Serological Study of Nocardiae and Mycobacteria by Using "Mycobacterium" *pellegrino* and *Nocardia corallina* Precipitation Reference Systems

MALIN RIDELL

Institute of Medical Microbiology, University of Göteborg, Göteborg, Sweden

The serological relationship between 125 strains of *Nocardia* and *Mycobacterium* was analyzed by means of the comparative immunodiffusion technique. Two reference precipitation systems, *N. corallina* and "M." *pellegrino*, were utilized. Most of the tested strains had one to three precipitinogens in common with the *N. corallina* and the "M." *pellegrino* reference strains. Most of the mycobacterial and most of the nocardia strains examined seemed to be serologically related to the two reference strains to an equally large extent. Eight strains, either designated *N. corallina*, *N. rubra*, or "M." *rhodochrous*, revealed a close serological relationship with the *N. corallina* reference strain, and so it might be justified to consider these strains as belonging to one taxonomic group. Ten other strains, designated *N. rubra*, "M." *pellegrino*, or "M." *rhodochrous*, displayed a close serological relationship with the "M." *pellegrino* reference strain, and they may therefore be regarded as belonging to another taxonomic group. Eight strains with the designations *N. corallina*, *N. rubra*, or "M." *rhodochrous* showed little serological similarity to either of the two reference strains. Thus, the present taxonomy of a number of strains of *Nocardia* and *Mycobacterium* seems questionable since some strains were found to be serologically different, although referred to the same species, whereas some strains, referred to different species, were serologically closely related. The classification and nomenclature of such strains should, therefore, be reconsidered.

The borderline between the two genera *Nocardia* and *Mycobacterium* is unclear, and the delineations between different species within these two genera are also incomplete. It is not settled, for example, whether the species tentatively designated "Mycobacterium" *rhodochrous* (Overbeck) Gordon and Mihm (12) should belong to *Nocardia*, *Mycobacterium*, or to some other genus (2, 8, 9, 11, 30). Strains designated "Mycobacterium" *pellegrino* Kofinek have also a position which is not clear (2, 14), and furthermore this name is not validly published (4). (Since it is unclear to which genus these species belong, the genus name is placed in quotation marks.)

It has been shown that strains from various species of the genera *Nocardia* and *Mycobacterium* have antigens in common. Serological analyses of the relationship between these two genera and of species within the genus *Nocardia* have been made by several authors, e.g., Castelnovo et al. (5, 6), Magnusson (20, 21), and others (15, 16, 26, 33). In a recent investigation by Ridell and Norlin (27), the precipitinogenic relationships between some

species of the genera mentioned above were studied. *Nocardia* strains and strains designated "M." *pellegrino* or "M." *rhodochrous* were analyzed by means of the comparative immunodiffusion technique using serological reference systems representing nine mycobacterial species. It was demonstrated that the tested strains and the mycobacterial reference strains cross-reacted, having up to three precipitinogens in common.

The aim of the present study was to obtain further knowledge of the antigenic relationships of strains from the genera *Nocardia* and *Mycobacterium*, with special regard to those species having unclear taxonomic positions. For this purpose two new reference systems were prepared, namely for the species *Nocardia corallina* (Bergey et al.) Waksman and Henrici (32) and "M." *pellegrino*.

MATERIALS AND METHODS

Bacterial strains. The number of strains tested was 125, 72 of which represented 16 different mycobacterial species (see Table 2). The number of strains

referred to the genus *Nocardia* was 42 (species are indicated in Table 4). In addition, seven strains designated "*M.*" *pellegrino* and four strains designated "*M.*" *rhodochrous* were studied. The sources and designations of these strains and of strains labeled *N. corallina* and *Nocardia rubra* (Kruse) Chalmers and Christopherson (7) are indicated in Table 1.

Antigen preparations. Antigens were prepared in two different ways as described previously (27). The two types of preparations are very similar as to precipitinogenic composition (23), and so they were employed interchangeably.

