Metabolism of Members of the *Spiroplasmataceae*

J. D. POLLACK,¹* M. C. McELWAIN,¹ D. DeSANTIS,¹ J. T. MANOLUKAS,¹ J. G. TULLY,² C.-J. CHANG,³
R. F. WHITCOMB,⁴ K. J. HACKETT,⁴ AND M. V. WILLIAMS^{1,5}

Department of Medical Microbiology and Immunology, The Ohio State University, Columbus, Ohio 43210¹; Mycoplasma Section, National Institute of Allergy and Infectious Diseases, Frederick Cancer Research Facility, Frederick, Maryland 21701²; Department of Plant Pathology, University of Georgia, Griffin, Georgia 30223³; Insect Pathology Laboratory, Plant Protection Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705⁴; and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210⁵

Cell-free extracts from 10 strains of *Spiroplasma* species were examined for 67 enzyme activities of the Embden-Meyerhof-Parnas pathway, pentose phosphate shunt, tricarboxylic acid cycle, and purine and pyrimidine pathways. The spiroplasmas were fermentative, possessing enzyme activities that converted glucose 6-phosphate to pyruvate and lactate by the Embden-Meyerhof-Parnas pathway. Substrate phosphorylation was found in all strains. A modified pentose phosphate shunt was present, which was characterized by a lack of detectable glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities. Spiroplasmas could synthesize purine mononucleotides by using pyrophosphate (PP_i) as the orthophosphate donor. All spiroplasmas except *Spiroplasma floricola* used adenosine triphosphate (ATP) to phosphorylate deoxyguanosine; no other nucleoside could be phosphorylated with ATP by any spiroplasma tested. These results contrast with those reported for other mollicutes, in which PP_i serves as the orthophosphate donor in the nucleoside kinase reaction. The participation of ATP rather than PP_i in this reaction is unknown in other mollicutes regardless of the nucleoside reactant. Deoxypyrimidine enzyme activities were similar but varied in the reactions involving deamination of deoxycytidine triphosphate and deoxycytidine. All *Spiroplasma* spp. strains had deoxyuridine triphosphatase activity. Uridine phosphorylase activity varied among strains and is possibly group dependent. As in all other mollicutes, a tricarboxylic acid cycle is apparently absent in *Spiroplasma* spp. Reduced nicotinamide adenine dinucleotide oxidase activity was localized in the cytoplasmic fraction of all *Spiroplasma* species tested. Our assays indicate that the members of the *Spiroplasmataceae* are essentially metabolically homogeneous in the highly conserved pathways which we studied, but differ from other mollicutes in several important respects. These differences are of probable phylogenetic significance and may provide tools for recognition of higher taxonomic levels of mollicutes.

The metabolism of the wall-less, helical, sterol-requiring members of the *Spiroplasmataceae* has been little studied. The insect and plant habitats of the spiroplasmas, as their unique phylogenetic position indicates (59), suggested to us that the metabolism of these organisms might differ from that described for other members of the class *Mollicutes* (10, 17, 39, 45, 55). Identification of such metabolic differences would aid in the characterization, classification, and study of the phylogeny of the spiroplasmas. This information can also identify metabolic steps or loci that are susceptible to chemical modulations that could inhibit the spiroplasmal diseases of corn, citrus, or other plants.

Most reports relating to the metabolism of spiroplasmas have concerned nutrition, noting, for example, the presence or absence of acid produced during growth with various sugars (8, 20, 43, 44, 58). Other reports have described optimal growth responses to various additives in formulations of semidefined and defined media (2, 4, 5, 18, 22, 24, 52). A number of studies have reported the chemical contents and the processing or appearance of radioactivity from either lipids or lipid precursors into membrane components of growing spiroplasmas (3, 13, 15, 23, 31, 32) or the uptake of compounds such as [¹⁴C]thymidine (50), [³²P]phosphate, or ¹⁴C-labeled amino acids (1). Other workers have performed enzymatic studies, noting the presence of adenosine triphosphatase (23, 31) or, in some spiroplasmas, uridine phosphorylase activity (29, 46). A number of reports concern the arginine metabolism of *Spiroplasma citri* and *Spiro-*

plasma kunkelii (24, 44, 48, 49). The endonucleases and polymerases of *S. citri* have also been studied (6, 7, 47). Saglio et al. (42) examined *S. citri* metabolism more generally by determining the energy charge values as a function of growth and metabolic activity. Only in reports of *S. citri* by Igwegbe and Thomas (21), who examined the arginine dihydrolase pathway, and by McElwain et al. (28), who examined purine and pyrimidine metabolism, have the enzyme components of major metabolic routes been systematically studied.

In this paper, we report the results of an attempt to determine the presence or absence and extent of a number of major interrelated metabolic pathways in members of the *Spiroplasmataceae*. We examined cytoplasmic extracts from 10 strains of *Spiroplasma* species, which represented eight of the nine named species and seven serogroups (including four subgroups of group I) and were isolated from ticks, bees, mosquitoes, and different plants (53). Extracts were assayed for 67 enzyme activities that are components of the Embden-Meyerhof-Parnas (EMP) pathway, pentose phosphate (PP) shunt, tricarboxylic acid (TCA) cycle, and purine and pyrimidine pathways.

(Some of the results were presented at the Seventh Congress of the International Organization for Mycoplasma, Baden, Austria, June 1988.)

MATERIALS AND METHODS

Spiroplasma strains. Ten spiroplasma strains were studied. The designations of these organisms, the growth media used, and the length of time which the bacteria were incubated

* Corresponding author.

TABLE 1. *Spiroplasma* species and groups studied^a

Binomial or common name	Group or subgroup	Strain	Medium	No. of days of incubation at 30°C
<i>S. citri</i>	I-1	R8A2 ^T	R2 ^b	3-4
<i>S. melliferum</i>	I-2	AS576	R2	2-3
<i>S. kunkelii</i>	I-3	I-747	C-3G ^c	6-7
Maryland flower spiroplasma	I-6	M55	BSS ^d	6
<i>S. floricola</i>	III	23-6 ^T	SP-4 ^e	2
<i>S. floricola</i>	III	OBMG	SP-4	2
<i>S. apis</i>	IV	SR3	R2	2-3
<i>S. mirum</i>	V	SMCA ^T	SP-4	5
<i>S. culicicola</i>	X	AES-1 ^T	BSS	2
<i>S. sabaudiense</i>	XIII	Ar-1343	BSS	4

^a See reference 53.^b See reference 9.^c See reference 25.^d BSS, Base serum sucrose broth.^e See reference 54.

statically at 30°C are shown in Table 1. Three spiroplasma strains were grown in a previously undescribed medium, base serum sucrose broth, which contains (per liter) 17.1 g of mycoplasma broth base (BBL Microbiology Systems), 91.34 g of sucrose, 0.71 g of penicillin G, 6.0 ml of 0.5% aqueous phenol red, and 100 ml of fetal bovine serum (final pH, 7.3). The mycoplasma broth base and sucrose were sterilized by autoclaving suspensions of 60 g of mycoplasma broth base and 320 g of sucrose in 2,930 ml of water; these suspensions were adjusted to pH 7.3 with 1 N HCl. Other components were prefiltered through 0.22- μ m filters, and the complete medium was sterilized by ultrafiltration through stacked 1.2-, 0.45-, and 0.22- μ m filters.

