Taxonomic Study of *Nocardia farcinica* Using Serological and Physiological Characters

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Thirty-four strains designated *Nocardia farcinica*, four strains of *N. asteroides*, and one of *N. brasiliensis* have been serologically analyzed by means of an immunodiffusion technique. These strains have also been studied concerning their ability to produce arylsulfatase, to grow in the presence of 7% NaCl, and to grow at 45 C. The *N. farcinica* strains were divided into two well-distinguished clusters. One of these clusters comprised 24 strains, which appear to belong to the genus *Mycobacterium*, whereas the other cluster comprised 10 strains, which seem to belong to the genus *Nocardia*.

The type species of *Nocardia* is *Nocardia farcinica* Trevisan. This organism was described for the first time by Nocard in 1888 as the causal agent of bovine farcy in the West Indies (19). Trevisan gave it the genus designation *Nocardia* and called the species *Nocardia farcinica* (30). There now exist at least two different strains believed to be Nocard's original strain, but neither of them can be proved to be the authentic one.

A number of investigators (1, 5-7, 11, 16, 26), using various methods, have shown that strains initially labeled *N. farcinica* differ considerably from strains of other *Nocardia* species and show similarity to mycobacterial strains. In an earlier investigation, Ridell and Norlin (26) studied the serological relationships of various nocardia and mycobacterial strains. It was observed that out of eight strains referred to as *N. farcinica*, five were more closely related to some of the mycobacterial reference strains than were the other three strains. This indicated that these strains probably belong to the genus *Mycobacterium*. The remaining *N. farcinica* strains tested reacted like nocardia strains.

The aim of the present study is to elucidate the taxonomic position of strains labeled *N. farcinica*. The methods used in the investigation were immunodiffusion and tests for arylsulfatase activity and the ability to grow at 45 C and in the presence of 7% NaCl.

MATERIALS AND METHODS

Immunodiffusion. (i) **Test strains.** Thirty-four strains designated *N. farcinica*, or which previously had been referred to by this name, four strains of *N. asteroides* (Eppinger) Blanchard, and one of *N. brasiliensis* (Lindenberg) Pinoy were included in the study. Table 1 gives the serial numbers of these strains, their

designations as received, and earlier designations and sources, etc. Strains with similar designations, but obtained from different laboratories, have been treated as different strains, even if they might be of the same origin. The strains N. 34 and B.F. 5 might have the same origin, as well as N. 58 and N. 66, N. 121 and B.F. 6, N. 124 and B.F. 10, and also the strains N. 33, N. 57, and B.F. 4.

(ii) **Antigen preparations.** Antigens were prepared in two different ways. One was a culture filtrate and the other was an extract of disintegrated bacterial cells. The methods used for the preparation of antigens have been described previously (26).

(iii) **Antisera.** Immune sera for reference purposes were prepared against 12 reference strains (see Table 2). The sera against the two true nocardiae, i.e., *N. asteroides* and *N. caviae*, and the serum against the *N. farcinica* strain, as well as those against the two "rhodochrous" (8, 25) strains, i.e., *N. corallina* and "*M.*" *pellegrino*, were obtained from rabbits. The sera against the mycobacterial strains were obtained from sheep. The animals were subcutaneously injected with a mixture containing 10 parts of antigenic material, nine parts of liquid paraffin, and one part of Arlcel A. The rabbits received 1 ml and the sheep received 8 ml of this mixture once a week for 7 weeks and were bled 2 weeks after the last injection. Test bleedings during the immunization time were performed to evaluate the progress of the antibody response.

(iv) **Serological technique.** The analyses were carried out by means of a microplate modification (32) of the immunodiffusion technique according to Ouchterlony (22, 23).

(v) **Reference precipitation systems.** Twelve reference precipitation systems were utilized. The strains from which the reference systems were prepared are given in Table 2. The mycobacterial systems and the systems prepared from strains of the "rhodochrous" taxon have been presented earlier (20, 21, 25, 26). The former have been prepared and established by Norlin et al. (20, 21), whereas the nocardia, *N. farcinica* and "rhodochrous" systems have been prepared and established by the author.