Antisera. Reference immune sera for *N. corallina* (strain N. 32) and "*M.*" *pellegrino* (strain N. 13) were prepared as follows. Rabbits were immunized by subcutaneous injections of a mixture containing 10 parts of antigen preparation (concentrated culture filtrate), 9 parts of liquid paraffin, and 1 part of Arlacel A. The rabbits received 1 ml of this preparation once a week for 7 weeks and were bled 2 weeks after the last injection. Test bleedings during the immunization were made to evaluate the progress of the antibody response.

Serological technique. The analyses were carried out by means of a microplate modification (31) of the comparative immunodiffusion technique according to Ouchterlony (24, 25).

Reference precipitation systems. Reference precipi-

tation systems for the species *N. corallina* and "*M.*" *pellegrino* were prepared with strain N. 32 and strain N. 13, respectively.

RESULTS

Reference precipitation systems for the species *N. corallina* and "*M.*" *pellegrino* were prepared. The precipitation pattern for *N. corallina* (strain N. 32) consisted of seven separate immunoprecipitates, labeled aA^{co}-gG^{co} (left pattern, Fig. 1); the pattern for "*M.*" *pellegrino* (strain N. 13) consisted of seven separate precipitates, labeled aA^{pe}-gG^{pe} (left pattern, Fig. 2). The two reference strains showed two common precipitinogens. When the "*M.*" *pellegrino* strain was analyzed by means of the *N. corallina* system, no reaction of identity could be revealed (see Table 5). In the *N. corallina* strain, however, the precipitinogen a^{pe} was demonstrated (see Table 7).

The number of precipitinogens revealed by the analyses of the 72 mycobacterial strains by means of the *N. corallina* system is indicated in Table 2. Antigen preparations from 15 of the

TABLE 1. Designations and sources of 28 of the strains studied

Laboratory no.	Strain designation as received	Received from ^a
N. 28	<i>Nocardia corallina</i> 13004/148	2
N. 29	<i>N. corallina</i> 13006/147	2
N. 30	<i>N. corallina</i> 2287	2
N. 31	<i>N. corallina</i> 2293	2
N. 32 ^b	<i>N. corallina</i> M-ö 5007	2
N. 52	<i>N. corallina</i> 5301	1
N. 53	<i>N. corallina</i> 5302	1
N. 19	<i>Nocardia rubra</i> 2295	2
N. 20	<i>N. rubra</i> 2298	2
N. 21	<i>N. rubra</i> 2299	2
N. 23	<i>N. rubra</i> 2302	2
N. 24	<i>N. rubra</i> 2303	2
N. 25	<i>N. rubra</i> 2304	2
N. 26	<i>N. rubra</i> 2305	2
N. 27	<i>N. rubra</i> 2306	2
N. 50	<i>N. rubra</i> 5201	1
N. 51	<i>N. rubra</i> 5202	1
N. 12	" <i>Mycobacterium</i> " <i>pellegrino</i> 59	2
N. 13 ^b	" <i>M.</i> " <i>pellegrino</i> 107	2
N. 14	" <i>M.</i> " <i>pellegrino</i> 129	2
N. 15	" <i>M.</i> " <i>pellegrino</i> 350	2
N. 16	" <i>M.</i> " <i>pellegrino</i> 906	2
N. 48	" <i>M.</i> " <i>pellegrino</i> 5101	1
N. 49	" <i>M.</i> " <i>pellegrino</i> 5102	1
N. 35	" <i>Mycobacterium</i> " <i>rhodochrous</i> 8139	2
N. 36	" <i>M.</i> " <i>rhodochrous</i> 8154	2
N. 45	" <i>M.</i> " <i>rhodochrous</i> NCTC 576	3
N. 46	" <i>M.</i> " <i>rhodochrous</i> EB 327	1

^a (1) R. Bönicke, Forschungsinstitut Borstel, Borstel, West Germany; (2) J. Juhlin, Malmö Allmänna Sjukhus, Malmö, Sweden; (3) National Collection of Type Cultures, London, England.

