Numerical Classification of *Rhodococcus* (Formerly *Gordona*) Organisms Recently Isolated from Sputa of Patients: Description of *Rhodococcus sputi* Tsukamura sp. nov.

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A total of 116 strains of *Rhodococcus* (formerly *Gordona*) recently isolated from sputa of patients with pulmonary disease were submitted to numerical classification together with 61 reference strains belonging to the genera *Rhodococcus*, *Nocardia*, and *Mycobacterium*. The numerical classification was carried out by two methods, one using 109 characters and another using 98 characters. Almost similar results were obtained by these two methods, and the following clusters were distinguished: (i) *Rhodococcus bronchialis* (synonym: *Gordona bronchialis*); (ii) *R. sputi* sp. nov.; (iii) *R. rubropertinctus* (synonym: *G. rubropertincta*); (iv) *R. rhodochrous* (synonym: *G. rhodochroa*); (v) *R. aurantiacus* (synonym: *G. aurantiaca*); (vi) *R. lentifragmentus* (synonym: *G. lentifragmenta*); (vii) *Nocardia rubra* strains received from M. Ridell; (viii) *N. asteroides*; (ix) *N. farcinica*; (x) *Mycobacterium fortuitum*. Clusters i to vii were combined into one large cluster, which was considered as the genus *Rhodococcus* (synonym: *Gordona*). In addition, two (method I) or four (method II) new clusters could be observed among the cluster *Rhodococcus*. Thus, it was considered that the *Rhodococcus* organisms form a genus that contains a number of species. *G. terrae* and *G. rosea* were incorporated into the cluster of *R. rubropertinctus* (*G. rubropertincta*), and were considered to be reduced to varieties of the species *R. rubropertinctus*. The largest one of the new clusters was named *Rhodococcus sputi* sp. nov. Tsukamura. Its definition is presented in this study. A proposal of changing the generic name from *Gordona* to *Rhodococcus* was made.

The so-called "rhodochrous" organisms were described by Gordon and Mihm (8), who differentiated some strains received as nocardiae from others and named *Mycobacterium rhodochrous*. Later Gordon (7) regarded this organism as an intermediate between *Mycobacterium* and *Nocardia*. Tsukamura (14) isolated a number of slightly acid-fast organisms from sputa of patients and soils, and stated that they form a genus, *Gordona*. The genus was considered to occupy an adjacent taxonomic position to the genus *Nocardia*. *M. rhodochrous* ATCC 13808, which previously had been named *R. rhodochrous*, was included in this new genus. Thereafter, other strains received as *M. rhodochrous*, too, were included in this genus (15). The majority of the strains formed a species, *G. rhodochroa*, and a few other strains formed a species, *G. rubropertincta*, together with strains of *G. rubra*, a species previously described. *G. rubra* was renamed *G. rubropertincta* to conform to the rules of nomenclature. At that time, the following species were described: *G. bronchialis* (14); *G. rubropertincta* (synonym *G. rubra*) (15); *G. terrae* (14); *G. rosea* (15); *G.*

rhodochroa (15); *G. aurantiaca* (19); *G. lentifragmenta* (20). Independently of these studies, Goodfellow and his associates (4, 5) reported that *M. rhodochrous* was differentiated from nocardiae and from rapidly growing mycobacteria. The rhodochrous taxon was considered to be distinguished from the genera *Mycobacterium* and *Nocardia* and to form a genus (6).

More recently, a number of strains considered to belong to the genus *Gordona* were isolated from sputa of patients with pulmonary disease. In an attempt to define the genus *Gordona* more accurately, a numerical classification using the recent isolates was carried out in the present study.

An appropriate name for the genus *Gordona* was considered to be *Rhodococcus* Zopf, as the type strain of *R. rhodochrous* Zopf (21) was contained in the genus (16). However, the generic name *Gordona* was tentatively used in the following studies, as the genus *Gordona* was the first name used for bringing this taxon up to its present form. Recently a similar proposal was made by Bousfield and Goodfellow (2), and, therefore, the generic name *Gordona* has been

changed to *Rhodococcus* in the present study.

MATERIALS AND METHODS

A total of 177 strains of *Rhodococcus* (formerly *Gordona*), *Nocardia*, and *Mycobacterium* were studied. Of these, 61 strains shown in Table 1 were used as reference strains, and the remaining 116 strains were isolated recently in this laboratory from sputa of patients with cavitory pulmonary tuberculosis or bronchiectasis. All 116 strains were considered to belong to the taxon *Rhodococcus*, as they showed common characters of the *Rhodococcus* that are shown below.

