

Table S2. Novel putative enzymes annotated onto the *M. bovis* metabolic map in this study.

Enzyme name	Enzyme abbr.	EC number	Gene number	Pathway	Equation	Evidence
A. Phosphotransferase glucose transporter subunitA Phosphotransferase glucose transporter subunit B	PTSI/G	2.7.1.69	MBOVPG45_0417 MBOVPG45_0573	Glycolysis	Glucose + protein N(pi)-phospho L-histidine = Glucose 6-phosphate + protein histidine	1) Sequence homology (Fig. S1)
B. Phosphotriesterase	ULAG	3.1.1.-	MBOVPG45_0738	Ascorbate metabolism	L-ascorbate 6-phosphate + H ₂ O = 3-dehydro-L-gulonate 6-phosphate	1) Ortholog of <i>M. pneumoniae</i> phosphotriesterase (1, 2) 2) Genomic context 3) Gap filling
C. D-allulose 6-phosphate 3-epimerase	ALSE	5.1.3.-	MBOVPG45_0627	Fructose and mannose metabolism	D-Allulose 6-phosphate = beta-D-Fructose	1) Ortholog of <i>E. coli</i> alsE 2) Homology in crystal structure in <i>E. coli</i> alsE 3) Alternative activity for this enzyme in <i>E. coli</i> (3)
D. Carbohydrate uptake ABC transporter-2 family	CUT-2	-	MBOVPG45_0018 MBOVPG45_0019 MBOVPG45_0020	Carbohydrate uptake	A Carbohydrate + ATP + H ₂ O → A Carbohydrate + ADP + phosphate	1) Biocyc database search 2) Operon structure 3) Conserved domain search
E. Pseudouridine kinase	PSUK	2.7.1.83	MBOVPG45_0026	Pyrimidine metabolism	ATP + Pseudouridine = ADP + Pseudouridine 5'-phosphate	1) Ortholog of <i>E. coli</i> psuK 2) Conserved domain search
F. Fructose-bisphosphate aldolase	FBA	-	MBOVPG45_0088	Pentose phosphate pathway	Sedoheptulose-1,7-phosphate = glycerine phosphate + erythrose-4-phosphate	1) Enzymatic reaction annotated in <i>M. gallisepticum</i> (4) 2) Alternative activity of this enzyme in yeast (5)
G. Acetaldehyde dehydrogenase	ADH	1.2.1.10	MBOVPG45_0543	Pyruvate metabolism	Acetyl-CoA + NADH + H ⁺ = acetaldehyde + CoA + NAD ⁺	1) Ortholog of <i>M. pneumoniae</i> MPN564 (2) 2) Alternative activity for this enzyme in <i>Thermoanaerobacter ethonolicus</i> (6) 3) Sequence homology (Fig. S1)
H. Glycerol ABC transporter	GTS A-2, B-2, C-2	-	MBOVPG45_0748 MBOVPG45_0749 MBOVPG45_0750	Glycerol uptake	Glycerol (out) + ATP = glycerol (in) + ADP	1) BioCyc database search

I. sn-glycerol-3-phosphate transport system	UGP A, C, E	-	MBOVPG45_0309 MBOVPG45_0308 MBOVPG45_0307	sn-glycerol 3-phosphate uptake	sn-glycerol-3-phosphate (out) + ATP = sn-glycerol 3-phosphate (in) + ADP	1) BioCyc database search 2) Orthologs with other mycoplasma species 2) Genomic context
J. Dihydroxyacetone kinase	DHAK	2.7.1.29	MBOVPG45_0523	Glycerolipid metabolism	Glycerone + ATP = Glycerone phosphate + ADP	1) Ortholog of <i>M. pneumoniae</i> dihydroacetone kinase 2) Conserved domain search 3) Sequence homology (Fig. S1)
K. Glycosyl transferase	GTF	2.4.1.157	MBOVPG45_0064	Glycerolipid metabolism	diacylglycerol + UDP-glucose = 3-D-glucosyl-1,2 diacyl-sn-glycerol + UDP	1) Ortholog of <i>M. pneumoniae</i> yibD (2) 2) Enzymatic activity (7)
L. Phosphatidylcholine acylhydrolase	PLDB	3.1.1.5	MBOVPG45_0331	Lipid metabolism	Phosphatidylcholine + 2 H ₂ O = sn-glycero 3-phosphocholine + 2 fatty acid	1) Ortholog of <i>M. pneumoniae</i> MPN445 (2)
M. Choline ethanolamine kinase	CHK	2.7.1.32	MBOVPG45_0333 MBOVPG45_0335	Glycerophospholipid metabolism	Choline + ATP = Choline-phosphate + ADP	1) Ortholog of <i>M. pneumoniae</i> MPN532 (2) 2) Conserved domain search
N. Choline-phosphate cytidyltransferase	PCT	2.7.7.15	MBOVPG45_0084	Glycerophospholipid metabolism	Choline-phosphate + CTP = CDP-choline + Diphosphate	1) Ortholog of <i>M. pneumoniae</i> MPN336 (2) 2) Conserved domain search
O. Hypoxanthine phosphoribosyltransferase	HPT	2.4.2.8	MBOVPG45_0476	Purine metabolism	PRPP + Guanine = GMP + diphosphate	1) Enzymatic reaction in <i>M. pneumoniae</i> and <i>M. gallisepticum</i> (2, 4)
P. Adenine phosphoribosyltransferase	APT	2.4.2.7	MBOVPG45_0796	Purine metabolism	PRPP + Adenine = AMP + diphosphate	1) Enzymatic reaction in <i>M. pneumoniae</i> and <i>M. gallisepticum</i> (2, 4)
P. Orotate phosphoribosyltransferase	APT	2.4.2.10	MBOVPG45_0796	Pyrimidine metabolism	Orotidine 5'-phosphate + diphosphate = orotate + PRPP	1) Conserved domain analysis 2) Protein structure prediction
Q. 5' -nucleotidase	NT5 (ushA)	3.1.3.5	MBOVPG45_0690	Purine metabolism	AMP + H ₂ O = adenosine + phosphate IMP + H ₂ O = Inosine+ phosphate XMP + H ₂ O = Xanthosine + phosphate	1) Ortholog of <i>E. coli</i> ushA 2) Sequence homolog (Fig. S1) 3) Conserved domain search

