Proposal of a New Genus, *Gordona*, for Slightly Acid-fast Organisms Occurring in Sputa of Patients With Pulmonary Disease and in Soil

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SUMMARY

A new genus *Gordona* has been proposed for slightly acid-fast organisms which occur in sputa of patients with pulmonary disease and in soil. This genus is considered to contain 'Mycobacterium' rhodochrous-like organisms. The genera *Mycobacterium* and *Gordona* are characterized by absence of mycelium and slight or strong acid-fastness. The genus *Gordona* shows characters intermediate between the genera *Mycobacterium* and *Nocardia*. It is distinguished from rapid-growing mycobacteria by its slight acid-fastness (weaker than *Mycobacterium*), absence of arylsulphatase activity at 2 weeks, ability to utilize sucrose as a sole carbon source and inability to utilize trimethylene diamine as a simultaneous nitrogen and carbon source. The genus is distinguished from nocardias by the absence of mycelium, ability to form acid from mannose, positive nitrate reduction and ability to utilize sucrose as a sole carbon source. It can be isolated from sputa of patients with lung cavities or bronchiectasis and from soil by prior treatment with alkali. The organisms are Gram-positive or variable; slightly acid-fast, that is, stained light pink or light violet by the Ziehl-Neelsen method; mycelium not formed; spores not formed; non-motile; aerobic; catalase-positive; oxidase-negative; acid formed from glucose by oxidation; growth occurs at 28° and 37° but not at 45°; growth at 2 to 3 days forming rough, reddish or pinkish colonies in air; occur as short rods; non-pathogenic for mice, rabbits, guinea pigs and chickens. Type species is *Gordona bronchialis*.

INTRODUCTION

During the course of studies on 'atypical' mycobacteria from clinical specimens, a number of organisms forming rough pinkish or reddish colonies on egg media as isolated from sputa of patients with pulmonary disease. Similar organisms were isolated from soils. These organisms showed intermediate characters between the genus *Mycobacterium* and the genus *Nocardia* but were distinguished from these genera by several common characters. The organisms most resembled 'Mycobacterium' *rhodochrous*, an organism whose taxonomy is not yet clarified (Gordon & Mihm, 1957, 1959; Gordon, 1966). A new genus *Gordona* is proposed for these organisms.

METHODS

Isolation. Sputum specimens from patients with pulmonary disease (cavitary pulmonary tuberculosis and/or bronchiectasis) were added to an equal volume of 4 % (w/v) NaOH solution and liquefied by incubation at 37° for 30 min. or by shaking vigorously at room temperature for 15 to 30 min. The sputum specimen was then inoculated on Ogawa egg

medium (Löwenstein-Jensen medium may be used) with a pipette or a spiral loop, and incubated at 37° for 4 to 8 weeks. The organisms growing on the medium were subcultured on Ogawa egg medium slopes containing 0.5 mg. sodium salicylate/ml. which excluded the growth of tubercle bacilli. After incubation at 37° for 3 weeks, strains growing on the salicylate medium were stained by the Ziehl-Neelsen method, the majority belonged to the 'atypical' mycobacteria group (mycobacteria other than tubercle bacilli) (Tsukamura, 1962). Some organisms showed only slight acid-fastness and occurred as short rods. These organisms formed rough reddish or pinkish colonies on egg medium when incubated in tubes with cotton-wool plugs.

Similar organisms were isolated from soils. Five g. of a soil sample were suspended in 25 ml. of distilled water and shaken vigorously in a 300 ml. Erlenmeyer flask at room temperature in a reciprocating shaker (stroke: 10 cm.; 60 cycles/min.) for 30 min. The suspension was allowed to stand for 10 min. and 15 ml. of the resulting supernatant was added to an equal volume of 8% (w/v) NaOH solution, shaken for 10 min. and then centrifuged at 500 *g* for 15 min. The residue was suspended in 10 ml. of 1% (w/v) NaH₂PO₄ solution and inoculated on Ogawa egg medium slopes with a spiral loop delivering 0.02 ml. The medium was incubated at 37° for 2 to 4 weeks and organisms growing as rough reddish colonies were selected and purified by single colony-isolation techniques.

