Presence of the Arginine Dihydrolase Pathway in Mycoplasma

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ABSTRACT

BARILE, MICHAEL F. (Division of Biologics Standards, National Institutes of Health, Bethesda, Md.), ROBERT T. SCHIMKE, AND DONALD B. RIGGS. Presence of the arginine dihydrolase pathway in Mycoplasma. J. Bacteriol. 91:189-192. 1966. — The presence of the arginine dihydrolase pathway was examined in 61 Mycoplasma strains representing at least 18 Mycoplasma species isolated from nine different sources: human, bovine, avian, murine, swine, goat, canine, sewage, and tissue culture origin. Some species were represented by only one or two strains. Different strains of the same species gave the same results. Ten species (56%) were positive. Many nonpathogenic Mycoplasma species (M. hominis, type 1 and 2, M. fermentans, M. salivarium, and M. gallinarum) were positive, whereas most pathogenic species (M. pneumoniae, M. gallisepticum, M. neurolyticum, and M. hyorhinis) were negative. The presence of arginine dihydrolase activity among Mycoplasma species may prove to be useful for purposes of identification and classification.

Schimke and Barile (25) reported that the rapid conversion of arginine to ornithine in tissue cultures contaminated with Mycoplasma and by the isolated Mycoplasma grown in broth culture occurred by the 3-enzyme arginine dihydrolase pathway. Barile and Schimke (3) subsequently developed a test for detection of Mycoplasma in contaminated tissue cultures based on the presence of arginine deiminase activity. The present study was undertaken to establish whether the arginine dihydrolase pathway was common to Mycoplasma from other sources, and whether this pathway could be used as a criterion for the presence of Mycoplasma. In addition, the presence of arginine dihydrolase activity is of interest, since this pathway may provide Mycoplasma with a significant source of high-energy phosphate, adenosine triphosphate (25, 28).

MATERIALS AND METHODS

Cultures. Mycoplasma cultures were collected from as many different investigators and laboratories as possible; in some cases, only one or two strains of a given Mycoplasma species was available. Sixty-one Mycoplasma strains representing at least 18 Mycoplasma serotypes (9) were obtained and examined for the presence of arginine deiminase activity. These include prototype Mycoplasma species isolated from human (13), bovine (20), avian (5, 10), murine (21), swine (30), goat (7), canine, sewage, and tissue culture (2) origins.

Culture media. M. pneumoniae, M. orale, M. hyorhinis, and strain 880 (12) were grown in a broth medium described earlier (6). All other Mycoplasma strains were grown in BYE broth (BBL) with 15% noninactivated horse serum added (4). Both media were supplemented with 0.2% L-arginine. In some cases, 10 μmoles of glutamine, 0.5% glucose, and vitamins as used in Eagle's (8) tissue culture medium were incorporated. Mycoplasma titers were estimated by a dilution-plate count method (25).

Arginine deiminase activity. Enzyme activity was estimated by a method described previously (3). The Mycoplasma were prepared for assay as follows: 100 ml of a 3-day-old broth culture was centrifuged at 30,000 × g for 20 min. The sedimented Mycoplasma were suspended in 1.0 ml of distilled water, then frozen and thawed twice. A 0.1-ml suspension of the Mycoplasma lysate was used in the enzyme assay, and the reaction mixture was incubated at 37 C for 30 min. Freezing and thawing were as effective as other methods of cell disruption, including sonic treatment and grinding with alumina, in yielding active enzyme preparations. Enzyme assays were repeated on three or more separate cultures before the Mycoplasma strain was considered to lack the enzyme. For each assay, the population of organisms was greater than 10⁶ per milliliter, a number of organisms previously found to be the lower limit for detection of activity.
M. hominis, type 1
Strains PG-21; PG-21C
Strain H-34
Strain HEp-2
M. hominis, type 2
Strains PG-27; PG-27C; 07; 39
M. fermentans
Strains PG-18; PG-18C
M. salivarum
Strains PG-20; PG-20C; B2
M. orale
M. pneumoniae
Strain FH-Lui
M. gallisepticum
Strains H-1344 (S6); 293 (S6); 8(S6)
M. gallinarum
Strain PG-16
M. pulmonis
Strains S; Ash
M. arthritidis
Strains JR-3*; Preston
M. neurolyticum
Strains PG-28; Type A; KSA
Strain T-15
M. hyorhinis
Strain 7
M. laidlawii A
Strain PG-8
M. laidlawii B
Strain PG-9
M. laidlawii
Strain Laidlaw
Strain 48
Strain B-15
M. spumans
Strain PG-13
M. canis
Strain PG-14
M. maculosum
Strain PG-15
Mycoplasma sp.
Strains 2R; 4R; 6R*

