Review Article

Catabolism in Mollicutes

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Introduction

Mollicutes are prokaryotic organisms characterized by the absence of a cell wall. They are osmotically fragile and exhibit plasticity and pleomorphism. Taxonomically, they are considered sufficiently distinct from cell-walled bacteria to be placed in a separate division, the Tenericutes. This has a single class, the Mollicutes, within which four families and six genera are generally accepted (Table 1). Genetically, however, the mollicutes are diverse. There is a wide variation in the guanine plus cytosine content of their DNA, and DNA–DNA hybridization between species is not generally detectable (Razin & Freundt, 1984).

Acholeplasmas, mycoplasmas and ureaplasmas occur primarily as pathogens or parasites of mammals and birds and are associated with many protracted respiratory, arthritic and urogenital diseases. Their ability to adhere to animal cells is widely reported and they are frequently associated with mucosal surfaces; some species are invasive and in AIDS-associated nephropathy, *M. fermentans* is apparently present within renal parenchyma cells (Bauer *et al.*, 1991). Anaeroplasmas and asteroleplasmas form part of the commensal rumen flora of cattle and sheep; their strict requirement for anaerobic conditions distinguishes them from other mollicutes which are facultatively anaerobic. Spiroplasmas have a distinctive helical morphology and have been isolated from the phloem and surfaces of plants, and from insects, including the honey bee, rabbit tick and mosquito. They cause such economically important plant diseases as corn stunt and citrus stubborn and are associated with lethargic diseases of beetles and insects, in which they infect nerve tissue and egg follicles. Their ability to infect and cause disease in mammals has also been demonstrated.

The role of mollicutes in plant and animal disease is well-established, but may currently be underestimated.

Many species have been isolated only in the past few years and adequate description of their pathogenicity awaits improved techniques for cultivation and characterization. There is, for example, recent evidence that mycoplasmas may be involved as co-factors in the progression of HIV infection to AIDS (Montagnier, 1991), and many mycoplasma-like organisms evident in plant tissues have yet to be grown *in vitro*. Mollicutes are also of importance as model organisms in which to study membrane structure and function and the relative ease with which they may be lysed has led to the demonstration of a cytoskeleton in *M. pneumoniae*. In addition, their structural simplicity and small genome (Table 1) is of general scientific interest. Some species have sufficient DNA to code for a maximum of only 600 proteins and are close to the minimal self-replicating organism proposed by Morowitz (1984). In size they are also close to the theoretical minimum, and viable units may be as small as 200–300 nm in diameter.

The intimate association of mollicutes with animal or plant cells enables their dependence on the host for a

Table 1. Families and genera of the class Mollicutes

Family/genus	Recognized species	Mol% G + C	Genome size (kbp)*	Sterol requirement
Mycoplasmataceae				
<i>Mycoplasma</i>	> 80	22–41	0.6–1.3	+
<i>Ureaplasma</i>	5	27–30	0.7–0.9	+
Acholeplasmataceae				
<i>Acholeplasma</i>	10	26–36	1.5–1.7	–
Anaeroplasmataceae				
<i>Anaeroplasma</i>	4	26–34	1.7	+
<i>Asteroleplasma</i>	1	40	1.7	–
Spiroplasmataceae				
<i>Spiroplasma</i>	10†	25–31	1.6	+

* Data cited by Weisburg *et al.* (1989) or Neimark & Lange (1990); methodology may affect values determined.

† The spiroplasmas have recently been divided into 23 groups, each of which may be equivalent to one or more species.

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wide array of organic nutrients. Defined media have been described for anaerobic mollicutes (Robinson, 1979) and some spiroplasmas (Chang, 1989), though minimal defined media have been developed for only a small number of *Mycoplasma* strains (Rodwell, 1983). Thus, mollicutes are generally grown in complex media containing high concentrations of serum or in defined media in which the role of the majority of constituents is uncertain. Even in such media growth rate and growth yield are typically low and broth cultures of many mollicutes do not reach visible turbidity.

