Numerical Taxonomy Study of the Taxonomic Position of *Nocardia rubra* Reclassified as *Gordona lentifragmenta* Tsukamura nom.nov.

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A numerical classification using a total of 105 characters showed that *Gordona* strains together with rhodochrous strains formed a cluster, suggesting that these organisms belong to the same genus. *Nocardia rubra* strains were incorporated into this cluster. *Nocardia rubra* has accordingly been transferred to the genus *Gordona*. As *Gordona rubra* would be a junior homonym of the name of a species already assigned to that genus and subsequently shown to be identical with *Gordona rubropertincta*, *Gordona lentifragmenta* Tsukamura nom.nov. has been proposed for the strains originally known under the name *Nocardia rubra*.

Previously, Tsukamura (20) gathered nocardiae having several specific names, *Nocardia rubra*, *N. lutea*, *N. globerula*, *N. minima*, *N. polychromogenes*, *N. erythropolis*, etc., into the species *Nocardia rubra*. When later (22) he proposed the genus *Gordona* for slightly acid-fast organisms lacking mycelium, *N. rubra* was found to resemble considerably *Gordona* organisms but was not included at that time in the genus *Gordona*, as it showed fragmenting mycelium. Later Tsukamura (23) transferred the rhodochrous group to the genus *Gordona* as *Gordona rhodochroa*. The presence of *N. rubra* hindered clearcut separation of *Gordona* from *Nocardia*. Elucidation of its taxonomic relationship to *G. rhodochroa* is the subject of the present study.

A total of 100 strains of *Nocardia*, *Gordona*, and *Mycobacterium* species (Fig. 1 and 2) was studied for a total of 105 characters. To the set of characters used previously (24), three enzyme tests, β -galactosidase activity (25), acid phosphatase activity (27), and semiquantitative catalase activity (14), were added, but 11 tests on the utilization of nitrogen compounds used previously were reduced to 4 in the present study. These four were the tests on utilization of benzamide, nicotinamide, NaNO_3 , and NaNO_2 as the sole source of nitrogen. The reason for the reduction was indicated previously (24). Of the 105 characters tested, 91 were effective for differentiating the strains. Fourteen characters were ineffective: nine showed positive matches (gram positiveness; glycerol as carbon source; growth at 37 °C; growth at 28 °C; resistance to 0.0625 mg of $\text{NH}_2\text{OH}\cdot\text{HCl}$ per ml; resistance to 0.1% NaNO_2 in Sauton agar; resistance to 0.5 mg of sodium salicylate per ml;

resistance to 10 mg of thiophene-2-carboxylic acid hydrazide per ml; glucose as carbon source); five showed negative matches in all strains (malonamidase; raffinose as carbon source; acid formation from raffinose; nicotinamide as simultaneous nitrogen and carbon sources; photochromogenicity). The methods used were described previously (1, 12, 14, 18, 19, 21, 24, 26-28).

Similarity values (matching coefficient = M value) between the strains were calculated by the following equation: M value = $[n_s/(n_s + n_d)] \times 100\%$, where n_s is the number of characters which show the same code symbols (+ + or - -) and n_d is the number of characters which show different code symbols (+ -). Clustering was made by the single-linkage method (17).

The results are shown in Fig. 1 and 2 as dendrograms. Probably due to the change of the characters used, three taxa, *Nocardia*, *Gordona* (rhodochrous group), and rapidly growing mycobacteria, were not so clearly separated as previously, but the three taxa could still be distinguished.

