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The Metabolic Pathways of *Acholeplasma* and *Mycoplasma*: An Overview

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The metabolism of the Mollicutes *Acholeplasma* and *Mycoplasma* may be characterized as restricted, for example, by virtue of the apparent absence of cytochrome pigments. Some Mollicutes have lowered EC_A values during their logarithmic growth phase, which we speculate may be related to insufficient substrate phosphorylation or insufficient ATP synthesis linked to glycolysis. We found that PEP is carboxylated by preparations of *A. laidlawii,* but not by other Mollicutes; thus in this organism oxaloacetate from PEP may be a link to other pathways. We found phosphoribosylpyrophosphate in *A. laidlawii,* which suggests that ribosylation of purines and pyrimidines occurs in Mollicutes other than *M. mycoides.*

The concept that microorganisms have considerable metabolic flexibility is ingrained in the study of biology, and this impression conjures the image of detailed metabolic maps and charts depicting many pathways by which these little engines can metabolize. It is not so certain to us that the class Mollicutes, excluding the *Thermoplasma,* has this metabolic flexibility. The metabolism of the Mollicutes, to our minds, is becoming, as Lewis Carroll's Alice in Wonderland said, "curiouser and curiouser." We are getting the impression that in Mollicutes catabolism and anabolism are limited; limited by virtue of the absence of, or gaps in, metabolic pathways. As an example, consider the apparent absence of cytochrome pigments, an observation which may serve as one distinguishing feature of the Mollicutes in the microbial world.

We believe that, to know more of how Mollicutes interact with their environment, we must know more of their chemistry. The metabolism of Mollicutes has not been an actively investigated area. What we know of it is based in good measure on the works of Professors Paul Smith and Alan Rodwell, their students and associates. There have been other significant contributors but the severe constraint of space prevents complete citation of all of the involved investigators. Much of the metabolic information known of the Mollicutes concerns the genera *Acholeplasma* and *Mycoplasma.* We will concentrate on these two groups, as the *Ureaplasma, Spiroplasma,* and *Anaeroplasma* have been less studied.

Our approach and our analyses have all been directed toward answering the questions: How do Mollicutes obtain energy? How do they synthesize ATP? Are their energetic demands reflected by both their predilection to interact with other cells and their ability to induce pathology?

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Tourtellotte and Jacobs indicated that glycolysis was the major source of energy for eleven fermentative mycoplasmas [1]. Paul Smith examined the production of ATP from glutamine by non-fermentative mycoplasmas [2]. ATP was produced by phosphorylytic deamidation of glutamine, but the reaction was considered to be of limited impact in the conservation or production of energy. The arginine dihydrolase pathway has been noted as a source of ATP in the arginine-utilizing mycoplasmas [3]. However, the work of Fenske and Kenny has indicated the absence during early growth of arginine deiminase, the initiating enzyme in the sequence [4]. This finding unsettles the view that arginine-utilizing Mollicutes generate most, if not all, of their energy by the metabolism of arginine. Kahane and coworkers emphasized the role of acetate kinase in the synthesis of ATP by *A. laidlawii* and *M. hominis* [5]. Muhlrad and associates later found that not all Mollicutes exhibit this activity, but indicated that the enzyme might be used to supplement ATP synthesis [6]. Earlier, it was indicated that the TCA cycle and an electron transport system was present in one non-fermentative Mollicutes [7,8]. However, recent workers have been unable to find cytochrome pigments in 14 Mollicutes [9]. Although the absence of a cytochrome-containing electron transport system reduces the number of sites where the energy of reducing equivalents can be converted into the energy of ATP, it does not settle the question of the presence of an electron transport system. This is because the absence of cytochrome pigments in Mollicutes does not diminish the possibility that an iron acid-labile sulfide containing flavoprotein, with an ATP generating function analogous to the action of the Site-one locus in mitochondria, exists in Mollicutes.

The involvement of flavins and flavoproteins was first indicated by Sylvia Smith and others describing the fermentative pathways of *M. gallisepticum* [10]. We now appreciate that many Mollicutes are properly categorized as having a flavin-terminated respiration. Reinards and colleagues recently reported that the NADH oxidase was in fact an iron acid-labile sulfide protein [11]. These data support the contention that some Mollicutes may have a truncated electron transport chain which is terminated at a flavin locus that may generate ATP as a consequence of the extrusion of protons. Substrate phosphorylation, as in glycolysis, gains more of our attention as the other possibilities are demonstrated or suspected of being unable to produce a sufficient amount of ATP.