Arylsulfatase activity and ability to grow at

TABLE 1. *Strains studied*

Laboratory no.	Designations, numbers, and sources
N. 33	<i>N. farcinica</i> 396/193. Received from Dr. Juhlin. ^a
N. 34	<i>N. farcinica</i> 397/194. Received from Dr. Juhlin.
N. 56	<i>N. farcinica</i> SN 5701. Received from Dr. Bönicke. ^b
N. 57	<i>N. farcinica</i> SN 5702. Received from Dr. Bönicke.
N. 58	<i>N. asteroides</i> EB 1957. <i>N. farcinica</i> ATCC 3318. Registered as type strain of <i>N. farcinica</i> . Received from Dr. Bönicke.
N. 66	<i>N. asteroides</i> RG 3318. <i>N. farcinica</i> ATCC 3318/NCTC 4524. Registered as type strain of <i>N. farcinica</i> . Received from Dr. Magnusson ^c (17) designated 752.
N. 67	<i>N. asteroides</i> ATCC 3399. Isolated by Dr. C. P. Fitch, N. Y. Received from Dr. Magnusson (17) designated <i>N. farcinica</i> 753.
N. 73	<i>N. farcinica</i> IP 736. Isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. Received from Dr. Magnusson (17) designated 749.
N. 74	<i>N. farcinica</i> IP 744. Isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. Received from Dr. Magnusson (17) designated 751.
N. 75	<i>N. farcinica</i> IP 378, ATCC 13781. Isolated from cattle with farcy in Dakar (17). Registered as authentic strain of <i>N. farcinica</i> (7). Received from Dr. Magnusson (17) designated 746.
N. 76	<i>N. farcinica</i> IP 740. Isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. Received from Dr. Magnusson (17) designated 750.
N. 118	<i>Actinomyces sumatrae</i> ATCC 6864. <i>N. asteroides</i> NCTC 1935. <i>N. cuniculi</i> . Isolated from rabbit in East Sumatra 1925. Received from Dr. Magnusson designated <i>N. farcinica</i> 645.
N. 119	<i>N. asteroides</i> M-ö 5011 S127S. Isolated from patient in Sweden (10). Received from Dr. Magnusson designated <i>N. farcinica</i> 844.
N. 120	<i>N. asteroides</i> M-ö 5010 S127R. Isolated from the same patient as N. 119 (10). Received from Dr. Magnusson designated <i>N. farcinica</i> 845.
N. 121	<i>N. farcinica</i> 434C. Isolated from cattle in Senegal. Received from Dr. Magnusson designated <i>Nocardia</i> sp. 874.
N. 122	<i>N. farcinica</i> 435A. Isolated from cattle in Senegal. Received from Dr. Magnusson designated <i>Nocardia</i> sp. 875.
N. 123	<i>N. farcinica</i> 435B. Isolated from the same animal as N. 122 in Senegal. Received from Dr. Magnusson designated <i>Nocardia</i> sp. 876.
N. 124	<i>N. farcinica</i> 435C. Isolated from cattle in Senegal. Received from Dr. Magnusson designated <i>Nocardia</i> sp. 877.
N. 125	<i>N. asteroides</i> 438C, 1015/167. Isolated in Yugoslavia. Received from Dr. Magnusson designated <i>N. farcinica</i> 878.
N. 126	<i>Nocardia</i> A 1758/68-69. Received from Dr. Magnusson designated <i>N. farcinica</i> 952.
B.F. 1	<i>N. farcinica</i> 1223 (M-Fa-4). Isolated from case of bovine farcy in Chad. Received from Dr. Gordon. ^d See ref. 16.
B.F. 2	<i>N. farcinica</i> 1226 (M-NF5). Isolated from case of bovine farcy in Chad. Received from Dr. Gordon. See ref. 16.
B.F. 3	<i>N. farcinica</i> 1242. Isolated from case of bovine farcy. Received from Dr. Gordon. See ref. 16.
B.F. 4	<i>N. farcinica</i> 1360, 5702, IP 396. Isolated from case of bovine farcy in French West Africa. Received from Dr. Gordon. See ref. 16.
B.F. 5	<i>N. farcinica</i> 1361, IP 397. Isolated from case of bovine farcy in French West Africa. Received from Dr. Gordon. See ref. 16.
B.F. 6	<i>N. farcinica</i> 1363, 434C. Isolated from cattle with bovine farcy in Senegal. Received from Dr. Gordon. See ref. 16.
B.F. 7	" <i>N.</i> " <i>farcinica</i> I 1224 (M-Fa-7). Chad. Received from Dr. Lechevalier ^e (16).
B.F. 8	" <i>N.</i> " <i>farcinica</i> I 1243. Sudan. Received from Dr. Lechevalier (16).
B.F. 9	" <i>N.</i> " <i>farcinica</i> I 1362 (M-433C). Senegal. Received from Dr. Lechevalier (16).
B.F. 10	" <i>N.</i> " <i>farcinica</i> I 1364 (M-435C). Senegal. Received from Dr. Lechevalier (16).
B.F. 11	" <i>N.</i> " <i>farcinica</i> NCTC 4524. Registered as type strain of <i>N. farcinica</i> , <i>N. asteroides</i> . Received from Dr. Lechevalier (16).
B.F. 12	" <i>N.</i> " <i>farcinica</i> I 1225 (M-Fa-17). Chad. Received from Dr. Lechevalier.
B.F. 13	" <i>N.</i> " <i>farcinica</i> I 1244. Sudan. Received from Dr. Lechevalier.
B.F. 14	" <i>N.</i> " <i>farcinica</i> I 931. Senegal. Received from Dr. Lechevalier.
N. 2	<i>N. asteroides</i> 320. Received from Dr. Juhlin.
N. 6	<i>N. asteroides</i> 633. Received from Dr. Juhlin.
N. 94	<i>N. asteroides</i> 432. Received from Dr. Gordon.
N. 114	<i>N. asteroides</i> ATCC 19247, RG 727. Suggested "working type." Received from ATCC. ^f
N. 115	<i>N. brasiliensis</i> ATCC 19296. Registered as reference strain. Received from ATCC.