With the exception of *G. auratiaca*, all *Gordona* strains formed a cluster at the 90% level. All strains of *N. rubra* tested (M-1, C-1, M-103, M-122, M-191, and M-192) were incorporated into the *Gordona* cluster, five forming a new subcluster and only one (strain M-191) being incorporated into another subcluster with *G. rubropertincta*, *G. rosea*, and *G. terrae*. *G. rubra*, described in 1971 (22), is now a synonym of *G. rubropertincta* (23). The *Gordona* organisms were classified into five subgroups: (i) *N. rubra*; (ii) *G. rhodochroa* (strain ATCC 13808, named *Rhodococcus rhodochrous* Zopf [15, 29], belongs to this taxon [23, 24]); (iii) *G. bron-*

FIG. 1. Dendrogram of the relationships between rapidly growing mycobacteria, *Gordona*, and *Nocardia*. The strains bearing the initial code A were received from the American Type Culture Collection, Rockville, Md.; those bearing the code N were from the National Collection of Type Cultures, London; those bearing the code R (and W) were from Ruth E. Gordon, Institute of Microbiology, Rutgers State University, New Brunswick, N.J.; those bearing the codes M and C were from I. Uesaka, Kyoto University, Kyoto, Japan; and those bearing the code MT belonged to the collection of our laboratory. The strains received from I. Uesaka belonged originally to the collection of N. M. McClung, University of South Florida, Tampa. The type strain of *Jensenia canicruria* was received from A. Shimizu, Kaken Chemical Company, Tokyo, Japan (KCC-AO132; Y. Okami; IMC; ATCC 11048; NCTC 8036).

chialis; (iv) *G. rubropertincta*, *G. rosea*, and *G. terrae*; and (v) *G. aurantiaca*.

The fourth subcluster could be divided into two subgroups, one consisting of *G. rubropertincta* and *G. rosea* and another consisting of *G. terrae*, at the 94% level. These three could previously be differentiated from each other (23,

24) but were lumped into one in the present study at the 92% level. The oldest specific epithet among these three is *roseus* (10).

In addition, four species of the genus *Nocardia* could be differentiated as different clusters—*N. brasiliensis*, *N. caviae*, *N. farcinica* (see Tsukamura [20]), and *N. asteroides*. The

FIG. 2. Dendrogram of the relationships between rapidly growing mycobacteria, *Gordona*, and *Nocardia* (see legend to Fig. 1 for additional information).

following species of rapidly growing mycobacteria were differentiated from each other at the 90% level as distinct clusters: *M. thermoresistibile*, *M. flavescens*, *M. fortuitum*, *M. aurum*, *M. parafortuitum*, *M. chelonae* subsp. *chelonae*, *M. chelonae* subsp. *abscessus*, *M. chitae*, *M. aichiense*, *M. chubuense*, *M. phlei*, *M. smegmatis*, *M. agri*, *M. vaccae*. Previously, *M. parafortuitum* was reported as a synonym of *M. vaccae* (13), but these two organisms could be differentiated from each other in the present study.

Distinguishing characters for differentiating

Nocardia, *Gordona* (rhodochrous group), and rapidly growing mycobacteria are shown in Table 1. The table includes not only the data of the present study but also those of other studies. Characters useful for differentiating between the species of the genus *Gordona* are shown in Table 2.

Taxonomic position and nomenclature of *N. rubra*. It has been shown that the strains of *N. rubra* cluster at a high level with the rhodochrous and other organisms placed in the genus *Gordona*.

TABLE 1. *Distinguishing characters for differentiating between the taxa rapidly growing mycobacteria, Gordona and Nocardia*

