asteroides* (that is, *N. farcinica*) as group Kyoto-I was carried out by determining S-values for three strains of the group B to various HMOs. These are shown in Table 8. Assuming that S-values to the HMO for individual members of a species show a normal distribution curve, it will be expected that *c.* 95 % of the members of a species will be included within the limits ((Mean) $\pm$ 2 $\times$ (Standard deviation)). Thus, a permissible low limit of the S-value for strains to be included in a species will be ((Mean) $-$ 2 $\times$ (Standard deviation)) %; this limit is calculated from the data of Table 2 and is shown in Table 8.

Table 20. *Pattern of utilization for growth of nitrogen compounds as simultaneous nitrogen and carbon sources and pattern of amidases in the species of Nocardia*

		<i>N. asteroides</i>	<i>N. rubra</i>	<i>N. farcinica</i>	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>
	Ratio of type* . . .	17/21	6/9	32/38	12/12	9/11	5/5	4/5
Utilization as N and C sources	L-Glutamate	+	+	+	+	+	+	+
	L-Serine	-	-	-	±	+	-	-
	Glucosamine	±	+	+	+	+	-	+
	Acetamide	-	+	+	-	-	-	±
	Benzamide	-	-	-	-	-	-	-
	Monoethanolamine	-	+	+	-	+	-	-
	Trimethylene diamine	-	-	-	-	-	-	-
	Ratio of type* . . .	18/21	8/9	32/38	10/12	11/11	5/5	4/5
Amidase pattern	Acetamidase	-	+	-	-	-	-	-
	Benzamidase	-	-	±	-	-	-	-
	Urease	±	±	+	+	+	-	-
	Isonicotinamidase	-	-	-	-	-	-	-
	Nicotinamidase	-	-	+	-	+	±	-
	Pyrazinamidase	-	±	+	-	+	±	-
	Salicylamidase	-	-	-	-	-	-	-
	Allantoinase	-	-	-	-	±	-	-
	Succinamidase	-	-	-	-	-	-	-
	Malonamidase	-	-	-	-	-	-	-

* (No. of strains showing the pattern indicated)/(no. of strains tested).

When S-values for the 3 strains of group B to various HMOs were determined, only S-values to the HMO of group Kyoto-I were within the permissible low limit (88.3 % or more); S-values to the other HMOs remained outside the limits (Table 8). Thus, strains ATCC 3318, W-3409B and R-784 were identified as a member of the group Kyoto-I.

Nomenclature of species. From the results obtained, 7 groups could be recognized in this study: (1) Kyoto-I = group B of *Nocardia asteroides*; (2) Kyoto-II; (3) group A of *N. asteroides*; (4) *N. brasiliensis*; (5) *N. caviae*; (6) *N. pelletieri*; (7) *N. madurae*. Of these, the nomenclature of the last four is no problem. In the following, nomenclature of the first three is discussed.

Strains of group A of *Nocardia asteroides* and group B of *N. asteroides* (= Kyoto-I) had been received bearing the name *N. asteroides*. These two groups, however, showed distinctly different clusters and were considered to be two different species. According

to the description of Gordon & Mihm (1957, 1959, 1962*a*), the group A seemed to be correctly named *N. asteroides* (Eppinger) Blanchard. This was discussed previously.

Group B (Kyoto-I) has been named *Nocardia farcinica* Trevisan for the following reasons: (a) Gordon & Mihm (1957, 1962*a*) recognized the priority of the name *N. farcinica* over the name *N. asteroides*, but they avoided the name *N. farcinica* because they considered that the name *N. asteroides* had already become established by usage; (b) the group containing strain ATCC 3318 was recognized as a distinct cluster clearly differentiated from other groups of *Nocardia*, including *N. asteroides* group A.

Table 21. *Distinguishing characters for the species of Nocardia*

Character	<i>N. asteroides</i>	<i>N. rubra</i>	<i>N. farcinica</i>	<i>N. brasiliensis</i>	<i>N. caviae</i>	<i>N. pelletieri</i>	<i>N. madurae</i>
Growth at 45°	—	—	+	—	+	+	+
Acetamidase	—	+	+	—	—	—	—
Benzamidase	—	—	+	—	—	—	—
Urease	+	+	+	+	+	—	—
Malate as C source	+	+	+	+	+	—	+
Succinate as C source	+	+	+	+	+	—	+
1,3-Butylene glycol as C source	—	—	+	—	—	—	—
Acid from arabinose	—	—	—	—	—	—	+
Acid from galactose	—	—	—	+	—	—	—
Acid from inositol	—	—	—	+	+	—	—
Acid from mannitol	—	+	—	—	+	—	+
Acid from rhamnose	—	—	+	—	—	—	+
Acid from sorbitol	—	+	—	—	—	—	—
Serine as N and C sources	—	—	—	+	+	—	—
Glucosamine as N and C sources	+	+	+	+	+	—	+
Acetamide as N and C sources	—	+	+	—	—	—	—
Monoethanolamine as N and C sources	—	+	+	—	+	—	—