Strains. Six strains were received as '*Mycobacterium*' *rhodochrous*. Among these, one strain (ATCC 13808) was received directly from the American Type Culture Collection, Rockville, Maryland, U.S.A., and five strains from Dr M. Goodfellow, Department of Microbiology, Medical School, University of Newcastle upon Tyne. The history of the strains are as follows: (1) tsu '*M.*' *rhodochrous* (without information of history); (2) N 30 '*M.*' *rhodochrous*; R. E. Gordon, A 12974; ATCC 12974; W. H. Burkholder, CF 17; P. E. Tilford; (3) N 31 '*M.*' *rhodochrous*; R. E. Gordon, W 21; S. A. Waksman; R. S. Breed; (4) N 55 '*M.*' *rhodochrous*; R. E. Gordon, 817; L. S. McClung, B 161 A; (5) N 56 '*M.*' *rhodochrous*; R. E. Gordon, 1256; A. I. Laskin, 2914; from soil. A strain of *Mycococcus luteus*, BKM B-963, was received from Dr M. Fateeva, All Union Collection of Micro-organisms, Moscow, U.S.S.R.

Tests. A total of 100 characters was recorded (see Table 4) by means of methods described previously (Tsukamura, 1966, 1967). Four additional tests, found to be useful for differentiation of mycobacteria (Tsukamura, 1969), were included in this study. They were not included in the tests used to calculate S-values, as they had not been used in the previous studies. These tests were: (1) growth on Sauton agar containing 0.1% (w/v) NaNO₂; (2) growth on Sauton agar containing 0.2% (w/v) NaNO₂; (3) growth on Sauton agar containing 1% (w/v) Tween 80; (4) growth on the Ogawa egg medium to which 5 µg. ethambutol/ml. was added.

The Sauton agar contained glycerol, 30 ml.; sodium glutamate, 4.0 g.; KH₂PO₄, 0.5 g.; MgSO₄·7H₂O, 0.5 g.; sodium citrate, 2.0 g.; ferric ammonium citrate, 0.05 g.; purified agar, 30.0 g.; distilled water, 970 ml. The medium (pH 7.0) was poured in 8 ml. quantities into tubes (170×17 mm.) and sterilized by autoclaving at 115° for 30 min., sloped and cooled. Slopes were surface inoculated with one loopful of the stock cultures and incubated at 37° unless especially noted. Results were read after incubation for 2 weeks.

The Ziehl-Neelsen stain was carried out according to the method used by Gordon & Smith (1953). In the present study, the term 'strong or complete' acid-fastness is used for organisms stained strongly red, and the term 'slight or weak' acid-fastness for organisms stained uniformly pinkish or feebly violet. Slight acid-fastness differs from 'partial' acid-fastness as observed in nocardias. Partial acid-fastness indicates a state in which the

majority of organisms are stained blue (non-acid-fast) and the minority stained red (acid-fast).

The diagnostic tests described by Cowan & Steel (1965) were also used to distinguish between the genera.

Numerical analysis. The 'hypothetical mean organism' (HMO), a modification of the 'hypothetical median organism' of Liston, Wiebe & Colwell (1963), is the term used to express a mean characterization of a species. HMOs were prepared by the method described previously (Tsukamura & Mizuno, 1968; Tsukamura, 1969). The characters of a species were expressed by symbols (+, -) and the S-value between two species 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 HMO's showed similar code symbols (+ + or - -) and n_d is the number of characters in which two HMO's showed different code symbols (+ -) (Tsukamura, 1966, 1969).

The characters used in the present study were identical to those used originally in a numerical taxonomic study of rapid- and slow-growing mycobacteria. In the present study, where rapid-growing mycobacteria and *Nocardia* strains were compared, 30 out of 100 proved to be ineffective, six showing negative matches and 24 showing positive matches.

Taxonomic distance was calculated according to the following equation (Lysenko & Sneath, 1959):

$$\text{Taxonomic distance } (d) = (100 - x)/x,$$

where x is a mean S-value (%) between the genera, and the dimension of a genus was expressed as the radius of a circle, $r = (100 - y)/y$, where y is a mean S-value (%) within a genus.

Pathogenicity tests. Mice were inoculated intravenously by a challenge dose of 2 mg. moist weight, and guinea pigs, rabbits and chickens were inoculated intravenously with a challenge dose of 2 mg. moist weight or intramuscularly with a challenge dose of 10 mg. moist weight. Viable organisms were counted in the lungs, spleen, liver and kidneys three weeks after inoculation. This was done by taking a piece of the organ, adding an equal weight of 2 % (w/v) NaOH solution, homogenizing and then inoculating from the suspension to Ogawa egg medium. The pathogenicity test was not included among the characters used in the calculation of S-values.

