Uric Acid Utilization by *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum* Isolates

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Forty-nine human and environmental isolates of *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum* were tested for their ability to grow on uric acid and a number of its degradation products. Nearly all (88 to 90%) strains used uric acid or allantoin as a sole nitrogen source; fewer (47 to 69%) used allantoate, urea, or possibly ureidoglycollate. Enzymatic activities of one representative isolate demonstrated the existence of a uric acid degradation pathway resembling that in other aerobic microorganisms.

Mycobacteriosis outbreaks in starlings (1), endemic mycobacteriosis in other birds with simultaneous isolation of *Mycobacterium avium* from feces-contaminated soil and mud of avian habitats (16), and recovery of related mycobacteria from still other birds and their habitats (20) provide cumulative evidence that nontuberculous mycobacteria of the *M. avium, Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum* (MAIS) group may utilize uric acid, a major avian nitrogen excretion product, or certain degradation products of uric acid as sources of carbon or nitrogen or both.

Previous metabolic studies have focused on degradation and utilization of uric acid or its degradation products by rapidly growing, nontuberculous mycobacteria (14) or on degradation of allantoin or urea (3, 5, 17–19). In these latter studies, the isolates which showed uricolytic activity were primarily rapidly growing nontuberculous mycobacteria. Klemperer et al. (9) demonstrated decomposition of radioactively labeled uric acid by various mycobacteria, including several MAIS isolates, and concluded that in *Mycobacterium butyricum* degradation is oxidative, involving the intermediate formation of allantoin, allantoic acid, ureidoglycollate, and urea, as suggested earlier by DiFonzo (6) for non-MAIS mycobacteria. Röhrscheidt et al. (13) cast doubt on these claims by showing that degradation in other rapidly growing mycobacteria did not follow the oxidative pathway. However, in none of these studies was a detailed examination of uric acid degradation and its pathway in MAIS isolates conducted.

In this report we describe the ability of 49 human and environmental isolates of *M. intracellulare* and *M. scrofulaceum* to utilize uric acid and its degradation products for growth. Furthermore, we examine the degradation pathway in one isolate.

MATERIALS AND METHODS

Bacterial strains. Of the 49 isolates of *M. intracellulare* or *M. scrofulaceum* used in this study, 27 were recovered from infected humans by the Mycobacteriology Laboratory of the New York State Department of Health, and 22 were isolated from fresh, brackish, or saline waters of the eastern United States (7). The isolates were identified to the species level and then assigned to one of the eight biotypes, based on their pigmentation and on the results of semiquantitative catalase and urease tests (8, 12). Isolates sharing two characteristics with either *M. intracellulare*, i.e., nonpigmented, urease negative, and catalase negative (– – –), or *M. scrofulaceum* (+ + +) were recorded as members of that species, although of different biotypes. One *M. scrofulaceum* isolate, W200 (+ + +), recovered from Lake Borgne, east of New Orleans, La., was used to study the enzymes of uric acid degradation.

Media. Strains were grown in Middlebrook and Cohn 7H9 medium containing 0.5% (vol/vol) glycerol and 10% (vol/vol) OADC Enrichment (BBL Microbiology Systems, Cockeysville, Md.). The basal medium used contained 0.5 g each of KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ per liter and 0.1 M glycerol. To this was added one of seven substrates, uric acid, allantoin, allantoic acid, ureidoglycollate, urea, L-glutamate, or $(NH_4)_2SO_4$, as the sole nitrogen source to a final concentration of 0.02 M (19). In some experiments, the concentrations of uric acid, allantoic acid, ureidoglycollate, and urea were lowered to 0.002 M to eliminate precipitate formation.

Preliminary tests established that liquid or solidified medium (Bacto agar, 20 g/liter; Difco Laboratories, Detroit, Mich.) gave similar results and that no significant differences in growth rates were observed at the different nitrogen source concentrations.

Cell extracts. Cells grown to late log phase in the basal medium were collected by centrifugation at 5,000 × *g* for 20 min at 4°C, washed twice, and suspended in

1/10 the original volume of 0.04 M potassium phosphate buffer (pH 7.2). Cells were disrupted with a French pressure cell until less than 10% of the cells were intact, as judged by microscopic examination. The suspension was then centrifuged at 10,000 × g for 20 min at 4°C, and the supernatant was used for enzyme assays. Assays were performed only on fresh extracts.