It is against this background that metabolic studies of mollicutes have been conducted. In the vast majority of species, knowledge of metabolism is limited to the results of a few biochemical tests of value in characterization. Major distinguishing features are the ability to hydrolyse arginine and to ferment glucose, fermentation in this context being the production of acid during metabolism. Within *Mycoplasma*, the majority of species either ferment glucose or hydrolyse arginine, though some species have neither activity and a small number have both. Ureaplasmas also lack both activities but hydrolyse urea. The remaining genera are fermentative, though some spiroplasmas additionally hydrolyse arginine.

Transport systems

In mollicutes it seems likely that, as in cell-walled bacteria, a number of fundamentally different substrate-uptake systems operate. Transport may be brought about via the generation of a proton-motive force. *M. gallisepticum* cells metabolizing glucose generated a membrane potential of 60 to 80 mV (inside negative) and a pH gradient of approximately one unit (inside alkaline) (Rottem *et al.*, 1981). A membrane-bound ATPase has been identified which functions as an electrogenic proton pump (Linker & Wilson, 1985) and may also have a role in the extrusion of sodium ions, via Na^+/H^+ exchange, and hence in the regulation of cell volume. Similar proton ATPases have been described in other *Mycoplasma* species and in acholeplasmas and spiroplasmas (Rottem *et al.*, 1987), and cation/proton antiport activity has been identified in *Ac. laidlawii* (Lelong *et al.*, 1989). Also in *Ac. laidlawii*, the high affinity uptake system (K_m 4-6 μM) for 3-O-methyl-D-glucose (3-MG) was sensitive to proton conductors; 3-MG was transported as free sugar and accumulated against a concentration gradient (Tarshis *et al.*, 1976).

A phosphoenolpyruvate-dependent phosphotransferase system (PEP:PTS), for which glucose and/or α -methyl-D-glucoside (α -MG) are substrates, has been demonstrated in fermentative mycoplasmas and spiroplasmas (Cirillo, 1979; Tarshis, 1991). In *M. capricolum*,

fructose is a substrate for this system and mutants lacking enzyme-II activity specific for glucose were isolated by their resistance to α -MG (Mugharbil & Cirillo, 1978); similar mutants of *M. mycoides* were isolated by their resistance to 3-deoxy-3-fluoro-D-glucose (Lee *et al.*, 1986). However, PEP:PTS activity is apparently absent in the non-fermentative mycoplasmas, *Ac. laidlawii* (Cirillo, 1979) and *U. urealyticum* (Cocks *et al.*, 1985).

In *M. hyorhina*, DNA sequence data suggest that the p37 gene product is a component of a high-affinity transport system and is analogous to periplasmic binding proteins of Gram-negative bacteria (Dudler *et al.*, 1988). Membrane location may be achieved by means of an N-terminal glyceride-cysteine 'anchor' (Gilson *et al.*, 1988). Substrates for this system have not been identified, though in Gram-negative bacteria periplasmic binding proteins are involved in the transport of sugars and amino acids. Cirillo (1979) suggested that the transport of L-histidine in *M. fermentans* and L-methionine in *M. hominis* may be directly energized by ATP.

Carbohydrate catabolism

Carbohydrate catabolism by whole cells

In *M. mycoides*, carbohydrates are oxidized aerobically to acetate and CO_2 . Anaerobically, pyruvate undergoes dismutation to lactate, acetate and CO_2 ; and lactate is the product of sugar metabolism in non-aerated cultures (Rodwell, 1960). Tourtellotte & Jacobs (1960) showed similar metabolic activities for a range of fermentative mycoplasmas and *Ac. laidlawii*. However, in *M. fermentans* and *M. canis*, oxygen uptake did not accompany glucose metabolism and lactate may be the product of sugar metabolism under aerobic and anaerobic conditions (Miles *et al.*, 1991). Some non-fermentative and non-arginine hydrolysing mycoplasmas have recently been shown to oxidize lactate, pyruvate and 2-oxobutyr-ate (Wadher *et al.*, 1990b) and pyruvate increased the growth yield of *M. agalactiae* (Miles *et al.*, 1988).