The group Kyoto-II included 7 strains. Two strains were identified later, and a total of 9 strains of this group is listed in Table 9. Among these, the following 5 had been identified by Gordon & Mihm (1957) as *Mycobacterium rhodochrous*: (1) *N. rubra* M-1; (2) *N. erythropolis* M-8; (3) *N. globerula* M-75; (4) *N. lutea* M-192; (5) *N. polychromogenes* M-6. One strain, *N. minima* M-103, had been identified by them as *N. asteroides*. Accordingly, this group might be identified as *M. rhodochrous*, but it showed several discrepancies from the characters of that species as described by Gordon & Mihm (1957). It is possible that, since the strains used by us had been transferred through two or three investigators and had been maintained in Dr Uesaka's laboratory for 7 years, some strains might be mislabelled or have changed their characters. Considering this possibility, strain ATCC 13808 of *M. rhodochrous* received directly from the American Type Culture Collection was studied and compared with the above strains. ATCC 13808 showed an S-value of 86 % to the HMO of the group Kyoto-II, a value outside the permissible limit for the group. This result made us hesitate to identify this group as *M. rhodochrous*, and we preferred to name it, provisionally, *Nocardia rubra*. This name had appeared first among 8 names for

this group, *N. rubra*, *N. erythropolis*, *N. globerula*, *N. convoluta*, *N. minima*, *N. lutea*, *N. polychromogenes* and *N. rubropertincta* (*Bergey's Manual*, 1957; *Index Bergeyana*, 1966).

Identification of strains not forming a cluster by preliminary sorting

It is supposed that, when S-values to the HMO for individual members of a species show a normal distribution curve, c. 95 % of the members of the species are included to the range $\{(\text{mean}) \pm 2 \times (\text{standard deviation})\}$. The permissible low limit of the S-value to an HMO is set for each species at $\{(\text{mean S-value to the HMO}) - 2 \times (\text{standard deviation})\}$ %. After establishing the permissible low limit for species, identification is made without subjective judgement (automatically) by determining S-values for the unknown strain to various HMOs. If an S-value to a HMO is within the permissible limit and S-values to the other HMOs are outside the permissible limits, the unknown strain is identified as a species of the first HMO. This method was applied to the strains received from Dr Uesaka and not forming a cluster in preliminary sortings, and the results are shown in Table 10. Adding the strains thus identified, the strains identified in the 7 species are shown in Table 9 and Tables 11 to 16, and the frequency of characters in the species summarized in Table 17.

Eleven strains remained unidentified (Table 18); some may be distinct species. S-values between these strains were less than 80 %, except in the following pairs: (1) *Nocardia intracellularis* M-170 and *N. bostroemi* M-159 (88 %); (2) *N. intracellularis* M-170 and *N. restricus* M-9 (84 %); (3) *N. leishmanii* M-82 and *N. alba* M-113 (87 %); (4) *N. madurae* C-637 and *N. coeliaca* M-122 (83 %).

Typical strains of the species. Strains showing a high similarity to the HMO are used as reference strains, and are shown in Table 19.

A simple method of identification

The principles of numerical taxonomy make an ideal basis for a method of identification, and a method of using HMOs has been presented in this paper. Laboratories not specialized for bacterial identification need a simpler method. In the identification of mycobacteria, two methods have been found useful, amidase pattern (Bönicke, 1962) and pattern of nitrogen compounds used as simultaneous nitrogen and carbon sources (Tsukamura, 1967*c*). By a combination of these two methods, identification is successful with most strains, and typical patterns of the species are shown in Table 20.

Schneidau (1963) stated that the amidase pattern was not as uniform in nocardias as in mycobacteria, and in the present study it was found that identification of nocardias was not successful by the use of the amidase pattern alone, but the test was useful in combination with utilization of nitrogen compounds as simultaneous nitrogen and carbon sources. A practical diagnostic table was prepared by a selection of distinguishing characters, with frequencies of more than 91 % in some species but in others at a frequency as low as 10 %. This is shown in Table 21, which included a few characters with a frequency less than 90 %.

Although both identification tables (Tables 20 and 21) include 17 tests, Table 20 could be more easily applied in practice. Table 21 contains more characteristic tests, and would provide a more accurate result.

DISCUSSION

Division of Nocardia asteroides into two species

The most important conclusion reached in this study is that the species *N. asteroides* can be divided into two species, *Nocardia asteroides* (Eppinger) Blanchard and *Nocardia farcinica* Trevisan (both in new senses). Among 112 strains received as *Nocardia* species, 21 strains of *N. asteroides* (in a new sense) and 38 strains of *N. farcinica* (in a new sense) were identified (Tables 11 and 12); the data of comparison between these two species are summarized in Table 22. Eighteen of 21 strains of the former and 28 of 38 strains of the latter had been named as *N. asteroides* when received.