Species	No. ^a	Percentage of strains showing positive reaction							
		AF	MYC	ARY	SUC	MAN	TMD	GAL	NO ₃
<i>Nocardia brasiliensis</i>	7	0	100	0	14	0	0	100	0
<i>N. caviae</i>	8	0	100	0	0	0	0	100	12
<i>N. farcinica</i>	32	0	100	0	28	6	0	100	0
<i>N. asteroides</i>	15	0	100	7	7	0	0	100	60
<i>N. rubra</i>	5	0	100	0	100	0	0	0	100
<i>Gordona bronchialis</i>	41	0	0	0	100	100	0	0	98
<i>G. rhodochroa</i>	10	0	10	0	100	60	0	0	100
<i>G. rosea-rubropertincta</i>	36	0	0	0	100	100	0	0	92
<i>G. aurantiaca</i>	6	17	0	0	100	100	0	100	0
<i>Mycobacterium thermoresistibile</i>	39	100	0	0	5	0	0	0	100
<i>M. flavescens</i>	10	100	0	100	0	0	0	0	100
<i>M. chitae</i>	7	100	0	100	0	0	0	0	100
<i>M. agri</i>	31	100	0	100	0	100	23	100	97
<i>M. chelonae</i> subsp. <i>chelonae</i>	20	100	0	100	0	100	0	100	0
<i>M. chelonae</i> subsp. <i>abscessus</i>	24	100	0	100	33	50	92	96	25
<i>M. fortuitum</i>	132	100	0	100	17	69	100	0	100
<i>M. aurum</i>	18	100	0	100	6	100	100	11	67
<i>M. parafortuitum</i>	6	100	0	100	17	100	100	0	83
<i>M. aichiense</i>	8	100	0	100	0	100	0	0	0
<i>M. chubuense</i>	15	100	0	100	100	100	100	7	100
<i>M. vaccae</i>	12	100	0	100	83	25	0	0	0
<i>M. phlei</i>	20	100	0	100	100	20	100	80	100
<i>M. smegmatis</i>	24	100	0	100	96	100	100	83	100

^a Number of strains tested.^b Abbreviations: AF, strong acid-fastness; MYC, fragmenting mycelium; ARY, two week-arylsulfatase; SUC, sucrose as sole carbon source; MAN, acid from mannose; TMD, trimethylenediamine as simultaneous nitrogen and carbon sources; GAL, β -galactosidase; NO₃, nitrate reduction (nitrite produced from nitrate).

Gordon and Mihm (9, 10) were the first to differentiate the rhodochrous group from nocardiae. They named the taxon *Mycobacterium rhodochrous*. Later, Gordon (7) regarded it as an intermediate between *Mycobacterium* and *Nocardia*. Goodfellow and his associates (5, 6) were able to differentiate "*Mycobacterium*" *rhodochrous* from nocardiae by applying numerical methods to their data. Tsukamura (22) proposed the genus *Gordona* for these and other slightly acid-fast organisms from sputum and soil.

The evidence provided by this study indicates that the species *N. rubra* should be transferred to the genus *Gordona* in association with *G. rhodochroa* and other species.

This transfer would result in the combination *G. rubra*. However the name was once used for another organism, *Gordona rubra* Tsukamura (22), which was subsequently shown to be identical to *Gordona rubropertincta*, which name had priority. Consequently, a new specific epithet *lentifragmenta* is proposed under Rule

34b Note 2 of the *Bacteriological Code*, giving the new name *Gordona lentifragmenta* Tsukamura for those organisms previously known under the name *Nocardia rubra* (Kruse) Chalmers and Christopherson (see reference 20). The characters of the species are given below.

(i) **Morphology.** Usually occur as gram-positive, slightly acid-fast or non-acid-fast rods or coccoids; mycelia form at early stage of growth but later fragment into rods, short rods, and coccoids; form pinkish-pigmented, rough colonies on egg media and Sauton agar.

(ii) **Biological.** Growth occurs at 28 C and 37 C, but not at 45 C; growth occurs at 3 days on egg media and Sauton agar; tolerant to 0.2% picric acid in Sauton agar; tolerant to 0.1% NaNO₂ in Sauton agar; tolerant to 0.25 mg of NH₂OH·HCl per ml in Ogawa egg medium; tolerant to 0.5 mg of *p*-nitrobenzoic acid per ml in Ogawa egg medium; tolerant to 1.0 mg of sodium salicylate per ml in Ogawa egg medium; susceptible to 5 μ g of ethambutol per ml in Ogawa egg medium (Löwenstein-Jensen me-