The range of substrates metabolized by the fermentative mycoplasmas is restricted and species-dependent. *M. mycoides* subspecies *mycoides* metabolized glucose, fructose, mannose, glucosamine, N-acetylglucosamine, glycerol, pyruvate and lactate (Miles *et al.*, 1985). Saturation constants were low (2-20 μM) except with glucosamine and mannose (130 μM and 1 mM respectively). Metabolism of these substrates required a glucose-specific component (Lee *et al.*, 1986) and although they were utilized with maximum rates similar to that of glucose, their metabolism is probably not significant at the low concentrations found *in vivo*. Reports that all glucose-fermenting mycoplasmas also ferment maltose,

starch and glycogen (see Razin & Freundt, 1984) are based on experiments using media containing serum or serum fractions, which contain saccharolytic enzymes. However, maltose was utilized by *M. mycoides* subspecies *capri* (Wadher & Miles, 1988).

In *Acholeplasma*, the range of sugars utilized is also restricted and includes sucrose, maltose, glucose, galactose, fructose and xylose; *Ac. parvum*, however, does not ferment sugars (Razin & Freundt, 1984). Reported patterns of sugar utilization vary within *Spiroplasma* species, though this may reflect the use of complex media containing fermentable sugars and serum enzymes (Chang, 1989). The anaerobic mollicutes ferment soluble starch, and in addition some strains may use maltose, galactose or glucose. The end-products of fermentation include ethanol, CO₂, H₂ and acetic, formic, lactic and propionic acids (Robinson, 1979).

The ability to use sugars is generally constitutive, though fructose metabolism in *M. mycoides* was four times higher in fructose-grown than in glucose-grown cells (Miles *et al.*, 1985). In *M. mycoides* subspecies *capri* and in *Ac. laidlawii*, maltose utilizing ability was also partly inducible (Slater & Folsome, 1971; Wadher & Miles, 1988).

Pathways of carbohydrate catabolism

The pathway of carbohydrate metabolism in *M. mycoides*, based on demonstrated enzyme activities in cell-free extracts, is given in Fig. 1. Catabolism of glucose by the Embden-Meyerhof-Parnas (EMP) pathway is initiated by phosphorylation to glucose 6-phosphate; hexokinase activity is present, though the possession of PEP:PTS activity suggests that extracellular glucose is phosphorylated during transport. The initial oxidative enzyme activities of the pentose phosphate (PP) pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, have not been demonstrated. However, the presence of transketolase and transaldolase activities would enable the conversion of fructose 6-phosphate and glyceraldehyde 3-phosphate into pentose phosphates and enable glucose to serve as a source of nucleotide pentoses. Ribo- and deoxyribonucleotides might also be catabolized to yield glycolytic intermediates, enabling nucleoside sugars to act as energy sources. *M. mycoides* strain Y may take up both ribonucleoside and deoxyribonucleoside 5-monophosphates and mutants deficient in nucleoside 5-monophosphate transport have been isolated (Youil & Finch, 1988).

Enzymes of the EMP pathway and the non-oxidative part of the PP pathway have also been demonstrated in other fermentative mycoplasmas, though many species lack hexokinase (DeSantis *et al.*, 1989). The non-

fermentative *M. hominis* and *M. bovis genitalium* also lacked hexokinase and, additionally, phosphofructokinase (PFK) and fructose-1,6-bisphosphate aldolase; however, enzyme activities for the pathway from glyceraldehyde 3-phosphate to phosphoenolpyruvate were present, except for 3-phosphoglycerate kinase in *M. bovis genitalium*.