Table 22. Comparison between *Nocardia asteroides* (group A of *N. asteroides*) and *N. farcinica* (group B of *N. asteroides*; group Kyoto-I)

Character	No. of strains showing a positive reaction* (%)		<i>N. asteroides</i> according to the description of Gordon & Mihm (1962)
	<i>N. asteroides</i>	<i>N. farcinica</i>	
No. of strains tested . . .	21	38	142
†Growth at 45°	0 (0)	35 (92)	(42)
†Acetamidase	2 (10)	38 (100)	.
†Benzamidase	0 (0)	27 (71)	.
Pyrazinamidase	4 (19)	33 (87)	.
†Acid from rhamnose	0 (0)	34 (90)	(32)
Rhamnose as C source	0 (0)	34 (90)	.
Ethanol as C source	3 (14)	37 (87)	.
Propylene glycol as C source	1 (5)	30 (79)	.
†1,3-Butylene glycol as C source	0 (0)	36 (95)	.
2,3-Butylene glycol as C source	4 (19)	37 (97)	.
†Acetamide as N and C sources	0 (0)	37 (97)	.
†Monoethanolamine as N and C source	1 (5)	37 (97)	.
Nitrate reduction	8 (38)	0 (0)	(88)
Two week-arylsulphatase	8 (38)	0 (0)	.

* Figures in parenthesis indicate percentage strains showing a positive reaction.

† Distinguishing characters shown in Table 12.

Relationships between species. Numerical classification of the strains received from Dr Gordon differentiated 6 clusters, (1) *Nocardia asteroides*, (2) *N. farcinica*, (3) *N. brasiliensis*, (4) *N. caviae*, (5) *N. pelletieri*, and (6) *N. madurae* (Fig. 1), and these results agreed well with the results obtained by classical methods, except that *N. asteroides* was divided into two species, *N. asteroides* (in a new sense) and *N. farcinica*. Numerical classification of the strains received from Dr Uesaka differentiated another cluster of *N. rubra*. Relationships between these species are shown in Table 3 as an S-value table of HMOs of these species and in Fig. 4 as a dendrogram.

The 100 characters used in this study were mainly composed of nutritional requirement tests and biochemical tests, and included only 9 morphological characters. Since morphological characteristics have been recognized as important in classical taxonomy of the genus *Nocardia*, we would like to add some comments on morphology. All strains received from Dr Gordon as *N. madurae* and *N. pelletieri* showed well-developed mycelium tending to fragmentation (designated permanent mycelium in this study). They showed only scant growth on Ogawa egg medium, possibly due to their sus-

ceptibility to malachite green (Uesaka, 1964), making it impossible to take into account in the S-value characters determined on this medium. These two species were distinct from other species in dendrograms (Fig. 2 and 4) and seemed to belong to a Streptomyces-like nocardia of Schneidau & Shaffer (1957). However, it has not been concluded whether or not these are *Streptomyces*.

Permanent mycelium was observed not only in these species but also, to some extent, in 16 strains of other species. Among these, 5 belonged to an unidentified group (Table 18) received as *N. madurae* C-637, *N. leishmanii* M-82, *N. alba* M-113, *N. asteroides* M-123 and *N. madurae* M-172. One belonged to *N. caviae* (M-180 received as *N. asteroides*), 2 belonged to *N. asteroides* (M-94 and M-161 received as *N. asteroides*), and 8 belonged to *N. farcinica* (M-72, M-158A, M-162, M-167, M-169, M-177, M-179 and M-181 received as *N. asteroides*). The presence of permanent mycelium seemed not to be sufficient to differentiate *N. pelletieri* and *N. madurae* from other species.

When observed by naked eye on glucose agar after incubation at 28° for 4 weeks aerial hyphae were observed at the following rates: *N. asteroides*, 5/21 (24 %); *N. farcinica*, 10/38 (26 %); *N. brasiliensis*, 2/12 (17 %); *N. caviae*, 1/11 (9 %); *N. rubra*, 0/9 (0 %); *N. pelletieri*, 0/5 (0 %); *N. madurae*, 0/5 (0 %); unidentified, 2/11.

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NOTE

After preparation of the manuscript (May 1968), the author was informed that there was a paper of Cerbón (1967) dealing with numerical taxonomy of *Nocardia*.

Recently, Magnusson & Mariat (1968) stated that *N. asteroides* seemed to be a separable species by an immunological method based on the specificity of delayed type skin reactions on guinea pigs. Their results are compatible with our results.

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