Numerical classification was carried out by two methods: the first (method I) used a total of 109 characters (Table 2) and the second (method II) used a total of 98 characters. In method II, the following were omitted from the set of characters of method I: acid from galactose, arabinose, xylose, rhamnose, trehalose, inositol, mannitol, and sorbitol; L-methionine as the N source; isonicotinamide as the N source; succinamide as the N source. Eight of these 11 characters were omitted because acid formation from carbohydrates may have a close correlation with the utilization of these carbohydrates as C source.

References to the tests were given in two previous papers (12, 18).

Numerical classification was carried out as follows. The results were recorded as + or -, and the matching coefficient (M-value) was calculated by the following equation: $M(\%) = [n_s / (n_s + n_d)] \times 100\%$, where n_s is the number of characters that give the same results between two strains, and n_d is the number of characters that give different results. The computer used was an IBM-S370 model 135 located in the Nagoya Factory of the Sumitomo Machinery Co., Obu, Aichi, Japan. The program (G90002) is written in English (CÔBOL). The complete sets of data have been deposited with the WFCF World Data Center for Microorganisms, Department of Microbiology, University of Queensland, Brisbane, Australia.

The results of the numerical classification are shown as dendrograms, which were prepared by the single-linkage method (11).

Four characters showed positive matches for all strains: gram-positiveness; growth at 37°C; growth on the TCH medium; growth on salicylate-Ogawa egg medium. Four characters showed negative matches for all strains: growth at 52°C; salicylamidase; succinamidase; utilization of benzamide as simultaneous N and C sources. Accordingly, the number of characters effective for differentiating the test strains were 101 and 90 in methods I and II, respectively.

RESULTS

Method I. The dendrogram (Fig. 1 to 3) showed the following 12 clusters at the 93% level: (A) *R. bronchialis* (formerly *G. bronchialis*); (B) *R. sputi* sp. nov.; (C) *R. rubropertinctus* (formerly *G. rubropertincta*), *R. terrae* (formerly *G. terrae*); and *R. rosea* (formerly *G. rosea*); (D) *R. aurantiacus* (formerly *G. aurantiaca*); (E) a new taxon; (F) *R. rhodochrous* (formerly *G. rhodochroa*); (G) *N. rubra* (Ridell);

(H) *R. lentifragmentus* (formerly *G. lentifragmenta*); (I) *N. asteroides*; (J) a new taxon; (K) *N. farcinica*; (L) *M. fortuitum*. Clusters B, E, and J were composed of recently isolated *Rhodococcus* organisms and did not belong to any known species.

Method II. The dendrogram (Fig. 4 to 6) showed 14 clusters at the 92% level (C and F at the 94% level). Of these, 12 were the same as shown in method I, and the other two clusters (M and N) appeared to be new. Hence, the clusters not encountered previously were B, E, J, M, and N. The number of strains forming the clusters was similar in both methods, except for the cluster B, which was composed of 25 strains in method I and 16 strains in method II. The unnamed clusters were considered to belong to new species. Only cluster B was named *R. sputi* sp. nov. Tsukamura. The range of the taxon *Rhodococcus* was considered to be a large cluster delineated by the 88 or 89% levels in methods I and II, respectively.

Characters useful for distinguishing the clusters are shown in Table 3.

The common characters of the *Rhodococcus* (formerly *Gordona*) organisms are as follows: gram positive; usually slightly acid fast; mycelium not formed; occur as short rods or coccoids; form rough, pinkish, reddish, brownish, or orange-pigmented colonies on egg media; pigmentation of colonies enhanced by light exposure; grow after 3 days on egg media and Sauton agar; grow at 28 and 37°C but do not grow at 45°C; grow on Ogawa egg medium containing 0.2% sodium *p*-aminosalicylate (PAS); grow on Ogawa egg medium containing 0.5 mg of sodium salicylate per ml; grow on Ogawa egg medium containing 0.25 mg of $\text{NH}_4\text{OH} \cdot \text{HCl}$ per ml; grow on Sauton agar containing 0.2% picric acid; grow on Sauton agar containing 0.1% NaNO_2 ; PAS and salicylate not degraded; arylsulfatase negative; utilize L-glutamate but do not utilize benzamide and trimethylene diamine as simultaneous N and C sources; utilize glycerol, glucose, mannose, fructose, sucrose, *n*-propanol, acetate, succinate, malate, and pyruvate as sole C sources in the presence of ammoniacal nitrogen; do not utilize arabinose and xylose as sole C sources and do not form acid from these pentoses; utilize L-serine, L-methionine, and pyrazinamide as sole N sources in the presence of glycerol carbon; β -galactoside negative.