In *U. urealyticum*, several enzyme activities required for the glycolytic sequence were also undetectable, including those of hexokinase, phosphoglycerokinase and lactate dehydrogenase (Cocks *et al.*, 1985). In addition, the low activity of other glycolytic enzymes suggested that they did not participate in any major energy-generating pathway, though the presence of non-oxidative enzymes of the PP pathway indicated that nucleoside sugars might be catabolized to glycolytic intermediates.

Spiroplasmas possess a number of key enzyme activities of the EMP pathway (Pollack *et al.*, 1989). PFK was ATP-dependent, though in two strains of *S. floridicola* no activity was detected and the possibility that fructose 6-phosphate might be metabolized to glyceraldehyde 3-phosphate via enzymes of the PP pathway was suggested. Enzymes of the PP pathway were demonstrated in all ten strains used in the study, though glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were absent. However, these activities were present in *Acholeplasma*, in which both the EMP and PP pathways appear to be functional (DeSantis *et al.*, 1989). In addition, acholeplasmas were distinguished from other facultatively anaerobic mollicutes in that PFK activity was PP_i-dependent (Pollack & Williams, 1986).

The anaerobic mollicutes possess enzyme activities of the non-oxidative portion of the PP pathway, and the EMP pathway (Petzel & Hartman, 1990; Petzel *et al.*, 1989b). PFK activity was ATP-dependent in *Asteroleplasma* and PP_i-dependent in *Anaeroleplasma* (Petzel *et al.*, 1989b). *Asteroleplasma*, however, differed from other mollicutes in that PP_i-dependent pyruvate, orthophosphate dikinase replaced ATP-dependent pyruvate kinase activity (PPDK; Petzel *et al.*, 1989a, b). Amongst cell-walled bacteria, PPDK activity is reported only in a restricted group of anaerobes (Petzel *et al.*, 1989a). The absence of hexokinase activity in *Asteroleplasma* and some anaeroleplasmas led Petzel & Hartman (1990) to suggest that glucose might be converted to glucose 1-phosphate using carbamyl phosphate or acetyl phosphate.

Glycerol catabolism

In *M. mycoides*, glycerol is required for growth in defined medium (Rodwell & Mitchell, 1979) and is phosphorylat-

Fig. 1. Carbohydrate catabolism in *Mycoplasma mycoides* (modified from Cocks *et al.*, 1985).

ed to L- α -glycerophosphate (GP), an essential precursor of lipid synthesis. However, during aerobic growth only a small quantity (10%) of the glycerol metabolized is incorporated into cellular material (principally glycerides), and GP is oxidized to triose phosphate by a GP oxidase. This reaction is essentially irreversible, consumes molecular oxygen and produces 1 mol of H_2O_2 per mol of GP (Rodwell & Mitchell, 1979). However, the *in vitro* growth characteristics of a GP oxidase-negative strain of *M. mycoides* were similar to those of the parent strain (Wadher *et al.*, 1990a). GP oxidase activity has also been demonstrated in other fermentative mycoplasmas (Miles *et al.*, 1991), but is not present in all species. In *U. urealyticum*, GP may be synthesized from dihydroxyacetone phosphate by a reversible NADH-linked dehydrogenase activity (Cocks *et al.*, 1985).

Pyruvate oxidation and the tricarboxylic acid cycle

In *M. mycoides*, pyruvate may be metabolized to acetate in a series of reactions involving pyruvate dehydrogenase, phosphate acetyltransferase and acetate kinase (Fig. 1). This pathway is potentially important in ATP generation (Kahane *et al.*, 1978) and high acetate kinase activities have been demonstrated in *Acholeplasma* and *Mycoplasma* species (Muhlrad *et al.*, 1981); however, activity in ureaplasmas and some mycoplasmas was undetectable or too low to suggest a key role in ATP generation. A pathway from pyruvate to acetate and involving ATP formation may also exist in anaeroplasmas (Petzel & Hartman, 1990). Acetate kinase activity was present and although NAD⁺-dependent pyruvate dehydrogenase was not detected, two species were able to convert pyruvate to acetyl-CoA via an FAD⁺-dependent pyruvate synthase.