At this juncture, perhaps parenthetically, it should be asked: how much ATP do Mollicutes produce?

Unlike values reported for bacteria [12], *Spiroplasma citri* [13], and *A. laidlawii* [14], we found that the *Mycoplasma* species and *A. morum* had 20 percent lower EC_A values during the mid-exponential growth phase than those observed in *A. laidlawii* B-PG9 ($p < 0.02$). This is also reflected by the percentage of ATP in the total adenylate pool. In *A. laidlawii* it was 97 percent; in *E. coli,* 92 percent. The values found in the other Mollicutes were from 45 to 63 percent ($p < 0.01$). These Mollicutes appear to be in a state of relative energy deficit during their mid-exponential growth phase. We speculate that the putative energy deficit of Mollicutes is manifested by their generally vexatious association with eukaryotic cells and also is associated with cytopathic effects. The lowered EC_A values reflect some metabolic restraint which we have, as an initial working hypothesis, cautiously attributed to insufficient substrate phyosphorylation or ATP synthesis linked to glycolysis.

Glucose is preferred by *Acholeplasma* and *Mycoplasma*, but it is not clear if they metabolize it by the Embden-Meyerhof-Parnas pathway (Fig. 1). In pursuit of these studies, Lanham and O'Brien and their colleagues have made significant contribu-

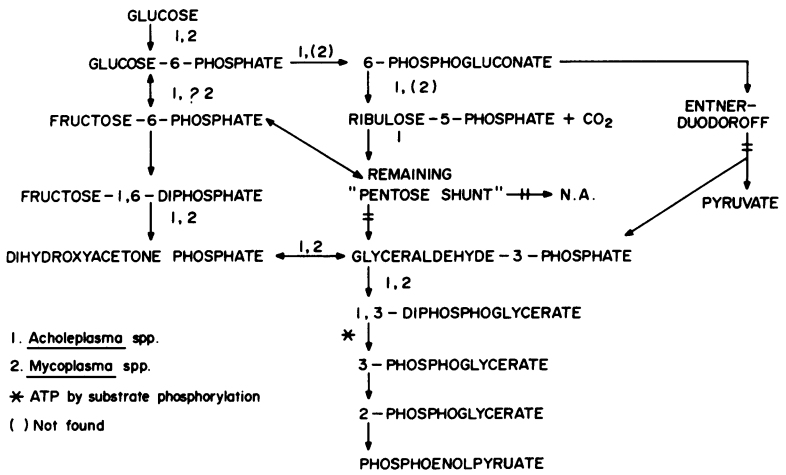

FIG. 1. Components of the Embden-Meyerhof-Parnas pathway in the Mollicutes.

tions studying Mollicutes isoenzymes by electrophoresis [15,16]. You can see that not all steps have been reported in the Mollicutes, specifically, the 6-phosphofructokinase. We have recently identified the presence of pyrophosphate-dependent phosphofructokinase activity in *A. laidlawii* B, and will report on this finding. (Parenthetically, one possible source of pyrophosphate is via the action of the purine phosphoribosyl transferases or of dUTPase. dUTPase activity was found by us in collaboration with Dr. Marshall Williams in three acholeplasmas.) The aldolase, isomerase, and dehydrogenase of the following three glycolytic steps have all been reported in both genera, but the subsequent steps, including the important phosphoglycerate kinase locus where ATP is synthesized, have not been reported. Nevertheless, this latter portion of the pathway is assumed to be present, based in some measure by the accumulation of lactate and pyruvate. It is possible that pyruvate accumulates through other routes, such as the hexose monophosphate pathway or pentose shunt in which glyceraldehyde-3-phosphate is also produced. More far-fetched and without any evidence in Mollicutes, is the production of pyruvate via the Entner-Duodoroff pathway, or the phosphoketolase pathway.

The pyruvate locus, the terminator of the Embden-Meyerhof-Parnas pathway, is of exceeding interest to us. In Fig. 1, phosphoenolpyruvate at this locus is shown as the specific end product of the pathway.

