Comparative Metabolism of *Mesoplasma*, *Entomoplasma*, *Mycoplasma*, and *Acholeplasma*

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Cytoplasmic fractions from species of the *Mollicutes* genera *Entomoplasma*, *Mesoplasma*, *Mycoplasma*, and *Acholeplasma* were assayed for NADH oxidase (NADH ox), ATP- and PP_i-dependent phosphofructokinase (PFK), ATP- and PP_i-dependent deoxyguanosine kinase (dGUOK), thymidine kinase (TK), TMP kinase (TMPK), glucose-6-phosphate dehydrogenase (G6Pde), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoenolpyruvate carboxylase, hypoxanthine-guanine phosphoribosyl transferase, dUTPase, and uracil-DNA glycosylase (UNG) activities. Membrane fractions were also examined for NADH ox activity. These activities were used as indicators of the presence and relative activities of major *Mollicutes* metabolic and DNA repair pathways. This was the first study to determine the presence of these enzymes in members of the genera *Entomoplasma* and *Mesoplasma*. Using the data obtained, we constructed a preliminary scheme for distinguishing genera of the class *Mollicutes* on the basis of the results of signature functional enzyme assays. This scheme includes phylogenetic relationships deduced from rRNA analyses, but is more informative with respect to metabolic potential. The criteria used include the presence of PP_i-dependent PFK, urease, dUTPase, and dGUOK activities. *Entomoplasma ellychniae* ELCN-1^T (T = type strain), *Entomoplasma melaleucae* M-1^T, *Mesoplasma seiffertii* F7^T, *Mesoplasma entomophilum* TAC^T, *Mesoplasma florum* L1^T, *Mycoplasma fermentans* PG18^T, and *Acholeplasma multilocale* PN525^T were similar in most respects. NADH ox activity was localized in the cytoplasm of these organisms. These strains had ATP-dependent PFK, MDH, LDH, ATP- and PP_i-dependent dGUOK, and UNG activities, but not dUTPase or G6Pde activities. In contrast, *Acholeplasma equifetale* C112^T, *Acholeplasma oculi* 19L^T, *Acholeplasma hippikon* C1^T, *Acholeplasma modicum* PG49^T, and *Acholeplasma morum* 72-043^T had membrane-localized NADH ox activity, PP_i-dependent PFK, G6Pde, and dUTPase activities, and significantly lower MDH and LDH activities and exhibited a faster rate with PP_i than with ATP in the dGUOK reaction. All of the members of the *Mollicutes* tested had hypoxanthine-guanine phosphoribosyl transferase, phosphoenolpyruvate carboxylase, and (except for *Mesoplasma entomophilum* TAC^T) UNG activities. All of the *Acholeplasma* strains except *Acholeplasma multilocale* PN525^T had TK, TMPK, and UNG activities. *Mesoplasma entomophilum* TAC^T was distinguished by having no detectable dUTPase, UNG, TK, and TMPK activities, indicating that there is a severe restriction in or an absence of a synthetic route to dTTP. Our data also suggest that *A. multilocale* PN525^T is a member of an unrecognized metabolic subgroup of the genus *Acholeplasma* or is not an *Acholeplasma* strain.

Distinguishing members of the class *Mollicutes* by their metabolic characteristics has been of very limited phylogenetic and taxonomic usefulness. Standards for describing *Mollicutes* taxa have been published previously (7). Characteristics such as gross cellular morphology, colonial appearance, genome size, and serological relatedness are more frequently emphasized as discriminating features. There are a few metabolic exceptions, including the ability to hydrolyze urea exhibited by *Ureaplasma* species, the growth requirement for sterol of some members of the *Mollicutes*, and the requirement for anaerobiosis. The usefulness of the various discriminating characteristics is associated with the taxonomic level being distinguished. Pertinent distinguishing metabolic characteristics at the order and family levels are a requirement for sterols for growth, a requirement for anaerobiosis, and optimum temperature. At the genus level, the sterols necessary for growth, anaerobiosis, temperature, and urea hydrolysis are emphasized. At the species level, serological relatedness presently overshadows all other fea-