Description of *Rhodococcus sputi* sp. nov. Tsukamura. Characters useful for distinguishing this species from others are as follows. This species without exception does not grow on Sauton agar containing 0.1% NaNO_2 ; does not grow on Sauton agar containing 0.1% sodium salicylate; grows on Ogawa egg medium containing 0.5

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

Cluster	Laboratory no.	Received as:		Source ^b	Habitat
		Name	Strain		
A	3410	<i>Gordona bronchialis</i>	ATCC 25592	1	Human sputum
	3411	<i>G. bronchialis</i>	50004	2	Human sputum
	3413	<i>G. bronchialis</i>	50006	2	Human sputum
	3885	<i>G. bronchialis</i>	50013	2	Human sputum
	3924	<i>G. bronchialis</i>	50017	2	Human sputum
	3926	<i>G. bronchialis</i>	50019	2	Human sputum
C	3602	<i>G. rubropertincta</i>	60002	2	Soil
	3605	<i>G. rubropertincta</i>	ATCC 25593	1	Soil
	3612	<i>G. terrae</i>	ATCC 25594	1	Soil
	5655	<i>Mycobacterium rhodochrous</i> ^c	ATCC 271	1	?
	5658	<i>M. rhodochrous</i> ^c	ATCC 4004	1	?
	5665	<i>M. rhodochrous</i>	ATCC 14343	1	?
	5674	<i>M. rhodochrous</i>	ATCC 19067	1	?
D	3462	<i>G. aurantiaca</i>	ATCC 25938	1	Human sputum
F	5657	<i>M. rhodochrous</i>	ATCC 4001	1	?
	5659	<i>M. rhodochrous</i>	ATCC 4276	1	?
	5660	<i>M. rhodochrous</i>	ATCC 4273	1	?
	5663	<i>M. rhodochrous</i>	ATCC 13808	1	?
	5664	<i>M. rhodochrous</i>	ATCC 14341	1	?
	5666	<i>M. rhodochrous</i>	ATCC 14347	1	?
	5667	<i>M. rhodochrous</i>	ATCC 14348	1	?
	5668	<i>M. rhodochrous</i>	ATCC 14349	1	?
G	7566	<i>Nocardia rubra</i>	N20	3	?
	7567	<i>N. rubra</i>	N21	3	?
	7568	<i>N. rubra</i>	N24	3	?
	7570	<i>N. rubra</i>	N26	3	?
	7572	<i>N. rubra</i>	N50	3	?
H	23002	<i>N. rubra</i> ^d	M-1	4	?
	23021	<i>N. minima</i> ^d	M-103	4	?
	23022	<i>N. coeliaca</i> ^d	M-122	4	?
	23024	<i>N. lutea</i> ^d	M-192	4	?
I	23056	<i>N. asteroides</i>	M-155	4	?
	23057	<i>N. asteroides</i>	M-156	4	?
	23088	<i>N. asteroides</i>	M-206	4	?
	23095	<i>N. asteroides</i>	R 443 (1)	5	?
	23097	<i>N. asteroides</i>	R 553	5	?
	23103	<i>N. asteroides</i>	ATCC 9970 ^e	1	?
K	7547	<i>N. farcinica</i> ^f	VAC 300	6	?
	7548	<i>N. farcinica</i> ^f	VAC 324	6	?
	7549	<i>N. farcinica</i> ^f	VAC 330	6	?
	7550	<i>N. farcinica</i> ^f	VAC 333	6	?
	7551	<i>N. farcinica</i> ^f	VAC 462	6	?
	7815	<i>N. farcinica</i> ^f	3318/AL	7	?
	23018	<i>N. globerula</i>	M-75	4	?
	23102	<i>N. farcinica</i>	ATCC 3318 ^e	1	?
	18001	<i>Mycobacterium fortuitum</i>	ATCC 19709	1	Soil
	18009	<i>M. fortuitum</i>	NCTC 8697	8	?
L	18112	<i>M. fortuitum</i>	ATCC 6841	1	Human
	42001	<i>M. minetti</i>	ATCC 19542	1	?
	5654	<i>M. rhodochrous</i>	ATCC 184	1	?
	5656	<i>M. rhodochrous</i>	ATCC 999	1	?
	5669	<i>M. rhodochrous</i>	ATCC 14350	1	?

