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A simple view of nocardial taxonomy

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INTRODUCTION

Nocardial taxonomy, like that of other actinomycete genera, has undergone all the viscissitudes of doubt associated with years of inadequate and sometimes inappropriate methodology. This is reflected by the great expansion of species names allocated to the genus over the first two thirds of this century (Buchanan, Holt & Lessel, 1966), followed by the contraction achieved in the list of approved bacterial names in 1980 (Skerman, McGowan & Sneath, 1980). Created by Trevisan in 1889 for five species, modern taxonomists would now allocate some 20 species to the genus, including only one of Trevisan's original five species (Table 1). As an actinomycete genus *Nocardia* has suffered from two particular disadvantages. First in having an apparently distinctive nocardioform morphology on the basis of which organisms were referred to this genus long after crude morphology lost its importance in many other genera. Second in having an unfortunate type species, *Nocardia farcinica*, with no really well accredited type strain.

THE NOCARDIOFORM APPEARANCE

Typical nocardial colonies consist of mycelial masses of Gram-positive and frequently partially acid fast bacilli of variable length and often covered with aerial hyphae. These aerial hyphae consist of chains of rather short bacilli with no real definition of spore forms. The masses of mycelium when young show frequent branching without separation of individual organisms, but with age these frequently disintegrate into bacillary masses. This appearance, in an aerobic organism, was for years sufficient to attribute organisms to the genus.

Nocardia farcinica

This organism, listed first by Trevisan, was assumed to be the type species of the genus, although this was not stated. It was originally isolated by Nocard from cases of bovine farcy in Guadalupe and was believed to be the sole cause of this disease (Nocard, 1888). However, in the 1970's it was discovered that cultures thought to be of the original strain held in the ATCC and the NCTC were of a *Nocardia* and of a *Mycobacterium* respectively (Chamoiseau & Asselineau, 1970). Resort to Guadalupe was no use, the disease has been eradicated there and no fresh isolate was available to confirm which was the organism really isolated by Nocard. However his description and drawings do not suggest that it was a *Mycobacterium* (Nocard, 1888). This situation was further complicated by Chamoiseau's description of *Mycobacterium farcinogenes* as the cause of bovine farcy in Tschad and Senegal (Chamoiseau, 1973), dividing it into two subspecies named for

Table 1. *Species of nocardiae*

Listed in Bergey (1974)		Approved list (1980)
N. farcinica (type species)		*N. farcinica*
*N. otitidis-caviarum**		*N. otitidis-caviarum**
N. brasiliensis		*N. brasiliensis*
N. asteroides		*N. asteroides* (type species)
N. transvalensis		*N. transvalensis*
N. coeliaca		*N. coeliaca*
N. petroleophila		*N. petroleophila*
N. saturnea		*N. saturnea*
N. vaccinii		*N. vaccinii*
N. cellulans		*N. cellulans*
N. globerula		*N. globerula*
N. calcarea		*N. calcarea*
N. restricta		*N. restricta*
6 species not in approved list	Not in Bergey	*N. amarae* *N. autotrophica* *N. carnea* *N. corynebacterioides* *N. hydrocarnonoxydans* *N. mediterranei* *N. orientalis*
4 species now considered synonyms of *N. asteroides*		
N. rhodnii		*Rhodococcus rhodnii*
N. rubropertincta		*R. rubropertincta*
N. rubra		*R. rubra*
N. erythropolis		*R. erythropolis*
4 species now considered '*M. rhodochrous*', but not in 1980 list		

* Often called *N. caviae* nowadays.

the two countries. Although his names for the subspecies broke the Code of Nomenclature for Bacteria, both organisms were certainly mycobacteria and appeared as two species, *M. farcinogenes* and *M. senegalese* (Chamoiseau, 1979) in the 1980 list (Skerman, McGowan & Sneath, 1980). The question might then be raised as to whether bovine farcy is ever caused by *Nocardia* and whether Nocard's strain must really have been a *Mycobacterium*. However, some strains from farcy in India do appear to be nocardiae. To cut this long story short, modern opinion is that the loosely defined disease called bovine farcy may be caused by either nocardiae or by the two mycobacterial species. In the lack of strong evidence to the contrary, the nocardial version of Nocard's organism has been accepted as genuine. However, because of nagging taxonomic doubt and the lack of a firmly based type strain for *Nocardia farcinica*, the Judicial Commission of the International Committee for Bacterial Taxonomy accepted the request of the nocardial study group to establish *N. asteroides* as the type species of the genus, and it appears thus in the 1980 list of approved bacterial names. Nevertheless a recent request has been made by Tsukamura (1982) that the name *N. farcinica* be rejected on the basis that the sole existing version of Nocard's isolate is of *N. asteroides*. A decision is awaited and if the request is supported the connection of modern *Nocardia* with Trevisan's genus becomes very tenuous.