^b Reference strain.

FIG. 1. Schematic illustration of immunodiffusion analysis of *N. rubra* strain (N. 20) by means of the *N. corallina* reference system.

FIG. 2. Schematic illustration of immunodiffusion analysis of *N. rubra* strain (N. 20) by means of the "*M.*" *pellegrino* reference system.

tested strains did not react with the reference antiserum, whereas antigen preparations from the other 57 strains tested formed one, two, or three precipitinogens with the antiserum. Table 3 shows the result of the same mycobacterial strains being analyzed by means of the "*M.*" *pellegrino* reference system. Antigen preparations from nine strains did not react with the anti-"*M.*" *pellegrino* serum, whereas one, two, or three precipitinogens were revealed in antigen preparations from the other 63 strains tested.

Table 4 shows the result obtained when 41 *Nocardia* strains and the 11 "mycobacterial" strains were analyzed by means of the *N. corallina* reference system. Antigen preparations from all of the strains reacted with the reference antiserum. Most of them formed one, two, or three precipitates, and an *N. polychromogenes* strain and a strain labeled *Nocardia* sp. formed four precipitates. Eight strains differed from the others by forming six or seven precipitates with the anti-*N. corallina* serum. Among these eight strains were four out of six *N. corallina* strains, two out of ten *N. rubra* strains, and two out of four "*M.*" *rhodochrous* strains. The precipitinogens of all strains labeled *N. corallina*, *N. rubra*, "*M.*"

pellegrino, or "*M.*" *rhodochrous* which were found identical with precipitinogens of the *N. corallina* reference strain are indicated in Table 5. Not all of the precipitinogens demonstrated

TABLE 2. Distribution of mycobacterial strains of various species according to the number of precipitates demonstrated by means of the *N. corallina* reference system

Run- yon group	Species	Distribution of strains				Total no. of strains tested
		0 ^a	1	2	3	
I	<i>M. kansasii</i>		3	5		8
	<i>M. marinum</i>	3	1	2	3	9
II	<i>M. gordonae</i>		1	1		2
	<i>M. scrofulaceum</i>		1		1	2
III	<i>M. avium</i>	5	1	2	1	9
	<i>M. gastri</i>	1	4	5		10
	<i>M. intracellulare</i>	1		2		3
	<i>M. terrae</i>	1	2			3
IV	<i>M. xenopi</i>		2			2
	<i>M. borstelense</i>		3			3
	<i>M. fortuitum</i>	2	1	3		6
	<i>M. phlei</i>		1	2	1	4
	<i>M. smegmatis</i>	1	2	1	1	5
	<i>M. vaccae</i>	1	3			4
	<i>M. microti</i>			1		1
	<i>M. bovis</i> var. BCG		1			1

^a Number of precipitates.

TABLE 3. Distribution of mycobacterial strains of various species according to the number of precipitates demonstrated by means of the "*M.*" *pellegrino* reference system

Run- yon group	Species	Distribution of strains				Total no. of strains tested
		0 ^a	1	2	3	
I	<i>M. kansasii</i>	1	3	4		8
	<i>M. marinum</i>		5	2	2	9
II	<i>M. gordonae</i>		2			2
	<i>M. scrofulaceum</i>	1		1		2
III	<i>M. avium</i>		9			9
	<i>M. gastri</i>		1	7	2	10
	<i>M. intracellulare</i>	1	2			3
	<i>M. terrae</i>		3			3
IV	<i>M. xenopi</i>			2		2
	<i>M. borstelense</i>		2	1		3
	<i>M. fortuitum</i>	3	2	1		6
	<i>M. phlei</i>		2	2		4
	<i>M. smegmatis</i>	2	3			5
	<i>M. vaccae</i>		1	2	1	4
	<i>M. microti</i>	1				1
	<i>M. bovis</i> var. BCG		1			1

^a Number of precipitates.