RESULTS AND DISCUSSION

General characters

All the 101 isolates, 71 from sputa and 30 from soils, showed a similar morphology, typically short rods (and sometimes as coccoids), 1 $\mu\text{m.}$ by 1.5 to 2.0 $\mu\text{m.}$ Most strains were Gram-positive, though some of those isolated from sputa were Gram-variable or only weakly positive. The organisms showed slight acid-fastness staining uniformly light pink or light violet by the Ziehl-Neelsen method. The organisms were resistant to acid and alkali, being more resistant than nocardias and as resistant as mycobacteria (Table 1).

Growth occurred after 2 to 3 days on egg media, Sauton agar and nutrient agar. The colonies growing on the first two media were pinkish or reddish (rarely orange) when incubated aerobically. The colonies remained light brownish or creamy if the air was limited (with paraffined cotton stopper or gum cap); grew at 28° and 37° but not at 45°;

no mycelium or branching observed; no spores were formed; grew only under aerobic conditions. The organisms were catalase-positive, oxidase-negative and formed acid from glucose by oxidation.

Table 1. *Comparison of tolerance to sodium hydroxide and sulphuric acid among Mycobacterium, Gordona and Nocardia*

The organisms growing on Ogawa egg medium were harvested after incubation at 37° for 7 days, homogenized by shaking with glass beads, suspended in distilled water (control), 1 % NaOH solution or 1 % H₂SO₄ solution to obtain a suspension of 2 to 5 mg. (moist weight)/ml., and allowed to stand at room temperature for 10 min. The suspension was then immediately diluted with saline, and a series of tenfold dilutions was prepared. Samples of each dilution (0.02 ml.) were inoculated onto Ogawa egg medium with a spiral loop and incubated at 37° for 5 days (alkali or acid are neutralized in medium).

Strain	Number of surviving organisms (%) after contact with:		
	Water	1 % NaOH	1 % H ₂ SO ₄
<i>M. phlei</i> SN 103	100.0	0.0219	9.52
<i>M. phlei</i> SN 106	100.0	0.0860	17.0
<i>M. smegmatis</i> SN 2	100.0	0.517	0.126
<i>M. fortuitum</i> ATCC 19709	100.0	33.2	36.0
<i>M. fortuitum</i> 334	100.0	16.6	102.4
<i>G. bronchialis</i> NCTC 10667	100.0	54.2	0.0189
<i>G. bronchialis</i> TAKESHITA	100.0	46.3	0.0810
<i>Gordona</i> sp. NORIMATSU	100.0	0.175	2.10
<i>G. rubra</i> NCTC 10668	100.0	0.214	0.536
<i>N. asteroides</i> ATCC 23824	100.0	< 0.0089	< 0.0089
<i>N. farcinica</i> ATCC 23825	100.0	< 0.0093	< 0.0093
<i>N. caviae</i> R-1315	100.0	0.0462	0.0923

Taxonomic consideration

In view of the above characters it was considered that the organisms occupy a taxonomic position near the genera *Mycobacterium* and *Nocardia* (Cowan & Steel, 1965). Thus a comparison was made between these organisms and representative strains of the taxa *Mycobacterium* (rapid-growing mycobacteria), '*Mycobacterium*' *rhodochrous* and *Nocardia*. The comparison was carried out using eight characters previously found to be useful in differentiating between rapid-growing mycobacteria and nocardias (Tsukamura, 1970), the genus *Mycobacterium* having been divided into two subgenera, which approximately correspond to slow-growing and rapid-growing mycobacteria (Tsukamura, 1967, 1970).

The organisms were differentiated from *Nocardia* by five of the eight characters (Table 2). They showed slight acid-fastness, lacked mycelium, formed acid from mannose, utilized sucrose as a sole carbon source, and reduced nitrate to nitrite. Alternatively, the organisms differed from *Mycobacterium* in four characters; they were not strongly acid-fast and showed no arylsulphatase activity at 2 weeks, they utilized sucrose as a sole carbon source, but were unable to grow on trimethylene diamine as a simultaneous carbon and nitrogen source (Table 2).

The organisms most resembled '*Mycobacterium*' *rhodochrous*, the taxonomic position of which is not yet established. They could be differentiated from the '*M.*' *rhodochrous* strains by only one of the eight distinguishing characters (Table 2). The organisms formed acid from mannose, showing growth in a medium containing mannose as the sole source of carbon, while the '*M.*' *rhodochrous* strains did not form acid from mannose, although growth occurred in the presence of mannose.