Preparation of cytoplasmic extracts and membrane fractions. Cells in log or late log phase were harvested by centrifugation at approximately $10,000 \times g$ for 30 min at 4°C. The cells were washed two to four times in kappa buffer by centrifugation at 4°C (35). Washed whole-cell pellets were kept frozen at -70°C for 2 to 5 days until they were extracted. Frozen pellets were thawed and suspended in about 15 ml of aqueous diluted kappa buffer (1:20). The whole-cell suspensions were subjected to disruptive decompression in a Parr-Bomb. Suspensions of broken cells were centrifuged at $250,000 \times g$ for 1 to 2 h at 4°C. The supernatants were dialyzed three or four times in 200 to 400 volumes of dialysis fluid at 4°C overnight (51) and were used for enzymatic studies. Washed membrane fractions of *S. citri*, *Spiroplasma floricola* 23-6^T (T = type strain), *Spiroplasma melliferum*, *Spiroplasma apis*, and *S. kunkelii* were prepared from the $250,000 \times g$ pellets (35). The membrane fractions were washed once in diluted kappa buffer (1:20) and then five or six times in kappa buffer.

Enzymatic analysis. The numbers in parentheses below identify the enzymes listed in Table 2. Assays for enzyme activities of the EMP pathway (enzymes 1 through 7) and the PP shunt (enzymes 21 through 27) and for deoxyribose-5-phosphate aldolase activity (enzyme 28) were performed as described previously (14, 56). The assays for enzymes involving pyruvate, phosphoenolpyruvate, malate, oxaloacetate, and aspartate and for citrate synthase, isocitrate dehydrogenase, and fumarase activities (enzymes 8 through 20) and the assay for adenosine triphosphate (ATP) formation were performed as described by Manolukas et al. (26). Reduced nicotinamide adenine dinucleotide (NADH) oxidase activity was assayed as previously described (38).

Assays for pyrimidine enzyme activities and for uracil-deoxyribonucleic acid glycosylase activity (enzymes 29 through 39) were performed as reported by Williams and Pollack (56, 57). The assay for deoxyribonuclease activity (enzyme 40) was performed by following the procedure of Hoffmann and Cheng (19), as described by Pollack and Hoffmann (37). The assays for purine enzyme activities (enzymes 41 through 67) were performed as described previously (28, 52). Membrane fractions were assayed only for NADH oxidase activity and protein content (35).

Most of the data in Table 2 are reported as the average numbers of nanomoles of product synthesized per minute per milligram of cell-free cytoplasmic protein. Enzymatic rates that were determined spectrophotometrically were calculated from periods when the reactions were linear (zero order) (i.e., when the substrate concentration was apparently not limiting and the reaction rate was proportional to the concentration of cell extract). When the number of different cell batches tested was three or greater, standard deviations were computed (Table 2). If the number of cell batches tested was less than three, no standard deviation was calculated.

To test for the appropriateness of our spectrophotometric reaction mixture when no activity was detected, 1×10^{-3} to 1×10^{-4} U of a commercial sample (Sigma Chemical Co.) of the enzyme being studied was added to the cuvette. Upon addition of the enzyme standard to the complete reaction mixture containing 10 to 80% nonreactive cell extract, enzyme activity was detected in every case.

RESULTS

The results of 67 assays are listed in Table 2. Only transaldolase (enzyme 27), deoxycytidine monophosphate (dCMP) deaminase (enzyme 30), and cytidine deaminase (enzyme 32) showed significant variation. In each of these three assays, about one-third of the responses determined from the 10 spiroplasmas were negative. The *S. citri* and *S. kunkelii* strains, the two plant pathogens, were the only strains that lacked both cytidine and dCMP deaminases (enzymes 30 and 32) and deoxyribose 5-phosphate aldolase (enzyme 28) activity; these two plant pathogens, as well as *Spiroplasma mirum*, lacked transaldolase (enzyme 27) activity. *S. mirum* had no detectable phosphoribose isomerase (enzyme 24) or pyrophosphate (PP_i)-dependent deoxyguanosine kinase (enzyme 63) activity. The two strains of *S. floricola* had neither ATP- nor PP_i-dependent phosphofructokinase (PFK) (enzymes 3 and 4) activity. All other spiroplasmas had only ATP-dependent PFK activity. Only the Maryland flower spiroplasma and *Spiroplasma culicicola* demonstrated deoxyuridine monophosphatase (enzyme 34) activity. With the exception of these responses, all of the spiroplasmas reacted identically; i.e., all possessed or lacked each of the 57 other enzyme activities which we studied (Table 2). All of the spiroplasmas tested had NADH oxidase activity localized in their cytoplasmic fractions; i.e., the ratio of the specific activity of NADH oxidase activity in each membrane fraction divided by the specific activity of NADH oxidase activity in the cytoplasmic fraction from the same batch of cells was less than 0.30 (34). This ratio was less than 0.02 in preparations from *S. citri*, *S. floricola* 23-6^T, *S. melliferum*, *S. apis*, and *S. kunkelii*. Also, all of the spiroplasmas listed in Table 1 produced ATP in the 3-phosphoglycerate kinase (enzyme 6) and pyruvate kinase (enzyme 8) assays.