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TABLE 2. Strains from which the antigens for the reference systems were prepared

Laboratory no.	Designations, numbers, and sources
N. 6	<i>N. asteroides</i> 633. Received from Dr. Juhlin. ^a
N. 41	<i>N. caviae</i> 318. Received from Dr. Juhlin.
N. 32	<i>N. corallina</i> M-ö 5007. Received from Dr. Juhlin.
N. 33	<i>N. farcinica</i> 396/193. Received from Dr. Juhlin.
N. 13	" <i>Mycobacterium</i> " <i>pellegrino</i> 107. Received from Dr. Juhlin.
MA 4	<i>M. avium</i> Ceppo Faisan IV, Runyon Group III. Received from Dr. Castelnuevo. ^b
MS 14	<i>M. fortuitum</i> 456, Runyon Group IV. Received from Dr. Castelnuevo.
MS 32	<i>M. kansasii</i> Atyp. 1, Runyon Group I. Received from Dr. Mantec.
MS 18	<i>M. marianum</i> (<i>scrofulaceum</i>) Ceppo chauviré. Received from Dr. Castelnuevo.
MS 8	<i>M. microti</i> SG 851. Received from Dr. Tauberneck. ^c
MS 1	<i>M. phlei</i> NCTC 8151 = ATCC 19249. Received from NBL. ^e
MC 1	<i>M. bovis</i> var. BCG Swedish substrain. Received from Dr. Sievers.

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45 C and in the presence of 7% NaCl. (i) **Test strains.** The 39 serologically analyzed strains and 53 other nocardia and "rhodochrous" strains were analyzed. The species designations which these strains had upon receipt are given in Table 5.

(ii) **Arylsulfatase activity.** Two different test methods were employed, the so-called phenolphthalein test according to Wayne et al. (34) and the Löwenstein-Jensen medium technique according to Tanzil and Bönicke (28).

(iii) **Growth at 45 C.** One loopful (approximately 10 mg) of bacterial mass from stock cultures on Löwenstein-Jensen medium was mixed with 2 ml of 0.85% saline. A 0.2-ml amount of this suspension was seeded on Löwenstein-Jensen medium in a screw-cap bottle and incubated at 45 C. The cultures were read after incubation for 3 weeks, and any colony formation observable by the naked eye was registered as growth.

(iv) **Growth in the presence of 7% NaCl.** One loopful (approximately 10 mg) of bacterial mass from stock cultures on Löwenstein-Jensen medium was

mixed with 2 ml of 0.85% saline. An 0.2-ml amount of this suspension was seeded on Löwenstein-Jensen medium containing 7% NaCl in a screw-cap bottle and incubated at 37 C. The cultures were read after incubation for 3 weeks, and any colony formation observable by the naked eye was registered as growth.