TABLE 2. Characters useful for differentiating the species of *Gordona*

Character	Percentage of strains showing positive reaction					
	<i>N. rubra</i>	<i>G. bronchialis</i>	<i>G. rhodochroa</i>	<i>G. terrae</i>	<i>G. rosea-rubroper-tincta</i>	<i>G. aurantiaca</i>
Acid phosphatase	0	90	30	0	0	0
α -Esterase	0	0	0	90	12	0
Tween hydrolysis (14 days)	0	0	100 ^a	0	0	100
Utilization as simultaneous nitrogen and carbon sources						
Acetamide	100	98	0	0	0	100
Monoethanolamine	80	0	0	95	100	100
Acid from:						
Galactose	0	0	0	0	0	100
Xylose	0	0	0	0	0	100
Rhamnose	0	0	0	100	0	0
Trehalose	0	98	0	100	70	100
Inositol	0	98	0	0	0	100
Mannitol	100	24	0	100	100	100
Sorbitol	100	0	0	100	100	100
Benzoate as sole carbon source	80	32	60	5	100	0
Malonate as sole carbon source	60	0	0	0	0	0
Acetamidase	100	98	20	0	0	100
Nicotinamidase	60	86	0	80	20	100
Pyrazinamidase	60	83	0	80	20	100
Allantoinase	0	0	0	95	100	100
No. of strains tested	5	41	10	20	17	6

^a Weakly positive.

dium may be substituted for Ogawa egg medium).

(iii) **Enzyme activities.** Tween not hydrolyzed at 14 days; α - and β -esterase activities not shown; β -galactosidase negative; catalase negative; nitrate reduced to nitrite; arylsulfatase activity not shown at 2 weeks; acetamidase activity shown but other amidase activities (Bönicke's series) not shown.

(iv) **Nutritional requirements.** Glucose, mannose, sucrose, *n*-propanol, *n*-butanol, isobutanol, propylene glycol, mannitol, and sorbitol utilized as carbon source in the presence of ammoniacal nitrogen, but galactose, arabinose, xylose, rhamnose, trehalose and inositol not utilized; acid formed from glucose, but acid not formed from mannose; acetate, citrate, succinate, malate, pyruvate, malonate, and fumarate utilized as carbon source; glutamate, glucosamine, acetamide, and monoethanolamine utilized, but serine, benzamide and trimethylene diamine not utilized as simultaneous nitrogen and carbon sources.

Transfer of some *N. rubra* strains to the rhodochrous group was reported recently also by other investigators, although the definition as a species was not yet made. Bradley (2) divided the rhodochrous complex into two subgroups;

one consisted of *N. corallina* and *N. rubra* and the other consisted of *N. erythropolis*. These two groups differed in their guanine plus cytosine content. Bradley and Huitron (3) observed that deoxyribonucleic acid (DNA) from a strain of *N. coeliaca* annealed extensively with DNA from *N. erythropolis* references, but DNA from a strain of *N. globetula* did not anneal appreciably with the *N. erythropolis* references. Ridell (16) studied the serological relationships between 125 strains of *Nocardia* and *Mycobacterium* by means of the comparative immunodiffusion technique and observed that eight strains, either designated *N. corallina*, *N. rubra* or *M. rhodochrous*, revealed a close relationship with the *N. corallina* reference strain. She also observed that strains designated *N. rubra* were heterogenous. Gordon et al. (8) reported that the type strain of *N. coeliaca* is a member of the rhodochrous group.

It was found recently that the test of β -galactosidase activity is a useful tool for differentiating *Gordona* organisms from nocardiae (25). The gordonae showed a negative reaction and the nocardiae a positive one. Strains identified previously as *N. rubra* (20) showed different reactions. Strains M-1 and C-1 received as *N. rubra*, strain M-103 received as *N. minima*,

strain M-192 received as *N. lutea*, strain M-122 received as *N. coeliaca*, and strain M-191 received as *N. rubropertincta* showed a negative reaction, and all, except for the strain, were incorporated into *Gordona lentifragmenta* Tsukamura. Strain M-75 received as *N. globetula*, strain M-79 received as *N. convoluta*, and strain M-6 received as *N. polychromogenes* showed a positive reaction. The latter three strains were not included as subjects of the present study.

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REPRINT REQUESTS

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