Heterogeneity of Thymidine Kinase Among Species of *Mycoplasma* and *Acholeplasma*

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The thymidine kinase activities present in nine strains (including four type strains and one neotype strain) of nine species of the genus *Mycoplasma* and two strains (including one type strain) of two species of the genus *Acholeplasma* were characterized with respect to migration in polyacrylamide gels and isoelectric point as determined by electrofocusing. There was a marked heterogeneity in the enzymes, not only between the two genera but also within each genus.

Previous investigators have shown that certain mycoplasmas have thymidine kinase (TK) activity (7, 9), and it has been suggested that these organisms might use the scavenger pathway to supply thymidine nucleotides for deoxyribonucleic acid synthesis.

The purpose of this study was to examine other mycoplasmas and some acholeplasmas for the presence of TK activity and to characterize the TKs by polyacrylamide gel electrophoresis and isoelectric focusing.

MATERIALS AND METHODS

The sources and designations of the strains of *Mycoplasma* and *Acholeplasma* used in this study are listed in Table 1.

Cells were grown at 37°C in 40 ml of a medium consisting of beef heart infusion supplemented with 20% whole horse serum, 10% yeast extract, 100 U of penicillin per ml, and 0.002% phenol red. Growth of the organisms and possible contamination were checked by inoculating 0.1 ml of the broth culture onto PPLO agar plates supplemented as described above. The growth from a 40-ml broth culture was harvested by centrifugation of the culture for 30 min at $20,000 \times g$ in a Ti 60 rotor in an L5-50 Beckman ultracentrifuge; the pellet was then resuspended in 1 ml of 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0) containing 0.5% Triton X-100. The resulting lysate was centrifuged for 15 min at $35,000 \times g$, and 100 μ l of the supernatant was assayed for TK activity. TK activity was assayed as described by Munyon et al. (3). The 200- μ l TK reaction mixture consisted of 0.07 M KCl, 1.3 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 4 mM adenosine triphosphate, 4.5 μ Ci of [3H]thymidine (60 Ci/mmol; New England Nuclear Corp.), 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0), and 100 μ l of the supernatant from the centrifuged lysate. This was allowed to react for 20 min at 38°C. The reaction was stopped with 35 μ l of 50% trichloroacetic acid, and 30- μ l portions were spotted onto DE-81 filters. After drying, the filters were washed four times in 0.001 M ammonium formate buffer (pH 3.6), four times in water, and once in 95%

ethanol. The papers were dried and counted in 5 ml of toluene scintillation fluid; 5% polyacrylamide gel electrophoresis and isoelectric focusing were then run on the lysate supernatants which contained TK activity.

Electrophoresis was carried out as described by Munyon et al. (3); 5% polyacrylamide was used for the separating gels. Electrophoresis was carried out in 7-cm gel columns at 4°C for approximately 2 h. A 100- μ l portion of the enzyme lysate plus 20 μ l of 50% glycerol and 0.2% bromophenol blue were added to the top of the gel. A constant current power supply was used, and this was set at 2 mA/gel. The electrophoretic migration was discontinued when the bromophenol blue band was approximately 3 mm from the anode end of the tube. The gels were removed from the tube and frozen for 15 min at -70°C. Gel slices (2 mm) were taken, placed on 0.7-cm squares of DE-81 paper saturated with 20 μ l of TK reaction mixture, and incubated for 4 h at 37°C in a humidified atmosphere. The TK reaction mixture consisted of 0.1 M maleate-tris(hydroxymethyl)aminomethane buffer (pH 6.5), 0.025 M KCl, 0.02 M $MgCl_2$, 0.0014 M 2-mercaptoethanol, 0.01 M adenosine triphosphate, and 0.1 mCi of [3H]thymidine (specific activity, 60 Ci/mmol) per gel column. After incubation, the papers were washed four times in 0.001 M ammonium formate buffer (pH 3.6), four times in water, and once in 95% ethanol. The papers were dried, placed in vials containing 5 ml of toluene scintillation fluid, and counted.

The isoelectric focusing gel solution consisted of the following: 26% (vol/vol) acrylamide-bis-acrylamide stock (28% [wt/vol] acrylamide plus 1.62% [wt/vol] bis-acrylamide); 3% (vol/vol) ampholytes, pH 3.5 to 10 (LKB); 2% (vol/vol) ampholytes, pH 7 to 9; 48.7% (vol/vol) glycerol (40% solution); 0.2% (vol/vol) riboflavin (4 mg/100 ml); 0.13% (vol/vol) N,N,N,N' -tetramethylethylenediamine; and 0.26% (vol/vol) ammonium persulfate (100 mg/ml). This solution was placed in gel tubes and photopolymerized for 30 min. Gel columns (10 cm) were run with 0.1 N NaOH as the upper electrode buffer and 0.01 N H_3PO_4 as the lower electrode buffer. The gels were overlaid with 0.05 ml of a solution consisting of the following: 0.06 ml of ampholytes, pH 3.5 to 10; 0.04 ml of ampholytes, pH 7 to 9; 0.95 ml of 40% (vol/vol) glycerol; and 0.4 ml of water. The gels were then prerun for 15 min at 200

TABLE 1. *Mycoplasma* and *Acholeplasma* strains used in this study

Serological type or species	Strain	Source
<i>M. arginini</i>	Tissue culture isolate	E. Stanbridge
<i>M. arthritidis</i>	PG6 ^a	E. Stanbridge
<i>M. fermentans</i>	PG18 ^b	E. Stanbridge
<i>M. gallinarum</i>	PG16 ^b	E. Stanbridge
<i>M. hominis</i>	PG21 ^b	E. Stanbridge
<i>M. hyorhinis</i>	ESCA-1	E. Stanbridge
<i>M. orale</i> type 1	ESE-1	E. Stanbridge
<i>M. pulmonis</i>	JB	J. Galpin
<i>M. salivarium</i>	PG20 ^b	E. Stanbridge
<i>A. laidlawii</i>	PG8 ^b	E. Stanbridge
<i>A. granularum</i>	No designation	G. Kenny via E. Stanbridge

^a Neotype strain.^b Type strain.

