***Mycobacterium* sp. strain 80: *Mycobacterium phlei* or *Mycobacterium smegmatis*?**

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Phage-induced changes in the bacterial host have been reported in several genera including *Mycobacterium* (Juhász, 1968). In general these changes affected only a few characters of the species and consequently did not interfere with the taxonomical standing of the organism involved. On the other hand *Mycobacterium* strain 80 which was obtained following lysogenization of *M. phlei* F89 with mycobacteriophage B2h·F89 (Juhász, 1968) differed from its *M. phlei* parent to such an extent that its taxonomic status became questionable.

Mycobacterium phlei F89 was obtained from Dr S. Froman, Olive View Sanatorium, Olive View, California, *M. smegmatis* SN 2 from Dr R. Bönicke, Research Institute for Experimental Biology and Medicine, Borstel, West Germany. Phage phlei B2 and phage smegmatis B1 were isolated from dung (Juhász & Bönicke, 1965); while phage B2h·F89 was obtained on reisolation of phage from lysogenic strain F89 (B2) (Juhász & Bönicke, 1966).

Table 1 shows the hybrid character of strain 80: properties shared by parental *Mycobacterium phlei* F89 along with newly acquired properties characteristic for *M. smegmatis*. A 22 hr amidase test characterized strain 80 as *M. smegmatis* (Bönicke, 1960; Juhlin, 1967). The strain could hydrolyse benzamide, isonicotinamide, salicylamide, and succinamide in addition to those amides which *M. phlei* is capable of hydrolysing. Similarly, the 2 hr nitrate reductase test (Juhász, Gelbart & Harize, 1969) and the 14-day phenolphthalein sulphatase test (Juhász *et al.* 1969) gave results more indicative of *M. smegmatis* than of *M. phlei*. On the other hand, Tween-80 hydrolysis (Wayne, 1962), pigmentation, heat resistance (Gordon & Mihm, 1959), and ability to grow at 52° were characteristic of *M. phlei*.

Sugar fermentation studies showed an intermediate position for strain 80: it could ferment rhamnose, considered a *Mycobacterium smegmatis* character, but like *M. phlei* F89 it could not ferment inositol (Gordon & Mihm, 1959). Drug resistance tests along with other enzymic tests such as tellurite reduction, as well as variations of the phenolphthalein sulphatase test failed to differentiate strain 80 from either *M. phlei* F89 or *M. smegmatis* SN2. Phage-sensitivity patterns (Table 1) showed that due to lysogenization with phage B2h·F89 strain 80 became immune to both B2h·F89 and B2. No sensitivity to smegmatis phage B1 could be observed.

The percentage guanine and cytosine content of the DNA of strain 80, as determined by the buoyant density method, was intermediate to that of *Mycobacterium*

phlei (69.9), and that of *M. smegmatis* (67.9) since the value of %GC obtained for strain 80 was 68.9. Because the DNA base composition values of *M. phlei* F89 and *M. smegmatis* SN2 differed by only 2%, a 1% difference could not be considered significant, yet another B2h·F89-lysogen, F89 (B2h) 2 (Juhász, 1968), which failed to reveal any of the changes reported for strain 80, had the same %GC value, 69.9, as parental *M. phlei* F89.

Table 1

	<i>M. smegmatis</i> SN2	Strain 80	<i>M. phlei</i> F89
22 hr Amidase test*			
Acetamide	+	+	+
Benzamide	+	+	0
Urea	+	+	+
Isonicotinamide	+	+	0
Nicotinamide	+	+	+
Pyrazinamide	+	+	+
Salicylamide	+	+	0
Allantoin	0	0	0
Succinamide	+	+	0
Malonamide	0	0	0
2 hr nitrate reduction (nmoles NO ₂ /ml.)	12	14	75
14-day phenolphthalein sulphatase test†	2+	3+	1+
Tween-80 hydrolysis (days till positive react.)	5	1	1
Phage sensitivity			
B1	+	0	0
B2	0	0	+
B2h	+	0	+

* The 22 hr amidase test is positive when spectrophotometric readings indicate production of over 3 µg. NH₃/ml. from a given amide bacterium mixture.

† A positive phenolphthalein sulphatase test indicates release of phenolphthalein from phenolphthalein disulphate salt.

1+ = 3-6 µg., 2+ = 6-16 µg., 3+ = 16-40 µg. phenolphthalein/ml.

Mycobacterial strains that do not conform to existing taxonomical criteria have previously been reported (Baess & Bentzon, 1969). The significance of strain 80 is that it was obtained from a well defined *Mycobacterium phlei* strain following lysogenization. Classification of such a strain is obviously difficult since reliance on the amidase spectrum and other enzymatic tests would assign strain 80 to *M. smegmatis*, while consideration of other characteristics, such as maximum growth temperature, heat resistance, colonial appearance, etc., would characterize the strain as an *M. phlei*. For taxonomists who rely on additional criteria such as DNA base composition, phage sensitivity, etc., the strain might even prove unclassifiable.

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REFERENCES

- BAESS, I. & BENTZON, M. W. (1969). Rapidly growing mycobacteria. *Acta path. microbiol. scand.* **75**, 331.
- BÖNICKE, R. (1960). Über das Vorkommen von Acylamidasen in Mycobakterien. IV. *Zentbl. Bakt. ParasitKde, Abt. I. Orig.* **178**, 209.
- GORDON, R. E. & MIHM, J. M. (1959). Comparison of some species of *Mycobacterium*. *J. gen. Microbiol.* **21**, 736.
- JUHASZ, S. E. (1968). Growth retardation, colonial changes and nutritional deficiency in *Mycobacterium phlei* due to lysogeny. *J. gen. Microbiol.* **52**, 237.
- JUHASZ, S. E. & BÖNICKE, R. (1965). Possible classification of rapidly growing mycobacteria on the basis of their phage susceptibility. *Can. J. Microbiol.* **11**, 235.
- JUHASZ, S. E. & BÖNICKE, R. (1966). Reciprocal genetic changes in mycobacterial host-virus systems: effect of lysogeny on the phage. *Nature, Lond.* **210**, 1185.
- JUHASZ, S. E., GELBART, S. & HARIZE, M. (1969). Phage induced alteration of enzymic activity in lysogenic *Mycobacterium smegmatis* strains. *J. gen. Microbiol.* **56**, 251.
- JUHLIN, I. (1967). Contribution to the classification of mycobacteria and nocardias. *Acta path. microbiol. scand.* **70**, Suppl. 189.
- WAYNE, L. G. (1962). Differentiation of mycobacteria by their effect on Tween 80. *Am. Rev. resp. Dis.* **86**, 579.