TABLE 2. Enzymatic activities of cell-free extracts of members of the *Spiroplasmataceae*^a

No.	Enzyme ^b	Name	Activity in %									
			S. citri	Maryland flower spiroplasma	S. mirum	S. sabaudiense	S. culicicola	S. apis	S. melliferum	S. kunkelii	S. floridcola OBMG	S. floridcola 23-6 ^c
2	Phosphoglucose isomerase		0.62(0.43)	0.08(0.03)	1.1(0.73)	3.9(0.68)	1.4(0.13)	1.3(0.56)	1.4(1.1)	0.61(0.42)	ND ^d	1.5(0.31)
3	ATP-dependent PFK		0.09(0.04)	2.7(0.53)	5.6(1.3)	1.1(0.02)	0.51(0.06)	0.57(0.15)	0.23(0.15)	0.09(0.04)	NA ^e	NA
5	Aldolase		0.04(0.01)	2.1(1.1)	0.71(0.45)	0.39(0.21)	0.23(0.12)	1.3(0.38)	0.95(0.82)	0.04(0.01)	ND	0.75(0.40)
6	3-Phosphoglycerate kinase		0.99(0.13)	9.1(5.6)	4.3(0.14)	1.1(0.08)	1.3(0.73)	1.3(0.66)	0.16(0.02)	0.99(0.13)	0.11(0.05)	1.6(0.73)
7	Enolase		0.87(0.15)	7.1(1.6)	0.92(0.29)	2.0(0.22)	0.25(0.03)	2.0(1.4)	1.9(0.61)	0.87(0.15)	ND	3.7(0.38)
8	Pyruvate kinase		82(11)	280(5.6)	43(15)	310(100)	89(12)	620(65)	269(79)	37(8.6)	ND	580(46)
9	Lactate dehydrogenase		7.7(0.09)	11(0.87)	0.10(0.01)	0.34(0.04)	0.16(0.04)	ND	8.4(0.64)	0.92(0.09)	2.4(0.35)	0.48(0.07)
10	Pyruvate dehydrogenase (PEP → PYR)		11(2.8)	9.8(1.2)	13(1.8)	4.1(0.10)	18(1.6)	38(2.1)	41(3.4)	11(1.5)	ND	30(13)
11	Pyruvate dehydrogenase (PYR → PEP)		4.1(1.6)	6.3(2.0)	9.5(0.10)	2.4(0.40)	12(5.4)	73(1.1)	74(2.3)	4.9(0.80)	ND	13(4.6)
13	Malate dehydrogenase (OAA → OAA)		38(7.2)	66(3.2)	9.0(4.4)	92(7.0)	83(13)	39(1.8)	37(5.0)	37(7.6)	ND	35(5.9)
14	Malate dehydrogenase (MAL → MAL)		220(59)	380(17)	170(72)	490(6.1)	500(96)	190(12)	180(41)	200(15)	ND	170(44)
16	Aspartate aminotransferase (OAA → ASP)		20(2.7)	10(0.70)	14(1.1)	23(0.90)	18(1.6)	6.8(4.1)	8.6(1.5)	15(4.7)	ND	16(4.6)
17	Aspartate aminotransferase (ASP → OAA)		39(12)	44(1.6)	13(1.6)	59(7.7)	61(4.3)	43(7.1)	44(6.0)	48(6.9)	ND	49(8.9)
23	Ribulose-5-phosphate epimerase		0.02(0.01)	2.3(1.6)	2.9(1.4)	0.42(0.11)	1.1(0.45)	0.30(0.02)	0.17(0.03)	0.02(0.01)	ND	0.28(0.10)
24	Phosphoribose isomerase		0.01(0.00)	1.8(0.41)	NA	0.02(0.01)	0.35(0.18)	0.30(0.14)	0.14(0.05)	0.01(0.00)	ND	0.48(0.29)
25	Transketolase (Xu5P + R5P → S7P + G3P)		0.01(0.00)	1.9(1.5)	0.23(0.19)	0.04(0.01)	0.27(0.19)	0.16(0.13)	0.08(0.05)	0.01(0.00)	0.17(0.11)	0.35(0.00)
26	Transketolase (Xu5P + E4P → F6P + G3P)		NA	0.58(0.21)	0.95(0.17)	0.06(0.04)	0.28(0.18)	0.18(0.02)	0.15(0.03)	NA	ND	0.52(0.05)
27	Transaldolase		NA	0.97(0.27)	NA	0.02(0.01)	0.26(0.18)	0.17(0.07)	0.19(0.12)	NA	ND	0.72(0.03)
28	Deoxyribose-5-phosphate aldolase		NA	0.98(0.48)	2.3(0.22)	0.21(0.18)	0.61(0.10)	0.21(0.11)	0.17	NA	0.11(0.01)	0.41(0.15)
29	dCMP kinase		0.13	0.19	0.01	0.02	NA	0.01	0.01	0.08	ND	0.09
30	dCMP deaminase		NA	NA	0.74(0.02)	NA	4.2(0.98)	1.3(0.47)	1.4(0.37)	NA	ND	2.5(0.05)
31	Deoxycytidine kinase		0.12(0.01)	0.24(0.01)	0.10(0.02)	0.02(0.01)	0.07(0.01)	0.07(0.05)	0.04(0.02)	0.05(0.04)	ND	0.04(0.02)
32	Cytidine deaminase		NA	7.2(2.4)	0.79(0.16)	13(3.9)	NA	NA	NA	NA	ND	NA
33	Deoxyuridine triphosphatase		0.23(0.05)	1.6(0.63)	0.21(0.06)	1.7(0.38)	1.8(0.51)	0.18(0.09)	0.16(0.03)	0.57(0.11)	ND	0.26(0.00)
34	Deoxyuridine monophosphatase		NA	1.1(0.34)	NA	NA	NA	NA	NA	NA	ND	ND
35	Uridine phosphorylase		NA	1.2	1.5	NA	NA	NA	NA	NA	ND	NA
36	Thymidine monophosphate kinase		0.09(0.01)	1.0(0.01)	0.10(0.00)	0.01(0.00)	0.02(0.00)	0.01(0.00)	0.01(0.00)	0.05(0.01)	ND	0.01(0.00)
37	Thymidine kinase		0.11(0.13)	0.01(0.00)	0.01(0.00)	0.21(0.02)	0.01(0.00)	0.01(0.00)	0.01(0.00)	0.30(0.02)	ND	0.05(0.00)
38	Thymidine phosphorylase		0.26(0.21)	3.8(1.0)	4.0(0.01)	3.4(0.49)	3.9(1.4)	0.10(0.01)	0.08(0.01)	0.38(0.02)	ND	0.05(0.00)
39	Uracil-deoxyribonucleic acid glycosylase		0.04(0.00)	0.04(0.01)	0.01(0.00)	0.04(0.00)	0.03(0.00)	0.10(0.00)	0.10(0.00)	0.01(0.00)	ND	0.01(0.00)
40	Deoxyribonuclease		0.57(0.23)	0.21(0.13)	0.78(0.13)	0.16(0.07)	1.4(0.37)	0.33(0.20)	0.15(0.07)	0.13(0.02)	ND	0.57(0.23)
41	Adenine phosphoribosyltransferase		140(0.27)	560(22)	670(9.0)	660(11)	610(22)	190(6.0)	240(9.0)	320(11)	160(11)	120(8.2)
42	Hypoxanthine phosphoribosyltransferase		76(9.8)	52(3.0)	44(2.0)	61(6.0)	60(1.0)	41(0.50)	48(0.60)	51(4.0)	19(3.7)	31(7.0)
43	Guanine phosphoribosyltransferase		99(24)	110(0.30)	90(1.0)	83(2.0)	83(2.0)	66(2.0)	69(1.0)	70(3.0)	21(3.6)	76(1.7)
44	Adenine phosphorylase, R-1-P (ADE → ADO)		57(5.8)	130(1.0)	22(1.0)	130(4.0)	120(2.0)	230(11)	220(4.0)	120(7.0)	64(7.1)	130(14)
45	Adenosine phosphorylase (ADO → ADE)		150(10)	110(11)	81(7.0)	90(8.2)	71(4.2)	81(10)	64(5.0)	110(9.2)	32(4.7)	50(4.3)
46	Adenosine phosphorylase, dR-1-P (ADE → dADO)		75(17)	190(9.0)	250(9.8)	150(5.0)	280(7.0)	220(5.0)	190(7.0)	92(5.1)	120(6.2)	110(8.2)
47	Deoxyadenosine phosphorylase (dADO → ADE)		78(11)	62(9.0)	54(6.2)	72(5.0)	58(6.2)	96(8.0)	52(4.0)	68(11)	9.0(2.6)	22(3.1)
48	Hypoxanthine phosphorylase, R-1-P (HPX → INO)		110(13)	150(4.0)	120(0.90)	180(4.0)	170(7.0)	140(7.0)	110(3.0)	130(4.2)	210(11)	120(14)
49	Hypoxanthine phosphorylase, dR-1-P (HPX → dINO)		140(34)	84(7.4)	95(2.0)	92(4.8)	89(1.0)	150(0.81)	150(4.0)	97(7.2)	76(6.0)	92(4.2)
50	Guanine phosphorylase, R-1-P (GUA → GUO)		340(33)	290(16)	360(6.0)	350(11)	310(10)	280(4.0)	310(6.0)	260(8.1)	93(5.4)	180(9.1)

