Immunodiffusion Studies of Some *Nocardia* Strains

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Forty-three strains of *Nocardia*, one of *Actinomadura* and two of *Nocardiopsis* were studied using the comparative immunodiffusion technique. Three reference precipitation systems were employed: one represented *Nocardia asteroides* N10, one *N. asteroides* ATCC 19247, and one *N. otitidis-caviarum* ATCC 14629. One tight cluster was formed by the *N. otitidis-caviarum* strains and another tight cluster was formed by some of the *N. asteroides* strains studied. However, other strains of *N. asteroides* were distinct from the latter cluster. Furthermore, *N. asteroides* ATCC 19247, which is the type strain, differed from most of the *N. asteroides* strains tested. Strains of the species *N. asteroides*, *N. brasiliensis*, *N. farcinica* and *N. otitidis-caviarum* were found to be closely related, while *N. amarae* strains differed slightly from this group. The strains referred to *Actinomadura* and *Nocardiopsis* were clearly distinct from the three *Nocardia* reference strains; nevertheless, three antigens common to these genera were revealed.

INTRODUCTION

During the last decade there have been several taxonomic studies on *Nocardia* and related organisms. Large amounts of data have been obtained from numerical taxonomic studies (Tsukamura, 1969, 1977; Goodfellow, 1971; Goodfellow *et al.*, 1974; Schaal & Reutersberg, 1978; Orchard & Goodfellow, 1980). Valuable information has also been obtained from genetical (Mordarski *et al.*, 1977, 1978), chemical (Alshamaony *et al.*, 1976; Minnikin *et al.*, 1977; Minnikin & Goodfellow, 1978, 1980), immunological (Magnusson & Mariat, 1968; Ridell & Norlin, 1973; Ridell, 1974, 1975, Ridell *et al.*, 1979) and phage sensitivity studies (Williams *et al.*, 1980). The results of these investigations have improved the classification of the genus *Nocardia*, but many problems still remain to be solved. For example, the species *N. asteroides* is not well defined and the relationship between *N. asteroides* and *N. farcinica* is not clarified. Furthermore, the genus *Nocardia* includes species which have been insufficiently studied and the delineation between this genus and other genera is not clear.

The aim of the present investigation was to study the antigenic relationships between some *Nocardia* strains with special reference to taxonomic problems.

METHODS

Test strains. Forty-three strains labelled *Nocardia*, one *Actinomadura* and two *Nocardiopsis* were studied (Table 1).

Antigens. Antigen preparations were made by artificial disintegration of the cells (Ridell & Norlin, 1973).

Antisera. Antigen preparations from the strains *N. asteroides* N10, *N. asteroides* N114, ATCC 19247 and *N. otitidis-caviarum* N112, ATCC 14629 were inoculated into rabbits for immune serum production (Ridell, 1975).

Serological technique. The analyses were carried out by means of a microplate modification (Wadsworth, 1962) of the immunodiffusion technique according to Ouchterlony.

Reference precipitation system. Three reference precipitation systems were utilized – one for *N. asteroides* N10, one for *N. asteroides* N114, ATCC 19247 (type strain of *N. asteroides*), and one for *N. otitidis-caviarum* N112.

ATCC 14629 (type strain of *N. otitidis-caviarum*). The principles for the establishment and the use of serological reference systems have been described previously (Ridell & Norlin, 1973; Ridell, 1974, 1975; Baker *et al.*, 1979; Ridell *et al.*, 1979).

RESULTS

The test strains were analysed by the comparative immunodiffusion technique employing the three reference systems. Table 1 shows the number of precipitinogens shared by the test strains and the reference strains. The figures give the number of precipitinogens which could be identified by means of the reference systems (see Ridell, 1975; Baker *et al.*, 1979). Thus, the total number of precipitates obtained by the reaction between the test strain preparations and reference antisera is not indicated in the table.

When the *N. asteroides* N10 reference system was used, one or more precipitates were identified in all 43 *Nocardia* strains analysed. In five of the strains labelled *N. asteroides* (N7, N60, N106, N107, N108), five or six precipitinogens were identified by means of this system. Furthermore, four precipitinogens were identified in one strain of *N. asteroides* (N110, NCTC 8595) and in one strain (N64) carrying the designation *N. farcinica*.

