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A STUDY OF THE RELATIONSHIP BETWEEN *NOCARDIA* AND *MYCOBACTERIUM DIERNHOFERI*—A TYPICAL FAST GROWING *MYCOBACTERIUM*

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Summary.—An antigenic analysis by immunodiffusion has been performed on representative strains of *Nocardia asteroides*, *N. brasiliensis*, *N. caviae*, *N. uniformis* and *Mycobacterium diernhoferi*. The findings have been related to those previously reported for mycobacteria. The 4 nocardial species were found to be internally homogeneous but antigenically distinct from each other. They possessed the Groups I and III antigens of mycobacteria and a group of 3 antigens shared among themselves alone. Thus, nocardiae are antigenically more closely related to the fast growing mycobacteria than to the slow growing mycobacteria.

***Mycobacterium diernhoferi* was found to be a typical fast growing mycobacterium possessing 3 serotypes distinguishable by minor differences in the Group IV (species specific) antigens.**

THERE has been considerable interest recently in the relationship between acid fast and partially acid fast genera. Increasing importance has been placed on the differences between the mycolic acids extractable from their cell walls as a means of generic differentiation. The nocardiae have been shown to possess nocardomycolic acids with a skeleton of 40–60 carbon atoms, whereas the true mycolic acids of mycobacteria have skeletons of 80–90 carbon atoms (Lechevalier, Horan and Lechevalier, 1971). Mordarska and Mordarski (1969) have shown nocardiae to possess a lipid fraction LCN-A incorporating nocardomycolic acid which is absent from mycobacteria (Goodfellow, Minnikin and Patel, 1973).

Although it is easy to distinguish nocardiae from slow growing mycobacteria by a multiplicity of methods, their differentiation from fast growing mycobacteria is more problematical. It might be argued that mycolic acid structure alone is insufficient to substantiate the existence of separate genera. The classic characteristic of nocardioform organisms—their ability to produce a mycelium which fragments into rods and cocci—is shared by at least some species of fast growing mycobacteria when cultured on certain liquid media.

Despite the multiplicity of nocardial names Goodfellow (1971), in a numerical analysis, has shown that the majority of strains belong to 3 species, *Nocardia asteroides*, *N. brasiliensis* and *N. caviae*. Among the few strains which could not be placed in these 3 species was a strain of *N. uniformis* which Goodfellow considered to be closely related to *N. caviae*. The absence of the type species for the genus, *N. farcinica* from this list of species is due to the doubtful authenticity of its type strains and the fact that organisms recoverable from farcy today are apparently mycobacteria (Chamoiseau, 1973) in most cases.

In an attempt to elucidate the serological relationship between nocardiae and mycobacteria, we have performed an immunodiffusion analysis of representative strains of Goodfellow's 3 main species, *N. uniformis* and of a fast growing *Mycobacterium*. *M. diernhoferi* (Bönicke and Juhasz) was selected as a typical example of the latter. A preliminary analysis of this species had shown it to possess a large number of demonstrable antigens and some serotype variation (Stanford, 1973).

MATERIALS AND METHODS

The bacteria studied were *Nocardia asteroides*: ATCC 3318, ATCC 727; *Nocardia brasiliensis*: ATCC 19295, IMRU 774B, IMRU 3407 (originally *M. rhodochrous*), N237 (originally *N. pretoriana*), N367; *Nocardia caviae*: NCTC 1934, N231, N232, N313, N314, N441; *Nocardia uniformis*: NC1B 9631; *Mycobacterium diernhoferi*: 424 ATCC 19340, 775 ATCC 19341, 425 ATCC 19344, 777 ATCC 25958, 778 ATCC 25959, 295 M 363, 423 SN 1402.