Nocardia asteroides

This is the second oldest named species in the genus, being originally called *Cladothrix asteroides* by Eppinger in 1891 (Buchanan & Gibbons, 1974) and transferred to *Nocardia asteroides* by Blanchard 5 years later (Lessel, 1960). It is a frequently encountered organism in soil, and a most important opportunistic pathogen of man and typifies many of the characteristics today associated with the genus. Many strains are available and they cluster together as a clear and distinct species, although of course with modern taxonomic methods subgroups can be distinguished which may later be recognized as subspecies.

THE GENUS *NOCARDIA* TODAY

Although the modern genus contains only one of Trevisan's original five species, and that is the dubious *N. farcinica*, the name of the genus was conserved by action of the Judicial Commission in 1954. It appears in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) as the type of two genera, the other *Pseudonocardia*, within the family Nocardiaceae Castellani and Chalmers, of the Order Actinomycetales. The pseudonocardiae, which have received little taxonomic attention, differ morphologically in their process of spore formation from the true nocardiae, and are not considered further in this review because of lack of information.

The great majority of organisms which at some time in their chequered careers have appeared as nocardiae started off with different generic titles, passed through *Nocardia* and into yet further genera in their search for hierarchical stability. Lessel in 1960, listed 25 genera that had participated in this form of blind mans buff. Perhaps the most difficult separation has been between *Nocardia* and the '*Mycobacterium rhodochrous*' group which is an even more illdefined cluster of probably several genera. At the moment, and in the 1980 list members of the '*M. rhodochrous*' group are placed in the single genus *Rhodococcus* Zopf (1981), although this genus is likely to follow with time a fate similar to that of *Nocardia*.

In the list of approved names (1980) 20 species of *Nocardia* appeared. In comparison with this, the *Index Bergeyana* of 1966 (Buchanan, Holt & Lessel, 1966) listed 171 legitimately published names for nocardial species. Most of the 20 species are, like the mycobacteria, organisms of moist soil, a few of which have developed opportunistic tendencies to invade mammalian tissues.

To belong to the genus *Nocardia* today, an organism must be Gram positive and may be partially or completely acid fast by the Ziehl–Neelsen stain. It must be aerobic and grow as a mycelium of rods varying according to age and species in the number of branches. Most produce aerial hyphae fragmented into short rods, but without any clearly separate spore structures. These simple features alone do not exclude some other related genera and in order to confirm nocardial identity at least some of the following criteria have to be taken into account.

Cell wall analyses of actinomycetes have shown that there are least seven types, differing in their diaminopimelic acid (DAP), amino acid and sugar content (Lechevalier & Lechevalier, 1970*a*). The nocardiae as typified by *N. asteroides* possess cell walls of type IV containing *Meso*-DAP, arabinose and galactose. Other

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Table 2. *Cell wall types of some actinomycete genera*

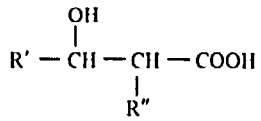
Type	Major constituents	Genera
I	L-DAP, glycine	*Norcardioides, Kineosporia, Streptomyces, Streptoverticillium, Microellobosporia, Sporichthya*
II	*Meso*-DAP, glycine (sometimes also hydroxy DAP) arabinose & xylose	*Micromonospora, Actinoplanes, Ampullariella, Amorphosporangium, Dactylosporangium*
III	*Meso*-DAP (and sometimes madurose)	*Actinosynnema, Actinomadura, Microbispora, Streptosporangium, Spirillospora, Planomonospora, Dermatophilus, Nocardiopsis, Thermoactinomyces, Geodermatiphilus*
IV	*Meso*-DAP, arabinose, galactose	*Mycobacterium, Nocardia, Pseudonocardia, Thermomonospora, Micropolyspora, Corynebacterium,* (animal pathogen type) *Rhodococcus*
V	Lysine, ornithine	*Actinomyces israelii*
VI	Lysine, aspartic acid	*Actinomyces bovis, Rothia, Oerskovia*
VII	DAB, glycine	*Agromyces*
—	*Meso*-DAP with numerous amino acids	*Mycoplana*

genera possessing cell walls of this type are *Mycobacterium*, *Corynebacterium* and *Rhodococcus*. However, excluded with cell walls of type III, *Meso*-DAP without arabinose or galactose, are the organisms that used to be called *N. madurae*, *N. pelletieri*, *N. flexuosa* and *N. dassonvillei* and which now belong to the genus *Actinomadura* (Lechevalier & Lechevalier, 1970*b*) (see Table 2).

Although simple cell wall structure does not distinguish between *Nocardia* and several other genera, study of the lipids of the outer part of the cell wall has enabled their separation. A unique class of lipid-containing substances project through the outer arabino–galactan layer of nocardiae, mycobacteria and the animal pathogenic corynebacteria. These are the mycolic acids which are β hydroxy acids with long chains at the α position (see Fig. 1). The corynemycolic acids have the smallest molecules with a carbon skeleton of 25–30 atoms. The mycolic acids of mycobacteria are the largest with 60–90 carbon atoms and the nocardomycolic acids are intermediate in size, 46–58 carbon atoms. Those of some rhodococci lie between those of nocardiae and corynebacteria (34–50 carbon atoms) (Minnikin & Goodfellow, 1976) and those of rhodococci previously in the genus *Gordona* (Tsukamura, 1971) may be slightly larger than those of nocardiae (52–66 carbon atoms) (Alshamaony, Goodfellow & Minnikin, 1976; Alshamaony *et al.* 1976).

These mycolic acids can be split in the process of pyrolytic gas chromatography into two parts, a fatty ester consisting of the acid radical plus the chain from the α position and a meroaldehyde from the rest of the mycolate. Mycolic acids can be extracted from whole dry cells and separated by thin layer chromatography. In this process those of nocardial origin can be clearly distinguished from those of mycobacteria and corynebacteria as an 'LCN-A' or lipid characteristic of *Nocardia* (Mordaska & Rethy, 1970).

Even with this further step in separation some of the rhodococci may still be difficult to separate from nocardiae. Further work indicated that thin layer chromatography of the products of methanolysis of mycolic acids could distinguish



Mycobacterial mycolic acids: R′ = 43–61* R″ = 19, 21 or 23*.

Nocardial mycolic acids: complete skeleton = 46 58*.

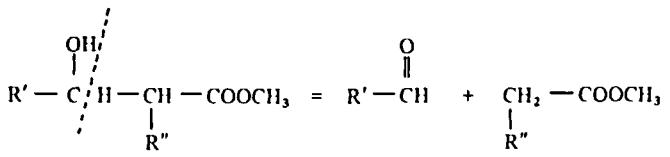
Corynebacterial mycolic acids: R′ = 14 18; R″ = 2, 4 or 6.

Rhodococcal mycolic acids:

'*M. rhodochrous*' group: complete skeleton = 34 50

'*Gordona*' group: complete skeleton 52–66

Pyrolytic breakdown of mycolic acids



Mycolate Meroaldehyde Fatty ester

Fig. 1. Mycolic acids and their pyrolysis products. * Numbers of carbon atoms.

mycobacteria, nocardiae, corynebacteria and rhodococci (Minnikin, Alshamaony & Goodfellow, 1975). The distinction was further supported by analysis of numbers of double bonds in the pyrolysis products of mycolic acids. This was done by linking a mass spectrometer to the outlet of a gas chromatograph (Minnikin & Goodfellow, 1976). By this system the mycolates of true nocardiae have 0–3 double bonds, those of rhodococci ('*M. rhodochrous*' group) 0–2 double bonds and those of rhodococci (*Gordona* species) have 1–4 double bonds (Alshamaony, Goodfellow & Minnikin, 1976; Alshamaony *et al.* 1976).

Besides these studies of mycolic acids and their pyrolytic products much work has been done on other lipids possessed by nocardiae. These vary between species but do not offer any easy means of distinction between the genera. For an introduction to them see the chapter by Minnikin & Goodfellow (1976) in *The Biology of the Nocardiae*.

Another chemotaxonomic procedure that looks useful for separating *Nocardia* from the other genera is chromatographic analysis of the lipid soluble iron-binding substances known as mycobactins and nocobactins (Ratledge & Patel, 1976). Although related in function and structure, there are chemical differences between these substances that are reflected in their chromatographic mobilities. Amongst mycobactins and nocobactins there are also differences according to the species from which they are extracted. True mycobactins have only been found in mycobacteria, and with the possible exception of a strain of *Rhodococcus* (ex '*Gordona*' group) nocobactins have only been found in nocardiae. This lack of nocobactins in at least most *Rhodococcus* species may prove a very useful marker.