Enzyme assays. Uricase (urate:oxygen oxidoreductase, EC 1.7.3.3) activity was measured by the disappearance of uric acid, which was assayed spectrophotometrically as described by Mahler (11).

Allantoinase (allantoin amidohydrolase, EC 3.5.2.5) activity was determined by measuring the utilization of allantoin. Allantoin was determined by conversion to glyoxylate by alkaline-acid hydrolysis with heat. The resulting glyoxylate was transformed to glyoxylic acid-phenylhydrazone upon reaction with phenylhydrazine, which was oxidized in the presence of strong acid and potassium ferricyanide. Azo coupling and decarboxylation of the oxidized product yielded the red 1,5-diphenylformazan, whose concentration was measured spectrophotometrically (21).

The combination of allantoicase (allantoate amidinohydrolase, EC 3.5.3.4) and allantoate deiminase [allantoate amidinohydrolase (decarboxylating), EC 3.5.3.9] activities was assayed by the enzymatic disappearance of allantoic acid. Allantoic acid was converted to glyoxylate by acid hydrolysis (2, 21).

Ureidoglycollate lyase (ureidoglycollate urea-lyase, EC 4.3.2.3) activity was determined by the utilization of ureidoglycollate, measured before and after the reaction period by conversion to glyoxylate with heat (21).

Urease (urea amidohydrolase, EC 3.5.1.5) activity was measured by determining the amount of ammonia formed enzymatically from urea by the phenolhypochlorite method of Russell (15).

Because commercial samples of allantoic acid contained ureidoglycollate, glyoxylate, and ammonia and because ureidoglycollate samples contained glyoxylate and ammonia, appropriate control mixtures were prepared, and the initial concentration of glyoxylate, the spontaneous rates of decomposition of the substrates, and the amount of glyoxylate formed spontaneously during the enzymatic reactions were measured. The enzyme activities reported in the tables have been corrected for the impurity and for the spontaneous decomposition of substrates; each value is an average of triplicate measurements. All specific activities are given as nanomoles of substrate converted per milligram of protein per minute at 37°C. Protein

concentrations of extracts were determined by the method of Lowry et al. (10).

RESULTS

Utilization of uric acid and degradation products. Preliminary experiments indicated that isolates of M. intracellulare and M. scrofulaceum could utilize uric acid or one of its degradation products as a sole nitrogen source, although not as a sole carbon source (data not shown). Of the 49 isolates, 88% utilized uric acid and 90% utilized allantoin as sole nitrogen sources (Table 1). Fewer isolates grew on allantoic acid, ureidoglycollate, and urea.

Because of the long periods of incubation required to grow these microorganisms in the basal medium (see above), the stability of each substrate was carefully monitored. The only apparently unstable substrate during the incubation periods used (even in enzyme analyses) was ureidoglycollate. Because of this instability, the isolates which appeared to utilize ureidoglycollate may have been growing on released urea.

Although differences in utilization of these nitrogen sources were found between M. intracellulare and M. scrofulaceum (Table 1) and differences among their biotypes occurred (data not shown), these differences were not significant at the 0.05% level (chi-square test). Also, no significant differences were noted between the human isolates and those recovered from eastern waters. M. scrofulaceum isolate W200, which grew on all substrates (albeit slowly on urate), was chosen for detailed studies of growth characteristics and enzyme activities.

Generation time with uric acid and degradation products. The generation times for isolate W200 on various substrates, as judged by the increase in colony-forming units and final growth yields, are given in Table 2. The rates and yields of growth on uric acid and allantoic acid were significantly poorer than on allantoin, ureidoglycollate, urea, and the control, L-glutamate. No conclusions concerning growth rates in ureidoglycollate can be drawn because of its instability.

TABLE 1. Utilization of uric acid or its degradation products as a sole nitrogen source by human and environmental isolates of M. intracellulare and M. scrofulaceum

Species	No. of isolates	% of isolates utilizing:[a]				
		Uric acid	Allantoin	Allantoic acid	Ureidoglycollate	Urea
M. intracellulare	31	84	87	58	65	48
M. scrofulaceum	18	94	94	56	78	44

[a] Percentage of isolates able to grow in glycerol basal medium containing one nitrogen source at a concentration of 0.02 or 0.002 M. Growth was scored as positive if a turbid suspension formed after 6 weeks at 37°C.