TABLE 4. *Distribution of Nocardia strains of various species and of strains designated "M." pellegrino and "M." rhodochrous according to the number of precipitates demonstrated by means of the N. corallina reference system*

Species	Distribution of strains								Total no. of strains tested
	0 ^a	1	2	3	4	5	6	7	
<i>N. asteroides</i>		7	4						11
<i>N. blackwellii</i>		1							1
<i>N. brasiliensis</i>		2							2
<i>N. caviae</i>		2	1						3
<i>N. corallina</i>				2			2	2	6
<i>N. farcinica</i>		1	1	1					3
<i>N. polychromogenes</i>				1	1				2
<i>N. rubra</i>			5	3				2	10
<i>N. rubropertincta</i>			1	1					2
<i>Nocardia</i> sp.					1				1
"M." <i>pellegrino</i>		1	2	4					7
"M." <i>rhodochrous</i>			1	1				2	4

^a Number of precipitates.TABLE 5. *Precipitinogens of strains designated N. corallina, N. rubra, "M." pellegrino, or "M." rhodochrous identified by the N. corallina reference system*

Strains	Total no. of precipitinogens	Identified precipitinogens of <i>N. corallina</i> reference (N. 32)						
		a ^{co}	b ^{co}	c ^{co}	d ^{co}	e ^{co}	f ^{co}	g ^{co}
<i>N. corallina</i>								
N. 28	3							
N. 29	3				+			
N. 30	6	+	+	+	+	+	+	
N. 31	7	+	+	+	+	+	+	+
N. 52	6		+	+	+	+	+	+
N. 53	7	+	+	+	+	+	+	+
<i>N. rubra</i>								
N. 19	2							
N. 20	3				+			
N. 21	2							
N. 23	7		+	+	+	+	+	+
N. 24	3							
N. 25	7	+	+	+	+	+	+	+
N. 26	2							
N. 27	2							
N. 50	2							
N. 51	3							
"M." <i>pellegrino</i>								
N. 12	2				+			
N. 13	2							
N. 14	1				+			
N. 15	3				+			
N. 16	3				+			
N. 48	3				+			
N. 49	3				+			
"M." <i>rhodochrous</i>								
N. 35	7	+	+	+	+	+	+	+
N. 36	7	+	+	+	+	+	+	+
N. 45	2							
N. 46	3				+			

in the tested strains were identified by means of the reference pattern. All seven precipitinogens of the reference strain were identified in five strains (N. 31, N. 53, N. 25, N. 35, and N. 36), six of them were identified in three strains (N. 30, N. 52, and N. 23), and only one was identified in nine strains. In 10 strains none of the precipitinogens of the reference strains was demonstrated. The precipitinogen d^{co} was the one most frequently demonstrated among the strains tested.

Figure 1, showing the result of a comparative immunodiffusion analysis by means of the *N. corallina* reference system, demonstrates three precipitinogens in the *N. rubra* strain N. 20. One of the precipitinogens demonstrated was identified as d^{co} .

The results obtained when antigen preparations from 42 *Nocardia* and 10 "mycobacterial" strains were analyzed by means of the "M." *pellegrino* reference system are given in Table 6. Antigen preparations from all strains except two (one *N. asteroides* and one *N. blackwellii* strain) reacted with the "M." *pellegrino* serum, forming from one up to seven precipitates. In 39 of the tested strains, one, two, or three precipitinogens were revealed. Four precipitinogens were revealed in one *N. corallina* strain, and five precipitinogens were revealed in one *N. rubra* and in one "M." *pellegrino* strain. Six or seven precipitinogens were revealed in eight strains. Among these eight strains were two of ten *N. rubra* strains, five of six "M." *pellegrino* strains, and one of four "M." *rhodochrous* strains. Table 7 demonstrates the identified precipitinogens in all tested strains that are referred to the species *N.*