TABLE 1—*continued*

Cluster	Laboratory no.	Received as:		Source ^b	Habitat
		Name	Strain		
— ^e	6038	<i>Nocardia</i> sp. ^f		2	Human sputum
	6618	<i>Jensenia canicruria</i>	KCC A-0132	9	?
	7565	<i>N. rubra</i>	N69	3	?
	7571	<i>N. rubra</i>	N27	3	?
	7573	<i>N. rubra</i>	N51	3	?
	7586	<i>N. sp.</i>		2	Human sputum
	7808	<i>R. coprophilus</i>	CUB 887	10	?
	23023	<i>N. rubropertincta</i>	M-191	4	?
	23030	<i>N. polychromogenes</i>	M-6	4	?

^a The other 116 strains not listed in this table were isolated from the sputum of patients by the present author and were considered to belong to *Gordona* sp. These strains appear in dendrograms showing our laboratory number.

^b (1) American Type Culture Collection (ATCC), Rockville, MD 20852; (2) M. Tsukamura, National Chubu Hospital, Obu, Aichi 474, Japan; (3) M. Ridell, Institute of Medical Microbiology, University of Göteborg, Sweden; (4) N. M. McClung, University of South Florida, Tampa, FL 33620 (received through I. Uesaka, Kyoto University, Kyoto, Japan); (5) R. E. Gordon, Institute of Microbiology, Rutgers, State University of New Jersey, New Brunswick, NJ 08903; (6) S. G. Bradley, Department of Microbiology, Virginia Commonwealth University, Richmond, VA 23219; (7) H. A. Lechevalier, Institute of Microbiology, Rutgers, State University of New Jersey, New Brunswick, NJ 08903; (8) National Collection of Type Cultures (NCTC), Central Public Health Laboratory, London NW9 5HT, England (received through K. Takeya, Department of Microbiology, Kyushu University, Fukuoka, Japan); (9) A. Seiya, Kaken Chemical Industries, Tokyo, Japan (the strain is equivalent to ATCC 11048; NCTC 8036); (10) T. Cross, Postgraduate School of Studies in Biological Sciences, University of Bradford, Bradford BD7 1DP, England.

^c These strains were identified as *G. rosea* by Tsukamura (14).

^d These strains were identified by Tsukamura et al. (19) as *G. lentifragmenta*.

^e These two ATCC strains were not received directly from the ATCC but from R. E. Gordon, Institute of Microbiology, Rutgers, State University of New Jersey, New Brunswick, NJ 08903.

^f These strains were identified by the present author as *N. farcinica* according to the definition previously described (12).

^g These strains did not form any cluster.

mg of *p*-nitrobenzoic acid per ml, 25 µg of rifampin per ml, and/or 5 µg of ethambutol per ml; Tween not hydrolyzed after 7 days but hydrolyzed after 14 days; catalase positive; α- and β-esterases negative; acid phosphatase positive; nitrate reduced to nitrite after 24 h; acetamidase, urease, nicotinamidase, pyrazinamidase, and allantoinase are produced, but benzamidase, isonicotinamidase, salicylamidase, succinamidase are not produced; acetamide and monoethanolamine utilized and L-serine, glucosamine hydrochloride, benzamide, and trimethylene diamine not utilized as simultaneous N and C sources; ethanol, *n*-butanol, isobutanol, citrate, and fumarate are utilized, and benzoate, malonate, propylene glycol, 1,3-, 1,4-, and 2,3-butylene glycols not utilized as sole C sources; acid formed from glucose, mannose, trehalose, mannitol, and sorbitol, but acid not formed from galactose, arabinose, xylose, rhamnose, and inositol; acetamide, urea, nicotinamide, succinamide, and nitrate utilized, but benzamide, isonicotinamide, and nitrite not utilized as sole N sources in the presence of glycerol carbon.

The type strain is ATCC 29627 (no. 3884 Tsukamura), which is deposited in the American Type Culture Collection, Rockville, Md.