In those mollicutes studied, there is no convincing evidence for a functional TCA cycle, though representative members of all groups, except *Acholeplasma*, possess malate dehydrogenase (Pollack *et al.*, 1989; Manolukas *et al.*, 1988). Anaeroplasmas also possess NADP⁺-dependent isocitrate dehydrogenase (Petzel & Hartman, 1990). However, the significance of this activity is unclear since citrate synthase was not detected. The literature contains evidence for a functional TCA cycle in *M. arthritidis* strain 07 (formerly referred to as *M. hominis* 07). However, Constantopoulos & McGarrity (1987) showed that this strain, in common with other mycoplasmas and acholeplasmas, lacked significant 2-oxoglutarate dehydrogenase activity.

Oxidases

With the exception of GP oxidase, substrate oxidation during carbohydrate catabolism by mollicutes is depen-

dent upon either NAD⁺- or NADP⁺-linked enzyme activities. Glucose-6-phosphate dehydrogenase activity in acholeplasmas (DeSantis *et al.*, 1989) and isocitrate dehydrogenase activity in anaeroplasmas (Petzel & Hartman, 1990) specifically require NADP⁺. Aerobically, reduced pyridine nucleotides may be oxidized by the flavin-containing oxidases of *Acholeplasma*, *Mycoplasma*, *Spiroplasma* and *Ureaplasma* species. In mycoplasmas, NADH oxidases contain either FMN or FAD, although in *Ac. laidlawii* both forms are apparently present (Pollack, 1979) and *M. pneumoniae* contains both flavin-dependent and -independent activities (Low & Zimkus, 1973). NADH oxidase activity is present predominantly in the soluble fraction of mycoplasma and spiroplasma cells, and the membrane fraction of acholeplasmas, where it is probably located on the inner surface (Pollack, 1979). NADPH oxidase is also present in the soluble fraction of mycoplasmas, but is apparently absent from acholeplasmas. Both NADH and NADPH oxidase activities have recently been detected in the cytosol fraction of *U. urealyticum* (De Silva & Quinn, 1991).

There is little evidence to suggest that mollicutes carry out oxidative phosphorylation, though *Acholeplasma* and *Mycoplasma* species possess NADH:ferricyanide oxidoreductase activity, a component of the mitochondrial energy-conserving site I locus (Pollack, 1979). There are no confirmed reports of the presence of quinones or cytochromes, and using difference spectroscopy at liquid nitrogen temperature, Pollack *et al.* (1981) were unable to obtain evidence for the presence of cytochromes in whole cell and membrane fractions of ten mollicute species. However, all species contained iron [up to 15 nmol (mg protein)⁻¹] and relatively small amounts of acid-labile sulphide [up to 1 nmol (mg protein)⁻¹], and it was suggested that iron and iron-sulphur proteins may play a role (with NADH oxidase) in electron transfer to oxygen.

Oxidation of NADH by cell-free extracts of mycoplasmas and acholeplasmas may yield H_2O_2 . However, Low & Zimkus (1973) demonstrated that in *M. pneumoniae*, H_2O_2 formation was associated only with flavin-dependent NADH oxidase activity, which in PAGE moved more quickly than flavin-independent activity. The presence of two oxidase activities was also indicated in other glucose and lactate oxidizing mycoplasmas, since during NADH oxidation by cell-free extracts, the ratio of H_2O_2 formed to oxygen consumed was significantly less than one (0.05 to 0.2; Miles *et al.*, 1991; Abugroun *et al.*, 1990). However, a ratio of one was found for mycoplasmas which did not oxidize glucose or lactate and for *Ac. laidlawii*.