Gordon & Mihm (1957, 1962) stated that most strains of *Nocardia asteroides*, *N. brasili-*

iensis and *N. caviae* produced acid from mannose but Tsukamura (1969, 1970) reported that nocardias did not form acid from mannose. This disagreement is probably due to Gordon & Mihm observing acid production after 28 days at 28° (Gordon & Smith, 1953), whereas Tsukamura recorded acid production after incubation at 28° for 14 days (Tsukamura, 1967). In addition, the media used are also different.

Table 2. *Comparison between strains of Nocardia, Gordona, 'Mycobacterium' rhodochrous and Mycobacterium (rapid-growing mycobacteria)*

Symbols (+ or -) are expressed by a feature shown by more than 51 % of species, the characters of a species being expressed as a HMO. Only the characters of '*Mycobacterium*' *rhodochrous* are expressed by the HMO itself.

Character*	<i>Nocardia</i>	<i>Gordona</i>	' <i>Mycobacterium</i> ' <i>rhodochrous</i>	<i>Mycobacterium</i>
(1) Strong acid-fastness	—	—	—	+
(2) Slight acid-fastness	—	+	+	+
(3) Fragmenting mycelium	+	—	—	—
(4) Nitrate reduction	—	+	+	+
(5) Arylsulphatase (2 weeks)	—	—	—	+
(6) Acid from mannose	—	+	—	+
(7) Sucrose as C source	—	+	+	—
(8) Trimethylene diamine as simultaneous N and C sources	—	—	—	+

* Except for the utilization of sucrose as sole C source and the slight acid-fastness, the characters shown here are the distinguishing characters previously described for *Mycobacterium* and *Nocardia* (Tsukamura, 1970).

The organisms were differentiated from *Mycococcus luteus*, BKM-B-963, by many characters: (1) the organisms were slightly acid-fast, while *Mycococcus* is not acid-fast; (2) the organisms occurred as rods, while *Mycococcus* occurs as coccoids; (3) the organisms reduced nitrate to nitrite, while *Mycococcus* does not; (4) the organisms grew well on egg media containing malachite green (for example, Ogawa egg medium), while *Mycococcus* does not grow on such media; (5) the organisms showed rough, reddish or pinkish colonies on Sauton agar, while *Mycococcus* shows smooth, yellowish colonies; (6) the organisms utilized glutamate as a simultaneous nitrogen and carbon source, while *Mycococcus* does not utilize it; (7) the organisms utilized serine, methionine, acetamide, urea, pyrazinamide, etc. as sole nitrogen sources, while *Mycococcus* does not utilize them.

The strain BKM-B-963 of *Mycococcus luteus* seems to be the only strain of the genus *Mycococcus* obtainable at present (personal communication of Dr M. Fateeva, All Union Collection of Micro-organisms, Moscow, U.S.S.R.). Two strains of the genus *Mycococcus* in the American Type Culture Collection are not *Mycococcus* but seem to belong either to the genus *Corynebacterium* or to *Arthrobacter* (personal communication from Dr M. Isono, Fermentation Research Institute, Osaka, Japan, who deposited these strains). According to *Bergey's Manual* (1957) the genus *Mycococcus* generally consists of coccoids variable in size and shape and is not acid-fast. Our organisms are rods and slightly acid-fast.

It would appear from these results that the organisms cannot be placed in any of the established genera. It was therefore considered appropriate to create a new genus for these organisms.

The generic name *Gordona* has been chosen for these organisms, taking the latinized name of Dr Ruth E. Gordon, who studied extensively '*Mycobacterium*' *rhodochrous* (included later as a member of *Gordona*).

The nomenclature of 'Mycobacterium' rhodochrous

An organism named *Micrococcus rhodochrous* (Zopf) Migula (1900) was moved by Gordon & Mihm (1957, 1959) into the genus *Mycobacterium* as *Mycobacterium rhodochrous*. The taxonomic position of this taxon was later questioned by Gordon herself. She stated that this organism showed characters intermediate between *Mycobacterium* and *Nocardia* but differed from *Corynebacterium*. The confused taxonomic position of this organism is expressed by the title of her paper: 'Some strains in search of a genus—*Corynebacterium*, *Mycobacterium*, *Nocardia* or what?' (Gordon, 1966). Following this paper, the name '*Mycobacterium*' *rhodochrous* has been widely used. The first problem is to decide whether or not our isolates belong to the same genus as '*Mycobacterium*' *rhodochrous*. A comparison was therefore carried out using the organisms isolated by us and several '*Mycobacterium*' *rhodochrous* strains. The results are shown in Table 3.