tures, and genome size and G+C content are recommended characteristics. Only rarely are fermentation of glucose and hydrolysis of arginine, esculin, and arbutin taxonomically consequential. Other discriminating metabolic activities have been described, but the usefulness of these characteristics suffers from the limited information about them for the approximately 150 known members of the *Mollicutes* (21, 26, 28). In this study we examined what we characterize as signature functional enzymatic activities in two recently described genera of the class *Mollicutes*. The data which we obtained are combined with other data to form a taxonomic scheme at the generic level that is based on metabolism and that to some extent parallels the phylogenetic relatedness scheme for the genera determined by rRNA analyses (30, 35, 42).

MATERIALS AND METHODS

Mollicutes, medium, and preparation of cytoplasmic and membrane fractions. The members of the *Mollicutes* included in this study are listed in Tables 1 through 3. The organisms were grown in 4-liter batches of SNE (26). All cultures were started with a 2 to 10% inoculum and were incubated at 30 or 37°C for 24 h. Cells were harvested and washed in kappa buffer (25). In some experiments washed cell pellets were frozen at -20°C for up to 4 months. Washed cells (fresh or thawed pellets) were broken by osmotic shock or sonication, and the resulting preparations were centrifuged to obtain membrane and cytoplasmic fractions as previously described (25). After the final centrifugation (144,000 × g, 2 h), the

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TABLE 1. NADH ox specific activities of *Mollicutes* membrane and soluble (cytoplasmic) fractions

Strain	NADH ox sp act ^a		M/S ^b
	Membrane fraction	Soluble fraction	
<i>Entomoplasma elychniae</i> ELCN-1 ^T	<0.001	740 ± 80	<0.001
<i>Entomoplasma melaleuca</i> M-1 ^T	<0.001	1,120 ± 81	<0.001
<i>Mesoplasma seiffertii</i> F7 ^T	<0.001	16 ± 4	<0.001
<i>Mesoplasma entomophilum</i> TAC ^T	<0.001	724 ± 61	<0.001
<i>Mesoplasma florum</i> L1 ^T	0.016	59 ± 3	<0.001
<i>Mesoplasma florum</i> GF	0.007	133 ± 18	<0.001
<i>Mycoplasma fermentans</i> PG18 ^T	0.020	11 ± 1	0.002
<i>Mycoplasma fermentans</i> MI (incognitus)	0.009	4.1 ± 0.4	0.002
<i>Acholeplasma equifetale</i> C112 ^T	142	10	14.2
<i>Acholeplasma oculi</i> 19L ^T	238	5	47.6
<i>Acholeplasma multilocale</i> PN525 ^T	<0.001	2.9 ± 2.3	<0.001
<i>Acholeplasma hippikon</i> C1 ^T	NT ^c	7	
<i>Acholeplasma modicum</i> PG49 ^T	431	8	53.9
<i>Acholeplasma morum</i> 72-043 ^T	NT	10	

^a Specific activities are expressed as nanomoles of NADH consumed per minute per milligram of cytoplasmic or membrane protein. For some assays three different batches of cells were used, and the mean ± standard deviation is given. If there is no standard deviation, the value is the mean of the values obtained with two different batches of cells. If activity could not be detected in two or three different batches of cells, the less than symbol is used and is followed by the lowest specific activity that could be detected by our assay.

^b M/S, ratio of membrane fraction specific activity to soluble (cytoplasmic) fraction specific activity.

^c NT, not tested.

supernatant fractions were clarified by filtration through a 0.22-μm-pore-size filter to produce the final fraction (cytoplasmic fraction). Cell fractions were prepared at 4°C. Membrane fractions were used only to assess NADH oxidase (NADH ox) activity.

