Delineation of *Nocardia farcinica* by Delayed Type Skin Reactions on Guinea Pigs

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SUMMARY

An immunological method based on the specificity of delayed-type skin reactions on guinea-pigs has been used for comparing 17 *Nocardia* strains. By this method, 4 isolates labelled *Nocardia farcinica*, four labelled *N. asteroides* and 1 strain labelled *N. blackwellii* could be grouped together with the type strain of *N. farcinica*. Three other strains labelled *N. farcinica* were clearly distinct from this group of 10 strains. A strain of *N. asteroides* used previously as reference strain for this species was clearly distinct from the *N. farcinica* strains, and this applied also to a recently proposed reference strain of *N. asteroides*. A strain of *N. brasiliensis* was clearly distinct from all the other strains, while the results obtained with a strain received without species designation were inconclusive. On this basis, the authors consider that all the 10 strains, which form a homogeneous group, should be classified as *N. farcinica*. The 3 other strains labelled *N. farcinica* should be classified into 2 other species. *Nocardia asteroides* would seem to be a separate species.

INTRODUCTION

In 1888 Nocard reported the isolation of a micro-organism which caused farcy in cattle in Guadeloupe (Nocard, 1888). The following year Trevisan described this organism under the name *Nocardia farcinica* (Trevisan, 1889). Based on a study of a culture (laboratory no. 752; see Table 1) believed to be Nocard's original and a similar strain (753) isolated and identified by Dr C. P. Fitch (New York) Waksman (1957) gave a more recent description of this species. Strain 752 is the type strain of *N. farcinica* (Sneath & Skerman, 1966).

Eppinger in 1891 described a new pathogenic micro-organism *Cladothrix asteroides* which was isolated from a man and which caused 'pseudotuberculosis' (Eppinger, 1891): this organism was named *Nocardia asteroides* by Blanchard (1896). A more recent description of *N. asteroides* is given by Waksman (1957). As an *ad hoc* reference strain of *N. asteroides*, strain 809 has recently been proposed (Sneath & Skerman, 1966).

Ørskov (1923) found differences between *Nocardia farcinica* (one isolate) and *N. asteroides* (three isolates) in morphology, and Erikson (1935), studying the same strain of *N. farcinica* as did Ørskov but two other strains of *N. asteroides*, later confirmed those observations. According to Waksman (1957), *N. farcinica* and *N. asteroides* can be distinguished by the colour of the vegetative mycelium. Mariat (1963) found some differences between *N. farcinica* and *N. asteroides* as regards capacity for synthesizing urease. On the other hand, in 1962 Gordon & Mihm published an expanded des-

cription of *N. asteroides* based on 142 strains, in which the 2 strains of *N. farcinica* mentioned above (752 and 753) could not be distinguished from those of *N. asteroides* by any of the observations or tests used.

Silvestri, Turri, Hill & Gilardi (1962) also referred *Nocardia farcinica* 752 to the same cluster ('sphere XI') as some strains of *N. asteroides*, including strain 809 mentioned above. However, this sphere XI also contained strains of several other species of *Nocardia* and one strain of *Mycobacterium phlei* (NCTC 8151). It is therefore probable that this sphere does not represent a single species in the sense in which this term has been used previously by other authors but is rather a taxon at a higher level. Thus it is not quite clear from previous studies whether *N. farcinica* and *N. asteroides* are, in fact, one or two species.

An immunological method, based on the specificity of the delayed type of skin reaction in guinea pigs, has been useful for classification of mycobacteria, and particularly for identification of species within this genus (Magnusson, 1961, 1965, Magnusson, Engbæk & Bentzon, 1961). By further application of this method on eight strains, it was found that distinction between *Nocardia asteroides* and *N. brasiliensis* was possible (Magnusson, 1962). The purpose of the present work was to see whether strains classified by other laboratories as *N. farcinica* and *N. asteroides* can be distinguished in the same way.

METHODS

Organisms. Strains classified as *Nocardia farcinica* or *N. asteroides* in various bacteriological laboratories or hospitals were included in the study, together with a strain of *N. brasiliensis* for comparison purposes. The origin and source of all the organisms used are shown in Table 1. Strain 752 is the type strain of *N. farcinica*, authentic co-type (Sneath & Skerman, 1966).

The cultures were maintained on Sauton medium in 250 ml. flasks at 38° and subcultured monthly.

Condition for growth and harvest. Cultures were inoculated by loop into 180 ml. Sauton medium in a 250 ml. flask and incubated at 38°. The cultures were incubated for 3–10 weeks, depending on the growth rate, and sterilized by heating in flowing steam for 1 hr. The organisms were harvested by paper filtration.

Immunological method. The experimental procedure was the same as that described previously (Magnusson, 1961; Magnusson *et al.* 1961), except for an amended method of sensitizing the guinea pigs and, in some cases culture filtrates instead of purified sensitins were used for the skin tests.

Sensitization of guinea pigs. One hundred and forty-eight albino guinea pigs (350–600 g.) were sensitized by intradermal injection of dried heat-killed organisms in liquid paraffin (Bayol 55). Each animal was given four simultaneous injections, each of 0.1 ml., of a suspension containing 0.4 mg. dried bacteria/ml.

Skin tests. Intradermal tests were done 3–4 weeks after sensitization, by using dilutions of filtrates of heat-sterilized cultures (cf) or purified sensitins (rs) prepared by a procedure similar to that used for tuberculin purified protein derivative. The culture filtrates were diluted 1/50 and 1/500 with isotonic phosphate-buffered saline (pH 7.38) containing 0.01 % chinosol and 0.005 % Tween 80 (Magnusson *et al.* 1958). Purified sensitins were used in doses of 2 µg. and 0.2 µg./0.1 ml. of the same diluent. Each injection consisted of 0.1 ml.

Table 1. *Strains studied*

Laboratory no.	Name when received, source* and culture collection numbers
80	<i>Nocardia</i> sp. ssc 261. Originally isolated from a Danish patient by Dr P. Holm, Statens Seruminstitut, Copenhagen. PH 184/45-46
81	<i>Nocardia asteroides</i> ssc 262. Originally isolated from a chicken by Dr O. J. Mayfield, D.V.M., Charles City, Iowa, U.S.A. EM 9935. M ^o CL 93. RG 404
84	<i>N. asteroides</i> PH 598/51-52. Originally isolated from placenta of a cow, see Case II in Fey <i>et al.</i> (1954)
85	<i>N. asteroides</i> PH 599/51-52. Originally isolated from a dog, see Case I in Fey <i>et al.</i> (1954)
117	<i>N. brasiliensis</i> RG 774A. Originally isolated from a submandibular abscess (Gordon & Mihm, 1959). M ^o CL 254
650	<i>N. asteroides</i> RG 784. Received at the NCTC, London, in June 1944 from N. F. Conant, Duke University Medical Center, Durham, North Carolina, U.S.A. NCTC 6761
677	<i>N. farcinica</i> G 396. IP 396
678	<i>N. farcinica</i> G 397. IP 397
745	<i>N. asteroides</i> E. Originally isolated from a Swedish patient (Bergström <i>et al.</i> 1966)
746	<i>N. farcinica</i> 378. Originally isolated from cattle at the Laboratoire élevage Curasson, Dakar. ATCC 13781. IP 378
749	<i>N. farcinica</i> 736. Originally isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. IP 736.
750	<i>N. farcinica</i> 740. Originally isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. IP 740
751	<i>N. farcinica</i> 744. Originally isolated from cattle with farcy at the Farcha Laboratory, Fort-Lamy, Chad. IP 744
752	<i>N. asteroides</i> RG 3318. Originally isolated from cattle with farcy by Nocard (Waksman, 1957). Type strain of <i>N. farcinica</i> , authentic co-type, see Sneath & Skerman (1966). ATCC 3318. NCTC 4524
753	<i>N. asteroides</i> RG 3399. Originally isolated by Dr C. P. Fitch, New York, U.S.A. (Waksman, 1957). ATCC 3399
787	<i>N. blackwellii</i> M ^o CL 81. Originally isolated from a foal (Erikson, 1935). ATCC 6846. NCTC 630. RG 503
809	<i>N. asteroides</i> RG 727. Originally isolated from sputum from a patient named Jackson by Dr W. M. Bowman, Georgia State Department of Public Health. Proposed as reference strain of <i>N. asteroides</i> (Sneath & Skerman, 1966).

* Cultures labelled SSC were received from Dr H. C. Engbæk, Statens Seruminstitut, Copenhagen, Denmark; those labelled PH were received from Dr P. Holm, Statens Seruminstitut, Copenhagen, Denmark; those labelled RG were received from Dr Ruth Gordon, Institute of Microbiology, Rutgers's State University, New Brunswick, New Jersey, U.S.A.; those labelled G were received from Dr Guilhon, Ecole Vétérinaire, Alfort, France; the one labelled E was received from Dr L. Edebo, Department of Bacteriology, University of Uppsala, Sweden, and the one labelled McCL from Dr N. M. McClung, Department of Bacteriology, University of Georgia, Athens, Georgia, U.S.A.

ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; EM, Dr C. W. Emmons, National Institute of Allergy and Infectious Diseases, Bethesda, Md., U.S.A.; IP, Institut Pasteur, Paris, France; NCTC National Collection of Type Cultures, London, England.

Experimental design. The data presented here were drawn from a series of 16 studies. In most cases the experimental design of the single studies was one of those described previously (Magnusson *et al.* 1961), 5 strains being compared with each other, or each of 4 strains compared with each of 4 other strains. However, results were also drawn from one study in which 10 strains were compared with each other, and from another in which each of 9 strains was compared with each of 9 other strains. In the two latter studies only the highest concentration of each sensitin was used.

Reading of reactions. The reactions were read after 24 hr and the longitudinal and transverse diameters of erythema recorded in millimetres. Each reaction was read by two readers. To avoid bias in reading the reactions, the dilutions were labelled by code.

Calculation of specificity differences (spd). Using averages of the reading of both readers, the difference in specificity of two sensitins was calculated as the difference between the homologous and the heterologous reactions as follows: $spd = (A_a + B_b) - (A_b + B_a)$, where A_a is the average homologous reaction obtained with sensitin A in animals sensitized with strain a , A_b is the average heterologous reaction obtained with sensitin A in animals sensitized with strain b , B_b and B_a denote the average homologous and heterologous reactions with B sensitin. Examples of the calculation have been published previously (Magnusson, 1961).

Each spd value reported below is the average of four determinations. In previous studies (Magnusson, 1961, Magnusson *et al.* 1961) where the same technique has been used, the standard deviation was 1.1–1.4 mm. for $spd \leq 3$ mm. and 2.0–2.2 mm. for $spd > 3$ mm.

RESULTS

The results of calculation of the specificity differences (spd) are shown in Table 2; sensitins showing $spd \leq 3$ mm are grouped together. The largest group contains 10 sensitins, including preparations from strain 752, which presumably originates from Nocard, and four other strains (749, 750, 751, 753) designated *Nocardia farcinica* as received. Sensitins from three strains (84, 85, 787), isolated from a cow, a dog and a

Table 2. *Specificity differences of sensitins*

The table shows the differences (in mm.) between homologous and heterologous reactions to intradermal tests in guinea pigs sensitized with the strains used for preparation of the sensitins. Italicized figures were published previously (Magnusson, 1962).

Strain	Sensitin															
	RS 84	RS 85	cf 752	cf 753	cf 749	cf 750	cf 751	cf 650	cf 745	cf 787	RS 80	cf 678	cf 746	cf 677	RS 81	cf 809
*RS 84 <i>Nocardia asteroides</i>	—	0	2	1	0	—1	0	3	3	2	4	12	15	13	13	3
RS 85 <i>N. asteroides</i>	0	—	2	3	—	—	—	—2	2	—	5	—	—	—	11	—
†cf 752 <i>N. farcinica</i>	2	2	—	3	0	1	3	—	3	1	2	11	—	6	11	6
cf 753 <i>N. farcinica</i>	1	3	3	—	1	0	0	—	—	2	3	14	—	11	13	6
cf 749 <i>N. farcinica</i>	0	—	0	1	—	—2	1	—	—	2	3	14	—	11	10	7
cf 750 <i>N. farcinica</i>	—1	—	1	0	—2	—	2	—	—	3	3	16	—	14	12	11
cf 751 <i>N. farcinica</i>	0	—	3	0	1	2	—	—	—	—	2	—	—	—	10	9
cf 650 <i>N. asteroides</i>	3	—2	—	—	—	—	—	—	—	—	4	—	—	—	10	—
cf 745 <i>N. asteroides</i>	3	2	3	—	—	—	—	—	—	—	2	—	—	—	12	—
cf 787 <i>N. blackwellii</i>	2	—	1	2	2	3	—	—	—	—	—	—	—	—	—	—
RS 80 <i>Nocardia</i> sp.	4	5	2	3	3	3	2	4	2	—	—	13	—	28	17	9
cf 678 <i>N. farcinica</i>	12	—	11	14	14	16	—	—	—	—	13	—	—2	9	10	14
cf 746 <i>N. farcinica</i>	15	—	—	—	—	—	—	—	—	—	—	—2	—	10	—	—
cf 677 <i>N. farcinica</i>	13	—	6	11	11	14	—	—	—	—	28	9	10	—	21	12
RS 81 <i>N. asteroides</i>	13	11	11	13	10	12	10	10	12	—	17	10	—	21	—	9
cf 809 <i>N. asteroides</i>	3	—	6	6	7	11	9	—	—	—	9	14	—	12	9	—
RS 117 <i>N. brasiliensis</i>	14	—	—	—	—	—	—	—	—	—	18	11	—	14	13	9

* RS = purified sensitin. † cf = heat-sterilized culture filtrate.

foal respectively, are also included in this group. This also applies to the sensitin of strain 745, isolated from man, and to strain 650, of unknown source.

Sensitins from three other strains (678, 746, 677) designated *Nocardia farcinica* on receipt cannot be included in this group (spd = 6–16 mm), but two of them (cf 678, cf 746) can be grouped together (spd = –2 mm).

Sensitin from strain 80 shows spd $\leq$ 3 mm. when compared with 6 of the sensitins in the main group and spd = 4–5 mm. when compared with the sensitins of three strains in this group. (The spd between RS 80 and RS 84 reported previously (Magnusson, 1962) is not correct, because of an error in calculation.) Therefore this sensitin 80 cannot be included in the main group by the criterion mentioned above.

Sensitins from all 14 strains show spd values varying from 3 to 21 mm. when compared with sensitins of *Nocardia asteroides* strain 81, *N. asteroides* strain 809 and *N. brasiliensis* strain 117, and therefore none of these preparations are grouped together.

The spd between sensitins of *Nocardia asteroides* strain 81 and *N. asteroides* strain 809 is 9 mm.

Classification of the strains on the basis of sensitin specificity

On the basis of the specificity differences of the sensitins and with the criteria for their utilization in classification used earlier (Magnusson *et al.* 1961; Magnusson, 1962), the strains can be classified as follows: strains 84, 85, 650, 745, 749, 750, 751, 752, 753, 787 as *Nocardia farcinica*; strains 678 and 746 as *Nocardia* sp.; strain 677 as *Nocardia* sp. and strain 80 as *Nocardia* sp.

DISCUSSION

The relationship between the strains found in this work by an immunological method can be supported by only a few results obtained by other methods. However, the method used here has given reproducible and reliable results in studies of more than 250 strains of mycobacteria. The relationships found between mycobacteria by this method have been confirmed by observations of the cultural, physiological and biochemical characteristics of those strains made in other laboratories. Identification at species level of a test strain by this method has been possible when an spd value of < 3 mm. between sensitin of a reference strain belonging to a known species and the test strain has been found. SPD values of 3 or 4 mm. have generally been considered inconclusive. These observations with mycobacteria have contributed to the following evaluation of the present results.

With one exception (strain 80) clear results have been obtained with all the 17 strains. Strain 80 has been classified as *Nocardia asteroides* (Dr Ruth Gordon, personal communication).

Ten of the strains, including the type strain of *Nocardia farcinica* (752) and 4 other strains (749, 750, 751, 753) previously identified as *N. farcinica*, form one group. Strains 749, 750, 751 were isolated from cattle with farcy and identified on the basis of their morphology and biochemical properties (Perpezat, Mariat, Destombes & Thomé, 1963). The five other members of this group are strains 84, 85, 650, 745, 787.

Strains 84 and 85 were identified as *Nocardia asteroides* when originally isolated from the placenta of a cow and from a dog, respectively (Fey, Holm & Teuscher, 1954).

Strain 650 was given by Professor N. F. Conant, North Carolina, U.S.A. Although its original source could not be traced, it cannot be the same isolate as any of the strains labelled *Nocardia farcinica*, since these strains were not kept in Conant's laboratory at the time when strain 650 was sent to the National Collection of Type Cultures, London (Professor N. F. Conant, personal communication).

Strain 745 originated from a human patient (Bergström, Edebo, Fors & Tegner, 1966). In the report describing its isolation, the strain was repeatedly referred to as *Nocardia asteroides*, although it was also mentioned that it had been identified as *N. farcinica* by the present writers.

Strain 787 has been classified as *Actinomyces blackwellii* by Erikson (1935); it is characterized by its medium growth consistency, good formation of aerial mycelium with frequent angular branching, no soluble pigment on protein media, no liquefaction of gelatine and no action on milk (Erikson, 1935). This description is based on the study of the properties of a single strain (787). Schneidau & Shaffer could neither distinguish this strain from 6 strains designated *Nocardia asteroides* in cultural studies (1957) nor by use of a slide agglutination test (1960).

Strains 650 and 787 have been classified as *Nocardia asteroides* by Gordon & Mihm (1957). The same applies to strains 84 and 85 (Dr Ruth Gordon, personal communication). As mentioned in the Introduction, Gordon & Mihm (1959) could distinguish neither the type strain of *N. farcinica* (strain 752) nor strain 753 from a series of strains which those authors called *N. asteroides*. In addition to strains 81 and 809 which were clearly distinguishable in the present work from the group of 10 strains (spd = 3-13 mm.) and from each other (spd = 9), the latter also included others which can be distinguished from this group, represented by strain 84 (unpublished data). These observations definitely do not support the opinion expressed by Gordon & Mihm (1962) that *N. farcinica* and *N. asteroides* are two different names for the same species.

It would appear justifiable to classify the 10 strains in the largest group only as *Nocardia farcinica*. In consequence, *N. blackwellii* and *Actinomyces blackwellii* are subjective synonyms of *N. farcinica*. The three remaining strains labelled *N. farcinica* (677, 678, 746) are quite distinct from the group of 10 strains in the present study. On the basis of the present findings, the writers consider strains 678 and 746 form one separate species and 677 another.

Six strains classified in this work as *Nocardia farcinica* (84, 85, 650, 752, 753, 787) have been examined by the methods described by Gordon & Mihm (1962). All of them grew at 50° and four (84, 85, 650, 787) were able to ferment rhamnose (Dr Ruth Gordon, personal communication). Among 8 other strains which were classified as *N. asteroides* according to Gordon & Mihm's criteria and which, according to the present method, are distinguishable from *N. farcinica* (unpublished data), none showed both the above-mentioned characteristics (Dr Ruth Gordon, personal communication). Of 142 strains classified as *N. asteroides*, only 21% grew at 50° (Gordon & Mihm, 1962). The strains classified as *N. farcinica* in the present work (except strain 749) have a higher growth rate at 45° than other strains classified as *N. asteroides* by Gordon & Mihm's criteria. It may thus be possible to demonstrate characteristic physiological and biochemical features of *N. farcinica* strains and to differentiate between that species and *N. asteroides* by conventional methods also. However, further studies are required.

It was reported earlier that 29 out of 30 strains of *Nocardia asteroides* were urease-positive, in contrast to 14 strains of *N. farcinica*, including 749, 750 and 751 which were urease-negative (Mariat, 1963). Strains 752 (the type strain of *N. farcinica*) and 753 are both urease-positive (unpublished observation). Thus the urease reaction may not always be of help in the distinction between *N. farcinica* and *N. asteroides*.

According to Waksman (1957), strains of *Nocardia farcinica* are transmissible to guinea pigs, cattle and sheep but not to rabbit, dogs or horses. However, two of the strains identified as *N. farcinica* in the present work (84, 85) were reported to be transmissible to rabbits (Fey *et al.* 1954). As mentioned earlier, one of these two strains was isolated from a dog, and strain 787, which has also been identified as *N. farcinica* in the present work, was originally isolated from a foal (Erikson, 1935). Therefore, according to the present findings, the description of *N. farcinica* given by Waksman (1957) and based on two strains only, should apparently be revised as regards the pathogenicity of the species. It is remarkable that strain 745, identified as *N. farcinica* in the present work, was isolated from a human patient (Bergström, *et al.* 1966). This is apparently the first known case of human infection caused by *N. farcinica*. Other such cases may be found, particularly where the causal agent has been identified as *N. asteroides* and where the isolate has been rhamnose-positive and able to multiply at 50°. Strains of *N. farcinica* isolated from human patients may also be identified among the strains which could not be distinguished from *N. blackwellii* in the study reported by Schneidau & Shaffer (1960).

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