Table 3. Comparison between *Gordona* strains and '*Mycobacterium*' *rhodochrous* strains

Character*	Gordona strains†	'Mycobacterium' <i>rhodochrous</i> ‡					
		1	2	3	4	5	6
Strong acid-fastness	—	—	—	—	—	—	—
Weak acid-fastness	+	+	—	+	+	+	+
Mycelium	—	—	—	—	—	—	—
Nitrate reduction	+	+	+	—	+	+	—
Arylsulphatase (2 weeks)	—	—	—	—	—	—	—
Acid from mannose	+	—§	+	+	—§	—§	—
Sucrose, sole carbon source	+	+	+	+	—	+	+
Trimethylene diamine, nitrogen and carbon sources	—	—	—	—	—	—	—
Acid from glucose	+	+	+	+	+	+	+
Tolerance to 0.2 % picric acid	+	+	+	+	+	+	+
Utilization as sole carbon source:							
acetate	+	+	+	+	+	+	+
succinate	+	+	+	+	+	+	+
malate	+	+	+	+	+	+	+
pyruvate	+	+	+	+	+	+	+
fumarate	+	+	+	+	+	+	+

* Characters shown in upper part are distinguishing ones for *Mycobacterium* (rapid-growing mycobacteria) and *Nocardia*. Characters shown in lower part are distinguishing ones for slow-growing and rapid-growing mycobacteria.

† All of the 101 strains of *Gordona* tested showed similar results.

‡ 1, ATCC 13808; 2, tsu; 3, N 30; 4, N 31; 5, N 55; 6, N 56. Strains, tsu and N 30 were tested at 28° as these two could not grow at 37°.

§ Acid not formed from mannose but growth occurs in presence of mannose.

All of the 101 isolates showed the same distinguishing characters. On the other hand, '*Mycobacterium*' *rhodochrous* strains differed by only one or two characters from the organisms isolated from sputum and soil. The '*Mycobacterium*' *rhodochrous* strains were also found to be similar to our isolates by means of the characters shown in the diagnostic table (p. 55) of Cowan & Steel (1965). These results suggested that our isolates and the '*M.*' *rhodochrous* strains belonged to the same genus.

Table 4. *Frequency of positive characters in each species of Gordona*

Character	<i>G. bronchialis</i>	<i>G. rubra</i>	<i>G. terrae</i>
No. of strains tested ...	41	10	20
1. Gram reaction	41	10	20
2. Strong acid-fastness	0	0	0
3. Slight acid-fastness	41	10	20
4. Permanent mycelium	0	0	0
5. Temporary or fragmenting mycelium	0	0	0
6. Rod shape	41	10	20
7. Compact grouping (like cord)	34	3	4
8. Rough colonial morphology	41	10*	20*
9. Colony pigmentation	41	10	20
10. Photochromogenicity	0	0	0
11. Rapid growth	41	10	20
12. Catalase	41	10	20
13. Nitrate reduction	40	8	19
14. Arylsulphatase (3 days)	0	0	0
15. Arylsulphatase (2 weeks)	0	0	0
16. Salicylate degradation	0	0	0
17. PAS degradation	0	0	0
18. Growth on 0.2 % sodium <i>p</i> -aminosalicylate	41	10	20
19. Growth on 62.5 µg. NH ₂ OH.HCl/ml.	41	10	20
20. Growth on 125 µg. NH ₂ OH.HCl/ml.	38	10	20
21. Growth on 250 µg. NH ₂ OH.HCl/ml.	5	9	16
22. Growth on 500 µg. NH ₂ OH.HCl/ml.	0	0	0
23. Tolerance to 0.1 % picric acid	41	10	20
24. Tolerance to 0.2 % picric acid	41	10	20
25. Growth at 28°	41	10	20
26. Growth at 37°	41	10	20
27. Growth at 45°	0	0	0
28. Growth at 52°	0	0	0
29. Acetamidase	40	0	0
30. Benzamidase	0	0	0
31. Urease	41	10	20
32. Isonicotinamidase	0	0	0
33. Nicotinamidase	35	0	16
34. Pyrazinamidase	34	0	16
35. Salicylamidase	0	0	0
36. Allantoinase	0	10	19
37. Succinamidase	0	0	0
38. Malonamidase	0	0	0
39. Acetate (as C source)	41	10	20
40. Citrate (as C source)	12	10	13
41. Succinate (as C source)	41	9	20
42. Malate (as C source)	41	9	20
43. Pyruvate (as C source)	41	10	20
44. Benzoate (as C source)	13	10	1
45. Malonate (as C source)	0	0	0
46. Fumarate (as C source)	41	9	20
47. Acid from glucose	41	10	20
48. Acid from mannose	41	10	20
49. Acid from galactose	0	0	0
50. Acid from arabinose	0	0	0
51. Acid from xylose	0	0	0
52. Acid from rhamnose	0	0	20
53. Acid from trehalose	40	7	20
54. Acid from raffinose	0	0	0
55. Acid from inositol	40	0	0
56. Acid from mannitol	10	10	20
57. Acid from sorbitol	0	10	20