RESULTS

The 39 strains listed in Table 1 were studied according to the comparative immunodiffusion technique employing the 12 reference systems indicated in Table 2. The result of this study is shown in Tables 3 and 4.

Figure 1 shows the principles of the comparative immunodiffusion analyses. The pattern obtained when the antigen preparation of the strain *N. farcinica* N. 76 was studied by means of the "*M.*" *pellegrino* reference system is shown. Four precipitinogens in total were demonstrated as being common to the test strain and the reference strain. Three of the precipitinogens in the test strain influenced the precipitates of the reference system. These three precipitinogens could thus be identified as corresponding to aA and eE (fusion of lines) and bB (deviation phenomena only) of the reference system.

Table 3 shows the number of precipitinogens demonstrated for each strain and each of the reference sera. The number of demonstrated precipitinogens which influenced the precipitates in the reference systems is indicated in parentheses. The far right column shows the result obtained by means of the four mycobacterial reference systems: *Mycobacterium kansasii*, *M. marianum*, *M. phlei*, and *M. microti*. The maximum total number of precipitinogens demonstrated and the maximum number of precipitinogens influencing the reference system by means of any of these four systems are given. In 24 of the tested *N. farcinica* strains, three

FIG. 1. Schematic illustration of the pattern obtained by comparative immunodiffusion analyses when the antigen preparation from the strain *N. farcinica* N. 76 was analyzed by means of the "*M.*" *pellegrino* reference system.

TABLE 3. Number of precipitinogens revealed in the 38 tested strains when analyzed by means of nocardia, rhodochrous, and mycobacterial reference systems

Cluster	Strain	Reference system								
		<i>N. asteroides</i> (7) ^a	<i>N. caviae</i> (10)	<i>N. corallina</i> (7)	" <i>M.</i> " <i>pellegrino</i> (7)	<i>N. farcinica</i> (8)	<i>M. avium</i> (7)	<i>M. fortuitum</i> (15)	BCG (12)	4 Myco- bacterial systems
Cluster A, <i>N. farcinica</i> strains	N. 33	—	—	1	1(1) ^b	8(8)	5(4)	8(4)	4	4 ^c [2] ^d
	N. 34	1	—	—	1	4(3)	5(2)	5(2)	5	4 [1]
	N. 56	1	2(1)	1	1	6(5)	5(2)	5(1)	5(1)	4 [1]
	N. 57	2	1(1)	—	1	6(4)	6(2)	7(2)	3(1)	5 [2]
	N. 73	1	3	1	1	5(3)	4(1)	6(5)	4(2)	4 [2]
	N. 75	1	3	1	1	4(2)	5(2)	5(3)	6(1)	3 [2]
	N. 121	—	2	1	—	5(3)	5(1)	4(3)	4(2)	4 [2]
	N. 122	1	3	—	1	5(3)	6(2)	5(3)	5(3)	4 [2]
	N. 123	1	4	—	2	5(4)	5(1)	6(5)	4(3)	4 [1]
	N. 124	1	1	—	—	5(2)	3(2)	5(4)	5(1)	3 [2]
	B.F. 1	—	2	—	—	5(5)	5(1)	3(2)	5(2)	5 [1]
	B.F. 2	1(1)	1	2	1	6(3)	5(2)	6(4)	5(2)	4 [1]
	B.F. 3	2	2	1	—	4(3)	3(2)	3(1)	3(2)	4 [2]
	B.F. 4	3	2	—	2	5(5)	5(2)	5(3)	6(1)	5 [2]
	B.F. 5	1	3	—	—	5(3)	6(2)	6(4)	3(2)	4 [2]
	B.F. 6	—	1	1	—	5(3)	5(2)	6(4)	5(2)	4 [2]
	B.F. 7	—	1	1	1	6(4)	5(2)	5(2)	6(2)	5 [2]
	B.F. 8	—	2	—	—	6(5)	5(1)	4(3)	5(2)	4 [2]
	B.F. 9	—	2	1	—	5(4)	5(2)	5(3)	4(1)	4 [2]
	B.F. 10	1	—	—	—	6(4)	7(2)	6(6)	4(2)	4 [2]
	B.F. 11	—	3	1	—	4(4)	4(1)	5(5)	2(1)	5 [2]
	B.F. 12	—	—	—	—	6(6)	5(1)	4(3)	4	5 [2]
	B.F. 13	—	3	1	—	6(3)	4	4(2)	5(2)	4 [2]
	B.F. 14	1(1)	2(1)	2	—	5(3)	4(1)	3(3)	5	3 [2]
Cluster B, <i>N. farcinica</i> strains	N. 58	4(2)	5(3)	1	4(1)	1(1)	2	2	2(1)	2 [1]
	N. 66	4(2)	5(2)	1(1)	2(1)	1	1(1)	—	2	2
	N. 67	3(2)	4(1)	2	—	—	1	—	2	2
	N. 74	4(2)	4(1)	1	—	1	2(1)	—	2	1
	N. 76	3(2)	3(2)	1(1)	4(3)	2	2(1)	—	2	1
	N. 118	4(1)	3(2)	—	1	1	2(1)	2	3	3 [2]
	N. 119	4(3)	4(3)	1	—	1	3(1)	1	2	3 [2]
	N. 120	2(1)	1	1	1	1	2	1	1	1
	N. 125	4(1)	5(3)	—	3(2)	2	3(1)	—	1	2 [1]
<i>N. asteroides</i> and <i>N. brasiliensis</i> strains	N. 2	4(4)	4(2)	2	2	1	3(2)	—	2(1)	2
	N. 6	7(7)	6(5)	1	1	2	1(1)	1	2(1)	2 [1]
	N. 94	4(3)	3(3)	3	4(1)	1	1(1)	1	2	2 [2]
	N. 114	3(2)	6(2)	—	2	1	1	—	2	2
	N. 115	4(2)	4(2)	—	3	2	3(2)	—	2	2 [1]