DISCUSSION

Previously, *G. rubropertincta*, *G. terrae*, and *G. rosea* (now *R. rubropertinctus*, *R. terrae*, and *R. roseus*, respectively) were regarded as different species, although they together were part of a large common cluster (15, 16). In contrast to this, the results of the present study showed that these three taxa can be combined into one species, namely *R. rubropertinctus*, and that *R. terrae* and *R. roseus* should be reduced to varieties of the species *R. rubropertinctus*. This lumping occurred probably because three enzyme tests, β-galactosidase, catalase, and acid phosphatase tests, which were not used in previous studies, were used in the present study and gave the same reactions for these taxa.

The type strain of *Jensenia canicruria* (no. 6618) was included to the *Rhodococcus* cluster at the 88% (method I) or 89% (method II) level.

Strains received as *N. rubra* from M. Ridell belonged to a cluster different from that of *R. lentifragmentus* (formerly *G. lentifragmenta*) which had been named *N. rubra* (13, 20). Ridell's *N. rubra* strains also were considered to belong to a species of the genus *Rhodococcus*. Ridell (10) reported previously that these strains and *M. rhodochrous* strains revealed a close serolog-

TABLE 2. *Characters tested*^a

1. Gram positiveness	56. Glucosamine as N and C sources
2. Acid fastness (complete)	57. Acetamide as N and C sources
3. Acid fastness (partial or slight)	58. Benzamide as N and C sources
4. Permanent mycelium	59. Monoethanolamine as N and C sources
5. Fragmenting mycelium	60. Trimethylene diamine as N and C sources
6. Rod shape	61. Glucose as C source
7. Cord formation	62. <i>n</i> -Propanol as C source
8. Rough colonies	63. <i>n</i> -Butanol as C source
9. Colony pigmentation (in dark)	64. Isobutanol as C source
10. Photochromogenicity	65. Acetate as C source
11. Growth after 3 days	66. Citrate as C source
12. Growth at 28°C	67. Succinate as C source
13. Growth at 37°C	68. Malate as C source
14. Growth at 45°C	69. Pyruvate as C source
15. Growth at 52°C	70. Benzoate as C source
16. Growth on 0.2% <i>p</i> -aminosalicylic acid	71. Malonate as C source
17. <i>p</i> -Aminosalicylic acid degradation	72. Fumarate as C source
18. Growth on NH ₂ OH · HCl (0.125 mg/ml)	73. Fructose as C source
19. Growth on NH ₂ OH · HCl (0.25 mg/ml)	74. Sucrose as C source
20. Growth on NH ₂ OH · HCl (0.5 mg/ml)	75. Ethanol as C source
21. Growth on Sauton agar	76. Propylene glycol as C source
22. Growth on salicylate agar (0.1%)	77. 1,3-Butylene glycol as C source
23. Salicylate degradation	78. 1,4-Butylene glycol as C source
24. 0.1% Picric acid-Sauton agar	79. 2,3-Butylene glycol as C source
25. 0.2% Picric acid-Sauton agar	80. Acid from glucose
26. Three-day arylsulfatase	81. Acid from mannose
27. Two-week arylsulfatase	82. Acid from galactose
28. TCH medium (10 µg/ml)	83. Acid from arabinose
29. Salicylate (0.5 mg/ml)-Ogawa medium	84. Acid from xylose
30. Ethambutol (5 µg/ml)-Ogawa medium	85. Acid from rhamnose
31. 0.1% NaNO ₂ -Sauton agar	86. Acid from trehalose
32. 0.2% NaNO ₂ -Sauton agar	87. Acid from inositol
33. 1% Tween-Sauton agar	88. Acid from mannitol
34. PNB medium (0.5 mg/ml)	89. Acid from sorbitol
35. Rifampin (25 µg/ml)-Ogawa medium	90. Mannose as C source
36. Tween hydrolysis (7 days)	91. Galactose as C source
37. Tween hydrolysis (14 days)	92. Arabinose as C source
38. Catalase (foam height >45mm)	93. Xylose as C source
39. α-Esterase	94. Rhamnose as C source
40. β-Esterase	95. Trehalose as C source
41. β-Galactosidase	96. Inositol as C source
42. Acid phosphatase	97. Mannitol as C source
43. Nitrate reduction (6 h)	98. Sorbitol as C source
44. Nitrate reduction (24 hours)	99. L-Serine as N source
45. Acetamidase	101. L-Methionine as N source
46. Benzamidase	101. Acetamide as N source
47. Urease	102. Benzamide as N source
48. Isonicotinamidase	103. Urea as N source
49. Nicotinamidase	104. Pyrazinamide as N source
50. Pyrazinamidase	105. Nicotinamide as N source
51. Salicylamidase	106. Isonicotinamide as N source
52. Allantoinase	107. Succinamide as N source
53. Succinamidase	108. Nitrate as N source
54. L-Glutamate as N and C sources	109. Nitrite as N source
55. L-Serine as N and C sources	

^a Method I includes all above 109 characters. Method II includes 98 characters, but excludes 82 to 89, 100, 106, and 107.

ical relationship. Bradley (3) also stated that *N. rubra* should be placed in the taxon rhodochrous. Their results agree with the results of the present study. On the other hand, strain 23030 received as *N. polychromogenes* M-6 (N.

M. McClung) was placed outside the *Rhodococcus* cluster.

In the present study as well as in previous studies (15, 16, 20), strains ATCC 14343 and ATCC 19067 were incorporated into the cluster

FIG. 1. Dendrogram (method I). (A) *Rhodococcus bronchialis* (formerly *Gordona bronchialis*); (B) *R. sputi* sp. nov.

of *R. rubropertinctus* and were distinguished from other strains that were received as *M. rhodochrous* but that belonged to another cluster, *R. rhodochrous*. The clusters of these two taxa could be distinguished by method I,

whereby they belonged to the same cluster, although they could be seen as two subclusters by method II. The result of method II is similar to that of Goodfellow et al. (6), who could not distinguish them as separate taxa.

FIG. 2. Dendrogram (method I). (C) *Rhodococcus rubropertinctus* (formerly *Gordona rubropertincta*); (D) *R. aurantiacus* (formerly *G. aurantiaca*).

By method I, three hitherto unnamed clusters could be found, whereas by method II, five new clusters could be found. Except for these differences, two methods of the numerical classification gave similar clusters. Method I contains eight characters of acid formation from carbohydrates which probably have the same significance as the tests for the utilization of the same carbohydrates. Therefore, method II is regarded as more appropriate. However, both methods gave similar results as a whole. This means that the use of a large number of characters as used in the present study can weaken the influence of linked characters. Furthermore, it is noteworthy that method II, despite its fewer characters, has given more clusters. It is suggested that a greater number of characters is not always advantageous, and that inclusion of characters with similar significance renders obscure some clusters.

It should be emphasized that the characters for numerical classification have to be unrelated to each other.

Problem of nomenclature. In 1971, Tsukamura (14) proposed to create a genus *Gordona* for slightly acid-fast organisms isolated from sputa of patients and soils. At that time, he showed that the type strain of *R. rhodochrous* ATCC 13808 was contained in the genus, although it did not belong to any existing species. Soon thereafter, Goodfellow and his associates (4, 5) showed that the rhodochrous complex was differentiated from mycobacteria and nocardia. Tsukamura (15, 16) reported that a number of strains of the rhodochrous complex were included in the genus *Gordona*. He considered *Rhodococcus* to be an appropriate name for this genus (16). However, the name *Gordona* was used successively to avoid confusion in nomen-

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FIG. 3. Dendrogram (method I). (F) *Rhodococcus rhodochrous* (formerly *Gordona rhodochroa*); (G) *Nocardia rubra* (Ridell); (H) *R. lentifragmentus* (formerly *G. lentifragmenta*); (I) *N. asteroides*; (K) *N. farcinica*; (L) *Mycobacterium fortuitum*.

clature. A further study of the relationships among *Mycobacterium*, *Gordona* including rhodochrous organisms, and *Nocardia* showed that *Gordona* is a taxon comparable with the genera

Mycobacterium and *Nocardia* (17). The results of the present study also showed that the taxon is clearly separated from *Nocardia*. Recently, Bousfield and Goodfellow (2) stated that the

FIG. 4. Dendrogram (method II). (A) *Rhodococcus bronchialis* (formerly *Gordona bronchialis*); (B) *R. sputi* sp. nov.