Phosphoenolpyruvate or PEP is viewed by us as a pivotal intermediate in Mollicutes metabolism (Fig. 2). Its ability to transfer its phosphate group is twice that of ATP; that is, the standard free energy of hydrolysis of PEP is double that of ATP. PEP may either be dephosphorylated to pyruvate or carboxylated to oxaloacetate. The former route results in the immediate production of pyruvate and ATP by the action of pyruvate kinase. This crucial step has only been demonstrated by Lanham and co-workers in *Acholeplasma* [15]. Pyruvate may also provide acetate and other components for the production of complex lipids, fatty acids, and butyrate through processes which have been well documented [17,18]. These latter activities can result in the linkage of the Embden-Meyerhof-Parnas pathway by acetyl-CoA to the tricarboxylic acid or TCA cycle. VanDemark and Smith have shown with *Mycoplasma arthritidis* 07 that acetyl-CoA can lead to the formation of citrate [8]. They have also clearly shown that the same strain possesses essentially all the enzymatic activities of the TCA cycle [8]. Unfortunately,

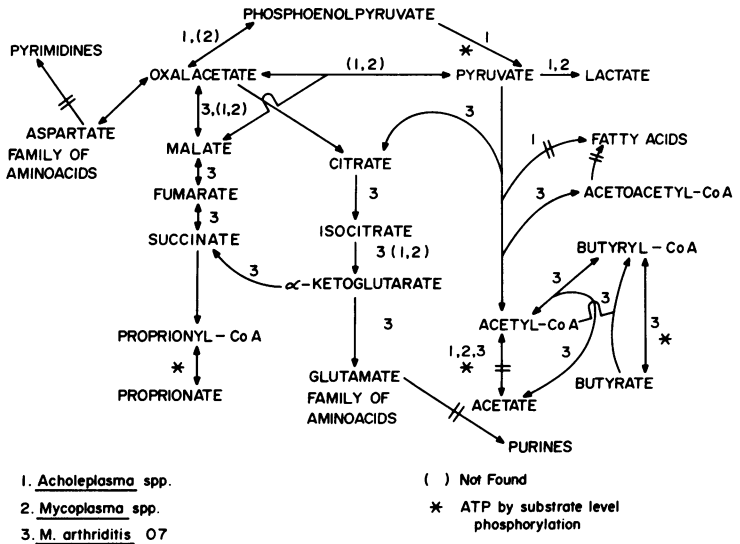

FIG. 2. Phosphoenolpyruvate metabolism in the Mollicutes.

the general presence of the TCA pathway in Mollicutes is unknown. This is of considerable import because if the TCA or part of the TCA cycle were present, it suggests the possibility that glutamate amino acids and thence purines can be synthesized, and also propionyl-CoA from which ATP might be formed in reactions analogous to acetate kinase activity.

The second possible fate of PEP involves carboxylation to oxaloacetate. We have reported at these meetings that *A. laidlawii* B-PG9 can carboxylate PEP. However, we cannot find this activity in *A. hippikon, M. gallisepticum, M. arginini, M. fermentans,* and *M. bovis.* By virtue of its ability to carboxylate PEP, *A. laidlawii* may be able to produce aspartate, an opinion supported by the fact that aspartate inhibits the carboxylase test. Therefore, in *A. laidlawii* we speculate that the formation of the aspartate family of amino acids and pyrimidines may be directly linked to the metabolism of glucose. We have not detected PEP carboxykinase, pyruvate carboxylase, or malic enzyme activities in any Mollicutes. Therefore, only in *A. laidlawii* could oxaloacetate formed from PEP be a link to other metabolic pathways. If other Mollicutes cannot convert PEP to oxaloacetate, then PEP may only lead to the production of lactate, pyruvate, or acetate, and their derivatives. Although we have not tested *M. arthritidis* 07 for carboxylations, linkage of glycolysis and the TCA cycle has been reported to occur in this organism via citrate through acetyl-CoA [8]. The presence of the TCA cycle in other Mollicutes is more problematic since the malic and isocitric dehydrogenases have not been found [16]. These data, coupled with our own studies with *Acholeplasma morum* and other *Mycoplasma* species, prompt us to suggest that *A. laidlawii* B-PG9 and *M. arthritidis* 07 are metabolically distinguishable from other species in their genera.

Glucose-6-phosphate may be metabolized by reactions associated with the pentose shunt (Fig. 3). Although the pentose shunt in Mollicutes has not been thoroughly examined, there are reports that *A. laidlawii* can convert glucose-6-phosphate to 6-phosphogluconate and then to ribulose phosphate [16]. Years ago, Castrejon-Diez and associates showed that 7, 6, and 3 carbon compounds accumulated in reactions with *A. laidlawii* extracts; these data suggested the active presence of the ketolase

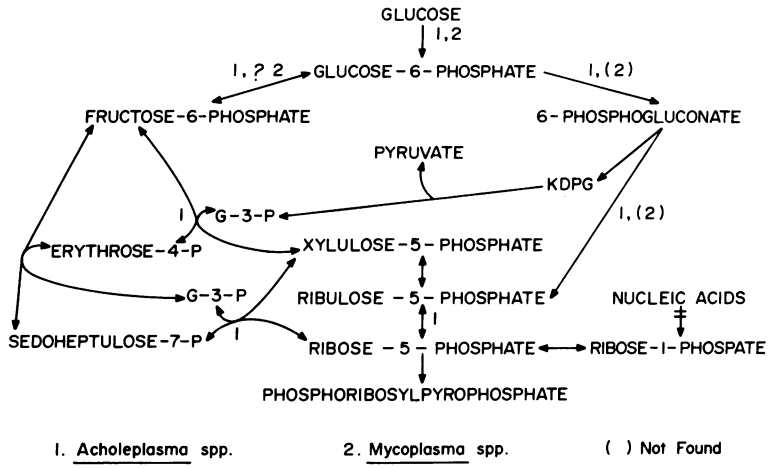

FIG. 3. Glucose-6-phosphate metabolism in the Mollicutes.

and aldolase of the pentose shunt [19]. The pentose shunt produces NADPH necessary for biosynthesis, offers an alternate route for the synthesis of ATP through glyceraldehyde-3-phosphate, and leads to the production of phosphoribosylpyrophosphate or PRPP. Nucleic acid degradation may be linked to this pathway through the formation of ribose-1-phosphate and then ribose-5-phosphate (R-5-P). The pivotal compound at this locus is PRPP. In other microorganisms, PRPP arises directly from ribose-5-phosphate. Conversion of glucose to R-5-P and subsequently to PRPP has not been demonstrated in the Mollicutes. The production of PRPP is important since it is involved in the *de novo* synthesis and in the salvage of purines, and also in complementary reactions in the synthesis of pyrimidines. We are studying the production and use of PRPP by Mollicutes, and our preliminary data indicate that PRPP is synthesized by *A. laidlawii* B.

The steps involved in purine synthesis and degradation are outlined in Fig. 4. The

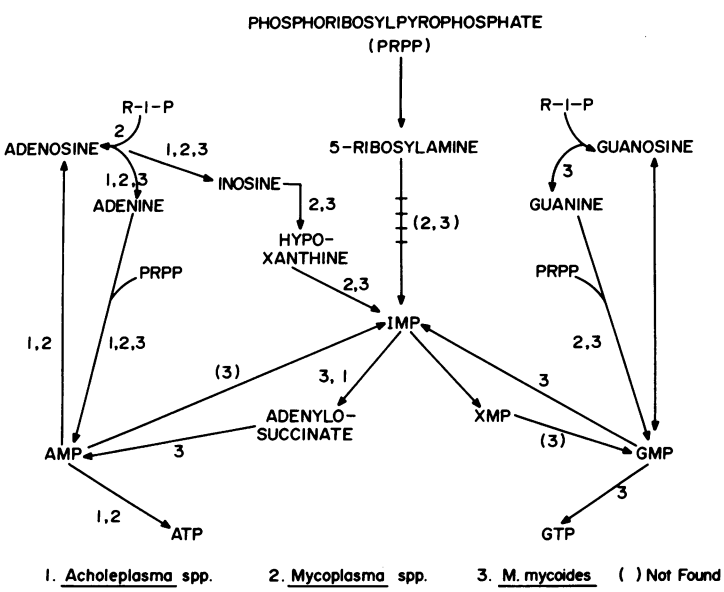

FIG. 4. Purine and pyrimidine metabolism in the Mollicutes.

