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INTERRELATIONSHIPS AMONG MYCOBACTERIA AND NOCARDIAE

R. E. MANION, S. G. BRADLEY, H. H. ZINNEMAN, AND W. H. HALL

Veterans Administration Hospital, and Department of Microbiology and Medicine, University of Minnesota, Minneapolis, Minnesota

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ABSTRACT

MANION, R. E. (Veterans Administration Hospital, Minneapolis, Minn.), S. G. BRADLEY, H. H. ZINNEMAN, AND W. H. HALL. Interrelationships among mycobacteria and nocardiae. J. Bacteriol. 87:1056-1859. 1964.—A total of 134 strains of mycobacteria and nocardiae were tested for susceptibility of 24 mycobacteriophages. On the basis of capability of diverse strains to serve as alternative propagating hosts for particular viruses, relationships among the actinomycetes were proposed. Cultures received as *Mycobacterium smegmatis*, *M. ranae*, *M. butyricum*, and *M. chelonei* were nearly identical, indicating that they are members of a single taxon. *M. phlei* and *M. fortuitum* strains constituted two distinct but related groups. Moreover, strains of *M. smegmatis* and *Nocardia asteroides* were susceptible to two variant actinophages originally isolated on *N. brasiliensis*.

Susceptibility to actinophages was used to demonstrate general relationships among actinomycetes (Bradley, Anderson, and Jones, 1961). The earlier work had shown that members of *Streptomyces* and most members of *Nocardia* were related to one another on the basis of overlapping patterns of viral sensitivity (Jones and Bradley, 1962). Moreover, strains of *Mycobacterium* were related to some cultures designated *N. farcinica*, but organisms in this group were not attacked by phages virulent for streptomycetes and kindred nocardiae cultures (Anderson and Bradley, 1962). However, nocardiae and mycobacteria were found to be closely allied when overall similarity in response to a large set of physiological and morphological criteria was used to establish kindredships (Jones, 1963).

To relate the mycobacteria to other actinomycetes by phage-typing analyses, possible intermediate forms or new phages must be introduced into the test system. The present study exploited both approaches. In addition, typing reagents having expanded host ranges were prepared by propagating particular viruses on alternative hosts (Manion and Bradley, 1962). As a result, many mycobacteria have been arranged into a related series which is connected to the nocardiae.

MATERIALS AND METHODS

The mycobacterial and nocardial strains used here were obtained as named cultures from several sources (Table 1) and as clinical isolates. A series of 15 mycobacteriophages was isolated from soil samples by the method of Hnatko (1953). Some of the phages studied by Anderson and Bradley (1962) were also included in our test battery (Table 2). All phages were grown to titers of 10^8 or higher in Bordet Gengou Broth (Difco) supplemented with 1% peptone and 4% glycerol. Lysates were filtered through Millipore membranes and stored at 4 C. Host-range determinations were carried out on modified Bordet Gengou medium containing 0.8% agar (Froman, Will, and Bogen, 1954). This overlay agar was heavily seeded with the test organism and poured into petri plates. After the medium had solidified, viral lysates were spotted on the agar surface. For slow-growing mycobacteria such as *M. tuberculosis* and Battey strains, Dubos Oleic Agar enriched with Dubos Oleic Albumin Complex (Difco) was used. This medium was inoculated by flooding the surface with 2 ml of a turbid broth suspension of the appropriate test microbe. After the fluid was absorbed, the plates were spotted with phage lysates. These cultures were incubated at 37 C for 2 to 5 days to allow the host to grow up and phage plaques to develop.

RESULTS

A total of 134 mycobacteria and nocardiae were tested for susceptibility to 24 mycobacteriophages. The strains were categorized as follows: 40 stock mycobacteria; 14 nocardiae; 17 Battey strains (Runyon et al., 1962); 13 scotochromogenic strains; 23 soil isolates; and 27 clinical isolates, including 5 *M. fortuitum* strains, 3 photochromogenic strains, and 19 Battey strains. Cer-

TABLE 1. *Principal stocks*

Received as	Source*	Preferred name†
Mycobacterium balnei	Steenken	—
M. butyricum S	Steenken	*M. smegmatis*
M. butyricum VA	MVAH	*M. smegmatis*
M. butyricum R	ATCC-356	*M. smegmatis*
M. chelonei	CDC	*M. smegmatis*
M. fortuitum Cruz	MVAH	*M. fortuitum*
M. fortuitum malta	Spink	*M. fortuitum*
M. friburgensis A403	Mankiewicz	*M. smegmatis*
M. kansasii	CDC	—
M. leprae (saprophyte)	CDC	—
M. phlei D	CDC	*M. phlei*
M. phlei R	MVAH	—
M. phlei T	ATCC-354	*M. phlei*
M. phlei Y	variant of R	*M. phlei*
M. rabinowitsch A402	Mankiewicz	*M. smegmatis*
M. ranae	CDC	*M. smegmatis*
M. rhodochrous 237	McClung	*Mycobacterium* sp.
M. rhodochrous 370	Gordon	*Nocardia* sp.
M. smegmatis 607	ATCC-607	*M. smegmatis*
M. smegmatis C	CDC	*M. smegmatis*
M. smegmatis A404	Mankiewicz	*M. smegmatis*
M. tuberculosis $H_{37}Ra$	CDC	—
M. tuberculosis $H_{37}Rv$	CDC	—
M. tuberculosis BCG	CDC	—
17 Battey strains	Runyon	—
13 Scotochromogens	Runyon	—
Nocardia asteroides 92	McClung	*Nocardia* sp.
N. asteroides 96	McClung	*Nocardia* sp.
N. farcinica 378	Hauduroy	*Mycobacterium* sp.
N. brasiliensis 301	Conant	*N. brasiliensis*

* ATCC, American Type Culture Collection; CDC, Communicable Disease Center; MVAH, Minneapolis Veterans Administration Hospital.

† The preferred name was assigned on the basis of phage-typing results; — = cultures not sensitive to test phages.

tain saprophytes, such as *M. ranae*, *M. chelonei*, *M. friburgensis*, and *M. butyricum*, were lysed by most of our mycobacteriophages, and *M. smegmatis* was uniformly sensitive to them. There were, however, significant differences in the efficiency of plating of particular phages on the alternative hosts. Other saprophytic mycobacteria, such as *M. phlei* D and *M. rhodochrous* 237, were attacked by only half of the phages tested. Some cultures bearing the same species designation displayed quite different patterns of susceptibility. *M. rhodochrous* 237 and 370 were unlike, physiologically and morphologically as well. In general, *M. rhodochrous* 237 and *N. farcinica* 378 behaved like typical mycobacteria, whereas *M. rhodochrous* 370 resembled the nocardiae (Table

TABLE 2. *Phages used and their propagating host*

Phage designation	Propagated on
101 to 115	*Mycobacterium smegmatis* 607
101	*M. fortuitum* Cruz
101	*M. phlei* D
110	*M. chelonei*
119	*M. rabinowitsch* A402
119	*M. phlei* D
MMP7	*M. friburgensis* A403
MMP10	*M. stercoides* A406
MMP14	*Mycobacterium* sp. A405
11728B	*M. phlei* T
MNP1	*Nocardia brasiliensis* N301
MNP1	*M. butyricum* VA
MNP2	*M. rhodochrous* 237

3). Not all disparate reactions between a set of phages and cultures bearing the same species designation can be attributed to mislabeling. *M. phlei* strain R was not attacked by any of our phages, whereas strains D, T, and Y possessed similar, but not identical, patterns of viral sensitivity (Table 4). Except for the fact that strain Y is a morphological variant of strain R, the latter mycobacterium might be considered incorrectly identified. All of our phages were unable to lyse *M. tuberculosis* $H_{37}Ra$, $H_{37}Rv$, and BCG. Battey strains, photochromogens, and scotochromogens could not serve as propagating hosts for any of the tested phages, but spots of clearing as described by Ward and Redmond (1962) were observed. *N. farcinica* 378 supported the growth of several mycobacteriophages, but *N. asteroides* was generally immune. Two phage preparations isolated on *N. brasiliensis* and modified by growth on alternative hosts attacked *N. asteroides*, *N. farcinica*, and some mycobacteria (Table 5). Accordingly, it is now possible to relate the true mycobacteria and the true nocardiae on the basis of overlapping susceptibility to phage. These relationships are best depicted as a much branched continuum (Fig. 1).

TABLE 3. *Susceptibilities of mycobacteria and nocardiae to mycobacteriophages**

Host	Phage				
	102	106	108	119	MMP14
Mycobacterium smegmatis 607	+	+	+	+	+
M. phlei D	+	0	+	0	+
M. fortuitum Cruz	0	+	+	0	0
Nocardia farcinica 378	0	0	0	+	+
M. rhodochrous 237	+	0	+	+	0
M. rhodochrous 370	0	0	0	0	0
N. asteroides 92	0	0	0	0	0

* Symbols: + = sensitive, 0 = resistant to test phages.

TABLE 4. *Diverse reactions of putative Mycobacterium phlei strains to mycobacteriophages**

Host	Phage				
	101	105	MMP10	MMP7	11723B
Mycobacterium smegmatis 607	+	+	+	+	+
M. phlei D	0	+	+	0	+
M. phlei T	+	+	+	+	+
M. phlei Y	+	+	0	+	+
Nocardia farcinica 378	0	0	0	+	0
M. phlei R	0	0	0	0	0

* Symbols: + = sensitive, 0 = resistant to test phages.

TABLE 5. *Overlapping sensitivity of nocardiae and mycobacteria to actinophages**

Host	Phage			
	MNP1	MNP1†	MNP2‡	115
Mycobacterium smegmatis 607	0	+	+	+
M. butyricum VA	0	+	0	+
Nocardia farcinica 378	0	+	+	0
M. rhodochrous 237	0	0	+	0
M. tuberculosis $H_{37}Ra$	0	0	0	0
N. asteroides 96	+	+	+	0
N. brasiliensis 301	+	0	0	0

* Symbols: + = sensitive, 0 = resistant to test phages.

† Single plaque isolated on, and then propagated on, *M. butyricum* VA.

‡ Single plaque isolated on, and then propagated on, *M. rhodochrous* 237.

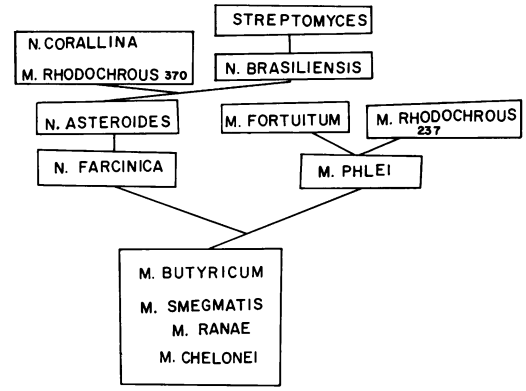

FIG. 1. *Relationships among mycobacteria and other actinomycetes based on phage susceptibility.*

DISCUSSION

Most mycobacteriophages are polyvalent, some attacking both saprophytic and pathogenic mycobacteria (Takeya et al., 1960; Froman et al., 1955). Phages which are reasonably specific for human strains of *M. tuberculosis* have also been described (Redmond, Cater, and Ward, 1963).

Both specific and polyvalent typing reagents have utility for identifying cultures; in addition, phages having broad host ranges provide insight into relationships among diverse groups. In this study, two strains were considered related only if they could serve as alternative propagating hosts for a particular virus. Ability of phage to attach to diverse microbes (Bowman, 1958) might reveal more remote affinities.

Our results with phage typing, like those of Bojalil, Cerbon, and Trujillo (1962) who used Adansonian classification, support the conclusions of Gordon and Smith (1953) and Gordon and Mihm (1959). All three groups agree that many strains designated *M. smegmatis*, *M. ranae*, *M. butyricum*, and *M. friburgensis* are so similar that they can reasonably be considered members of the same species. Moreover, we all found *M. phlei* to be a distinct entity closely allied to *M. smegmatis*. *M. fortuitum* strains were recognizably different from, although clearly related to, *M. phlei*.

Based upon viral susceptibility, the actinomycetes have now been arranged so that the streptomycetes and mycobacteria are joined by the nocardiae. The nocardiae with persistent mycelia seem to be the intermediate type, whereas those which fragment rapidly are depicted as a branch.

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