Mycobactins from *Mycobacterium avium*

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Mycobactins and exochelins have been isolated from four strains of *Mycobacterium avium* indicating this species to be unexceptional in the production of compounds involved in iron assimilation.

Mycobactins, which are lipid-soluble, iron-binding compounds, have been isolated from a wide variety of mycobacteria and have even been suggested as specific chemotaxonomic characters (6). Only *Mycobacterium paratuberculosis* and certain related strains have been found devoid of this compound, but they do require mycobactin as an essential growth factor (6). The recent discovery of L- α -asparaginy-L- α -(N-hydroxy)-asparagine in cultures of a strain of *Mycobacterium avium* by McCullough and Merkal (1) prompted these authors to suggest that this species of mycobacteria uses a different iron-sequestering system from that of other members of the genus. As we have been examining several strains of this species and have found them all to contain substantial levels of mycobactin, we wish to urge a note of caution before *M. avium* is regarded as an exceptional species.

Four *M. avium* strains, two from the National Collection of Type Cultures (8559 and 8562) and two field isolates (one from a deer and the other from a pheasant), were grown at 37°C on a modified Proskauer and Beck medium under iron-deficient conditions (3) for 5 to 8 weeks, depending on their vigor. Cells were harvested by filtration under vacuum, sucked dry, and extracted in 95% ethanol for 24 h in the usual manner for mycobactin isolation (6). The mycobactins were converted into their ferri complexes and purified as previously described (5).

Table 1 summarizes the yields and R_f values of these mycobactins after thin-layer chromatography. The mycobactins were readily separable from those from other mycobacteria in appropriate solvent systems. Reference mycobactins were from our laboratory collection. Nuclear magnetic resonance spectroscopy indicated the same nucleus was present in the mycobactins from *M. avium* as in all other previously described mycobactins (6). The long aliphatic side chain was in the more usual R_1 position (6) rather than attached to the centre of the molecule as is the case with the mycobactins M and N from *M. marinum*.

As mycobactin is only a part of the system elaborated by mycobacteria for iron uptake, we also examined culture filtrates for the presence of exochelins, which are water-soluble iron-binding compounds also involved in this process (2). In each case an exochelin was detected and could be extracted, as its ferri-complex, into chloroform. Thin-layer chromatography showed that these were similar, though not identical with those exochelins previously recorded from *Mycobacterium bovis* BCG (2).

We thus find that these strains of *M. avium* are unexceptional with respect to the iron-binding compounds which they elaborate and by inference in the manner by which they assimilate iron. The occurrence of asparaginyL-hydroxyasparagine in another *M. avium* (1) is of interest, however, as it could be related metabolically or functionally to the exochelin, type of molecule of which several types are already known to exist. The possible absence of mycobactin in this isolate is obviously of importance as, if confirmed, this would change our views of the constancy of iron metabolism patterns in mycobacteria. Mycobactins and related compounds have so far been found in all species of mycobacteria and nocardia thus far examined, although curiously no similar compound has been found in members of the rhodochrous complex (4).

We propose to term the mycobactin from *M. avium* mycobactin Av as it is no longer possible to keep to the single-letter designation originated by Snow (6).

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REPRINT REQUESTS

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TABLE 1. Yields of mycobactin from *Mycobacterium avium* species grown deficient of iron and comparison of them with mycobactins from other species

Species (and origin)	Cell yield (g/100 ml)	Mycobactin recovered (mg/g [dry wt])	Chromatographic mobilities ^a	
			System I (R_f values)	System II (R_R values)
<i>M. avium</i> NCTC 8559	0.42	24.5	0.73	1.00
<i>M. avium</i> NCTC 8562	0.42	6.3	0.72	1.00
<i>M. avium</i> deer isolate	0.53	1.5	0.71	1.22
<i>M. avium</i> pheasant isolate	0.32	7.4	0.75	1.04
<i>M. smegmatis</i> NCIB 8548			0.15	0.12
<i>M. bovis</i> BCG (Glaxo; P691)			0.26	0.18
<i>M. tuberculosis</i> H37Rv			0.27 ^b	0.16 ^b
<i>M. marinum</i> NCTC 10424			0.53, 0.64 ^{b, c}	0.39, 0.84 ^{b, c}

^a Thin-layer chromatography (I) on alumina using cyclohexane/*tert*-butanol (9:1 vol/vol) and (II) on silica gel using three successive developments of benzene/ethyl acetate (1:1, vol/vol). For the latter system, mobilities relative to the mycobactin from *M. avium* NCTC 8559 are given.

^b Mycobactins kindly supplied by G. A. Snow.

^c Two spots are visible: the slower running is the major component (i.e., mycobactin M), the other is mycobactin N.

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