H_2O_2 production resulting from the oxidation of NADH (and GP) may be detected in cell suspensions oxidizing carbohydrates. In addition, some *Mycoplasma*

species and *Ac. laidlawii* produce superoxide during glucose metabolism (Almagor *et al.*, 1984; Meier & Habermehl, 1990). The potential of these products to cause oxidative damage to host cell structures may be augmented by the inhibition of host cell catalase by superoxide, and of superoxide dismutase by H_2O_2 (Almagor *et al.*, 1984). There are conflicting reports of the presence of catalase in mollicutes (Pollack, 1979), which according to Meier & Habermehl (1990) may be heterogeneously distributed amongst species.

Regulation of carbohydrate catabolism and the provision of precursors for anabolism

Regulation of carbohydrate catabolism in mollicutes has been little investigated. However, as in other organisms, fructose 1,6-bisphosphate (FBP) appears to play a crucial but possibly diverse role. It accumulates in metabolically active cells of *M. gallisepticum* (Egan *et al.*, 1986), but its formation is apparently the rate-limiting step of glycolysis in *Ac. laidlawii* (Pollack & Williams, 1986). In *Acholeplasma* (Neimark, 1979), *Anaeroplasm*a and *Asteroleplasma* species (Petzel *et al.*, 1989a), FBP is also an allosteric activator of NAD^+ -dependent lactate dehydrogenase (LDH) activity [L(+)] specific in *Acholeplasma*, which appears to function essentially in the direction of pyruvate reduction. *Ac. laidlawii* also possesses NAD^+ -independent D(−)-lactic dehydrogenase of relatively low activity and uncertain function (Neimark, 1979). The LDHs of those *Mycoplasma* species that have been studied are NAD^+ -dependent but do not, however, require FBP for activity; in most species LDH was L(+)-lactate specific, though in *M. fermentans* it was D(−) specific and lactate-oxidizing ability was not detectable.

Mollicutes have limited synthetic capabilities. However, Manolukas *et al.* (1988) have demonstrated potential pathways whereby the products of carbohydrate catabolism in mycoplasmas and acholeplasmas may be used in the synthesis of lipids, nucleic acids and certain amino acids. Synthesis of aspartate may occur via oxaloacetate which may be produced from glycolytic intermediates by the activity of malate synthase (in mycoplasmas) or by anaplerotic reactions involving PEP or pyruvate carboxylases in mycoplasmas, acholeplasmas and perhaps anaeroplasmas (Petzel *et al.*, 1989b). However, all of these activities are apparently absent in spiroplasmas (Pollack *et al.*, 1989) and *Ast. anaerobium* (Petzel *et al.*, 1989b). The PEP carboxylase of *Ac. laidlawii* was activated by FBP and low (0.5 mM) bicarbonate levels, but non-competitively inhibited by aspartate suggesting that it may be an important function in the coordination of protein, lipid and nucleic acid precursor synthesis (Manolukas *et al.*, 1989).

Glycosidases

Glycosidases may have roles in processing cellular glycoconjugates and the utilization of extracellular di- and polysaccharides. In animal pathogens, extracellular or membrane-bound activity may also modify host cell glycoconjugates leading to auto-immune disease. α -Glucosidase was detected in some of the bovine and caprine mycoplasma strains investigated by Salih *et al.* (1983), and activity in *Ac. laidlawii* A (Slater & Folsome, 1971) and *M. mycoides* subsp. *capri* (Wadher & Miles, 1988) was partly inducible by maltose. Membrane-associated β -glucosidase activity has also been demonstrated in *Ac. laidlawii* (strain B) and *M. gallinarum* (Smith, 1979). More recently, Kahane *et al.* (1990) used 4-methylumbelliferyl substrates to demonstrate the presence of α and β -glucosidase, β -galactosidase and β -N-acetylglucosaminidase (NAGase) in four *Mycoplasma* species and *Ac. laidlawii*. Activities were partly membrane-bound, but generally low. Significantly, however, neuraminidase and α - and β -mannosidase activity in *M. pneumoniae* could not be detected and the glycosidase activity of avirulent *M. pneumoniae* strains was similar to that of a virulent strain.

