Identification of *Mycobacterium phlei* TMC 1523 and *Mycobacterium smegmatis* SN40 as Members of *Mycobacterium smegmatis* and *Mycobacterium fortuitum*, Respectively: Selective Inhibition by Trimethoprim

ADALBERT LASZLO AND LESLIE EIDUS

Bureau of Bacteriology, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2

On the basis of a study of the effect of trimethoprim on the growth of 131 rapidly growing mycobacteria identified at the species level, it was found that two of the stains were possibly misidentified. This suspicion was confirmed by checking the salient phenotypic characters of the strains. It was found that strain TMC 1523 is not a strain of *Mycobacterium phlei* but of *M. smegmatis* and that strain SN40, previously identified as a member of *M. smegmatis*, is a strain of *M. fortuitum*.

In a biochemical study carried out on mycobacteria, Hendren (7) noticed that *Mycobacterium phlei* ATCC 356 was actually a strain of *M. smegmatis*. We recently experienced similar errors during our attempts to differentiate the various mycobacterial species by selective inhibition. The first antibiotic we investigated was ρ -nitro- α -acetyl-amino- β -hydroxy propiophenone, which, at a concentration of 10 μ g/ml, exclusively inhibits strains of both *M. tuberculosis* and *M. bovis* (3, 4). Boenicke (2) utilized thio-phen-2-carbonic acid hydrazide to differentiate between strains of *M. tuberculosis* and *M. bovis*.

In continuation of these antibiogram studies, we recently used trimethoprim (Tmp) in a study of the selective inhibition of rapidly growing mycobacteria. In modified fluid Sauton medium containing 5% bovine serum, 131 rapidly growing mycobacteria (Table 1) were tested against Tmp at concentrations of 15 and 30 μ g of drug per ml. The composition (per liter) of the medium was the following: L-asparagine, 2.00 g; magnesium sulfate $\cdot 7\text{H}_2\text{O}$, 0.25 g; sodium citrate, 1.25 g; ferric ammonium citrate, 0.05 g; sodium phosphate (dibasic anhydrous), 3.40 g; potassium phosphate (monobasic), 1.60 g; and glycerol, 20 ml.

The results of the inhibition study are shown in Table 2. With one exception, all strains identified as belonging to *M. smegmatis* were inhibited by Tmp during 3- and 6-day incubation periods. The strains of *M. fortuitum*, *M. chelonae* subsp. *abscessus*, *M. chelonae* subsp. *chelonae*, and *M. peregrinum* grew freely in both the 15- and the 30- μ g/ml Tmp concentrations. Of the 30 *M. phlei* strains, 29 grew and 1 was inhibited. The 12 *M. diernhoferi* strains were more susceptible to Tmp than those of the *M. fortuitum*

complex, *M. peregrinum*, and *M. phlei*: in the first 3 days, eight *M. diernhoferi* strains were inhibited by 15 and by 30 μ g of Tmp per ml. However, during the 6-day incubation period, all strains continued to grow in a 15- μ g/ml concentration, and only two strains were inhibited by 30 μ g of Tmp per ml.

The strains which did not fit into the selective inhibition scheme were *M. smegmatis* SN40, received from the Forschungsinstitut, Borstel, Germany, and *M. phlei* TMC 1523 of the Trudeau Institute, Saranac Lake, N.Y. (8). However, an examination of these strains revealed that TMC 1523 is not a strain of *M. phlei* but of *M. smegmatis* (this may explain why it was inhibited by trimethoprim) and that SN40, previously regarded as a member of *M. smegmatis*, is a strain of *M. fortuitum*. According to the results of our investigation, strains of the latter organism are not inhibited by Tmp.

The identification of mycobacteria as members of *M. phlei*, *M. smegmatis*, or *M. fortuitum* was based on the following methods and results. With the methods of Gordon and Mihm (5), strains of *M. phlei* grew at 52°C, did not produce acid from inositol or rhamnose, and failed to utilize benzoate as a carbon source; they gave negative amidase results with benzamide and succinamide (6). In contrast, strains of *M. smegmatis* did not grow at 52°C, produced acid from both inositol and rhamnose, utilized benzoate as a carbon source, and hydrolyzed benzamide and succinamide in the amidase test. Unlike strains of *M. smegmatis*, the *M. fortuitum* strains did not grow at 45°C, did not produce acid from arabinose, galactose, rhamnose, sorbitol, or xylose, were unable to use benzoate as a carbon

TABLE 1. Source and designation of mycobacterial strains used in this study

Organism	Source ^a	Designation of strains ^b
<i>M. smegmatis</i>	BI	SN1, SN2, SN3, SN4, SN6, SN8, SN10, SN11, SN12, SN14, SN40, SN41, SN43
	RU	W113, NCTC 523, NCTC 8150, IMRU 3, IMRU 25, IMRU 101, IMRU 392, IMRU 393, IMRU 447, IMRU 471, IMRU 476, IMRU 497, IMRU 519, IMRU 541
	TI	TMC 1515, TMC 1533, TMC 1546
	SMC	ATCC 23011
<i>M. fortuitum</i>	BI	SN201, SN203, SN209, SN212, SN217, SN220, SN227, SN229, SN231, SN234, SN236, SN238, SN239, SN242, SN256, SN259, SN222
	RU	NCTC 3630, NCTC 6929, IMRU 12, IMRU 389, IMRU 395, IMRU 448, IMRU 457, IMRU 478, IMRU 480, ATCC 6841, ATCC 6842, IMRU 48
	TI	TMC 1508, TMC 1530, TMC 1529
	NJH	581-Israel
	RIVV	MIS 102, MIS 107
	BI	SN270, SN271, SN272, SN273, SN274, SN275
<i>M. chelonae</i> subsp. <i>abscessus</i>	TI	TMC 1544, TMC 1543
	SMC	ATCC 23003
	RIVV	MIS 104
	LMP	YOUNG 8830/75
<i>M. chelonae</i> subs. <i>chelonae</i>	BI	SN281R, SN282, SN283, SN285, SN288R
	RU	IMRU 1277
<i>M. peregrinum</i>	RU	ATCC 14467
	NJH	Starr-Glidden, 16632 Welsh
	TI	TMC 1545
	SMC	ATCC 23001, ATCC 23015
<i>M. phlei</i>	BI	SN102, SN103, SN105, SN106, SN108, SN109, SN110, SN111, SN112, SN113, SN118, SN119
	RU	NCTC 525, NCTC 8151, NCTC 10008, ATCC 10142, ATCC 11758, IMRU 5, IMRU 8a, IMRU 45, IMRU 451, IMRU 500, IMRU 501, IMRU 594, IMRU 811, IMRU 855
	TI	TMC 1516, TMC 1523
	SMC	ATCC 19249, ATCC 23042
<i>M. diernhoferi</i>	BI	SN1401, SN1406, SN1409, SN1410, SN1413, SN1416, SN1418, SN1421, SN1422, SN1424, SN1425
	RU	ATCC 19340

^a BI, Forschungsinstitut, Borstel, Germany; RU, Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, N.J.; TI, Trudeau Institute, Saranac Lake, N.Y.; SMC, Shimane Medical College, Shimane, Japan; NJH, National Jewish Hospital and Research Center, Denver, Colo.; RIVV, Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands; LMP, Laboratory of Microbiology and Pathology, Department of Health, Brisbane, Australia.

^b ATCC, American Type Culture Collection; IMRU, Institute of Microbiology, Rutgers—The State University; MIS, International Study on Determination of Minimal Mandatory Methods; NCTC, National Collection of Type Cultures; SN, Stamm Number; TMC, Trudeau Mycobacterial Collection; W, Waksman.

source (5), and produced negative amidase tests with benzamide and succinamide. In addition, the 3-day arylsulfatase test of Wayne (10) was negative with strains of *M. smegmatis* and positive with strains of *M. fortuitum*. In these tests, strains TMC 1523 and SN40 behaved as members of *M. smegmatis* and *M. fortuitum*, respectively. These strains were also submitted to R. E. Gordon (Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, N.J.), who confirmed our results with regard to the identification of these strains.