Assays. The enzymatic assays used in this study were essentially identical to the standard pyrimidine-coupled spectrophotometric and radioisotopic assays that we have described previously. We performed assays for NADH ox (28), hexokinase (2), ATP- and PP_i-dependent phosphofructokinase (PFK) (29), glucose-6-phosphate dehydrogenase (G6Pde) (5, 28), lactate dehydrogenase (LDH) (16), phosphoenolpyruvate carboxylase (10), malate dehydrogenase (MDH) (9), hypoxanthine-guanine phosphoribosyl transferase (32), ATP- and PP_i-dependent deoxyguanosine kinase (dGUOK) (12), dUTPase (38), thymidine kinase (39), thymidine monophosphate kinase (TMPK) (8), and uracil DNA glycosylase (UNG) (40) activities.

RESULTS

The membrane fraction/soluble (cytoplasmic) fraction ratios indicate localization of activity; ratios higher than 0.3 indicate membrane localization, while lower values indicate soluble or cytoplasmic fraction localization (21, 22) (Table 1). We found that the NADH ox activities of *Acholeplasma equifetale*, *Acholeplasma oculi*, and *Acholeplasma modicum* were localized in the membrane fractions (Table 1). *Acholeplasma multilocale* PN525^T (T = type strain) had a low level of NADH ox activity in its cytoplasmic fraction, and no activity was detected in the membrane fraction. The NADH ox activities of all other organisms examined were localized in the cytoplasmic fractions.

All of the *Acholeplasma* strains except *A. multilocale* PN525^T had both hexokinase and G6Pde activities (Table 2). The *Mesoplasma* strains and *Mycoplasma fermentans* did not have hexokinase activity. *Mesoplasma florum* GF had a relatively low level of G6Pde activity, and all other *Mollicutes* strains had no G6Pde activity.

All of the *Acholeplasma* strains except *A. multilocale* PN525^T had PFK activity that was predominantly PP_i dependent; i.e., the PP_i-dependent PFK activity was 10- to 30-fold greater than the ATP-dependent PFK activity (Table 2). On the other hand,

TABLE 2. Enzymatic activities of members of the *Mollicutes*

Strain	Sp act ^a								
	ATP-dependent PFK	PP _i -dependent PFK	G6Pde	LDH	Phosphoenolpyruvate carboxylase	MDH	Hypoxanthine-guanine phosphoribosyl transferase	ATP-dependent dGUOK	PP _i -dependent dGUOK
<i>Entomoplasma elychniae</i> ELCN-1 ^T	1,220 ± 160	19	<0.008	980 ± 81	<1	430 ± 33	0.019 ± 0.001	0.002	0.017 ± 0.001
<i>Entomoplasma melaleuca</i> M1 ^T	772 ± 49	<0.005	<0.008	4,900 ± 413	8	1,170 ± 98	0.008 ± 0.0005	0.022 ± 0.002	0.003 ± 0.0003
<i>Mesoplasma seiffertii</i> F7 ^T	3,500 ± 160	<0.005	<0.008	5,800 ± 266	1 ± 0.9	17 ± 2	0.018 ± 0.001	0.020 ± 0.002	0.010 ± 0.0006
<i>Mesoplasma entomophilum</i> TAC ^T	1,840 ± 90	<0.005	<0.008	3,400 ± 255	2 ± 0.2	968 ± 59	0.032 ± 0.001	0.002 ± 0.0001	0.005 ± 0.0004
<i>Mesoplasma florum</i> L1 ^T	570 ± 22	17 ± 1	<0.008	3,400 ± 130	4 ± 0.2	316 ± 4	0.0026 ± 0.0003 ^b	NT ^c	NT
<i>Mesoplasma florum</i> GF	363 ± 24	140	0.63 ± 0.07	3,370 ± 119	5 ± 0.4	220 ± 16	NT	NT	NT
<i>Mycoplasma fermentans</i> PG18 ^T	50 ± 7	<0.005	<0.008	60 ± 3	<1	3 ± 0.5	0.034 ± 0.004	0.063 ± 0.002	0.006 ± 0.0005
<i>Mycoplasma fermentans</i> MI (incognitus)	72 ± 5	3 ± 0.04	<0.008	107 ± 7	NT	6 ± 0.3	0.024 ± 0.002	0.028 ± 0.0002	0.001 ± 0.0001
<i>Acholeplasma equifetale</i> C112 ^T	31	333 ± 10	18	12	5	10	0.019	0.002	0.0003
<i>Acholeplasma oculi</i> 19L ^T	14	200	14	135	0.3	5	0.023	<0.000075	0.009
<i>Acholeplasma multilocale</i> PNS25 ^T	190 ± 4	<0.005	<0.008	6 ± 0.05	2	3	0.045	0.029	0.022
<i>Acholeplasma hippikon</i> C1 ^T	53	635	24	10	<1	25	0.038	0.006	0.011
<i>Acholeplasma modicum</i> PG49 ^T	9	290	23	53	4	4	0.023	<0.000075	0.006
<i>Acholeplasma morum</i> 72-043 ^T	21	365	21	15	<1	4	0.005	<0.000075	0.00053

^a Activities are expressed as nanomoles of product synthesized or reactant consumed (depending on the assay) per minute per milligram of cytoplasmic protein. For some assays three different batches of cells were used, and the mean ± standard deviation is given. If there is no standard deviation, the value is the mean of the values obtained with two different batches of cells. If activity could not be detected in two or three different batches of cells, the less than symbol is used and is followed by the lowest activity that could be detected by our assay.

^b Data from reference 32.

^c NT, not tested.

TABLE 3. Pyrimidine enzymatic activities of members of the *Mollicutes*

Strain	Sp act ^a			
	dUTPase	Thymidine kinase	TMPK	UNG
<i>Entomoplasma elychniae</i> ELCN-1 ^T	<1	<0.001	56 ± 49	0.316 ± 0.073
<i>Entomoplasma melaleucaae</i> M1 ^T	<1	30 ± 26	85	0.275 ± 0.114
<i>Mesoplasma seiffertii</i> F7 ^T	<1	<0.001	107 ± 82	<0.001
<i>Mesoplasma entomophilum</i> TAC ^T	<1	<0.001	<0.001	<0.001
<i>Mesoplasma florum</i> L1 ^T	<1	0.3 ± 0.2	NT ^b	NT
<i>Mycoplasma fermentans</i> PG18 ^T	<1	96 ± 73	124 ± 115	0.074
<i>Mycoplasma fermentans</i> MI (incognitus)	<1	0.3 ± 0.1	1.6 ± 0.3	0.436 ± 0.058
<i>Acholeplasma equifetale</i> C112 ^T	15 ± 11	5.4 ± 2.1	65 ± 59	0.611 ± 0.277
<i>Acholeplasma oculi</i> 19L ^T	29 ± 4	2.8	43	0.352
<i>Acholeplasma multilocale</i> PN525 ^T	<1	3.9	19	<0.001
<i>Acholeplasma hippikon</i> C1 ^T	0.7 ± 0.2	28	285	1.806
<i>Acholeplasma modicum</i> PG49 ^T	2 ± 0.1	5	38	0.475
<i>Acholeplasma morum</i> 72-043 ^T	3 ± 0.9	7	21	0.233

^a Specific activities are expressed as nanomoles of product synthesized or reactant consumed (depending on the assay) per minute per milligram of cytoplasmic protein. For some assays three different batches of cells were used, and the mean ± standard deviation is given. If there is no standard deviation, the value is the mean of the values obtained with two different batches of cells. If activity could not be detected in two or three different batches of cells, the less than symbol is used and is followed by the lowest specific activity that could be detected by our assay.

^b NT, not tested.

A. multilocale PN525^T, like the *Entomoplasma*, *Mesoplasma*, and *Mycoplasma* species, had ATP-dependent PFK activity. In most of these organisms the PFK specific activity with ATP was 1,000-fold greater than any PP_i-dependent specific activity. All of the members of the *Mollicutes* tested had LDH and MDH activities. It is worth noting that the levels of LDH and MDH activities in the *Entomoplasma* and *Mesoplasma* species were generally higher than the levels found in the other organisms, even though quantitative comparisons were not generally valuable in this study because our fractions were crude. Hypoxanthine-guanine phosphoribosyl transferase activity was detected in all strains, and phosphoenolpyruvate carboxylase activity was detected in most strains.

All of the *Acholeplasma* strains except *A. multilocale* PN525^T had higher specific activities when PP_i was used than when ATP was used in the dGUOK reaction (Table 2). No uniform preference for either ATP or PP_i in the dGUOK reaction was observed with *Mesoplasma* and *Entomoplasma* strains. Both strains of *Mycoplasma fermentans* examined had higher specific activities in the dGUOK reaction when ATP was used than when PP_i was used.

dUTPase activity was found only in *Acholeplasma* species, but it was not found in *A. multilocale* PN525^T (Table 3). Thymidine kinase activity was detected in all of the strains tested except the *Entomoplasma elychniae*, *Mesoplasma entomophilum*, and *Mesoplasma seiffertii* strains. TMPK activity was detected in all of the strains tested except the *Mesoplasma entomophilum* strain. UNG activity was found in all of the strains examined except the *A. multilocale*, *Mesoplasma entomophilum*, and *Mesoplasma seiffertii* strains.

DISCUSSION

Traditionally, identification and classification of members of the class *Mollicutes* have been based on phenotypic characteristics, such as the ability to produce acid from glucose, a requirement for urea for growth, and utilization or hydrolysis of arginine, on serological associations, and on rRNA sequences (30, 35). We suggest that assays for gene products (that is, functional enzyme assays) may be used with confidence to augment 16S rRNA gene analysis data to develop a simple and practical taxonomic scheme for identification and classification of members of the *Mollicutes*. Our concept is rooted in the view

that the presence of enzymatic function is a reflection of inheritable traits and that an assay for function may be more informative for individual species than rRNA analysis or even primary gene sequence analysis when it is used alone.

Our suggestion is based on a series of observations. First, the members of the class *Mollicutes* have undergone a remarkable series of genome reductions regardless of the phylogenetic lineage, as determined by rRNA analysis (30, 35, 42). As a result, this class of organisms contains some of the smallest genomes presently known among free-living organisms. This large-scale genome reduction has probably reduced the metabolic potential of these organisms in an as-yet unpredictable manner. Second, even when the complete genomic DNA sequence is known, the primary DNA sequence is not completely determinative with regard to function; that is, although function is dependent on the primary sequence, tertiary effects, such as the degree of oligomerization and peptide-peptide interactions, and the relative concentrations of substrates and cofactors also affect the function of the gene product. In addition, the extent of posttranslational modification of metabolic enzymes in prokaryotes other than modification through the action of toxins remains largely undescribed. Pancholi and Fischetti (18) have recently described the novel functional effects of ADP ribosylation of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GADPH) from group A streptococci. It is also interesting that these researchers suggested earlier that GADPH (17) is exposed on the surface of group A streptococci and that through multiple binding capacities for human host proteins the members of a family of GADPH molecules may play different roles in the colonization, internalization, and proliferation of streptococci subject to minor sequence variations. Similarly, human GADPH has been shown to exhibit alternative enzymatic functions depending on its oligomeric state. For example, depending on the state of oligomerization, identical gene products may result in either GADPH activity or UNG activity (13).

In an alternative approach to our functional studies both Wasinger et al. (34), who analyzed *Mycoplasma genitalium* G37, and Cordwell et al. (3), who analyzed *Spiroplasma meliferum*, used a two-dimensional gel system to isolate and characterize specific proteins. Protein sequence data were compared with public database data to determine levels of

TABLE 4. Distinguishing metabolic activities of genera of the class *Mollicutes*

Genus	Enzyme activities				
	PP _i -dependent PFK	Urease	dUTPase	ATP-dependent dGUOK	PP _i -dependent dGUOK
<i>Acholeplasma</i>	+	—	+	—	+
<i>Anaeroplasm</i>	+	—	+	—	+
<i>Ureaplasma</i>	—	+	—	—	—
<i>Spiroplasma</i>	—	—	+	+	+
<i>Mesoplasma</i> ^b	— ^c	—	—	Variable	Variable
<i>Mycoplasma</i> ^b	—	—	— ^d	— ^e	—
<i>Entomoplasma</i> ^b	—	—	—	Variable	Variable
<i>Asteroleplasma</i>	—	—	—	—	+

^a *Spiroplasma mirum* is PP_i-dependent dGUOK negative.

^b *Mesoplasma* species can be distinguished from *Mycoplasma* and *Entomoplasma* species because *Mesoplasma* species grow on sterol-free media containing Tween 80, whereas *Mycoplasma* and *Entomoplasma* species do not (33). Provisionally, the UNG assay may be a discriminating assay, as the two *Entomoplasma* species have UNG activity and the two *Mesoplasma* do not (Table 3). Our data also indicate that *Entomoplasma* and *Mesoplasma* species have much higher MDH specific activities than *Mycoplasma* species.

^c *Mesoplasma florum* GF exhibits a low level of PP_i-dependent PFK activity.

^d *Mycoplasma mycoides* subsp. *mycoides* is dUTPase positive (14) and exhibits phylogenetic and taxonomic affinity to the dUTPase-positive genus *Spiroplasma* (35, 36).

^e *Mycoplasma fermentans* PG18 and MI are ATP-dependent dGUOK positive.

similarity and putative identity. This approach, however, emphasizes gene expression, not gene function, which must be assessed independently.

In the present study we found MDH activity in all *Mesoplasma* and *Entomoplasma* species tested. These findings extend our observation that except for the *Acholeplasmas*, every member of the *Mollicutes*, including *Mycoplasma genitalium* G37^T (9), has MDH activity (9, 20, 23, 27). However, no one has described a *Mollicutes* open reading frame or gene sequence that exhibits sufficient sequence homology to any MDH sequence or MDH consensus sequence in the data banks to claim identity or some level of similarity (1, 6, 19). The MDH gene may be one of the gene sequences that have not been putatively assigned, perhaps because the open reading frames assigned to MDHs in other prokaryotes are insufficiently similar to *Mollicutes* sequences, as detected in multiple alignments. The supposed high mutability of the *Mollicutes* may be related to or the cause of an atypical gene sequence (40).

The data presented in this paper provide further evidence that six *Mollicutes* genera can be distinguished by their enzyme contents (4, 5, 9, 11, 20, 23, 27). In addition, this study extended our previous findings to include species belonging to the two recently described genera of the class *Mollicutes*, the genera *Entomoplasma* and *Mesoplasma* (33). The total number of species in the eight genera that have been described is about 150. Since we have studied only 66 species, including representatives of all eight genera, our comparative analysis is incomplete and the results are provisional. Using our data, we constructed a preliminary scheme, a scheme that was suggested in a recent report but was offered there without any supporting data (25); this scheme separates all of the *Mollicutes* genera on the basis of their enzyme contents and appears to be consistent with previous phylogenetic opinion at that level (30). The distinguishing enzymatic characteristics, based on this and other studies, are listed in Table 4. In this arrangement the phylogenetically related members of the facultatively anaerobic genus *Acholeplasma* and the strictly anaerobic genus *Anaeroplasm* have indistinguishable characteristics.

The three *Mesoplasma* species and two *Entomoplasma* species that we examined had no dUTPase activity. We consider this remarkable, since before this study the only cells in nature that had been reported to lack dUTPase activity were the cells of members of the *Mollicutes* genera *Mycoplasma*, *Ureaplasma*, and *Asteroleplasma* (20, 37, 38, 40). The only exception within this group is the dUTPase-positive organism *Mycoplasma mycoides* subsp. *mycoides*, the type subspecies of the type species of the genus (14). However, this mycoplasma is now recognized as an organism that is more closely related phylogenetically to the dUTPase-positive spiroplasmas (35); the merits of retaining the name *Mycoplasma mycoides* subsp. *mycoides* for this organism rather than placing it in the genus *Spiroplasma* have been discussed previously (36).

Mesoplasma entomophilum TAC^T is unusual in that it lacks not only UNG activity (UNG is a DNA repair enzyme), but also dUTPase, thymidine kinase, and TMPK activities. The absence of TMPK, represented by an inability to synthesize TDP, means that in order to gain TTP for DNA synthesis the organism must successfully scavenge for TDP or TTP itself. The ability to take up phosphorylated purines or pyrimidines (nucleoside diphosphates or nucleoside triphosphates) is relatively rare in prokaryotes, although it has been reported in *Mycoplasma mycoides* subsp. *mycoides* (15). The absence of UNG and dUTPase suggests that this organism may have a higher incidence of mutations involving either the incorporation of dUTP into DNA or an apparent inability to excise uracil from its DNA (39–41).

The relatively high levels of ATP-dependent PFK, LDH, and MDH and the absence of G6Pde activities in the *Entomoplasma* and *Mesoplasma* species suggest that the members of these genera rely on glycolysis and are fermentative. Also, they may not be capable of ribose synthesis via the pentose phosphate pathway and may be dependent on the maintenance of intracellular NAD concentrations and NADH ox activity to maintain LDH and MDH activities or other reactions involving the terminal transfer of electrons.

Of interest is our observation that two human isolates of *Mycoplasma fermentans*, the *Mycoplasma* species associated with AIDS, have ATP-dependent nucleoside kinase activity. One of our isolates, *Mycoplasma fermentans* MI, was originally isolated from an AIDS patient. We have shown that all *Mycoplasma* strains except certain *Mycoplasma hominis* strains are unable to phosphorylate any nucleoside tested to form the respective nucleoside monophosphate (31, 32). Four strains isolated from humans with clinical symptoms of disease exhibited nucleoside kinase activity, but these strains used PP_i, not ATP, as the phosphorus donor (11). Four nonhuman isolates could not phosphorylate nucleosides with either ATP or PP_i. On the other hand, the relatively common human *Mollicutes* pathogen *Mycoplasma pneumoniae* and two organisms frequently associated with human disease, *Mycoplasma genitalium* and *Ureaplasma urealyticum*, have no detectable nucleoside kinase activity (11). The significance of the association of nucleoside phosphorylation in only some strains isolated from patients with clinical disease is not known, but we feel that this association deserves further study.

A. multilocale PN525^T was found to lack hexokinase, G6Pde, PP_i-dependent PFK, dUTPase, and UNG activities and could use both ATP and PP_i in the dGUOK reaction. This isolate lacked membrane-associated NADH ox activity and cannot reduce benzyl viologen (26). All of these responses are unlike those found in any of the other 12 known *Acholeplasma* species (5, 9–12, 26–29). These differences represent aberrations in the following six metabolic pathways: glycolysis, pentose phosphate, pyrimidine and purine metabolism, DNA repair, and

respiration. We suggest that *A. multilocale* PN525^T is a member of an unrecognized metabolic subgroup of the genus *Acholeplasma* or is not an *Acholeplasma* strain. The bulk of our data suggests that *A. multilocale* PN525^T is either a *Mycoplasma* strain or an *Entomoplasma* strain.

NADH ox activity is localized in the membrane of almost every prokaryote. *Acholeplasma* species also have this characteristic, but *Mycoplasma*, *Spiroplasma*, and *Ureaplasma* species are known to be exceptions (21–23, 27). The localization of NADH ox activity in the cytoplasmic fraction of the *Mesoplasma* and *Entomoplasma* species examined in this study metabolically relates these genera to the genera *Mycoplasma*, *Spiroplasma*, and *Ureaplasma*. The *Mollicutes* strain formerly called *Acholeplasma florum* L1 and now called *Mesoplasma florum* L1^T initially disrupted the concept that all *Acholeplasma* species had membrane-localized NADH ox activity since the activity in this organism was found to be localized in the cytoplasm (24). In the present study we confirmed this observation. However, with the reassignment of this species to the genus *Mesoplasma* (33) the remaining *Acholeplasma* species tested are the only *Mollicutes* species with membrane-localized NADH ox activity, and the concept that membrane localization of NADH ox activity can be used to identify *Acholeplasma* species is supported.

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