* Intermediate between S- and R-types.

Table 4 (*cont.*)

Character	<i>G. bronchialis</i>	<i>G. rubra</i>	<i>G. terrae</i>
No. of strains tested ...	41	10	20
58. Glycerol (as C source)	41	10	20
59. Glucose (as C source)	41	10	20
60. Mannose (as C source)	41	10	20
61. Galactose (as C source)	0	0	0
62. Arabinose (as C source)	0	0	0
63. Xylose (as C source)	0	0	0
64. Rhamnose (as C source)	0	0	20
65. Trehalose (as C source)	41	10	20
66. Raffinose (as C source)	0	0	0
67. Inositol (as C source)	40	0	0
68. Mannitol (as C source)	12	10	20
69. Sorbitol (as C source)	5	10	20
70. Fructose (as C source)	41	10	20
71. Sucrose (as C source)	41	10	20
72. Ethanol (as C source)	41	10	20
73. Propanol (as C source)	41	10	20
74. Propylene glycol (as C source)	40	0	0
75. 1,3-Butylene glycol (as C source)	8	0	0
76. 1,4-Butylene glycol (as C source)	6	8	0
77. 2,3-Butylene glycol (as C source)	11	10	1
78. L-Glutamate (as N-C sources)	41	10	20
79. L-Serine (as N-C sources)	3	0	0
80. Glucosamine HCl (as N-C sources)	18	10	18
81. Acetamide (as N-C sources)	40	0	0
82. Benzamide (as N-C sources)	0	0	0
83. Monoethanolamine (as N-C sources)	0	10	19
84. Trimethylene diamine (as N-C sources)	0	0	0
85. L-Glutamate (as N source)	41	10	20
86. L-Serine (as N source)	40	10	20
87. L-Methionine (as N source)	39	7	19
88. Acetamide (as N source)	41	6	19
89. Benzamide (as N source)	1	5	18
90. Urea (as N source)	41	0	20
91. Pyrazinamide (as N source)	41	6	20
92. Isonicotinamide (as N source)	30	7	20
93. Nicotinamide (as N source)	38	3	20
94. Succinamide (as N source)	31	10	20
95. Nitrate (as N source)	25	10	20
96. Nitrite (as N source)	0	0	0
97. Nicotinic acid production	0	0	0
98. Growth on TCH medium (10 µg./ml.)	41	10	20
99. Growth on salicylate medium (0.05 %)	40	10	20
100. Growth on salicylate medium (0.1 %)	38	10	18

Species of the genus Gordona

The strains belonging to the genus *Gordona* were examined for the 100 characters shown in Table 4. Of the 101 strains isolated from sputum and soil, 71 were classified into three species. The 41 strains from sputum formed one species, which was called *Gordona bronchialis*. Thirty strains isolated from the soil constituted the remaining two species, *Gordona rubra* and *G. terrae*.

Among the remaining 30 strains, which were isolated from sputum and did not belong to *Gordona bronchialis*, 14 seemed to be a variety of the species *G. bronchialis*, but this was not certain. Four strains seemed to form a possible fourth species, but this is not presented in the present paper. The other strains seemed to belong to miscellaneous species

but specific names were not proposed, as such species would have included only one or two strains. Strains received as '*Mycobacterium*' *rhodochrous* seemed to belong to these miscellaneous species.

The frequency of occurrence of positive characters in the three named species are shown in Table 4. The characters which are not shown in Table 4 are as follows: (1) tolerant to 5 µg. ethambutol/ml.; (2) tolerant to 0.1 % NaNO₂; (3) tolerant to 0.2 % NaNO₂; (4) tolerant to 1 % Tween 80. The type strains and two other strains of each species were tested for their pathogenicity in mice, guinea pigs, rabbits and chickens. All of the strains tested failed to produce any visible lesions in lungs, spleen, liver and kidneys, and no viable organism was found in the organs.