When the other *N. asteroides* reference system (based on strain N114, ATCC 19247, which is the type strain) was employed, it was also possible to identify one or more precipitinogens in all the 43 *Nocardia* strains. Four precipitinogens were identified in one strain of *N. asteroides* (N77), while only one, two or three precipitinogens were identified in the other strains referred to this species. In several strains referred to *N. farcinica* (N64, N65, N67, N120, N125) four precipitinogens were identified. In the remaining strains of the different species examined, no more than three precipitinogens were identified by the N114, ATCC 19247 reference system.

The *N. otitidis-caviarum* reference strain (N112, ATCC 14629, the type strain) also shared precipitinogens with most of the *Nocardia* strains tested. Five, six or seven precipitinogens were identified in all of the test strains designated *N. otitidis-caviarum* by means of this system. Furthermore, two strains referred to *N. asteroides* (N5 and N6) shared six precipitinogens and two strains (N4 and N77) shared four precipitinogens with the reference strain of *N. otitidis-caviarum*, and all of these precipitinogens were identified.

The number of precipitinogens identified in the three *N. amarae* strains by means of the three reference systems was generally smaller than that identified in *N. asteroides*, *N. brasiliensis*, *N. farcinica* or *N. otitidis-caviarum*.

Table 1. Number of precipitinogens identified by comparative immunodiffusion analyses employing three *Nocardia* reference systems

Lab. no.	Strain	Reference system		
		<i>N. asteroides</i> N10	<i>N. asteroides</i> N114, ATCC 19247	<i>N. otitidis-caviarum</i> N112, ATCC 14629
N133*	<i>Nocardia amarae</i> , Lechevalier Se 6, ATCC 27808	2	1	1
N134	<i>N. amarae</i> , Lechevalier Se 61	2	1	0
X6	<i>N. amarae</i> , Goodfellow N667	1	1	1
N1	<i>N. asteroides</i> , Juhlin 44	1	2	1
N4	<i>N. asteroides</i> , Juhlin 320	2	3	4
N5	<i>N. asteroides</i> , Juhlin 333	2	3	6
N6	<i>N. asteroides</i> , Juhlin 633	3	2	6
N7	<i>N. asteroides</i> , Juhlin 714	6	1	1
N8	<i>N. asteroides</i> , Juhlin 732	3	2	1
N9	<i>N. asteroides</i> , Juhlin 875	3	3	1
N10†	<i>N. asteroides</i> , Juhlin M-65006	6‡	2	1