TABLE 2. Generation time during exponential growth and final growth yield of *M. scrofulaceum* isolate W200 on various sole nitrogen sources[a]

Nitrogen source	Generation time (days)	Growth yield (10^8 CFU/ml)
L-Glutamate (control)	3.5	8.1
Uric acid	6	1.1
Allantoin	3.1	24.0
Allantoic acid	4.6	2.3
Ureidoglycollate	3.6	7.5
Urea	3.6	11.0

[a] Isolate W200 was grown in glycerol minimal medium containing one nitrogen source at a concentration of 0.02 M and at 37°C with shaking.

Enzyme activities. All enzyme activities tested were expressed by isolate W200 (Table 3). Ureidoglycollate lyase activity was increased when the cells were grown on L-glutamate; allantoinase and urease activities were present in lower amounts in L-glutamate-grown cells. Allantoicase-allantoate deiminase activities did not appear to be stimulated by growth on allantoin. The enzyme activities of extracts of cells grown on urate and allantoate were not measured, because growth yields were too low to permit accurate measurement. Extracts of ureidoglycollate-grown cells were not assayed because of the possibility that such cells were growing on urea.

DISCUSSION

This study has established that many isolates of *M. intracellulare* or *M. scrofulaceum* or both can utilize uric acid and its degradation products as sole sources of nitrogen, but not carbon, for growth. The fact that environmental and human isolates are equally capable of utilizing these compounds demonstrates a possible similarity between MAIS from human infection and from the environment (23). Whether or not uric acid utilization is unique to rapidly growing mycobacteria and MAIS or occurs also in other mycobacteria is beyond the scope of this work.

Other workers have demonstrated that rapidly growing mycobacteria are capable of either utilizing these compounds for growth or synthesizing the enzymes necessary for aerobic decomposition (3, 5, 6, 9, 14, 17–19). Some of these enzymes are present in lower amounts in glutamate-grown cells, implying that the enzymes could be regulated. Finally, the enzymes are representative of those of the pathway common to most aerobic microorganisms (22).

Because the *M. scrofulaceum* isolate (W200) used for the enzymatic studies synthesizes urease, no conclusion can be drawn concerning the involvement of allantoicase or allantoate deiminase in the pathway (22). The low growth rate and

TABLE 3. Specific activities of uricolytic enzymes in *M. scrofulaceum* isolate W200

Enzyme	Cells grown on:		
	Allantoin	Urea	L-Glutamate (control)
Uricase	6.0[a]	8.2	<0.1
Allantoinase	1.1	0.19	0.07
Allantoicase and allantoate deiminase	<0.05	0.29	<0.05
Ureidoglycollate lyase	0.38	0.38	0.48
Urease	20.0	7.0	1.2

[a] Enzyme activity is expressed as nanomoles of substrate converted per milligram of protein per minute at 37°C. The limits of detection in the enzyme assays were 0.1 for uricase; 0.05 for allantoinase, allantoicase-allantoate deiminase, and ureidoglycollate lyase; and 0.04 for urease. Assays were done in triplicate; the values shown are means, with variation of <10% of the mean.

yield of this isolate on allantoic acid are not unique to mycobacteria, as two of seven isolates of the uricolytic *Bacillus fastidiosus* failed to grow only on this compound (2). The poor growth of isolate W200 on uric acid and allantoic acid, as well as the growth patterns of some other isolates tested, could be due to transport barriers.

The failure of earlier workers to demonstrate the presence of uricolytic-specific enzymes in MAIS isolates (3, 5, 13, 17) may be explained by the growth conditions. The cells were usually grown in complex media containing either ammonia or amino acid nitrogen. The ready availability of other nitrogen sources, such as ammonia or L-glutamate, probably repressed uricolytic enzyme synthesis, even in the presence of substrates. Such inhibition involving amino acid nitrogen has been reported for the allantoin-degradative enzymes of *Saccharomyces cerevisiae* (4).

Not only is the breakdown and utilization of uric acid and its degradation products potentially useful as a taxonomic tool, but this physiological feature of MAIS organisms also has potential ecological importance. MAIS avian infections and the apparently frequent presence of MAIS in avian habitats, which are undoubtedly rich in uric acid and its derivatives, may be due to the ability of these organisms to utilize uric acid and its degradation products as nitrogen sources.

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