^a Number of precipitinogens in the reference system.^b Number in parentheses represents number of precipitinogens which influenced precipitates in the reference system.^c Maximum total number of precipitinogens revealed with any of the following reference systems: *M. kansasii*, *M. marianum*, *M. microti*, and *M. phlei*.^d Number in brackets is the maximum number of precipitinogens influencing the reference system, revealed by any of the following reference systems: *M. kansasii*, *M. marianum*, *M. microti*, and *M. phlei*.

precipitinogens at maximum were demonstrated by means of the nocardia (*N. asteroides*, *N. caviae*) reference systems. Only a few of these precipitinogens were identified. Strain N. 123 was an exception, with four precipitinogens

being demonstrated with the *N. caviae* reference system. A maximum of two precipitinogens was obtained when these 24 strains were analyzed by means of the two "rhodochrous" (*N. corallina*, "*M.*" *pellegrino*) reference sys-

tems. When the same 24 strains were analyzed by means of the *N. farcinica* reference system and the seven mycobacterial reference systems, five precipitinogens or more (up to eight) were revealed in most cases. A number of these precipitinogens influenced the precipitates in the reference systems. These 24 *N. farcinica* strains are discussed as strains of cluster A. It should be mentioned that strain N. 33, which is used as a reference strain, belongs to this cluster.

The remaining strains consisted of 10 *N. farcinica* strains, which are discussed below as strains of cluster B, and four *N. asteroides* and one *N. brasiliensis* strain. Twelve of these 15 strains demonstrated four or more (up to six) common precipitinogens, and two of them (N. 126 and N. 76) demonstrated three precipitinogens when tested with either of the reference strains for *N. asteroides* or *N. caviae*. When these 15 strains were analyzed by means of the two "rhodochrous" reference systems, none, one, two or three, and, in three cases, four precipitinogens were revealed. When the same strains were analyzed by means of the *N. farcinica* and mycobacterial reference systems, a maximum of three precipitinogens was revealed. One strain (N. 120) did not display more than one or two precipitinogens with any of the reference systems.

The distribution of strains according to the number of precipitinogens revealed by each reference system is summarized in Table 4. It can be seen that the *N. farcinica* strains of cluster A had more precipitinogens in common with the mycobacterial reference strains than with the true nocardia and "rhodochrous" reference strains. The *N. farcinica* strains of cluster B, and the strains of *N. asteroides* and *N. brasiliensis*, are distributed in a different way. These strains proved to have more precipitinogens in common with the reference strains of *N. asteroides* and *N. caviae* than with the other reference strains.

When the arylsulfatase activity (see Table 5) was tested, 16 of the tested strains, all belonging to cluster A of the *N. farcinica* strains, reacted positively. None of the cluster B strains or the other strains tested gave a positive reaction. The same result was obtained with both methods used. In Table 5 it is also shown that none of the 24 cluster A strains grew at 45 C, whereas all 10 cluster B strains grew at this temperature. Further, the ability to grow in the presence of 7% NaCl is shown. Nine of the 24 cluster A strains and all cluster B strains grew under such conditions.