corallina, *N. rubra*, "M." *pellegrino*, and "M." *rhodochrous*. Not all precipitinogens demonstrated in the tested strains were identified by means of the reference pattern. In three strains (N. 24, N. 12, and N. 16), however, all seven precipitinogens of the reference strain could be identified, and in six strains (N. 20, N. 14, N. 15, N. 48, N. 49, and N. 45), five or six of them could be identified. In most of the remaining strains, one, two, or three precipitinogens were identified. The precipitinogens a^{pe} and g^{pe} were the factors most frequently demonstrated in the analyzed strains.

Figure 2, showing the result of a comparative immunodiffusion analysis by means of the "M." *pellegrino* reference system, demonstrates six precipitinogens in the *N. rubra* strain N. 20 (the same strain as in Fig. 1). All six precipitinogens in the *N. rubra* strain were identified by means of the reference pattern.

DISCUSSION

The results of this study are based on comparative immunodiffusion analyses with reference systems. This method offers a possibility to identify individual precipitates. However, precipitates obtained by the test strain and the reference antiserum can be unfavorably situated in relation to corresponding precipitates in the reference system, and no identity reaction can thus be revealed (compare Fig. 1). There are further reasons for failure to demonstrate identity reactions. However, the presence of a precipitate can be regarded as a demonstration of a precipitinogen common to the test strain and the reference strain.

Standardization of antigen preparations is

TABLE 6. Distribution of *Nocardia* strains of various species and of strains designated "M." *pellegrino* and "M." *rhodochrous* according to the number of precipitates revealed by means of the "M." *pellegrino* reference system

Species	Distribution of strains								Total no. of strains tested
	0 ^a	1	2	3	4	5	6	7	
<i>N. asteroides</i>	1	9		1					11
<i>N. blackwellii</i>	1								1
<i>N. brasiliensis</i>		2							2
<i>N. caviae</i>		1	1	1					3
<i>N. corallina</i>		2	2	2	1				7
<i>N. farcinica</i>		2	1						3
<i>N. polychromogenes</i>			2						2
<i>N. rubra</i>		1	1	5		1	1	1	10
<i>N. rubropertincta</i>				2					2
<i>Nocardia</i> sp			1						1
"M." <i>pellegrino</i>						1	3	2	6
"M." <i>rhodochrous</i>		1	1	1			1		4

^a Number of precipitates.

TABLE 7. *Precipitinogens of strains designated N. corallina, N. rubra, "M." pellegrino, or "M." rhodochrous identified by the "M." pellegrino reference system*

Strains	Total no. of precipitinogens	Identified precipitinogens of <i>M. pellegrino</i> reference (N. 13)						
		a ^{pe}	b ^{pe}	c ^{pe}	d ^{pe}	e ^{pe}	f ^{pe}	g ^{pe}
<i>N. corallina</i>								
N. 28	3	+						+
N. 29	2							+
N. 30	1	+						
N. 31	2	+						
N. 32	1	+						
N. 52	3							
N. 53	4	+						
<i>N. rubra</i>								
N. 19	3							+
N. 20	6	+	+	+		+	+	+
N. 21	3					+		+
N. 23	2							
N. 24	7	+	+	+	+	+	+	+
N. 25	1							
N. 26	5			+		+		+
N. 27	3	+						
N. 50	3	+						+
N. 51	3	+						+
<i>"M." pellegrino</i>								
N. 12	7	+	+	+	+	+	+	+
N. 14	6	+	+	+		+	+	+
N. 15	5	+	+	+		+	+	
N. 16	7	+	+	+	+	+	+	+
N. 48	6	+		+		+	+	+
N. 49	6	+	+	+		+	+	+
<i>"M." rhodochrous</i>								
N. 35	1							
N. 36	2	+						
N. 45	6	+	+	+		+	+	+
N. 46	3							+

difficult, and small variations in cultivation conditions may partially explain the variations in the number of demonstrated precipitinogens. Therefore, differences of one or two precipitinogens have a limited value for differentiation purposes.