Stock cultures of all strains were maintained on Löwenstein-Jensen medium. For production of antigen, strains were cultured on Sauton's medium solidified with 1% of agar and incubated at 32° for approximately 2 weeks. The organisms were scraped from the medium and suspended in normal saline. The suspensions were centrifuged and the deposits resuspended in twice their own volume of saline ready for treatment in the ultrasonic disintegrator. The methods followed beyond this point were those previously described (Stanford and Gunthorpe, 1971). Antigens were prepared from each strain and antisera were raised in pairs of rabbits to *Nocardia asteroides* ATCC 727, *N. brasiliensis* IMRU 774B and ATCC 19295, *N. caviae* N441, *N. uniformis* NC1B 9631 and *Mycobacterium diernhoferi* strains 423 (SN 1402) and 424 (ATCC 19340).

Because of the serotype variation known to occur in *M. diernhoferi*, colonies of each strain of this species were examined for rough–smooth variation. It was found that strains 295, 423, 425 and 775 were uniformly smooth; strains 424 and 777 were mainly smooth but produced a few rough colonies and strain 778 was entirely rough. The rough and smooth variants of strains 424 and 777 were separated and it was found that the rough variants were stable on repeated subculture whereas the smooth strains continued to produce an occasional rough colony. Separate antigens were prepared from the rough and smooth variants of the 2 strains.

The nocardial antigens were tested by immunodiffusion with each of the pairs of antisera raised to nocardiae and patterns of shared and species specific antigens were recorded. Antigens prepared from 2 strains of *M. diernhoferi* (423 and 424) were also tested with these sera and the number of antigens shared between this species and the nocardiae was determined.

Similarly the antigens of each strain of *M. diernhoferi* and of the rough and smooth variants were tested with their homologous antisera and the antigenic pattern of this species determined. Antigens of representative strains of the nocardial species were also tested with the antiseria to *M. diernhoferi*.

For the determination of antigens shared between the present study strains and other mycobacteria, antigens of representative strains of several fast growing and slow growing species were tested with the antisera to the nocardial species and *M. diernhoferi* (Stanford, 1973).

RESULTS

When tested with their homologous antisera, all strains of *Nocardia asteroides*, *N. brasiliensis*, *N. caviae* and *N. uniformis* could be shown to possess 11, 11, 9 and 12 antigens respectively. Seven of these antigens could also be demonstrated when each strain was tested with the antisera to the other nocardial species. Thus, there appears to be at least 7 antigens shared between the 4 species and each species possesses from 2 to 5 specific antigens. Four of the common nocardial antigens demonstrable with the antisera to nocardiae were found to be shared by *Mycobacterium diernhoferi* and also by other fast growing mycobacteria. Only 2 of these antigens, however, could be demonstrated in the representative slow growing mycobacteria.

When the antigens prepared from each strain of *M. diernhoferi* were tested with either pair of antisera raised to this species, a minimum of 13 precipitation lines were formed. With the antiserum raised to strain 423, this strain and 2 others (295 and 777) produced at least one line more than the other strains. With the other antiserum (raised to 424) all strains except 423 and 777 produced 2 extra lines. The antigens produced from the rough and smooth variants of strain 777 could not be distinguished from each other, but the rough variant of strain 424 differed from the smooth variant (predominating in the original strain) by the loss of 3 demonstrable antigens (Fig. 1).

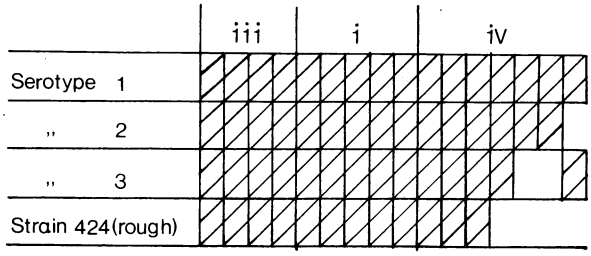

FIG. 1.—Histogram showing the antigenic structure of the 3 serotypes of *Mycobacterium diernhoferi* and of the rough colonial variant of strain 424. Antigens of Groups I, III and IV are shared vertically in this figure.

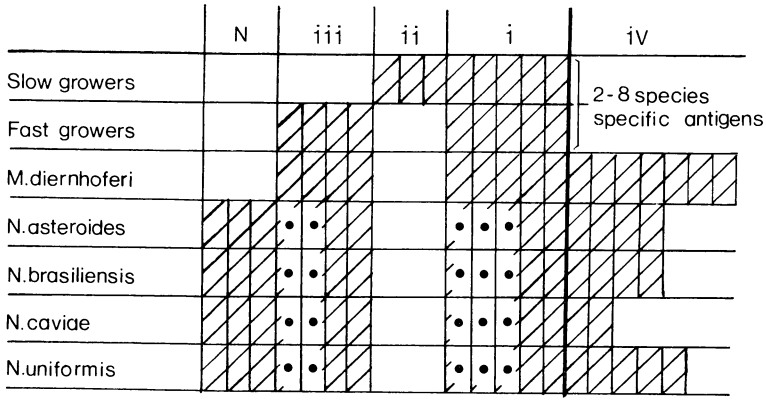

FIG. 2.—Histogram showing the antigenic relationships between 4 species of nocardiae, *Mycobacterium diernhoferi* and other fast growing and slow growing mycobacteria. The antigens of Groups I, II, III and N are shared vertically, but the Group IV antigens are limited to individual species.

Antigens prepared from representative strains of other fast growing mycobacterial species possessed 9 antigens in common with *M. diernhoferi* corresponding to the Group I and III antigens described previously (Stanford, 1973). Strains of the nocardial species were shown also to possess the same 9 antigens in this test system. The results are shown in Fig. 2. The difference between the number of antigens demonstrable in *M. diernhoferi* using the antisera to nocardial species and the number of antigens demonstrable in the nocardial species using the antisera to *M. diernhoferi* is thought to be due to the better quality of the latter sera.

DISCUSSION

Our results show that constant antigenic differences accompany the speciation achieved by Goodfellow (1971) in numerical analyses of *Nocardia*. Each of the 4 nocardial species possesses a group of antigens shared by members of that species alone. All 4 species possess a group of antigens (N in Fig. 2) which they share among themselves but not with mycobacteria, and additionally they share the antigens of Groups I and III with the fast growing mycobacteria. The apparent antigenic homology between individual members of each species conflicts with the observation of Kwapinski *et al.* (1973) who found a great deal of variation in their system.

The strains of *Mycobacterium diernhoferi* all shared 13 demonstrable antigens, 9 of which were found to be common to other fast growing mycobacteria and to the nocardiae. A small amount of variation among the species specific (Group IV) antigens enabled 3 serotypes to be distinguished but these are probably of no practical value, perhaps representing small deletional mutations (Grange, 1973) or the effect of mycobacteriophage (Grange and Bird, personal communication). The relationships between the serotypes (Fig. 1) should be compared with those between the serotypes of *M. fortuitum* (Grange and Stanford, 1974). The lack of antigenic differences between the rough and smooth forms of one strain (777) and the difference of 3 antigens between the rough and smooth forms of another strain (424) are also comparable with observations made on *M. fortuitum* (Stanford and Grange, 1974).

The probable antigenic relationships between the 4 species of nocardiae, *M. diernhoferi* and the other fast and slow growing mycobacteria are shown in Fig. 2. Since the nocardiae and fast growing mycobacteria share Groups I and III antigens, their relationship appears to be closer to one another than to the slow growing mycobacteria with which they share only Group I antigens. The antigens called " N " in Fig. 2 which are shared only by the nocardial species might alternatively be interpreted as Group IV antigens, making the 4 named species serotypes of a single species as in the case of *M. diernhoferi*. Against this interpretation, however, is the absence of nocardial strains with antigenic characteristics of 2 or more of these postulated serotypes (cf serotype 1 of *M. diernhoferi* and the serotype II, III, IV complex of *M. fortuitum* described by Pattyn *et al.*, 1974), and the clarity of separation achieved in Goodfellow's numerical study (1971). Nevertheless, extended studies of more nocardial strains and of intraspecific variation within this genus are needed to confirm their species status.

In conclusion, the 4 nocardial species delineated by numerical analysis have been found antigenically distinct and their close relationship with the fast growing mycobacteria has been established. The study of *M. diernhoferi* showed this to be a distinct mycobacterial species, of which 3 serotypes were recognized.

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