DISCUSSION

With immunodiffusion analyses, antigens (precipitinogens) shared by two strains can be demonstrated. Individual precipitates may also be identified. However, there are several reasons for failure to demonstrate identity reactions using this technique; e.g., the pertinent precipitinogen might exist in very different concentrations in the preparations of the strains compared. The presence of precipitates can be regarded as a demonstration of precipitinogens which are common to the test strain and the reference strain even though no identity reaction occurs. It should also be mentioned that the presence of a common antigen might be demonstrated by the presence of deviation phenomena. It can be said that the greater the number of precipitinogens shared by a group of strains becomes, the closer their relationship. Such conclusions should only be drawn if a considerable number of strains have been analyzed and the results are in agreement or when a smaller number of strains have been analyzed with the aid of a relatively large number of reference systems. Further, the number of precipitinogens in the reference system constitutes an important factor. Differences of one or two precipitinogens can have a limited value for differentiation purposes, as small variations in preparing antigens and antisera may result in variations in the number of precipitinogens demonstrated.

In earlier investigations (25, 26) it has been shown that many strains from different species of the genera *Nocardia* and *Mycobacterium*, and of the "rhodochrous" taxon, had one to three precipitinogens in common. However, some strains shared a greater number of precipitinogens, indicating a closer affinity and possibly a species relationship. On the basis of such results, it was shown that, among eight strains referred to as *N. farcinica*, five were closely related to mycobacterial strains, whereas three reacted like nocardiae. All of these eight strains are included in the present study.

The *N. farcinica* strains were divided into two different groups, cluster A and cluster B. Cluster A, consisting of 24 strains, showed greater serological similarity to the mycobacterial reference strains and to the *N. farcinica* reference strain N. 33 than did the other strains tested. The serological similarity with the *N. asteroides*, *N. caviae*, *N. corallina*, and "*M.*" *pellegrino* reference strains was, on the other hand, relatively small. These results indicate that the strains of cluster A are closely related to strains of various species of the genus *Mycobacterium*

TABLE 4. Distribution of the 39 test strains according to number of precipitates, demonstrated by means of each reference system

Cluster	No. of precipitates	No. of strains reacting with the reference system								4 Mycobacterial systems ^b
		<i>N. asteroides</i> (7) ^a	<i>N. caviae</i> (10)	<i>N. corallina</i> (7)	" <i>M.</i> " <i>pellegrino</i> (7)	<i>N. farcinica</i> (8)	<i>M. avium</i> (7)	<i>M. fortuitum</i> (15)	BCG (12)	
Distribution of <i>N. farcinica</i> strains of cluster A	8					1		1		
	7						1	1		
	6					8	3	6	3	
	5					11	14	9	10	6
	4		1			4	4	4	7	15
	3	1	6				2	3	3	3
	2	2	8	2	2				1	
	1	11	5	11	9					
	0	10	4	11	13					
Distribution of <i>N. farcinica</i> strains of cluster B	8									
	7									
	6		3							
	5									
	4	6	3		2					
	3	3	3		1		3		1	2
	2	1		1	1	3	5	2	6	4
	1		1	6	2	6	2	2	2	4
Distribution of 4 <i>N. asteroides</i> and 1 <i>N. brasiliensis</i> strains	0			3	4	1		6	1	
	8									
	7	1								
	6		2							
	5									
	4	3	2		1					
	3	1	1	1	1		2			
	2			1	2	2			5	5
	1			1	1	3	3	2		
	0			2				3		

^a Number in parentheses indicates the number of precipitinogens in the reference system.

^b Distribution of strains according to the maximum number of precipitinogens revealed by any of the following reference systems: *M. kansasii*, *M. marianum*, *M. microti*, and *M. phlei*.

and that they should probably be classified in this genus. It should be mentioned that the reference strain N. 33 is also included among the test strains and belongs to cluster A.

The other group, cluster B, consisted of 10 strains displaying a greater serological affinity to the reference strains of *N. asteroides* and *N. caviae* than did strains of cluster A. The cluster B strains also showed a relatively low degree of serological affinity to the mycobacterial reference strains. Furthermore, the four tested strains of *N. asteroides* (one of which was the *N. asteroides* reference strain and another the suggested working type of *N. asteroides*) and the *N. brasiliensis* strains gave similar results when tested. Therefore, it is probable that the strains of cluster B are nocardia strains. In this

connection it should be mentioned that several of the strains in cluster B have also been referred to as *N. asteroides*.