<table>
<thead>
<tr>
<th>Identification</th>
<th>Source</th>
<th>Arginine deiminase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. hominis, type 1</td>
<td>Human urethra</td>
<td>+</td>
</tr>
<tr>
<td>M. fermentans</td>
<td>Human oral</td>
<td>+</td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>Human pneumonia</td>
<td>-</td>
</tr>
<tr>
<td>M. gallisepticum</td>
<td>Avian CRD†</td>
<td>+</td>
</tr>
<tr>
<td>M. pulmonis</td>
<td>Murine, lung</td>
<td>+</td>
</tr>
<tr>
<td>M. neurolyticum</td>
<td>Murine, arthritidis</td>
<td>-</td>
</tr>
<tr>
<td>M. hyorhinis</td>
<td>Murine, conjunctiva</td>
<td>-</td>
</tr>
<tr>
<td>M. laidlawii</td>
<td>Sewage</td>
<td>-</td>
</tr>
<tr>
<td>M. spumans</td>
<td>Canine</td>
<td>+</td>
</tr>
</tbody>
</table>

* These Mycoplasma strains were isolated by the senior author.
† CRD = chronic respiratory disease.
‡ RD = "rolling disease."

**RESULTS**

The results on the presence or absence of arginine deiminase activity in the Mycoplasma tested are summarized in Table 1. Attempts to determine specific activities of arginine deiminase, i.e., activity per milligram of cell protein, for each of the Mycoplasma tested were not made because of difficulties in adequately washing the cells free of protein in the medium without also lysing cells. Where specific activities were determined, however, the values were markedly similar for different Mycoplasma species, varying from 3 to 5 micromoles of citrulline formed per min per mg of cell protein at 37°C.

Different strains of the same Mycoplasma species isolated by different investigators gave the same results. There were no inconsistencies (Table 1). Ten of the Mycoplasma species tested (56%) contained arginine deiminase activity. Five of the six human Mycoplasma species were positive (M. hominis, type 1 and 2; M. orale; M. salivarum; and M. fermentans). M. pneumoniae was negative. The other positive Mycoplasma species were M. gallinarum and M. iners (avian), M. spumans and M. maculosum (canine), and M. arthritidis (murine). The negative Mycoplasma species were M. gallisepticum (avian), M. canis (canine), M. hyorhinis (swine), M. pulmonis...
and *M. neurolyticum* (murine), and *M. laidlawii* (saprophyte).

The presence of ornithine transcarbamylase, another enzyme of the arginine dihydrodrolase pathway, was surveyed in selected arginine deiminase-positive and -negative *Mycoplasma*, including strains Laidlaw, calf, NTF, JR-3, PG-21, H-1344, 07, and 48. When assayed as described by Schimke and Barile (25), ornithine transcarbamylase activity was found only in those *Mycoplasma* species which also contained the arginine deiminase activity.

The effect of growth conditions on demonstrating the presence of arginine deiminase activity was examined in selected positive and negative *Mycoplasma* strains, including Laidlaw, calf, kid, ERKS, 07, and 48. Growth of *Mycoplasma* in media with or without supplemental arginine or glucose, and harvesting cells in log or stationary phase of growth, did not affect the presence of arginine deiminase activity. The cell yields of *Mycoplasma* containing the arginine dihydrodrolase pathway were increased consistently when media were supplemented with additional L-arginine, whereas no increase was observed with *Mycoplasma* which did not contain the arginine dihydrodrolase pathway (25).

**DISCUSSION**

Of the 18 *Mycoplasma* species studied, 10 (56%) were positive for the arginine dihydrodrolase pathway. Both positive and negative *Mycoplasma* species were obtained from human, murine, canine, and avian sources. It is of interest that the nonpathogenic *Mycoplasma* species were usually positive, whereas most of the pathogenic *Mycoplasma* species were negative. For example, of the six known human *Mycoplasma* species, *M. pneumoniae*, the only species definitely known to be pathogenic for man, exhibited no arginine deiminase activity. Moreover, negative results were also obtained with the nonhuman *Mycoplasma* species known to be highly pathogenic, including *M. neurolyticum*, *M. hyorhinis*, and *M. gallisepticum*. Whether there is any correlation between pathogenicity and lack of arginine dihydrodrolase activity remains to be determined. In any case, the use of the arginine deiminase assay may prove to be of value for purposes of identification and classification of *Mycoplasma* species and may be useful as a diagnostic tool.

The arginine dihydrodrolase enzyme system has not been found in tissue cell cultures, in mammalian tissue, or in several rickettsiae and one psittacosis virus studied, including the Breinl and E strains of epidemic typhus, *Rickettsia pro-

wazekii*, the Wilmington strain of murine typhus, *Rickettsia mooseri*, and the P-4 strain of ornithosis virus (Schimke, Hopps, and Barile, unpublished data). The pathway has been found in only a few species of microorganisms, including *Streptococcus* (11, 15), *Lactobacillus* (32), *Clostridium* (26), *Pseudomonas* (16), yeast (24), and *Mycoplasma* (25, 28, 29). The results given in the present report indicate that the arginine dihydrodrolase enzyme system is a common pathway among a number of species of *Mycoplasma*. This may suggest that the positive *Mycoplasma* could have been derived from bacteria containing this pathway.

Schimke and Barile (25) suggested that the metabolism of arginine by the arginine dihydrodrolase pathway may constitute a significant energy source for *Mycoplasma*. Preliminary findings have shown that arginine deiminase, ornithine transcarbamylase, and carbamate kinase constitute approximately 10, 4.0, and 2% respectively, of the total soluble protein of *Mycoplasma* strain 07 (Berlin and Schimke, unpublished data). The presence of such large amounts of the enzymes involved in converting arginine to ornithine with the generation of adenosine triphosphate further supports the suggested importance of the arginine dihydrodrolase pathway in the generation of energy for certain *Mycoplasma*.

Each of 39 contaminated cell cultures and each of the 39 *Mycoplasma* isolates were found to contain arginine deiminase activity. Previously we concluded that a positive arginine deiminase test presents strong evidence of *Mycoplasma* contamination. A negative test would not rule out the possible presence of *Mycoplasma* lacking this pathway and would require standard culture procedures to establish freedom from *Mycoplasma*.

The common occurrence of arginine dihydrodrolase activity in *Mycoplasma* isolated from contaminated tissue cell cultures strengthens the suggestion that the laboratory worker is one source of contamination. The *Mycoplasma* recovered from contaminated cell cultures serotype as human oral or genital *Mycoplasma* species (1, 14, 18, 19, 22). *Mycoplasma* species are common inhabitants of the human oral (23, 27, 31) and urogenital tracts (4, 17). We have shown that the human *Mycoplasma* species isolated from and associated with cell culture contamination contain this pathway, whereas other human *Mycoplasma* species, such as *M. pneumoniae*, which have not been associated with cell culture contamination, do not have this pathway.
Literature Cited