In a previous study (27), it was demonstrated that there are at least three precipitinogens common to many species of the genera *Mycobacterium* and *Nocardia* and that two of them correspond to the mycobacterial cross-reacting factors α and β . The results of the present study showed that most of the mycobacterial strains cross-reacted with the anti-*N. corallina* and the anti-*"M." pellegrino* reference sera, forming one, two, or three precipitates (see Tables 2 and 3), and thus confirmed the earlier results. The different mycobacterial species seemed to be serologically related to the two reference strains to a similar degree, and

the *"M." pellegrino* strain was not more closely related to any of the mycobacterial species than was the *N. corallina* strain.

All of the *Nocardia* strains and the strains designated *"M." pellegrino* and *"M." rhodochrous* cross-reacted with the anti-*N. corallina* reference serum, and all except two cross-reacted with the anti-*"M." pellegrino* reference serum. Most test strains of the *Nocardia* species, as for example *N. asteroides*, did not seem to be serologically more closely related to either the *N. corallina* or the *"M." pellegrino* reference strain than were strains of the genus *Mycobacterium* as they had not more than three precipitinogens in common with the reference strains (see Tables 4 and 6). It should be mentioned that the tested strains labeled *N. farcinica* in an earlier study (27) reacted like *Nocardia* and not like *Mycobacterium* strains.

The precipitation patterns formed by four *N.*

corallina strains, two *N. rubra* strains, and two "*M.*" *rhodochrous* strains, when analyzed by means of the *N. corallina* reference system, indicate a high degree of serological similarity between these strains and the reference strain (see Table 5). Other strains, however, with the same species designations did not show the same high degree of serological similarity to the reference strain. The results obtained when the *Nocardia* and "mycobacterial" strains were analyzed by means of the "*M.*" *pellegrino* reference system show the same pattern concerning serological relationships (see Table 7). It might be appropriate to place together in one group the strains *N. rubra* (N. 23, N. 25), *N. corallina* (N. 30, N. 31, N. 32, N. 52, and N. 53), and "*M.*" *rhodochrous* (N. 35 and N. 36). Another group can be formed by the strains "*M.*" *pellegrino* (N. 12, N. 13, N. 14, N. 15, N. 16, N. 48, and N. 49), *N. rubra* (N. 20, N. 24), and "*M.*" *rhodochrous* (N. 45). Strain N. 26 might also be placed in this group. This strain has, however, a more uncertain position as indicated by the demonstration of five precipitinogens, of which three were identified when analyzed with the "*M.*" *pellegrino* reference system. Eight of the tested strains referred to the species *N. corallina*, *N. rubra*, or "*M.*" *rhodochrous* were not closely related to any of the two reference strains (having at most three precipitinogens in common). Thus, it seems that these eight strains should be referred to taxonomic groups other than those represented by the two reference strains.

It is of interest to note that the *N. corallina* strains N. 30, N. 31, N. 32, N. 52, and N. 53, all of which have been placed in the same serological group, display the same amidase pattern—they decompose urea and allantoin—whereas the serologically different strains N. 28 and N. 29, also designated *N. corallina* upon receipt, decompose only urea (unpublished results). Hence, strains designated *N. corallina* form at least two different taxonomic groups.

It should be mentioned that the two strains *N. corallina* (N. 32) and "*M.*" *pellegrino* (N. 13), which were chosen as the reference strains, are not the type strains of these species and that no type strains seem to exist for these species. (28).