51	Guanosine phosphorylase (GUO → GUA)	72(8.2)	64(7.6)	93(10)	82(9.0)	78(11)	110(15)	97(4.2)	88(7.0)	21(3.1)	42(0.90)
52	Guanine phosphorylase, dR-1-P (GUA → dGUO)	380(41)	280(22)	350(5.0)	210(7.0)	230(6.0)	460(4.0)	340(5.0)	310(9.2)	72(5.1)	110(10)
53	Deoxyguanosine phosphorylase (dGUO → GUA)	34(7.1)	62(7.8)	71(6.2)	42(6.0)	31(6.2)	92(10)	82(6.0)	72(6.8)	18(5.3)	71(8.0)
54	Adenosine kinase, ATP (ADO → AMP)	NA	NA	NA	NA	NA	NA	NA	NA	VAR ^c	VAR
55	Adenosine kinase, PP _i (ADO → AMP)	43(6.7)	11(0.10)	20(0.70)	46(2.0)	28(0.10)	18(1.0)	13(1.0)	17(2.6)	VAR	VAR
56	Deoxyadenosine kinase, ATP (dADO → dAMP)	NA	NA	NA	NA	NA	NA	NA	NA	VAR	VAR
57	Deoxyadenosine kinase, PP _i (dADO → dAMP)	27(3.5)	11(0.20)	7.0(0.80)	18(0.20)	4.0(0.20)	17(0.10)	10(0.60)	8.0(1.2)	VAR	VAR
58	Inosine kinase, ATP (INO → IMP)	NA	NA	NA	NA	NA	NA	NA	NA	VAR	VAR
59	Inosine kinase, PP _i (INO → IMP)	9.9(2.1)	10(0.40)	15(2.0)	26(1.0)	14(0.10)	16(0.01)	23(2.0)	9.0(3.4)	VAR	VAR
60	Guanosine kinase, ATP (GUO → GMP)	NA	NA	NA	NA	NA	NA	NA	NA	VAR	VAR
61	Guanosine kinase, PP _i (GUO → GMP)	5.0(0.57)	6.1(0.70)	6.0(0.60)	10(1.8)	9.0(0.36)	30(5.0)	16(3.0)	15(4.1)	VAR	VAR
62	Deoxyguanosine kinase, ATP (dGUO → dGMP)	80(2.7)	88(5.0)	94(6.0)	54(5.0)	14(0.20)	5.5(0.66)	5.7(0.20)	17(1.2)	VAR	VAR
63	Deoxyguanosine kinase, PP _i (dGUO → dGMP)	5.3(0.80)	18(7.0)	NA	23(2.0)	11(2.6)	14(3.0)	16(0.70)	11(2.1)	VAR	VAR
64	Adenosine monophosphate nucleotidase	23(2.5)	78(4.0)	71(1.0)	54(3.0)	46(2.0)	100(3.0)	110(6.0)	62(5.4)	8.0(1.2)	7.4(1.3)
65	Deoxyadenosine monophosphate nucleotidase	47(3.6)	60(2.0)	86(8.0)	59(1.0)	58(1.0)	97(7.0)	108(2.0)	84(7.2)	2.1(0.30)	5.2(0.90)
66	Inosine monophosphate nucleotidase	0.88(0.21)	4.0(0.30)	4.8(0.40)	4.4(0.10)	9.0(0.60)	3.0(0.10)	2.8(0.10)	5.2(0.90)	1.4(0.70)	0.90(0.60)
67	Guanosine monophosphate nucleotidase	34(5.5)	39(1.0)	56(5.0)	39(3.0)	31(1.0)	62(2.8)	59(1.0)	41(6.6)	0.80(0.20)	2.1(0.90)

^a Activities for the following enzymes were not detected in any of the strains studied: glucokinase (enzyme 1), PP_i-dependent PFK (enzyme 4), malate synthase (enzyme 12), phosphoenolpyruvate carboxylase (enzyme 15), citrate synthase (enzyme 18), isocitrate dehydrogenase (enzyme 19), fumarase (enzyme 20), glucose 6-phosphate dehydrogenase (enzyme 21), and 6-phosphogluconate dehydrogenase (enzyme 22).
^b Abbreviations: PEP, phosphoenolpyruvate; PYR, pyruvate; MAL, malate; OAA, oxaloacetate; ASP, aspartate; Xu5P, xylulose 5-phosphate; R5P, ribose 5-phosphate; S7P, sedoheptulose-7-phosphate; G3P, glyceraldehyde 3-phosphate; E4P, erythrose-4-phosphate; F6P, fructose 6-phosphate; ADE, adenine; ADO, adenosine; R-1-P, ribose 1-phosphate; dR-1-P, deoxyribose 1-phosphate; dADO, deoxyadenosine; HPX, hypoxanthine; INO, inosine; dINO, deoxyinosine; GUA, guanine; GUO, guanosine; dGUO, deoxyguanosine; AMP, adenosine monophosphate; dAMP, deoxyadenosine monophosphate; IMP, inosine monophosphate; GMP, guanosine monophosphate; dGMP, deoxyguanosine monophosphate.

^c For all enzymes (except lactate dehydrogenase) activity is reported as the number of nanomoles of product synthesized per minute per milligram of protein ($n = 3$). For lactate dehydrogenase activity is reported as the number of micromoles of product synthesized per minute per milligram of protein ($n = 2$ or 3). The values in parentheses are standard deviations.

^d ND, Not done.

^e NA, No activity detected. We could detect the activity of 0.1×10^{-3} to 1.0×10^{-3} IU of commercial enzyme.

^f VAR, Variable or questionable (see text).

DISCUSSION

In this paper we report the presence or absence of different enzymatic activities associated with carbohydrate, purine, and pyrimidine ribo- and deoxyribonucleotide metabolism in various *Spiroplasma* species. As we and other workers have discussed previously, problems associated with studies in which researchers use crude cell extracts from organisms grown in rich undefined media may lead to incorrect conclusions concerning the presence or absence of enzymatic activity (11, 28). Such errors may be due to low assay sensitivity, contaminating and interfering enzymes, or perhaps, in certain cases, failure to induce an inducible enzyme.

Furthermore, we cannot be certain that the reaction sequences which we detected in our in vitro studies with cell-free extracts are functional in actively metabolizing whole cells; such functionality must be proved by assays in which whole cells are used, similar to the assays described by McIvor and Kenny (30). Furthermore, although the rates shown in Table 2 are indicative of the presence or absence of enzymes, they may not reflect enzyme mass or the magnitude of in situ activity. For example, the higher specific activities which we obtained when we studied purine enzymes may not mean that the purine pathways are more active than the PP shunt, whose specific enzyme activities were relatively lower. It is likely that the variability in rates shown in Table 2 are strong reflections of assay sensitivity and interfering enzyme activities.

Notwithstanding potential difficulties in interpretation, we have made certain operating, but only qualitative, assumptions concerning spiroplasma metabolism, based on our studies with cell-free extracts. We found no qualitative metabolic differences in the spiroplasma extracts that could be attributed to growth in the four different media which we used.

We believe that spiroplasmas are fermentative; i.e., they convert glucose 6-phosphate to pyruvate and lactate by reactions that appear to constitute the classical EMP pathway. In spiroplasmas, as in *Mycoplasma* species, PFK activity, the rate-limiting step of glycolysis, is ATP dependent; i.e., PP_i cannot substitute for ATP (14). In *Acholeplasma* species, the PFK activity is PP_i dependent (40). The synthesis of ATP in the 3-phosphoglycerate kinase and pyruvate kinase assays confirms the capability of spiroplasmas to perform substrate phosphorylation.

Our inability to detect PFK activity in all batches of both *S. floricola* strains may reflect a methodological error, since the absence of PFK activity in a fermentative or apparently fermentative organism that possesses all of the other EMP enzymes is unknown to us. Possibly in *S. floricola* the EMP pathway, although involving glucose 6-phosphate as a substrate and phosphoglucose isomerase activity to form fructose 6-phosphate (Table 2), lacks PFK activity. The absence of PFK activity is circumvented by connecting with the PP shunt at fructose 6-phosphate. After the carbons of glucose 6-phosphate pass through the PP shunt, they re-enter the EMP pathway at glyceraldehyde 3-phosphate and proceed to pyruvate and lactate. However, this hypothesis concerning *S. floricola* was not tested.

The PP shunt appears to be present but incomplete in spiroplasmas, since we did not detect glucose 6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase activity in any strain. The absence of these two activities may indicate some limitation on pathways requiring reducing equivalents such as reduced nicotinamide adenine dinucleotide phosphate (e.g., the apparent inability of growing spiroplasmas, such as *S. citri*, to synthesize lipids from

[^{14}C]acetate [15] and their growth requirement for cholesterol). All spiroplasmal extracts had transketolase activity in one or two directions. The presence of this activity, coupled with transaldolase activity in six of nine strains, indicates that linkage of the EMP pathway and the PP shunt in spiroplasmas, as in acholeplasmas, can occur at fructose 6-phosphate or glyceraldehyde 3-phosphate (14). This linkage may permit the synthesis of nucleic acid precursors from glucose and allow degradation products of ribonucleic acid and deoxyribonucleic acid metabolism, with the involvement of deoxyribose 5-phosphate aldolase activity, to reenter the glycolytic pathway. The latter course would probably also require phosphoribose mutase activity, which we have not studied but which has been reported in *Ureaplasma urealyticum* and *Mycoplasma mycoides* subsp. *mycoides* (11).

Adenosine monophosphate, inosine monophosphate, and guanosine monophosphate are known to be synthesized in mollicutes in the following ways: (i) in a one-step reaction, from the respective nucleobase and phosphoribosyl pyrophosphate, and (ii) in a two-step reaction (in the first part, ribose 1-phosphate or deoxyribose 1-phosphate is used to form the ribo- or deoxyribonucleoside, and then in the second part, ATP or PP_i is used as the orthophosphate donor to form the ribo- or deoxyribomononucleotide) (52). All spiroplasmas were able to synthesize these mononucleotides by the one-step reaction or by the two-step reaction in which PP_i was used as the orthophosphate donor. The first two routes (the phosphoribosyl one-step pathway and the PP_i -dependent two-step pathway) are both also found in all *Acholeplasma* species and in *Anaeroplasmia intermedium* (28), as well as in *Asteroleplasma anaerobium* (J. Petzel, M. McElwain, D. DeSantis, J. Manolukas, M. V. Williams, P. A. Hartman, M. J. Allison, and J. D. Pollack, unpublished data). Species of the *Mycoplasmataceae*, except *U. urealyticum* and some pathogenic *Mycoplasma hominis* strains, can synthesize the mononucleotides only by the phosphoribosyl one-step pathway, from the nucleobase and phosphoribosyl pyrophosphate, as they apparently have no cytoplasmic purine nucleoside kinase activity (28).

