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Use of Phage F-φWJ-1 of *Mycobacterium fortuitum* to Discern More Phage Types of *Mycobacterium tuberculosis*

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A total of 125 strains of *Mycobacterium tuberculosis* from the Southeastern area of the United States was subjected to phage typing. In addition to the five major mycobacteriophages, a new phage, F-φWJ-1, was used in the study. The results obtained with the five major phages were: type A_0, 35.2%; type B, 29.6%; and type C, 4.0%. The remaining 21.2% of the strains phaged typed as subgroups A_1 through A_6. These percentages were similar to the typing results of earlier studies. The new phage, F-φWJ-1, subdivided each of the phage types, with the exception of type C, into two subgroups. The possible role of host modification-restriction of the phages used in phage typing of strains of *M. tuberculosis* is discussed.

Since the first observation of bacteriophages lytic on *Mycobacterium tuberculosis* (3), other investigators have reported the isolation of mycobacteriophages that subdivide the species on the basis of their sensitivity to the mycobacteriophages (1, 2, 10, 12). The species *M. tuberculosis* comprises a single homogenous serogroup (4); therefore, the use of mycobacteriophages to subdivide the species has been recognized as a potentially important epidemiological tool. The World Health Organization International Reference Center for the Diagnosis of Tuberculosis has sponsored a series of cooperative studies on the use of mycobacteriophages, and the results of the 1971-1972 and 1972-1973 studies have been recently reported (9).

In the present phage typing system, three of the five major mycobacteriophages use species of mycobacteria other than *M. tuberculosis* for their indicator host strains (9). Since the phages are replicated on species other than *M. tuberculosis* but are used for phage typing isolates of *M. tuberculosis,* the phage patterns obtained by the use of these phages may well be a reflection of a modification of the phages by their alternate host strains. With this hypothesis in mind, a lytic phage F-φWJ-1, isolated and maintained on a strain of *Mycobacterium fortuitum* (R-113), was incubated with the major phages used in the typing procedure.

The present report deals with the phage typing results obtained using 125 isolates of *M. tuberculosis* from the southeastern United States. The typing results obtained by using the new phage F-φWJ-1 are also included.

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MATERIALS AND METHODS

Mycobacterial isolates. The 125 isolates of *M. tuberculosis* used in this study were obtained from the Drug Reference Section of the Mycobacteriology Branch, Center for Disease Control. The isolates originated from the southeastern area of the United States and were confirmed as *M. tuberculosis* by the niacin and nitrate reductase tests (13).

Mycobacteriophages and their lysates. The five major mycobacteriophages, MTPH no. 2 through MTPH no. 6, were obtained from the National Institute of Public Health, Netherlands. Mycobacteriophage F-φWJ-1 was isolated in this laboratory from the soil of a flower bed by the enrichment method (3) in which *M. fortuitum* (R-113) was used. The phage has been maintained with strain R-113 for the indicator host strain. The phages, their host strains, and their routine test dilutions are listed in Table 1.

Media and procedures. The media used for the propagation of the phages and the methods for determination of their respective routine test dilutions were those described by Redmond and Ward (11). The preparation of the mycobacterial isolates to be tested, the phage testing procedures, and the reading of the results were outlined by the Study Group (9). To avoid loss of titer during the preparation of the phage lysates, all centrifugation of the phage lysates was carried out at 4 C. Also, although the mycobacteriophages are usually quite stable in the undiluted lysates when stored at 4 C, it was found that to obtain reproducible phage typing results the tubes containing the routine test dilutions of the phages had to be placed in crushed ice while being used for the spotting of the bacterial lawns. These

two observations are of special importance for the phages MTPH no. 5 and MTPH no. 6.

RESULTS

The phage typing results obtained with the 125 isolates of *M. tuberculosis* and the phages MTPH no. 2 through MTPH no. 6 are listed in Table 2. The most common phage type encountered was type A_0 which accounted for 35.2% of the isolates. Phage type B comprised 29.6% of the isolates, and type C only 4.0% of the isolates. The remaining 21.2% of the isolates were distributed among the remaining A phage types. Isolates belonging to the phage type A_2 were not found among the 125 isolates tested. Seven of the isolates were not lysed by either MTPH no. 4 or MTPH no. 6 and are listed in Table 2 as phage type A_6. A repeat of the phage typing of each isolate after a 1-month interval yielded the same results as in Table 2.

The phage typing results obtained with phage F-ϕWJ-1 are listed in Table 3. Phage F-ϕWJ-1 subdivided each phage type with the exception of phage type C into two further subgroups based on the lysis or absence of lysis of the isolates by this phage. Although phage type A has been previously subdivided, this is the first phage reported that subdivides phage type B isolates into two subgroups. In accordance with the Study Group's policy, phage F-ϕWJ-1 was sent to another laboratory for evaluation, and our results were confirmed. A description and characterization of phage F-ϕWJ-1 will be published at a later date.

TABLE 1. *Mycobacteriophages, their indicator host strains, and routine test dilutions (RTD) used for typing isolates of M. tuberculosis*

Phage	Host	RTD
MTPH no. 2	*M. tuberculosis* $H_{37}Rv$	10^6
MTPH no. 3	*M. tuberculosis* $H_{37}Rv$	10^5
MTPH no. 4	*M. smegmatis* ATCC 607	10^6
MTPH no. 5	*M. intracellulare* P-17	4.0×10^3
MTPH no. 6	Froman's F-130[a]	10^7
F-ϕWJ-1	*M. fortuitum* (R-113)	10^6

[a] Unidentified rapid grower.

TABLE 2. *Subdividing the 125 isolates of M. tuberculosis by mycobacteriophages no. 2 through no. 6*

Phage type	Phage pattern of MTPH phages[a]					No. of isolates	Strains lysed (%)
	2	3	4	5	6		
A_0	+	−	−	−	−	44	35.2
A_1	+	−	−	+	−	17	13.6
A_2	+	−	+	−	−	0	0.0
A_3	+	+	−	−	−	12	9.6
A_4	+	−	+	+	−	2	1.6
A_5	+	+	+	−	−	1	0.8
A_6	+	+	−	+	−	7	5.6
B	+	+	+	+	−	37	29.6
C	+	+	+	+	+	5	4.0

[a] Symbols: (+), Lysis; (−) no lysis.

TABLE 3. *Subdivision of the 125 isolates of Mycobacterium tuberculosis by mycobacteriophage F-ϕWJ-1*[a]

Phage type	WJ (+)	WJ (−)	Total no. of strains
A_0	27	17	44
A_1	12	5	17
A_2	0	0	0
A_3	4	8	12
A_4	1	1	2
A_5	0	1	1
A_6	6	1	7
B	23	14	37
C	5	0	5

[a] Symbols: (+), Lysis; (−) no lysis.

DISCUSSION

The subdivision of *M. tuberculosis* isolates into their various phage types has been reproducible in this laboratory, although the procedure has been somewhat unsuccessful in international studies (5, 9). Phage types C appear to be uncommon. In a study of 92 isolates of *M. tuberculosis*, only three isolates belonging to type C were reported (2), and in a recent study of the phage types found among Eskimo patients there were no phage type C found among 200 isolates (5). The present study found only five isolates in the 125 tested as belonging to type C. The influence of temperature on the phage lysates of MTPH no. 6, as described earlier in this report, could play an important role in the identification of this phage type. Although phage typing could prove an important tool in the epidemiological study of tuberculosis infections, considerably more investigation is needed to insure reproducibility among different laboratories.

By using phage F-ϕWJ-1 and *M. fortuitum* as the indicator host strain, we could discriminate six more phage subgroups of *M. tuberculosis* than the investigators in the international study could with their phages (9). The latter study included five phages, one of which, MTPH no. 2, attacks all strains of *M. tuberculosis*. The indicator host strain for each of the four remaining MTPH phages is a different species of bacteria, one of which is *M. tuberculosis* (MTPH no. 3). This would suggest that each species of mycobacteria exerts a modification of phage deoxyribonucleic acid which is differen-

tially restricted by different isolates of *M. tuberculosis*. This would also suggest that the phages replicated on *M. tuberculosis* might result in the phage deoxyribonucleic acid being modified to a form unrestricted by all strains of *M. tuberculosis*. This has been reported by Baess (1) and may explain the uniform action of MTPH no. 2 on all strains of *M. tuberculosis*. Phage MTPH no. 3 (host *M. tuberculosis* $H_{37}Rv$) constitutes an exception to this generalization and may require an explanation other than restriction. Other observations on host-directed modification of phages have been reported for mycobacteria (6, 8).

The restriction and modification hypothesis suggests that testing phages grown on other species of mycobacteria might be more useful in finding new typing phages than the indiscriminate screening of all mycobacteriophages. In this hypothesis, it is assumed that each species constitutes a population with no variation in its ability to modify phage deoxyribonucleic acid; therefore, caution is required when data from presumably a single species are interpreted. Work is currently underway in which we are attempting to adapt known phages to alternative hosts as well as to isolate new phages from such hosts.

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