Catabolism of proteins and amino acids

Proteolytic activity, as detectable by liquefaction of inspissated animal serum or gelatin or casein hydrolysis by growing cells, is characteristic of few mollicute species. However, *M. mycoides* strain Y can obtain amino acids for growth by the degradation of bovine serum albumin (Rodwell, 1983) and *M. capricolum* and *Ac. laidlawii* grew in a medium in which free amino acids were effectively removed by dialysis (Sjostrom & Kenny, 1990). In addition, a specific peptidase cleaving at leucine residues was demonstrated in *M. capricolum*, suggesting that the source of leucine for this organism might be a peptide more readily transported than free leucine, and alanine may be provided as L-Ala₃ or L-Ala₄ in growth media for *M. mycoides* strain Y (Rodwell & Mitchell, 1979).

Peptidases may be intrinsic membrane proteins and membrane fractions of *Ac. laidlawii* contained aminopeptidase, dipeptidase and smaller amounts of carboxypeptidase (Choules & Gray, 1971). Activity was also present in the cytoplasm and to a lesser extent in the medium; however, essentially all of the activity which cleaved the N- α -terminal L-alanyl residue of alanine oligopeptides was membrane bound (Pecht *et al.*, 1972). Aminopeptidase activity has also been demonstrated in mycoplasmas and *U. urealyticum* (Vinther & Black, 1974), and Neill & Ball (1980) showed that the specificity

of the aminopeptidase activity of *Ac. laidlawii* and three bovine mycoplasmas towards a range of aminoacyl- β -naphthylamides distinguished between species. Carboxypeptidase activity specific for arginine has been demonstrated in whole cell suspensions of arginine-hydrolysing mycoplasmas (Shibata & Watanabe, 1986), but not in fermentative strains. The *M. salivarium* enzyme is membrane-associated and has been purified and characterized (Shibata & Watanabe, 1988). *M. salivarium* membranes also contain aminopeptidase activity specific for N-terminal arginine and leucine residues (Shibata & Watanabe, 1987).

Proteases may be involved in pathogenicity and the role of *M. arthritis* in arthritic disease of animals, and the potential role of mycoplasmas in human arthritis, has prompted investigation of collagenase activity. Woolcock *et al.* (1973) purified two enzymes from *M. arthritis* which released free amino groups and oligopeptides from gelatin (a denatured form of collagen), and one of these enzymes additionally released hydroxyproline. Activity was present in whole cells and culture supernatants (Czekalowski *et al.*, 1973), although native collagen and α -casein were not hydrolysed. In ureaplasmas, extracellular host-specific IgA proteases have been demonstrated (e.g. Kapatais-Zoumbos *et al.*, 1985).

Arginine is catabolized in many *Mycoplasma* and *Spiroplasma* species by the arginine dihydrolase pathway (Pollack, 1979).

This pathway generates 1 mol ATP per mol arginine and considering the likely energy requirement for arginine transport, the energetic advantage of the pathway appeared doubtful until the demonstration of an arginine-ornithine antiport system in *Spiroplasma melliferum* (Rottem & Shirazi, 1990). Arginine would appear to be the sole energy source in the majority of mycoplasmas possessing this pathway. However, a number of arginine-utilizing strains also ferment sugars, and *M. hominis* (arginine-utilizing, non-fermentative) apparently possesses an additional (unknown) energy-yielding mechanism (Fenske & Kenny, 1976); in growing cells, arginine deiminase activity did not increase until late in the exponential phase, possibly after exhaustion of the other energy substrate.