The relatively unusual ability of some mollicutes and spiroplasmas to use deoxyribose 1-phosphate to synthesize deoxyribonucleosides may be taxonomically significant (28). The use of deoxyribose 1-phosphate by the purine phosphorylases (Table 2, enzymes 46, 50 and 51) suggests a reduced need for ribonucleotide reductase activity or perhaps the existence of a route without ribonucleotide reductase for the synthesis of nucleic acid precursors of deoxyribonucleic acid.

Our findings regarding enzymes of deoxypyrimidine metabolism were similar for all spiroplasmas except in the reactions involving the deamination of dCMP and deoxycytidine. Although failure to demonstrate cytidine deaminase could possibly be artifactual (11), we believe that the lack of both dCMP and cytidine (deoxycytidine) deaminase activities only in *S. kunkelii* and *S. citri*, the two plant pathogens, reflects the inability of these organisms to use deoxycytidine or dCMP as a source of deoxyribose 1-phosphate or cytosine for the synthesis of thymidine nucleotides. Similarly, the lack of detectable levels of dCMP kinase in *S. culicicola* extracts suggests that this organism may not salvage deoxycytidine for deoxycytidine triphosphate synthesis.

Like all *Acholeplasma* species (28), *Anaeroplasmia intermedium* (28), *M. mycoides* subsp. *mycoides* (33), and *Asteroleplasma anaerobium* (Petzel et al., unpublished data), the spiroplasmas have deoxyuridine triphosphatase (enzyme 33) activity, whereas all other *Mycoplasma* species and *U.*

urealyticum do not (57). All other procaryotic and eucaryotic cells that have been examined have deoxyuridine triphosphatase activity (57). This notable absence of deoxyuridine triphosphatase activity in *U. urealyticum* and *Mycoplasma* species other than *M. mycoides* subsp. *mycoides* (33) may be of phylogenetic significance and suggests that the *Ureaplasma* species and almost all *Mycoplasma* species may be more closely related to each other than either taxon is to the spiroplasmas. In fact, previously published phylogenies of members of the *Mollicutes* (41, 59) do suggest that *M. mycoides* and perhaps other *Mycoplasma* species represent a significant synapomorphy defining the clade of monophyletic *Mycoplasma* and *Ureaplasma* species.

Steiner et al. (46) first reported that some spiroplasmas lack uridine phosphorylase activity. In a more extensive study, McGarrity et al. (29) reported that uridine phosphorylase activity is absent in 8 groups and variously present in 1 of 20 groups of spiroplasmas. We studied nine of the same serogroups, and our results agree with those of McGarrity et al. (29) in seven instances. In the case of *S. melliferum* (subgroup I-2) and *S. culicicola* (group X), we failed to detect uridine phosphorylase activity, as reported previously (29). The differences may be technical; i.e., we used cell-free cytoplasmic extracts, while McGarrity et al. used whole unfractionated cell lysates and considered the activity to be membrane associated (29).

An unusual activity of spiroplasma preparations is that they can phosphorylate deoxyguanosine but no other nucleoside with ATP (Table 2, enzyme 62). This feature may be taxonomically useful, because no other mollicute tested (i.e., *Acholeplasma* or *Mycoplasma* species, *U. urealyticum*, *Anaeroplasmia intermedium* [28], and *Asteroleplasma anaerobium* [Petzel et al., unpublished data]) has ATP-deoxyguanosine kinase activity or can phosphorylate any nucleoside with ATP. All other mollicute purine nucleoside kinase activities require PP_i as the orthophosphate donor.

We found pyruvate dehydrogenase activities (enzymes 10 and 11) in both directions in all of the spiroplasma samples which we studied. These activities have been reported in other members of the *Mollicutes* (12). In preliminary experiments, we have not found pyruvate carboxylase activity in any *Spiroplasma* species (unpublished data), and we did not detect (Table 2) phosphoenolpyruvate carboxylase (enzyme 15) or malate synthase (enzyme 12) activity in any spiroplasma extract. We did not assay for malic enzyme(s) (EC 1.1.1.38-40) or lactate-malate transhydrogenase (EC 1.1.1.99.7) (16). If further studies prove these observations to be correct, it may be that in *Spiroplasma* species there is no direct link between the pyruvate or phosphoenolpyruvate of the EMP pathway and amino acids via oxaloacetate. This linkage apparently exists in *Mycoplasma* and *Acholeplasma* species (26).

Although we assayed for only four enzymes of the tricarboxylic acid cycle and found malate dehydrogenase activities (enzymes 13 and 14) in extracts of all strains and citrate synthase, isocitrate dehydrogenase, and fumarase activities (enzymes 18 through 20) in none, we determined that the tricarboxylic acid cycle is absent from the *Spiroplasma* species which we studied, as it is from all other mollicute genera that have been examined (26). Jones et al. (22) first suggested the absence of some of the tricarboxylic acid cycle in *S. citri*. Also, the absence of isocitrate dehydrogenase and malate synthase activities suggests the absence of the glyoxylate cycle. Aspartate may be an important modulating intermediate that could be formed by the action of aspartate aminotransferase activities (enzymes 16 and 17), which we

detected in both directions in all spiroplasma preparations (Table 2). This reaction may be involved in maintaining oxaloacetate levels and hence may affect malate dehydrogenase activity, as well as the concentration of cellular nicotinamide adenine dinucleotide and NADH. There is no de novo synthesis of purines in members of the *Mollicutes*, so aspartate presumably does not contribute its nitrogen to the purine ring, but the nitrogen of aspartate has been reported to be transferred to the amino group of inosine monophosphate in the synthesis of adenosine monophosphate by extracts of a number of mollicute species (52).

The cytoplasmic localization of NADH oxidase activity in spiroplasmas has been reported previously only for *S. citri* (23, 31). In this study, the cytoplasmic localization of NADH oxidase activity was extended to four other *Spiroplasma* spp. These observations support the results of phylogenetic studies that associate *Spiroplasma* and *Mycoplasma* species (59) since all of the strains of both genera which have been tested have NADH oxidase activity localized in their cytoplasmic fractions, while the activity is localized in the membranes of *Acholeplasma* species (34). NADH oxidase activity is apparently absent from *U. urealyticum* (27, 36).

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of Edward A. Clark, R. Donaldson, and Nancy Teders.