Table 5. *Mean S-values and their standard deviations to the hypothetical mean organism (HMO) of itself and to the HMO of other species*

The value in table shows [(mean S-value) ± (standard deviation)], and the number in parentheses the number of test strains.

Species	Mean S-value (%) to the HMO of		
	<i>G. bronchialis</i>	<i>G. rubra</i>	<i>G. terrae</i>
<i>G. bronchialis</i>	94.76 ± 2.52* (n = 41)	79.59 ± 2.58 (n = 41)	81.68 ± 2.70 (n = 41)
<i>G. rubra</i>	75.60 ± 2.36 (n = 10)	93.40 ± 1.26* (n = 10)	86.46 ± 2.80 (n = 10)
<i>G. terrae</i>	83.00 ± 1.78 (n = 20)	88.35 ± 1.39 (n = 20)	97.30 ± 1.81* (n = 20)

* Intraspecies mean S-value.

Organisms of the genus *Gordona* can be found in the sputum of patients with cavitary pulmonary tuberculosis and bronchiectasis and are resistant to alkali. Though growing rapidly on successive subcultures, the organisms grow only slowly on primary isolation. Moreover, the colonies of the organisms look like tubercle bacilli when cultivated in containers with a gum cap or paraffined cotton stopper. It is possible, therefore, that the *Gordona* organisms are sometimes mistaken for tubercle bacilli. In a co-operative study undertaken by 13 Japanese national sanatoria, 57 strains (0.34 %) of *Gordona* organisms were found amongst 16,795 strains which grew after treatment with alkali. These 57 strains of the *Gordona* organisms were sent to our laboratory as 'atypical' acid-fast organisms.

To confirm that the three species were well differentiated, the following calculation was made. Intraspecies mean S-values (a mean S-value for the membership of a species to the HMO of itself) and interspecies mean S-values (a mean S-value for the membership of a species to the HMO of another species) are shown in Table 5. The difference between intra- and inter-mean S-values was greater than the addition of both standard deviations in any combination of two species. This finding indicated that these species were clearly separable from each other (with a statistically significant difference, $p = 0.001$, by 't' test).

Gordona bronchialis is proposed as the type species. The following type strains are deposited in the National Collection of Type Cultures, London, and the American Type Culture Collection, Rockville, Maryland, U.S.A.: *Gordona bronchialis* strain 3410; NCTC 10667; ATCC 25592; *Gordona rubra* strain 3605; NCTC 10668; ATCC 25593; *Gordona terrae* strain 3612; NCTC 10669; ATCC 25594.

Species-distinguishing characters

Distinguishing characters for the species of the genus *Gordona* are shown in Table 6. These characters were previously found useful for the differentiation of rapid-growing mycobacteria (Gordon & Mihm, 1959; Bönicke, 1962; Tsukamura, 1966, 1967; Tsukamura, Mizuno & Tsukamura, 1968) and nocardias (Tsukamura, 1969).

Table 6. *Distinguishing characters for differentiation of the species of the genus Gordona*

Character	<i>G. bronchialis</i>	<i>G. rubra</i>	<i>G. terrae</i>
Number of strains tested ...	41	10	20
Acetamidase	+	—	—
Urease	+	+	+
Nicotinamidase	+	—	+
Pyrazinamidase	+	—	+
Allantoinase	—	+	+
L-Glutamate as N and C sources*	+	+	+
L-Serine as N and C sources*	—	—	—
Glucosamine as N and C sources*	±	+	+
Acetamide as N and C sources*	+	—	—
Benzamide as N and C sources*	—	—	—
Monoethanolamine as N and C sources*	—	+	+
Trimethylene diamine as N and C sources*	—	—	—
Citrate as sole C source	±	+	+
Benzoate as sole C source	±	+	—
Propylene glycol as sole C source	+	—	—
Acid from rhamnose	—	—	+
Acid from inositol	+	—	—
Acid from mannitol	±	+	+
Acid from sorbitol	—	+	+

+ = Positive in more than 81 %; ± = positive in 51 to 80 %; ± = positive in 21 to 50 %; — = positive in less than 20 %.

* Utilization for growth as simultaneous nitrogen and carbon sources (Tsukamura, 1966, 1967).

Relationship between Mycobacterium, Gordona and Nocardia observed by numerical analysis

Comparing HMO's, a S-value was prepared for the species of *Mycobacterium* (rapid-growing mycobacteria), *Gordona* and *Nocardia*, and used to calculate the taxonomic distance between the genera (Table 7 and Fig. 1).

The taxonomic distance between *Mycobacterium* and *Gordona* (0.387) is greater than that taxonomic distance between *Gordona* and *Nocardia* (0.255). This finding shows that the genera *Gordona* and *Nocardia* have a higher phenetic similarity than the genera *Gordona* and *Mycobacterium*, and suggests that the genus *Gordona* should be included in the family Actinomycetaceae to which the genus *Nocardia* belongs.

The greatest difficulty we met in the present study was that the similarity coefficient between the species of the same genus was occasionally less than that between the species of different genera (Table 7). According to the current concept of genera, the species of a genus should have shown a higher S-value than the species of different genera. At present, an acceptable answer to this difficulty remains to be found. The odd result shown in Table 7 might have been caused by overlap between genera (Fig. 1) or by uneven selection of characters (though the characters used were selected without bias) in which case the numerical taxonomy would require more characters than are considered as sufficient at present.

Since a species named *Nocardia rubra* by the present author (Tsukamura, 1969) contained several strains named '*Mycobacterium*' *rhodochrous* by Gordon & Mihm (1957), the author would like to make some comment on the relationship between the genus *Gordona* and the species *N. rubra*. On the basis of the distinguishing characters shown in Table 2, *N. rubra* differs from the genus *Gordona* as follows: (1) *N. rubra* does not show slight

Fig. 1. Taxonomic relationship among *Mycobacterium* (M) (rapid-growing mycobacteria), *Gordona* (G) and *Nocardia* (N). Taxonomic distance between the genera was expressed as $d = (100 - x)/x$, where x is a mean S-value (%) between the genera. Extent of a genus was expressed as the radius of a circle, $r = (100 - y)/y$, where y is a mean S-value (%) within a genus. Taxonomic distances are written in figures. Values of r are as follows: $r_M = 0.351$; $r_G = 0.220$; $r_N = 0.244$.

Table 7. S-value table composed of S-values between the HMOs of species of *Mycobacterium* (rapid-growing mycobacteria), *Gordona* and *Nocardia*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>Mycobacterium abscessus</i>	100	—	—	—	—	—	—	—	—	—	—	—	—	—
2. <i>M. fortuitum</i>	85	100	—	—	—	—	—	—	—	—	—	—	—	—
3. <i>M. phlei</i>	73	76	100	—	—	—	—	—	—	—	—	—	—	—
4. <i>M. aurum</i>	71	83	77	100	—	—	—	—	—	—	—	—	—	—
5. <i>M. smegmatis</i>	58	67	76	74	100	—	—	—	—	—	—	—	—	—
6. <i>Gordona rubra</i>	69	76	71	73	64	100	—	—	—	—	—	—	—	—
7. <i>G. terrae</i>	70	73	76	74	73	87	100	—	—	—	—	—	—	—
8. <i>G. bronchialis</i>	75	76	77	79	68	78	85	100	—	—	—	—	—	—
9. ' <i>M.</i> ' <i>rhodochrous</i> *	74	73	76	70	55	79	80	83	100	—	—	—	—	—
10. <i>Nocardia asteroides</i>	77	72	77	67	56	78	81	84	87	100	—	—	—	—
11. <i>N. brasiliensis</i>	73	68	71	67	62	76	77	80	81	88	100	—	—	—
12. <i>N. caviae</i>	75	72	77	73	64	76	79	78	79	84	84	100	—	—
13. <i>N. farcinica</i>	69	74	71	75	64	70	77	78	79	80	74	78	100	—
14. <i>N. rubra</i>	72	79	74	76	63	85	86	81	82	81	79	77	79	100

* This is not a HMO but a feature of characters of strain ATCC 13808 of '*Mycobacterium*' *rhodochrous*.

acid-fastness but shows partial acid-fastness; (2) *N. rubra* shows fragmenting mycelium; (3) *N. rubra* shows negative nitrate reduction. Like the *Gordona* species and unlike the other species of the genus *Nocardia*, *N. rubra* usually utilizes sucrose as a sole carbon source and forms acid from mannose (Tsukamura, 1969). Furthermore, among the *Nocardia* species, *N. rubra* shows the highest S-values to the HMOs of the *Gordona* species (Table 7). In view of these findings, *N. rubra* is regarded as an intermediate between the

genera *Gordona* and *Nocardia*. *Nocardia rubra* has been differentiated from the genus *Gordona* by the distinguishing characters and there is no decisive reason for eliminating it from the genus *Nocardia*.

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