Serological and antigenic studies of nocardiae and related genera have proved disappointing. Although precipitation in gel methods clearly distinguish between

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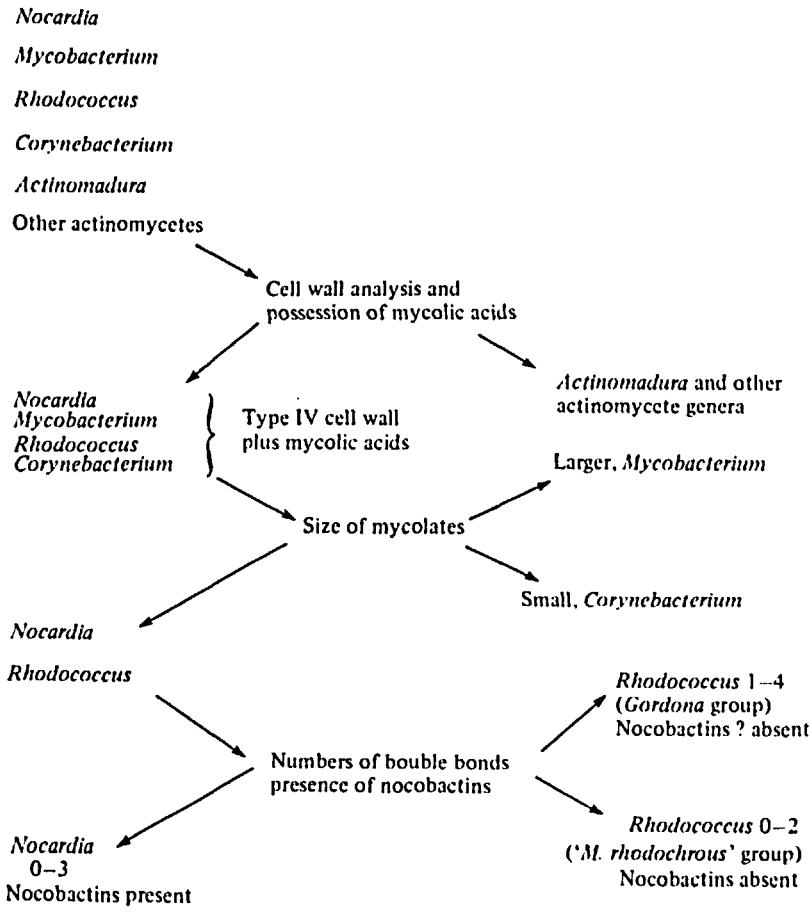

Fig. 2. Scheme based on cell wall type, mycolate analysis and presence of nocobactins for distinguishing *Nocardia* from other genera.

individual species, and sometimes between subspecies within each of the genera, separation between the genera themselves has not been clearcut (Kwapinski, 1964; Lind & Ridell, 1976; Chaparas *et al.* 1982). Everyone agrees that there are antigens common to nocardiae, mycobacteria, rhodococci of both types and corynebacteria, and that there are species specific antigens. The report of antigens closely relating nocardiae to the fast growing mycobacteria and demonstrating further antigens apparently shared by the nocardial species alone (Stanford & Wong, 1974) have unfortunately not been supported by other workers (Ridell, 1977, 1983). Although I am personally convinced that the genera could be separated by antigenic analysis, such a view is poorly supported in the literature.

Numerical taxonomy separates reasonably clearly between species and subspecies, but like the antigenic studies it has been much less successful at clearly separating genera (Goodfellow, 1971; Goodfellow, Fleming & Sackin, 1972; Tsukamura *et al.* 1979). Techniques of DNA analysis look promising (Mordaski *et al.* 1976, 1980), but insufficient data on differentiation at the generic level has been published so far. Nevertheless, there is no reason to doubt that such techniques will prove successful.

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In conclusion, the genus *Nocardia*, as typified by *N. asteroides*, *N. brasiliensis* and *N. caviae* upon which most of the work has been based, is moving towards a state of stability. It shares with *Mycobacterium*, *Corynebacterium* and *Rhodococcus* a cell wall of *Meso*-DAP, Arabinose and galactose and the special long chain fatty acids called mycolic acids. Analyses of these acids, especially by the recognition of LCN-A, clearly distinguish mycobacterium and corynebacterium from nocardiae and rhodococci. Distinguishing *Nocardia* from the two genera probably incorporated in *Rhodococcus* may be achieved by measuring numbers of double bonds, but this is not a readily available technology (Fig. 2). As an interim measure identification at the species level and relying on the list of approved names for generic title is all that is readily available. Species identification is much less problematic than generic identification. Methods based on conventional taxonomy using key criteria and numerical and antigenic analyses are freely available in the literature and have not been reviewed here. In 1982 the Subcommittee on the Taxonomy of Nocardia and Related Organisms of the International Committee on Systematic Bacteriology promised a new definition of *Nocardia*. This is eagerly awaited.

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