V (constant voltage), for 30 min at 300 V, and for 1 h at 400 V. After prerunning, 55 μ l of the enzyme lysate plus 8 μ l of ampholytes, pH 3.5 to 10, 6 μ l of ampholytes, pH 7 to 9, 130 μ l of 100% glycerol, and sheep erythrocyte hemoglobin were layered below the overlay buffer with a Hamilton syringe. The gels were run in a cold room (4°C) for 15 h at 400 V and then for 1 h at 600 V. The gels were removed, frozen, sliced, and assayed as described above for the 5% polyacrylamide gels. Slices (4 mm) from the control gel were placed in 1 ml of water and shaken for 2 h at 4°C, and the pH was determined with a Beckman Phasar-I digital pH meter.

RESULTS AND DISCUSSION

Figure 1A shows the electrophoretic profiles of *Mycoplasma pulmonis* strain JB and *Mycoplasma arthritidis* PG6 TKs, and Fig. 1B shows the electrophoretic profiles of the *Acholeplasma granularum* strain and *Acholeplasma laidlawii* PG8 TKs. The *M. pulmonis* JB TK had an R_f (migration relative to bromophenol blue) value of less than 0.1, whereas *M. arthritidis* PG6 TK had an R_f value which ranged from 0.14 to 0.21. The *A. granularum* strain TK had an R_f value between 0.34 and 0.37, and the *A. laidlawii* PG8 TK R_f ranged from 0.41 to 0.47. Polyacrylamide gel electrophoreses were also run on lysate supernatants from strains of *Mycoplasma arginini*, *M. fermentans*, *M. gallinarum*, *M. hominis*, *M. hyorhinis*, *M. orale*, and *M. salivarium* (Table 2). The TK R_f values for these different strains varied over a wide range; there were even differences within each genus (i.e., *Mycoplasma* and *Acholeplasma*).

The results of the isoelectric focusing gel determinations for *A. laidlawii* PG8 and *M. arthritidis* PG6 TKs are shown in Fig. 2A and B, respectively. The isoelectric point (pI) for *A. laidlawii* PG8 TK was between 7.0 and 7.2, and

that for *M. arthritidis* PG6 TK was ≥ 7.9 . The pI values for these and the other strains are shown in Table 2. The two *Acholeplasma* strains had similar pI values (approximately 7.2), which were quite different from those of the *Mycoplasma* strains. The TK activities of *M. arthritidis* PG6 and the other mycoplasmas remained at the tops of the isoelectric focusing gels; therefore, gels with higher pH ranges must be used to determine accurately the pI values of these strains.

The heterogeneity of the TKs found in different species of *Mycoplasma* is not surprising considering the lack of deoxyribonucleic acid homology among them, as determined by McGee

FIG. 1. Polyacrylamide gel electrophoresis of *Mycoplasma* and *Acholeplasma* TK activities. (A) Dashed line, *M. pulmonis*; solid line, *M. arthritidis*. (B) Dashed line, *A. granularum*; dotted line, *A. laidlawii*. dTMP, Deoxyribosylthymine monophosphate.

TABLE 2. R_f values and isoelectric points of TKs from strains of *Mycoplasma* and *Acholeplasma*

Strain	R_f	pI
<i>M. arginini</i> tissue culture isolate	0.3-0.33	≥ 8.0
<i>M. arthritidis</i> PG6	0.14-0.21	≥ 7.9
<i>M. fermentans</i> PG18	0.2-0.33	≥ 8.0
<i>M. gallinarum</i> PG16	0.5	
<i>M. hominis</i> PG21	0.36-0.4	≥ 7.5
<i>M. hyorhinis</i> ESCA-1	0.1-0.2	≥ 7.8
<i>M. orale</i> type 1 ESE-1	0.2-0.27	≥ 7.7
<i>M. pulmonis</i> JB	<0.1	
<i>M. salivarium</i> PG20	0.3-0.38	≥ 8.0
<i>A. laidlawii</i> PG8	0.41-0.47	7.0-7.2
<i>A. granularum</i>	0.34-0.37	7.2-7.5

^a As determined by 5% polyacrylamide gel electrophoresis.

FIG. 2. Isoelectric focusing profiles of TKs. (A) Bottom curve, *A. laidlawii* TK profile. (B) Bottom curve, *M. arthritidis* TK profile. The top curves in both (A) and (B) are the pH profiles of the gels. dTMP, Deoxyribosylthymine monophosphate.

et al. (2) and Reich et al. (5, 6). There were no *Mycoplasma* species examined in this study which did not contain TK activity. Differences in electrophoretic mobility of hypoxanthine-guanine phosphoribosyl transferase activity between *Mycoplasma* species have already been demonstrated by Stanbridge et al. (8) and Van Diggelen et al. (10).

TK, together with other enzyme activities (such as hypoxanthine-guanine phosphoribosyl transferase, HGPRT), is frequently studied in cultures of mammalian cells. The cryptic contamination of tissue cultures by mycoplasmas not readily propagated on artificial culture media is a proven problem in such studies. The heterogeneity of the TKs from mycoplasmas, although of some taxonomic interest, must also be considered in such studies as those dealing

with the selection of mutant phenotypes of tissue culture cells or the biochemical transformation of cells by viruses (1, 4).

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REPRINT REQUESTS

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