LITERATURE CITED

1. Bové, J. M., and C. Saillard. 1979. Cell biology of spiroplasmas, p. 83-153. In R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 3. Plant and insect mycoplasmas. Academic Press, Inc., New York.
2. Chang, C.-J. 1984. Vitamin requirements of three spiroplasmas. *J. Bacteriol.* **160**:488-490.
3. Chang, C.-J. 1985. Lipid utilization of two flower spiroplasmas and honeybee spiroplasma. *Can. J. Microbiol.* **31**:173-176.
4. Chang, C.-J., and T. A. Chen. 1982. Spiroplasmas: cultivation in chemically defined medium. *Science* **215**:1121-1122.
5. Chang, C.-J., and T. A. Chen. 1982. Nutritional requirements of two flower spiroplasmas and honeybee spiroplasma. *J. Bacteriol.* **153**:452-457.
6. Charron, A., C. Bebear, G. Brun, P. Yot, J. Latrille, and J. M. Bové. 1979. Separation and partial characterization of two deoxyribonucleic acid polymerases from *Spiroplasma citri*. *J. Bacteriol.* **140**:763-768.
7. Charron, A., M. Castroviejo, C. Bebear, J. Latrille, and J. M. Bové. 1982. A third polymerase from *Spiroplasma citri* and two other spiroplasmas. *J. Bacteriol.* **149**:1138-1141.
8. Chen, T. A., and C. H. Liao. 1975. Corn stunt spiroplasma: isolation, cultivation, and proof of pathogenicity. *Science* **188**:1015-1017.
9. Chen, T. A., J. M. Wells, and C. H. Liao. 1982. Cultivation *in vitro*: spiroplasmas, plant mycoplasmas, and other fastidious walled prokaryotes, p. 417-446. In M. S. Mount and G. H. Lacy (ed.), *Phytopathogenic prokaryotes*, vol. 2. Academic Press, Inc., New York.
10. Clark, T. B. 1982. Spiroplasmas: diversity of arthropod reservoirs and host-parasite relationships. *Science* **217**:57-59.
11. Cocks, B. G., R. Youil, and L. R. Finch. 1988. Comparison of enzymes of nucleotide metabolism in two members of the *Mycoplasmataceae* family. *Int. J. Syst. Bacteriol.* **38**:273-278.
12. Constantopoulos, G., and G. J. McGarrity. 1987. Activities of oxidative enzymes in mycoplasmas. *J. Bacteriol.* **169**:2012-2016.
13. Davis, P. J., A. Katznell, S. Razin, and S. Rottem. 1985. Spiroplasma membrane lipids. *J. Bacteriol.* **161**:118-122.
14. DeSantis, D., V. V. Tryon, and J. D. Pollack. 1989. Metabolism of Mollicutes: the Embden-Meyerhof-Parnas pathway and the hexose monophosphate shunt. *J. Gen. Microbiol.* **135**:683-691.
15. Freeman, B. A., R. Sissenstein, T. T. McManus, J. E. Wood-