The 10 strains which were found to be serologically closely related to the "*M.*" *pellegrino* reference strain also showed the same amidase pattern as the reference strain, i.e., they decompose acetamide, nicotinamide, and pyrazinamide (unpublished results). Juhasz and Bönicke (14) reported the same pattern when analyzing seven "*M.*" *pellegrino* strains. Thus, all the "*M.*" *pellegrino* strains formed a distinct

cluster and might therefore be referred to a single species. It is also very probable that the strains *N. rubra* (N. 20, N. 24, and N. 26) and "*M.*" *rhodochrous* (N. 45) belong to this species. The tested strains designated *N. rubra* could be divided into at least three distinct clusters—strains closely related to each of the two reference strains and strains which could not be grouped in the present study. The same heterogeneity was found among strains labeled "*M.*" *rhodochrous*.

The results of the present study imply either that the present taxonomy of the genus *Nocardia* is unsuitable for the above-mentioned species or that the strains included in this study were incorrectly labeled when received. Castelnovo et al. (5) have demonstrated a close serological relationship between some strains of *N. rubra*, "*M.*" *pellegrino*, and "*M.*" *rhodochrous*, and Lind et al. (19) and Adams et al. (1) have shown that "*M.*" *rhodochrous* is a very heterogeneous species. Bradley (3) found that the "rhodochrous complex" contained at least two species—one typified by *N. corallina* or *N. rubra* and the other by *N. erythropolis*. In a numerical taxonomy study of a great number of nocardioform strains, Goodfellow (8) placed within one cluster, which he called the *M. rhodochrous* group, strains designated *N. corallina*, *N. rubra*, or *N. pellegrino*, among others. This cluster was divided into several subgroups. He concluded that the present taxonomy of nocardioform bacteria is illogical.

Two serological groups, represented by the two reference strains, have been distinguished among the strains used in this study. There is no indication, however, to which genus—*Nocardia*, *Mycobacterium* or some other genus—the strains included in the two groups should be referred. Several investigators have discussed to which genus strains designated "*M.*" *pellegrino* and "*M.*" *rhodochrous* should belong. Bönicke et al. (2, 14, 29) have studied these species and suggested the designation *Nocardia pellegrino*. Gordon (11-13) placed the "*M.*" *rhodochrous* strains, tentatively, among the mycobacteria. Castelnovo et al. (5, 17) proposed placing in one group some strains identified as belonging to *N. corallina*, *N. rubra*, "*M.*" *rhodochrous*, or "*M.*" *pellegrino*, referring this group of strains to the genus *Nocardia*. Bradley (3) found that strains of the "rhodochrous complex" were more allied to *N. asteroides* and *N. farcinica* than to *M. tuberculosis*. By studying lipid composition, Lechevalier et al. (18) suggested that all of the strains of "*M.*" *rhodochrous* that they tested should be assigned to *Nocardia*. Tsukamura (30), on the other hand, has proposed that "*M.*" *rhodochrous*-like organisms

should be referred to a new genus, *Gordona*. Goodfellow et al. (8, 9) stated that numerical taxonomy indicates that the "*M.*" *rhodochrous* complex forms a taxon clearly distinct from the genus *Mycobacterium* as well as from the genus *Nocardia*. It is interesting to note that their studies (10, 22) have shown that strains of the "*M.*" *rhodochrous* complex contain a lipid similar to, but not identical with, a lipid characteristic of *Nocardia*. The present study indicates that some strains designated *N. rubra*, *N. corallina*, "*M.*" *pellegrino*, or "*M.*" *rhodochrous* are probably not more closely related to the true nocardiae (i.e., species like *N. asteroides*) than to the true mycobacteria.

It is obvious that the specific identification of a fairly large number of strains referred to *Nocardia* or *Mycobacterium* should be reconsidered because there are strains which are very different but which have been placed in the same species and there are also strains which have been placed in different species but which are closely related.

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REPRINT REQUESTS

Address reprint requests to: Dr. Malin Ridell, Institute of Medical Microbiology, Department of Bacteriology, Guldhedsgatan 10, S-413 46 Göteborg, Sweden.

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