According to the Trudeau Mycobacterial Cul-

ture (TMC) Collection catalog (8), *M. phlei* TMC 1523 was deposited in the TMC Collection by D. W. Smith, Madison, Wis., who presumably obtained it from the American Type Culture Collection (ATCC) as ATCC 19249 (= ATCC 11758). ATCC 11758 is the suggested "working type" of *M. phlei* (1), and at this stage it is very difficult to determine where the mislabeling or contamination of strain TMC 1523 occurred. In 1968, Tsukamura et al. (9) included ATCC 19249 in a taxonomic study of rapidly growing mycobacteria. They found that their culture of this strain did not exhibit the expected

TABLE 2. Screening of 131 rapidly growing mycobacteria for inhibition by 15 and 30 µg of Tmp per ml during 3- and 6-day incubation periods

Organism	No. of strains	No. of strains inhibited in:			
		3 days		6 days	
		15 ^a	30	15	30
<i>Mycobacterium smegmatis</i>	31	30 ^b	30	30	30
<i>M. fortuitum</i>	35	0	0	0	0
<i>M. chelonae</i> subsp. <i>abscessus</i>	11	0	0	0	0
<i>M. chelonae</i> subsp. <i>chelonae</i>	6	0	0	0	0
<i>M. peregrinum</i>	6	0	0	0	0
<i>M. phlei</i>	30	1 ^c	1	1	1
<i>M. diernhoferi</i>	12	8	8	0	2

^a Tmp concentration (micrograms per milliliter).

^b One *M. smegmatis* strain (SN40) grew in the presence of Tmp.

^c One *M. phlei* strain (TMC 1523) was inhibited by Tmp.

characteristics of *M. phlei*, and concluded: "The strain ATCC 19249 of *M. phlei* showed some similarity with *M. smegmatis* and *M. parafortuitum*. For better representation of the species *M. phlei* we included strain SN102." However, fast-growing mycobacteria may show test variations within the same species, and therefore reference strains displaying unexpected behavior should not be replaced by others without first confirming their identification. Moreover, whenever possible, preference should be given in taxonomic studies to the utilization of strains received directly from recognized culture collections rather than through indirect sources.

The findings of this laboratory supplement the observations made by Hendren (7). Selective inhibition by Tmp of growth of mycobacteria detected two erroneously identified strains. Such incorrectly identified strains may occasionally be maintained and utilized for decades without detection of the mistake. Therefore, it is necessary that the identification of reference strains be confirmed before their use, especially in taxonomic studies.

REPRINT REQUESTS

Address reprint requests to: Dr. Leslie Eidus, Bureau of Bacteriology, Laboratory Centre for Disease Control, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2.

LITERATURE CITED

1. American Type Culture Collection. 1974. American Type Culture Collection catalogue of strains, 11th ed., p. 76. American Type Culture Collection, Rockville, Md.
2. Boenicke, R. 1958. Differenzierung humaner und boviner Tuberkelbakterien mit Hilfe von Triphen-2-Carbonsau-rehydrazid. *Naturwissenschaften* 45:392.
3. Eidus, L., B. B. Diena, and L. Greenberg. 1959. The tuberculostatic activity of intermediate compounds of chloramphenicol. *Antibiot. Chemother.* IX:491-496.
4. Eidus, L., B. B. Diena, and L. Greenberg. 1960. The use of *p*-nitro- α -acetyl-amino- β -hydroxy-propiophenone (NAP) in the differentiation of mycobacteria. *Am. Rev. Respir. Dis.* 81:759-760.
5. Gordon, R. E., and J. M. Mihm. 1959. A comparison of four species of mycobacteria. *J. Gen. Microbiol.* 21:736-748.
6. Helbecque, D. M., V. Handzel, and L. Eidus. 1975. Simple amidase test for identification of mycobacteria. *J. Clin. Microbiol.* 1:50-53.
7. Hendren, R. W. 1974. Identification of *Mycobacterium phlei* ATCC 356 as a strain of *Mycobacterium smegmatis*. *Int. J. Syst. Bacteriol.* 24:491-492.
8. Trudeau Mycobacterial Culture Collection. 1972. Trudeau Mycobacterial Culture Collection catalogue, p. 171. Trudeau Institute, Saranac Lake, N.Y.
9. Tsukamura, M., S. Mizuno, and S. Tsukamura. 1968. Classification of rapidly growing mycobacteria. *Jpn. J. Microbiol.* 12:151-166.
10. Wayne, L. G. 1961. Recognition of *Mycobacterium fortuitum* by means of a 3-day phenolphthalein sulfatase test. *Am. J. Clin. Pathol.* 36:185-187.