FIG. 5. Dendrogram (method II). (C) *Rhodococcus rubropertinctus* (formerly *Gordona rubropertincta*); (D) *R. aurantiacus* (formerly *G. aurantiaca*); (F) *R. rhodochrous* (formerly *G. rhodochroa*).

rhodochrous complex should be regarded as a genus and its generic name should be *Rhodococcus*. Their proposal agrees with a previous proposal of the present author (16). In consideration of these agreements, it is now the time to change the generic name from *Gordona* to *Rhodococcus*.

The relationship between the genus *Gordona* and the rhodochrous complex is troublesome. Alshamaony et al. (1) reported that the mycolic acids from the *Gordona* strains formed a group intermediate in size between nocardomycolic acids and mycolic acids *sensu stricto*, and they regarded *Gordona* as a different taxon from the

taxa *Mycobacterium*, rhodochrous, and *Nocardia*. Mordarski et al. (9) reported that the values for deoxyribonucleic acid (DNA) relatedness between the taxon *Gordona* and the taxon rhodochrous were comparatively low. However, conclusions of Tsukamura (14, 17) and Alshamaony et al. (1) agree in that they regard *Gordona* as a taxon more closely related to *Nocardia* than to *Mycobacterium*. The findings of Alshamaony et al. (1) and Mordarski et al. (9) show that *Gordona sensu stricta* and the rhodochrous complex have some differences. If one regards, however, these differences as those within a genus, one would be able to regard

FIG. 6. Dendrogram (method II). (G) *Nocardia rubra* (Ridell); (H) *Rhodococcus lentifragmentus* (formerly *Gordona lentifragmenta*); (I) *N. asteroides*; (K) *N. farcinica*; (L) *Mycobacterium fortuitum*.

TABLE 3. Characters useful for differentiating *Gordona* and *Nocardia* species studied in the present study

Character	Strains (%) showing positive reaction												
	A ^a	B	C	D	E	F	G	H	I	J	K	M	N
	33 ^b	16	10	11	3	8	5	4	6	3	9	4	5
Partial or slight acid fast-ness	76	88	90	73	0	13	0	100	100	0	55	0	100
Fragmenting mycelium	3	0	0	0	0	13	80	100	100	0	89	0	0
Growth at 45°C	0	0	0	0	0	0	40	0	0	0	100	0	0
Growth on salicylate agar	21	6	20	91	100	75	0	0	0	67	0	100	40
Ethambutol-Ogawa medium	100	94	100	100	100	100	100	0	100	100	100	100	100
0.1% NaNO ₂ -Sauton agar	48	6	100	91	100	100	100	100	83	67	33	0	0
PNB medium	79	88	100	91	100	100	0	100	100	100	100	100	100
Rifampin-Ogawa medium	58	100	100	100	100	100	100	0	100	100	100	100	100
Tween hydrolyzed after 14 days	3	69	0	91	100	0	80	0	33	0	55	75	80
Catalase (semiquantitative)	97	100	100	91	33	100	80	50	50	0	44	100	80
α-Esterase	0	38	30	0	0	0	0	0	83	0	0	50	100
β-Esterase	97	13	90	45	0	100	20	0	83	0	67	0	60
β-Galactose	3	0	0	91	0	0	0	0	100	0	100	0	0
Acid phosphatase	94	88	0	9	100	0	0	0	17	100	22	25	100
Nitrate reduction (24 h)	91	44	100	9	0	100	100	50	0	33	0	0	80
Acetamidase	100	69	20	91	100	25	100	100	50	100	100	25	20
Benzamidase	0	0	0	0	0	0	0	0	0	0	89	0	0
Urease	97	100	100	100	100	38	20	0	83	100	100	100	40
Nicotinamidase	94	100	40	82	100	0	100	75	50	100	100	100	0
Pyrazinamidase	97	100	40	82	100	0	100	75	50	100	100	100	0
Allantoinase	6	69	80	100	0	0	20	0	17	67	22	25	40
L-Serine as N and C sources	12	13	0	91	0	0	0	25	0	0	0	0	0
Glucosamine as N and C sources	18	13	90	91	0	100	20	75	0	0	100	50	0
Acetamide as N and C sources	97	88	20	100	100	0	100	100	0	0	100	0	40
Monoethanolamine as N and C sources	6	88	80	100	0	0	80	100	0	0	100	0	60
n-Butanol as C source	33	69	100	91	100	100	100	100	100	0	78	0	20
Isobutanol as C source	36	69	100	91	100	100	100	100	100	0	78	0	0
Citrate as C source	52	81	80	91	100	50	100	100	17	100	22	100	80
Benzoate as C source	0	0	60	18	0	63	0	75	0	0	11	0	0
Fumarate as C source	100	88	100	100	67	100	80	100	100	0	100	100	60
Sucrose as C source	100	100	100	100	100	100	80	100	0	67	44	100	100
Ethanol as C source	58	94	100	91	100	100	100	100	33	0	89	0	80
Propylene glycol as C source	48	6	60	82	0	100	100	100	17	0	67	0	0
Acid from glucose	100	100	100	100	100	100	100	100	67	0	100	100	100
Acid from mannose	97	100	100	100	100	88	100	0	0	0	67	100	100
Mannose as C source	100	100	100	100	100	100	100	100	0	0	89	100	100
Galactose as C source	3	0	0	91	0	0	0	0	0	0	0	0	0
Rhamnose as C source	0	6	30	0	0	0	0	0	0	0	67	0	0
Trehalose as C source	100	100	100	100	100	0	0	0	0	0	44	100	100
Inositol as C source	100	0	0	91	0	0	0	0	0	0	22	0	0
Mannitol as C source	42	100	100	100	0	0	100	100	0	33	0	100	100
Sorbitol as C source	0	100	100	100	0	0	100	100	0	0	0	100	100
Acetamide as N source	100	100	100	100	100	100	100	100	100	0	100	0	100
Benzamide as N source	0	0	0	0	0	0	40	0	83	0	33	0	0
Urea as N source	88	56	100	100	100	100	100	100	100	0	100	0	0
Nicotinamide as N source	88	88	100	100	100	100	100	100	100	0	100	0	80
Nitrate as N source	70	100	100	100	100	100	80	100	100	0	100	0	80