Some of the strains in cluster B had, at most, four (two strains had only three) precipitinogens in common with any of the reference strains. It should, however, be emphasized that the number of precipitates of the nocardia reference systems, and also of the "rhodochrous" reference systems, was generally smaller than the number of precipitates of the mycobacterial reference systems.

Two of the tested strains placed in cluster B and one *N. asteroides* strain had four precipitinogens in common with the reference strains for "*M.*" *pellegrino*. All the other tested strains, however, revealed a relatively low serological

TABLE 5. Results of the arylsulfatase test and the tests of ability to grow at 45 C and in the presence of 7% NaCl

Cluster or group of strains	Total no. of tested strains	No. of strains					
		Aryl-sulfatase activity		Growth at 45 C		Growth with 7% NaCl	
		+	-	+	-	+	-
<i>N. farcinica</i> strains of cluster A	24	16	8	0	24	9	15
<i>N. farcinica</i> strains of cluster B	10	0	10	10	0	10	0
<i>Nocardia</i> and rhodochrous strains ^a	58	0	58	14	44	46	12

^a Strains carrying the designations *N. asteroides*, *N. blackwellii*, *N. brasiliensis*, *N. caviae*, *N. corallina*, *N. polychromogenes*, *N. rubra*, *N. rubropertincta*, "M." *pellegrino*, and "M." *rhodochrous*.

relationship to the two "rhodochrous" reference strains. In an earlier investigation by Ridell (25) it was stated that some "rhodochrous" strains were not more closely related to the true nocardiae (i.e., species like *N. asteroides*) than they were to the mycobacteria. The result of the present study confirms this view. No further conclusions concerning the taxonomical position of the "rhodochrous" taxon can be made based on the results of this study.

Strain N. 120 had only one or two precipitinogens in common with any of the reference strains. However, it has tentatively been placed in cluster B. This strain and strain N. 119 (referred to cluster B) were both isolated from a patient suffering from hemolytic anemia and treated with steroids (10). The two strains had primarily been designated *N. asteroides* (10) but were later designated *N. farcinica* by Magnusson (personal communication).

The demonstration of arylsulfatase (see Table 5) shows that most strains of cluster A reacted positively, whereas such activity was not revealed in any of the other nocardia or rhodochrous strains tested. Mycobacterial strains have also been studied for arylsulfatase activity at our laboratory. Strains referred to as *M. borstelense*, *M. fortuitum*, and *M. marianum* reacted positively, whereas other tested strains, representing all four Runyon groups, reacted negatively (unpublished data). A number of investigators (12-15, 24, 28, 29, 33-35) have used the arylsulfatase test to differentiate mycobac-

terial species, and it has been shown that this enzyme is easily demonstrated in several species of mycobacteria. Tanzil and Bönicke (28), investigating nocardia strains, showed that, out of seven *N. farcinica* strains tested, five exhibited a comparatively high degree of arylsulfatase activity whereas the remaining two strains of this species, as well as strains of other species, showed little or no activity. The results from arylsulfatase tests lend further support to the view that the strains of cluster A should be referred to the genus *Mycobacterium*. The dissimilarity between the strains of the two clusters, A and B, is also reflected by the findings obtained with the growth temperature and saline resistance tests (see Table 5) performed in this study.

The results of the present tests justify the division of the examined *N. farcinica* strains into two groups, one of which might be referred to as the genus *Mycobacterium* and the other as *Nocardia*. Various investigators (1, 5-7, 11, 16, 26), using different methods, have shown that certain *N. farcinica* strains should be placed among the mycobacteria, whereas others (2, 3, 9, 31) have shown that certain *N. farcinica* strains are closely related to strains of *N. asteroides*.

Chamoiseau et al. (1, 5-7) showed that bacteria isolated from zebu with farcy in Africa, as well as the *N. farcinica* strain ATCC 13781 (N. 75), should be classified with the mycobacteria, and the name *Mycobacterium farcinogenes* was proposed (6). It is of interest to note that strain ATCC 13781 has been discussed as possibly being Nocard's original strain (7, 16). Fourteen of the strains included in the present study and placed in cluster A (B.F. 1-14) have been analyzed earlier by Lechevalier et al. (16; personal communications) concerning the presence of mycolic and nocardiomycolic acids. They placed all these strains in the genus *Mycobacterium*.