					GMP + H ₂ O = Guanosine + phosphate	
					dGMP + H ₂ O = Deoxyguanosine + phosphate	
					dAMP + H ₂ O = Deoxyadenosine + phosphate	
Q. 5' -nucleotidase	NT5 (USHA)	3.1.3.5	MBOVPG45_0690	Pyrimidine metabolism	CMP + H ₂ O = Cytidine + Orthophosphate	1) Ortholog of <i>E. coli ushA</i> 2) Sequence homology (Fig. S1) 3) Conserved domain search
					UMP + H ₂ O = Uridine + Orthophosphate	
					dCMP + H ₂ O = Deoxycytidine + Orthophosphate	
					dUMP + H ₂ O = Deoxyuridine + Orthophosphate	
					dTMP + H ₂ O = Thymidine + Orthophosphate	
Q. Nicotinamide ribonucleotide phosphohydrolase	USHA	3.1.3.5	MBOVPG45_0690	Nicotinate and Nicotinamide metabolism	Nicotinate D-ribonucleotide + H ₂ O = Nicotinate D-ribonucleoside + Orthophosphate	1) Gap filling 2) Ortholog of <i>E. coli ushA</i>
					Nicotinamide D-ribonucleotide + H ₂ O = Nicotinamide-beta-riboside + Orthophosphate	
R. Pyruvate kinase	PYK	-	MBOVPG45_0158	Pyrimidine metabolism	UTP + pyruvate = UDP + phosphoenolpyruvate	1) Ortholog of <i>M. pneumoniae</i> pyrK MPN303 2) Enzymatic reaction (2, 4)
					CTP + pyruvate = CDP + phosphoenolpyruvate	
					dCTP + pyruvate = dCDP + phosphoenolpyruvate	
					dUTP + pyruvate = dUDP + phosphoenolpyruvate	

					dTTP + pyruvate = dTDP + phosphoenolpyruvate	
S. Orotidine 5'-phosphate decarboxylase	PYRF	4.1.1.23	MBOVPG45_0734	Pyrimidine metabolism	Orotidine 5'-phosphate + H ⁺ = CO ₂ + UMP	1) Gap filling 2) BioCyc database search
T. Pyrimidine phosphorylase Uridine phosphorylase	PDP (UPP)	2.4.2.3	MBOVPG45_0301	Pyrimidine metabolism	Uridine + phosphate = Uracil + alpha -D-ribose 1-phosphate	1) Gap filling 2) BioCyc database search 3) Ortholog of <i>M. pneumoniae</i> MPN064 4) Enzymatic activity (2, 8, 9)
		2.4.2.4			Thymidine + Phosphate = Thymine + 2-deoxy -alpha -D-ribose - 1 phosphate	
U. Malate transporter	AEC family	-	MBOVPG45_0327	Malate uptake	(R,S)-Malate (out) + H ⁺ = (R, S)-malate (in) + H ⁺	1) Ortholog of <i>M. fermentans</i> malate permease MFE_01530 2) Ortholog of <i>Clostridium cadaveris</i> malate permease protein 3) BioCyc and BLAST database search
V. Dicarboxylate/amino acid cation symporter family amino acid transporter	DAACS family	-	MBOVPG45_0568	Amino acid and dicarboxylate uptake	Substrate (dicarboxylate or amino acid) (out) + nM ⁺ (M ⁺ =H ⁺ or Na ⁺) (out) = Substrate (In) + nM ⁺ (in)	1) Conserved domain search 2) Protein structure of a member of the DAACS family (10, 11) 3) Biocyc, Uniprot, transporter classification database search
W. Neutral amino acid transporter, L-type amino acid transporter	LAT family	-	MBOVPG45_0533	Amino acid uptake	Amino acid (out) = amino acid (in)	1) BLAST database search 2) Conserved domain search
X. Glycine cleavage system H protein	GCSH	-	MBOVPG45_0459	Amino acid metabolism	Glycine + H ₄ folate + NAD ⁺ = 5,10-methylene-H ₄ folate + CO ₂ + NH ₃ + NADH + H ⁺	1) Conserved domain search 2) Sequence homology (Fig. S1)
Y. Acyl carrier protein (ACP) phosphodiesterase	ACPH	3.1.4.14	MBOVPG45_0347	Pantothenate and CoA biosynthesis	Acyl-carrier protein + H ₂ O = Pantetheine 4'-phosphate + Apo-[acyl-carrier-protein]	1) Ortholog of <i>M. pneumoniae</i> MPN479
Z. Pyridine nucleotide transhydrogenase, soluble	STHA	1.6.1.1	MBOVPG45_0108	Nicotinate and Nicotinamide metabolism	NADPH + NAD ⁺ = NADP ⁺ + NADH	1) Ortholog of <i>M. hyopneumoniae</i> pdhD-1 MHP168_678 2) Ortholog of <i>E. coli</i> sthA with <i>M. hyopneumoniae</i> pdhD-1 3) Conserved domain search