Arginine deiminase may also have a role in pathogenicity. Arginine-utilizing mycoplasmas are toxic to mammalian cells in culture due to arginine depletion from the

culture medium, an effect which may be reproduced by mycoplasma cell extracts or arginine deiminase. In *M. arthritis*, multiple metabolic roles for arginine deiminase were suggested by the presence of two cytoplasmic forms (Weickmann & Fahrney, 1977). A high-activity form was present in exponentially growing cells but towards the end of the growth phase a low-activity form was additionally produced. Lin (1986) similarly showed two distinct enzyme forms in *M. hominis*; however, the low-activity form was membrane-associated. Matsuura *et al.* (1990) have also proposed that NH_3 produced by the arginine dihydrolase pathway may be primarily responsible for the tissue damage observed following the intracutaneous inoculation of rabbits with *M. salivarium*.

There are few reports concerning catabolism of amino acids other than arginine. Glutamine was metabolized to glutamic acid by some human mycoplasmas (Smith, 1960), and 1 mol ATP per mol glutamine was generated suggesting a possible role in energy conservation (Pollack, 1979). The only amino acids oxidized by *M. mycoides* cells suspended in a salts solution were threonine and serine (Rodwell, 1960), which were metabolized via the corresponding keto acids (pyruvate and 2-oxobutyrate) to acetate and propionate respectively. Low levels of aspartate and alanine aminotransferase have also been demonstrated in a range of achleplasmas and mycoplasmas (e.g. Constantopoulos & McGarrity, 1989; Manolukas *et al.*, 1988; Salih *et al.*, 1983) and glutamate dehydrogenase activity is present in *Ac. laidlawii* (Yarrison *et al.*, 1972). However, it appears unlikely that these activities are of significance in catabolism in the absence of a functional TCA cycle and particularly 2-oxoglutarate dehydrogenase (Constantopoulos & McGarrity, 1987). *U. urealyticum* possesses L-histidine ammonia-lyase (Romano & La Licata, 1978).

Catabolism of lipids

The restricted ability of mollicutes to metabolize lipids is indicated by the extent to which their growth is dependent upon exogenous sterol (Table 1), fatty acids and in some mollicutes, phospholipids. Fatty acid uptake is energy-linked and protein-mediated (Dahl, 1988). Mycoplasmas and spiroplasmas are unable to synthesize or alter the chain length of fatty acids; however, ureaplasmas synthesize both saturated and unsaturated fatty acids, whilst achleplasmas are able to synthesize saturated acids and elongate exogenously supplied unsaturated acids (Smith, 1979).

Lipolytic activity in mollicutes is indicated by the ability of some species to form pearly films and spots on serum agar and to cause zones of clearing on egg-yolk medium. Rottem & Razin (1964) showed that three

Mycoplasma species and *Ac. laidlawii* hydrolysed tributyrin, and cell extracts of *M. gallisepticum* were additionally shown to hydrolyse long-chain fatty acid esters (i.e. trilaurin and triolein) slowly, confirming the presence of lipase activity. In *M. gallisepticum*, tributyrin-hydrolysing ability was soluble and optimal at 37 °C and pH 7.5. However, in *M. arthritidis* 07 lipase activity was membrane-associated (Smith, 1979). This strain also possessed membrane-associated esterase activity towards the cholesteryl esters of fatty acids. Activity was distinct from lipase activity and required a micellar substrate. Cholesterol esterase activity was detected in other mycoplasmas and acholeplasmas, but not in all strains.

Attempts to detect phospholipase activity in mollicutes have generally given negative results (Smith, 1979). However, *U. urealyticum* possessed endogenous phospholipase A₁, A₂ and C activity which was essentially membrane-bound (De Silva & Quinn, 1991). Low levels of membrane-bound phospholipase A activity were also present in *M. gallisepticum*; this activity was increased fourfold by detergents and it was suggested that in intact cells activation was brought about by soluble regulatory proteins (Rottem *et al.*, 1986). Phospholipase A₂ has been demonstrated in culture supernatants of *M. mycoides* and *Ac. laidlawii* (Bhandari & