NOTES

Identification of *Mycobacterium phlei* ATCC 356 as a Strain of *Mycobacterium smegmatis*

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Mycobacterium phlei ATCC 356 has been shown to be a strain of *Mycobacterium smegmatis*.

A strain of bacteria listed as *Mycobacterium phlei* ATCC 356 (hereafter referred to as strain 356) was obtained from the American Type Culture Collection, Rockville, Md., and has been used in these laboratories for several years in the study of fatty acid biosynthesis (4 and references therein). It was assumed that this strain was representative of the species *M. phlei*.

Since it was known that 6-methylsalicylic acid (6-MSA) is synthesized via the polyketide pathway and excreted into the growth medium by *M. phlei* ATCC 354 and *M. phlei* ATCC 10142 (3), the study of 6-MSA biosynthesis in strain 356 was undertaken. It was subsequently found that crude extracts of this organism failed to synthesize 6-MSA from acetyl-coenzyme A and malonyl-coenzyme A in the presence of reduced nicotinamide adenine dinucleotide phosphate. In contrast, crude extracts of both *M. phlei* ATCC 354 and *M. phlei* ATCC 10142 were found to exhibit 6-MSA synthetase activity under the same conditions.

Mycobactin P, which is made exclusively by strains of *M. phlei*, is known to contain 6-MSA (7). It might be expected that mycobacterial species which do not produce a mycobactin containing 6-MSA would not synthesize 6-MSA. Indeed, *Mycobacterium smegmatis* is known to produce mycobactin S, which contains salicylic acid instead of 6-MSA (7), and extracts of *M. smegmatis* show salicylic acid synthetic capacity, whereas extracts of *M. phlei* are completely inactive in this respect (4, 6). The absence of 6-MSA synthetic ability in a mycobacterial strain presumed to be *M. phlei* seemed curious. To resolve this issue the mycobactins were isolated from strain 356 and *M. phlei* ATCC 354 by the methods described in ref. 3 and identified by ultraviolet absorption spectroscopy

(Fig. 1) and thin-layer chromatographic comparison with authentic samples of mycobactins P and S. *M. phlei* ATCC 354 was found to produce only mycobactin P, as expected, but

FIG. 1. Comparison of ultraviolet absorption spectra of mycobactins isolated from strain 356 and *M. phlei* ATCC 354 with authentic mycobactins S and P. Samples were dissolved in methanol at a concentration of approximately 6×10^{-5} M, and the spectra were recorded with a Cary 15 spectrophotometer.

strain 356 was found to produce only mycobactin S. This observation was indicative that strain 356 was actually a strain of *M. smegmatis* rather than *M. phlei*.

Several diagnostic tests known to differen-

TABLE 1. *Comparative tests of strain 356 and M. phlei ATCC 354*

Strain	Test ^a					
	Growth on glycerol agar 30 C	Growth on glycerol agar 52 C	Acid production from inositol	Acid production from sorbitol	Utilization of oxalate	Utilization of mucate
Strain 356	+	—	+	+	+	+
<i>M. phlei</i> ATCC 354	+	+	—	+	—	—

^a These tests were performed as described in reference 1.

tiate between *M. phlei* and *M. smegmatis* (1) were performed on *M. phlei* ATCC 354 and the acid-fast strain 356, and the results are shown in Table 1. These results support the hypothesis that strain 356 is a strain of *M. smegmatis*. The identification of strain 356 as *M. smegmatis* has since been confirmed by a more complete series of tests in another laboratory (R. E. Gordon, personal communication). It appears that the original classification (2) of strain 356 as a strain of *M. phlei* rather than *M. smegmatis* was due to the accidental mislabeling of a culture.

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