- ward, I. M. Lee, and J. B. Mudd. 1976. Lipid composition and lipid metabolism of *Spiroplasma citri*. *J. Bacteriol.* **125**:946-954.
16. Gottschalk, G. 1986. Bacterial metabolism, 2nd ed., p. 208-282. Springer-Verlag, New York.
17. Hackett, K. J., and T. B. Clark. 1989. Ecology of spiroplasmas, p. 113-200. In R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 5. Spiroplasmas. Academic Press, Inc., New York.
18. Hackett, K. J., A. S. Ginsberg, S. Rottem, R. B. Henegar, and R. F. Whitcomb. 1987. A defined medium for a fastidious spiroplasma. *Science* **237**:525-527.
19. Hoffmann, P. J., and Y.-C. Cheng. 1978. The deoxyribonuclease induced after infection of KB cells by herpes simplex virus. I. Purification and characterization of the enzyme. *J. Biol. Chem.* **253**:3557-3562.
20. Igwegbe, E. C. K., C. Stevens, and J. J. Hollis, Jr. 1979. An *in vitro* comparison of some biochemical and biological properties of California, USA, and Morocco isolates of *Spiroplasma citri*. *Can. J. Microbiol.* **25**:1125-1132.
21. Igwegbe, E. C. K., and C. Thomas. 1979. Occurrence of enzymes of arginine dihydrolase pathway in *Spiroplasma citri*. *J. Gen. Appl. Microbiol.* **24**:261-269.
22. Jones, A. L., R. F. Whitcomb, D. L. Williamson, and M. E. Coan. 1977. Comparative growth and primary isolation of spiroplasmas in media based on insect tissue culture formulations. *Phytopathology* **67**:738-746.
23. Kahane, I., S. Greenstein, and S. Razin. 1977. Carbohydrate content and enzymatic activities in the membrane of *Spiroplasma citri*. *J. Gen. Microbiol.* **101**:173-176.
24. Lee, I.-M., and R. E. Davis. 1984. New media for rapid growth of *Spiroplasma citri* and corn stunt spiroplasma. *Phytopathology* **74**:84-89.
25. Liao, C. H., and T. A. Chen. 1977. Culture of corn stunt spiroplasma in a simple medium. *Phytopathology* **67**:802-807.
26. Manolukas, J. T., M. F. Barile, D. K. F. Chandler, and J. D. Pollack. 1988. Presence of anaplerotic reactions and transamination, and the absence of the tricarboxylic acid cycle in Mollicutes. *J. Gen. Microbiol.* **134**:791-800.
27. Masover, G. K., S. Razin, and L. Hayflick. 1977. Localization of enzymes in *Ureaplasma urealyticum* (T-strain *Mycoplasma*). *J. Bacteriol.* **130**:297-302.
28. McElwain, M. C., D. K. F. Chandler, M. F. Barile, T. F. Young, V. V. Tryon, J. W. Davis, Jr., J. P. Petzel, C.-J. Chang, M. V. Williams, and J. D. Pollack. 1988. Purine and pyrimidine metabolism in *Mollicutes* species. *Int. J. Syst. Bacteriol.* **38**:417-423.
29. McGarrity, G. J., L. Gamon, T. Steiner, J. Tully, and H. Kotani. 1985. Uridine phosphorylase activity among the class mollicutes. *Curr. Microbiol.* **12**:107-112.
30. McIvor, R. S., and G. E. Kenny. 1978. Differences in incorporation of nucleic acid bases and nucleosides by various *Mycoplasma* and *Acholeplasma* species. *J. Bacteriol.* **135**:483-489.
31. Mudd, J. B., M. Ittig, B. Roy, J. Latrille, and J. M. Bové. 1977. Composition and enzyme activities of *Spiroplasma citri* membranes. *J. Bacteriol.* **129**:1250-1256.
32. Mudd, J. B., I.-M. Lee, H.-Y. Liu, and E. C. Calavan. 1979. Comparison of the membrane composition of *Spiroplasma citri* and the corn stunt *Spiroplasma*. *J. Bacteriol.* **137**:1056-1058.
33. Neale, G. A. M., A. Mitchell, and L. R. Finch. 1983. Enzymes of pyrimidine deoxyribonucleotide biosynthesis in *Mycoplasma mycoides* subsp. *mycoides*. *J. Bacteriol.* **156**:1001-1005.
34. Pollack, J. D. 1979. Respiratory pathways and energy yielding mechanisms, p. 188-211. In M. F. Barile and S. Razin (ed.), *The mycoplasmas*, vol. 1. Cell biology. Academic Press, Inc., New York.
35. Pollack, J. D. 1983. Localization of enzymes in mycoplasmas: preparatory steps. *Methods Mycoplasmol.* **1**:327-332.
36. Pollack, J. D. 1986. Metabolic distinctiveness of ureaplasmas. *Pediatr. Infect. Dis.* **5**:S305-S307.
37. Pollack, J. D., and P. J. Hoffmann. 1982. Properties of the nucleases of Mollicutes. *J. Bacteriol.* **152**:538-541.
38. Pollack, J. D., S. Razin, and R. C. Cleverdon. 1965. Localization of enzymes in *Mycoplasma*. *J. Bacteriol.* **90**:617-622.
39. Pollack, J. D., V. V. Tryon, and K. D. Beaman. 1983. The metabolic pathways of *Acholeplasma* and *Mycoplasma*: an overview. *Yale J. Biol. Med.* **56**:709-716.
40. Pollack, J. D., and M. V. Williams. 1986. PP_i-dependent phosphofructotransferase (phosphofructokinase) activity in the Mollicutes (mycoplasma) *Acholeplasma laidlawii*. *J. Bacteriol.* **165**:53-60.
41. Rogers, M. J., J. Simmons, R. T. Walker, W. G. Weisburg, C. R. Woese, R. S. Tanner, I. M. Robinson, D. A. Stahl, G. Olsen, R. H. Leach, and J. Maniloff. 1985. Construction of the mycoplasma evolutionary tree from 5S rRNA sequence data. *Proc. Natl. Acad. Sci. USA* **82**:1160-1164.
42. Saglio, P. H. M., M. J. Daniels, and A. Pradet. 1979. ATP and energy charge as criteria of growth and metabolic activity of Mollicutes: application to *Spiroplasma citri*. *J. Gen. Microbiol.* **110**:13-20.
43. Saglio, P. H. M., R. E. Davis, R. Dalibart, G. Dupont, and J. M. Bové. 1974. *Spiroplasma citri*: L'espece type des spiroplasmes. *Colloq. INSERM (Inst. Natl. Sante Rech. Med.)* **33**:27-34.
44. Saglio, P. H. M., M. L'hospital, D. Laffèche, G. Dupont, J. M. Bové, J. G. Tully, and E. A. Freundt. 1973. *Spiroplasma citri* gen. and sp. n.: a mycoplasma-like organism associated with "stubborn" disease of citrus. *Int. J. Syst. Bacteriol.* **23**:191-204.
45. Saglio, P. H. M., and R. F. Whitcomb. 1979. Diversity of wall-less prokaryotes in plant vascular tissue, fungi, and invertebrate animals, p. 1-36. In R. F. Whitcomb and J. G. Tully (ed.), *The mycoplasmas*, vol. 3. Plant and insect mycoplasmas. Academic Press, Inc., New York.
46. Steiner, T., G. J. McGarrity, and D. M. Phillips. 1982. Cultivation and partial characterization of spiroplasmas in cell cultures. *Infect. Immun.* **35**:296-304.
47. Stephens, M. A. 1982. Partial purification and cleavage specificity of a site-specific endonuclease, *SciNI*, isolated from *Spiroplasma citri*. *J. Bacteriol.* **149**:508-514.
48. Stevens, C., R. M. Cody, and R. T. Gudauskas. 1980. Arginine metabolism of the corn stunt spiroplasma. *Curr. Microbiol.* **4**:139-142.
49. Townsend, R. 1976. Arginine metabolism by *Spiroplasma citri*. *J. Gen. Microbiol.* **94**:417-420.
50. Townsend, R., P. G. Markham, K. A. Plaskitt, and M. G. Daniels. 1977. Isolation and characterization of a non-helical strain of *Spiroplasma citri*. *J. Gen. Microbiol.* **100**:15-21.
51. Tryon, V. V., and J. D. Pollack. 1984. Purine metabolism in *Acholeplasma laidlawii* B: novel PP_i-dependent nucleoside kinase activity. *J. Bacteriol.* **159**:265-270.
52. Tryon, V. V., and J. D. Pollack. 1985. Distinctions in *Mollicutes* purine metabolism: pyrophosphate-dependent nucleoside kinase and dependence on guanylate salvage. *Int. J. Syst. Bacteriol.* **35**:497-501.
53. Tully, J. G., D. L. Rose, E. Clark, P. Carle, J. M. Bové, R. B. Henegar, R. F. Whitcomb, D. E. Colflesh, and D. L. Williamson. 1987. Revised group classification of the genus *Spiroplasma* (class *Mollicutes*), with proposed new groups XII to XXII. *Int. J. Syst. Bacteriol.* **37**:357-364.
54. Tully, J. G., R. F. Whitcomb, H. F. Clark, and D. L. Williamson. 1977. Pathogenic mycoplasmas: cultivation and vertebrate pathogenicity of a new spiroplasma. *Science* **195**:892-894.
55. Whitcomb, R. F. 1980. The genus *Spiroplasma*. *Annu. Rev. Microbiol.* **34**:677-709.
56. Williams, M. V., and J. D. Pollack. 1985. Pyrimidine deoxyribonucleotide metabolism in *Acholeplasma laidlawii* B-PG9. *J. Bacteriol.* **161**:1029-1033.
57. Williams, M. V., and J. D. Pollack. 1988. Uracil-DNA glycosylase activity. Relationship to proposed biased mutation pressure in the class *Mollicutes*, p. 440-444. In R. E. Moses and W. C. Summers (ed.), *DNA replication and mutagenesis*. American Society for Microbiology, Washington, D.C.
58. Williamson, D. L., and R. F. Whitcomb. 1975. Plant mycoplasmas: a cultivable spiroplasma causes corn stunt disease. *Science* **188**:1018-1020.
59. Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221-271.