de novo pathway of purine formation is committed at the conversion of PRPP to 5-phosphoribosylamine (PRA) which is converted by numerous steps to inosine monophosphate (IMP). Adenosine monophosphate (AMP) and guanosine monophosphate (GMP) arise from IMP in separate, two-step reactions. AMP is formed via adenylsuccinate. GMP is formed through xanthine monophosphate (XMP). AMP and GMP may be converted back to IMP by different reactions. Through these steps guanylates and adenylates may be interconverted. Conversion of AMP to ATP and GMP to GTP is effected by adenylate or guanylate kinases, respectively. Adenylate kinase activity has been demonstrated in both *Acholeplasma* and *Mycoplasma* species [16,20,21]. Guanylate kinase has only been reported in *M. mycoides, mycoides* [20,21].

The salvage pathway is characterized by the conversion of free bases or nucleosides to the corresponding nucleoside monophosphates. For example, adenine or adenosine are converted to AMP. Incorporation of the free base, as adenine, into AMP is catalyzed by the enzyme adenine phosphoribosyltransferase. Transferases for adenine, hypoxanthine, and guanine have been demonstrated in *Acholeplasma* and *Mycoplasma* species [20–24]. Conversion of purine nucleosides to the 5′-monophosphate has not been reported in the Mollicutes.

In defined media Mollicutes have a requirement for purine precursors [25–27]. This suggests that purine metabolism in Mollicutes primarily involves the salvage and interconversion enzymes rather than *de novo* synthesis. Rodwell demonstrated that guanosine can serve as the sole purine source for *M. mycoides, mycoides* [25]. In important studies, Mitchell and her colleagues demonstrated the conversion of GMP to AMP in *M. mycoides, mycoides* [20,21]. Their work also shows that AMP is not deaminated to IMP and that IMP is not converted to GMP. Thus, in *M. mycoides, mycoides* guanylates may be converted to adenylates, but adenylates cannot be converted to guanylates.

There are other studies of nutritional data concerning purine interconversions in *Acholeplasma* [26–28]. Gabridge and Stahl suggested that competition for adenine or AMP may be involved in the host-parasite relationship during *Mycoplasma* infections [29]. New evidence from our laboratory demonstrates that in cell-free extracts from *Acholeplasma laidlawii* B-PG9, the steps involved in the conversion of IMP to AMP are present. As yet, we have not been able to demonstrate *de novo* synthesis of purines from glycine. These data suggest that as with *M. mycoides, mycoides* there is no *de novo* synthesis of purines, but that conversion of guanylates to adenylates may occur [20,21]. Therefore, all the adenylates of *A. laidlawii* B may arise from salvage pathways involving purine bases or nucleosides derived from the medium or host tissues.

In our overview of the metabolic pathways of *Acholeplasma* and *Mycoplasma* we have focused on compounds with high potential to transfer their phosphate group. Phosphoenolpyruvate, phosphoribosylpyrophosphate, and pyrophosphate, as well as ATP, are considered to be pivotal in, and descriptive of, Mollicutes metabolism. They are pivotal because they are associated with entrée to other energetically or biosynthetically useful pathways, and are also useful sentinels of such connections. Fluctuations in their content or their presence often indicate gaps or functional deficiencies in metabolic pathways. Only *A. laidlawii,* for example, has been found by us to carboxylate PEP. Other Mollicutes apparently do not carboxylate PEP or pyruvate and, therefore, cannot enter the TCA cycle from glycolysis via oxaloacetate. To compensate for this and other deficiencies, such as the absence of a cytochrome-containing electron transport system, the relatively low energy values

during mid-exponential growth, or the presumed inability to synthesize *de novo* adenylates from glucose, Mollicutes require a compensatory environment, such as a rich milieu, or one containing living tissue or substances with high group energy transfer potential like phosphoenolpyruvate, phosphoribosylpyrophosphate, or pyrophosphate. These deficiencies and those already recognized further distinguish the Mollicutes from other prokaryotes.

What does not seem contradictory to us is that many features distinguishing Mollicutes from other prokaryotes connote a metabolic homogeneity and validity to the class. Although to Alice these newly appreciated features might only generate curiosity, to us they generate excitement.

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