A special problem in discussing the taxonomy of strains labeled *N. farcinica* are cultures designated ATCC 3318 and NCTC 4524. In the list by Sneath and Skerman of 1966 (27) the type strain of *N. farcinica* is registered by the two collection numbers NCTC 4524/ATCC 3318. In the NCTC catalogue, NCTC 4524 and ATCC 3318 are treated as identical and are designated *N. asteroides*. However, Lechevalier et al. (16) obtained different results when analyzing the lipids of the two strains, one of which was designated NCTC 4524 and the other ATCC 3318. They placed the former strain among the mycobacteria and the latter among the nocardiae. In this connection it should be

mentioned that the description of *N. farcinica* in *Bergey's Manual* (4) is based on the strain ATCC 3318.

In 1962 Gordon and Mihm (9) studied a specimen labeled ATCC 3318 and placed this strain with *N. asteroides*. Magnusson and Mariat (17), however, analyzed later a specimen with the double designation ATCC 3318/NCTC 4524 by the delayed-type skin reaction and found that this strain was clearly distinct from *N. asteroides*. They kept the designation *N. farcinica* for this strain and referred several other strains to the same species. Some of these strains were included in the present study and were all classified in cluster B. It should also be mentioned that in an earlier investigation by Ridell and Norlin (26) other strains received from Magnusson et al. have been analyzed. In that study all tested strains, which they referred to the same group as *N. farcinica* ATCC 3318/NCTC 4524, reacted like most tested nocardia strains, e.g., *N. asteroides*. Mordarska et al. (18), analyzing NCTC 4524, stated that it should not be retained in the genus *Nocardia*. Tsukamura (31), as well as Berd (2), divided strains of *N. asteroides* into two subgroups, placing the strain ATCC 3318 in one of these groups. Berd found, however, that among several *N. farcinica* strains five strains were distinct from any of these groups.

Thus, it has been shown by several investigators that some strains called *N. farcinica* (including strains designated NCTC 4524) should be classified as mycobacteria (16), whereas other *N. farcinica* strains (including strains designated ATCC 3318 and strains carrying the double designation ATCC 3318/NCTC 4524) seem to be nocardiae (2, 9, 16, 17, 26, 31). The latter shared great similarities with *N. asteroides*. However, this species has been shown to be heterogenous (2, 31).

In the present study were included one specimen designated ATCC 3318 (N. 58), obtained from Bönicke, one specimen designated ATCC 3318/NCTC 4524 (N. 66), obtained from Magnusson, and one specimen designated NCTC 4524 (B.F. 11), obtained from Lechevalier. Strains N. 58 and N. 66 were referred to cluster B (nocardia-like strains), whereas strain B.F. 11 was placed in cluster A (mycobacteria-like strains). Thus, the results obtained in the present study were in accordance with those concerning the type strain confusion, which have been presented by earlier investigators.

It is of interest to note that 18 out of the 24 cluster A strains with known origin were isolated in Africa, whereas only two out of the 10 cluster B strains came from that continent.

Further studies must, however, be carried out to elucidate a possible geographic difference concerning the prevalence of cluster A and cluster B strains.

Even if the results of the present study, as well as other studies, indicate that some *N. farcinica* strains should be referred to the genus *Mycobacterium*, it is still not settled which taxonomic position within this genus these strains should have. Chamoiseau (6) has proposed a new species divided into two subspecies: *Mycobacterium farcinogens* subsp. *sene-galense* and subsp. *tchadense*. In the present study some of the strains of cluster A showed serological similarities to the reference strains of *M. fortuitum*, whereas others demonstrated a relatively close relationship to *M. avium* or *M. bovis* var. BCG. However, these similarities are not great and unequivocal enough to suggest a close affiliation of the actual *N. farcinica* strains to any of these species.

The result of the serological study, supported by the results obtained with the other three tests, shows that strains labeled *N. farcinica* form two distinct clusters, one of which might be referred to the genus *Nocardia* and the other to the genus *Mycobacterium*. However, their relationship to already recognized species within these genera remains unsettled. Further, the question of the nomenclature is complex, due to, among other things, the fact that no strain has been proven to be Nocard's authentic strain.

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