Table 1 – continued

Lab. no.	Strain	Reference system		
		<i>N. asteroides</i> N10	<i>N. asteroides</i> N114, ATCC 19247	<i>N. otitidis-caviarum</i> N112, ATCC 14629
N60	<i>N. asteroides</i> , Magnusson 81, SSC 262	5	2	3
N77	<i>N. asteroides</i> , Magnusson 80	2	4	4
N94	<i>N. asteroides</i> , Gordon 432	3	2	3
N106	<i>N. asteroides</i> , Gordon 443(1)	6	2	3
N107	<i>N. asteroides</i> , Gordon 443(2)	6	2	1
N108	<i>N. asteroides</i> , NCTC 659	6	2	2
N110	<i>N. asteroides</i> , NCTC 8595	4	3	3
N114*†	<i>N. asteroides</i> , ATCC 19247	3	7‡	1
N68	<i>N. brasiliensis</i> , Magnusson 119, Gordon 746	1	3	1
N69	<i>N. brasiliensis</i> , Magnusson 728, Emmons 10011	2	2	1
N78	<i>N. brasiliensis</i> , Gordon 605	2	2	2
N115*	<i>N. brasiliensis</i> , ATCC 19296	2	2	2
N58*	<i>N. farcinica</i> , ATCC 3318, Bönicke EB1957	2	2	2
N61	<i>N. farcinica</i> , Magnusson 84, <i>N. asteroides</i> , PH598/51-52	1	3	1
N62	<i>N. farcinica</i> , Magnusson 85, <i>N. asteroides</i> , PH599/51-52	3	3	2
N64	<i>N. farcinica</i> , Magnusson 650, <i>N. asteroides</i> , RG784	4	4	2
N65	<i>N. farcinica</i> , Magnusson 745, <i>N. asteroides</i> , E	3	4	1
N67	<i>N. farcinica</i> , Magnusson 753, RG3399	2	4	2
N70	<i>N. farcinica</i> , Magnusson 787, <i>N. blackwellii</i> , McCl 81	3	3	2
N118	<i>N. farcinica</i> , Magnusson 645, ATCC 6864, NCTC 1935	2	3	2
N119	<i>N. farcinica</i> , Magnusson 844, <i>N. asteroides</i> , M-65011 S127R	3	3	3
N120	<i>N. farcinica</i> , Magnusson 845, <i>N. asteroides</i> , M-65010 S127R	2	4	0
N125	<i>N. farcinica</i> , Magnusson 878, <i>N. asteroides</i> , 1015/167 Mariat 438C	2	4	3
N41	<i>N. otitidis-caviarum</i> , Juhlin 318	2	2	6
N54	<i>N. otitidis-caviarum</i> , Bönicke SN5601	3	3	6
N55	<i>N. otitidis-caviarum</i> , Bönicke SN5602	2	3	5
N89	<i>N. otitidis-caviarum</i> , Gordon 416	2	2	5
N90	<i>N. otitidis-caviarum</i> , Gordon 424	3	3	5
N91	<i>N. otitidis-caviarum</i> , Gordon 737	3	2	6
N92	<i>N. otitidis-caviarum</i> , Gordon 1316	2	2	5
N93	<i>N. otitidis-caviarum</i> , Gordon 1370	3	2	7
N112*†	<i>N. otitidis-caviarum</i> , ATCC 14629	3	3	7‡
N116	<i>Actinomadura madurae</i> , ATCC 19425	0	1	0
N84	<i>Nocardioopsis dassonvillei</i> , Gordon 575	0	0	1
N117	<i>Nocardioopsis dassonvillei</i> , ATCC 23219	1	0	0

* Type strain. † Reference strain. ‡ Number of precipitinogens of the reference system.

Sources: NCTC, National Collection of Type Cultures, London, England; ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; R. Bönicke, Forschungsinstitut Borstel, Borstel, Germany; M. Goodfellow, Department of Microbiology, The University, Newcastle-upon-Tyne, England; R. E. Gordon, Institute of Microbiology, Rutgers University, New Brunswick, N.J., U.S.A.; I. Juhlin, Malmö Allmänna sjukhus, Malmö, Sweden; M. Lechevalier, Institute of Microbiology, Rutgers University, New Brunswick, N.J., U.S.A.; M. Magnusson, Statens Seruminstitut, Copenhagen, Denmark.

In the strains of *Actinomadura* and *Nocardioopsis* only one precipitinogen was identified with any of the three reference systems, and in six of the nine test combinations no precipitinogen at all could be identified. However, in several test combinations, additional unidentified precipitates were found (not presented in Table 1). For example, when *Nocardioopsis dassonvillei* N84 and *A. madurae* N116, ATCC 19425 were analysed by the *N. asteroides* N10 system, three precipitates were demonstrated in each test combination.

DISCUSSION

The comparative immunodiffusion method can be used to classify bacteria. In previous studies (Ridell & Norlin, 1973; Ridell, 1974, 1975) mycobacteria, nocardiae and rhodococci have been studied by this method, employing the same standardized techniques as in the present study. For example, two reference systems, each consisting of seven precipitates, were employed by Ridell (1974): strains for which it was possible to identify all, or all but one or two, precipitinogens of a reference strain were classified together with the reference strain and were considered to belong to the same taxonomic group. In the present study the same principle has been used for the taxonomic delineations.

The immunodiffusion analyses clearly demonstrated that two of the strains designated *N. asteroides* (N5 and N6) are serologically closely related and indistinguishable from strains of *N. otitidis-caviarum*. Thus, these two strains designated *N. asteroides* do not belong to this species but should be referred to the species *N. otitidis-caviarum*.

Apart from strains N5 and N6, 14 strains referred to *N. asteroides* were studied. The results demonstrated that six of them (N7, N10, N60, N106, N107, N108) are serologically very similar and can be grouped together, while the remaining eight *N. asteroides* strains are distinct from this group. The results thus indicate that *N. asteroides* is a heterogeneous species in which at least one homologous group exists. Other workers studying *N. asteroides* using numerical (Tsukamura, 1969, 1977; Goodfellow, 1971; Schaal & Reutersberg, 1978; Orchard & Goodfellow, 1980) as well as genetical analyses (Mordarski *et al.*, 1977) have demonstrated that this species is clearly heterogeneous comprising two or more groups. Only a limited number of strains are, however, common to the various studies which makes it difficult to compare the groups revealed.

The reference strain N114, ATCC 19247 is the type strain of *N. asteroides*. This strain shared comparatively few precipitinogens with the other *N. asteroides* strains tested. The present results thus point to the possibility that the type strain of *N. asteroides* clearly diverges from most strains of this species.

It was not possible in the present study to distinguish *N. farcinica* from *N. asteroides*, and, furthermore, five of the *N. farcinica* strains (N64, N65, N67, N120 and N125) demonstrated a relatively close serological relationship with the type strain of *N. asteroides*. Other workers have grouped strains of *N. farcinica* with those labelled *N. asteroides* (e.g. Tsukamura, 1969; Schaal & Reutersberg, 1978), but it has also been found that *N. farcinica* represents a fairly homogeneous group (Mordarski *et al.*, 1978; Orchard & Goodfellow, 1980). It is of interest to note that six of the *N. farcinica* strains included (N61, N62, N64, N65, N67 and N70) were analysed by Magnusson & Mariat (1968) using delayed type skin reaction. They found, in contrast to the present result, a clear difference between these strains and the type strain of *N. asteroides*. The delayed type skin reaction is, however, a much more sensitive method than immunodiffusion, which might explain the different results.

All strains of *N. otitidis-caviarum* formed a tight serological group together with the *N. asteroides* strains N5 and N6. This group was clearly distinct from all other nocardia strains tested apart from *N. asteroides* strains N4 and N77 which showed a certain serological relatedness to *N. otitidis-caviarum*. These findings are in agreement with those of Goodfellow (1971) and Orchard & Goodfellow (1980) who considered *N. otitidis-caviarum* to be a good taxospecies.

The results of the present study indicate that strains of the species *N. asteroides*, *N. brasiliensis*, *N. farcinica* and *N. otitidis-caviarum* are all serologically closely related, sharing several precipitinogens. The strains of *N. amarae* also shared precipitinogens with the reference strains but this species occupied a position some distance from the closely related group of nocardia species. This result agrees with those of Minnikin & Goodfellow (1980) and Williams *et al.* (1980), who demonstrated that *N. amarae* differs from other nocardiae.

One strain of *Actinomadura madurae* and two of *Nocardioopsis dassonvillei* were included in the present study. These species were once classified as *Nocardia* (Gordon, 1966; Gordon & Horan, 1968), but Lechevalier & Lechevalier (1970) found them different from this genus, and proposed the genus *Actinomadura* to accommodate them. Later, Meyer (1976) proposed the genus name *Nocardioopsis* for strains labelled *A. dassonvillei*. The separation of *Actinomadura* and *Nocardioopsis* from *Nocardia* has been supported by several investigations (Tsukamura, 1969; Goodfellow, 1971; Goodfellow *et al.*, 1974, 1979) and likewise the serological result here demonstrated that strains of *Actinomadura* and *Nocardioopsis* are distinct from *N. asteroides* and *N. otitidis-caviarum*. However, three antigens are common to strains of *Actinomadura*, *Nocardioopsis*, and *N. asteroides*, although these antigens were not identified by the reference system (and they are therefore not given in Table 1). The presence of three common antigens is not unusual for organisms of different but related genera. For example, the serological technique used here has demonstrated that nocardiae, mycobacteria and rhodococci share at least three antigens (Ridell & Norlin, 1973; Ridell, 1974). Thus, the presence of three common antigens is no reason *per se* to group strains of *Actinomadura* and *Nocardioopsis* in the genus *Nocardia*.

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