^a Cluster. (A) *Rhodococcus bronchialis* (*Gordona bronchialis*); (B) *R. sputi*; (C) *R. rubropertinctus* (*G. rubropertincta*); (D) *R. aurantiacus* (*G. aurantiaca*); (E) *R. rhodochrous* (*G. rhodochroa*); (G) *Nocardia rubra* (Ridell); (H) *R. lentifragmentus* (*G. lentifragmenta*); (I) *N. asteroides*; (K) *N. farcinica*. E, J, M, and N are unnamed *Rhodococcus* clusters. The clusters shown in this table are those obtained by method II (98 characters).

^b Number of strains.

Gordona sensu stricta and the rhodochrous complex as belonging to the same genus, *Rhodococcus*, based on the phenetic data presented in our previous studies (15–17) and in the present study. In this case, nomenclature of the *Rhodococcus* species will be as follows: *R. rhodochrous* Zopf 1891 (21) (synonym: *G. rhodochroa* Tsukamura 1973 [15]); *R. bronchialis* Tsukamura (synonym: *G. bronchialis* Tsukamura 1971 [14]); *R. rubropertinctus* (Hefferan) comb. nov. Tsukamura (synonym: *G. rubropertincta* [Hefferan] Tsukamura 1973 [15]; *G. rubra* Tsukamura 1971 [14]); *R. aurantiacus* Tsukamura and Mizuno (synonym: *G. aurantiaca* Tsukamura and Mizuno 1971 [19]); *R. lentifragmentus* Tsukamura, Mizuno, and Murata (synonym: *G. lentifragmenta* Tsukamura, Mizuno, and Murata 1975 [20]); *R. sputi* Tsukamura sp. nov.

In conclusion, the *Rhodococcus* cluster consisting of *Gordona sensu stricta* and the rhodochrous complex could be distinguished from the clusters of *N. asteroides*, *N. farcinica*, and *M. fortuitum*. It contained more than several clusters. This finding supports the opinion that the *Rhodococcus* (synonym: *Gordona*) taxon is a genus containing a number of species and is distinguished from *Nocardia* and *Mycobacterium*.

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ADDENDUM

After preparation of this manuscript, two papers of Goodfellow and Alderson (J. Gen. Microbiol. 100:99–122, 1977) and Rowbotham and Cross (J. Gen. Microbiol. 100:123–138, 1977) appeared. These authors also concluded that both the rhodochrous taxon and *Gordona* taxon should be combined into one genus, *Rhodococcus*. Their proposals agree with the opinion expressed in the present paper.

REPRINT REQUESTS

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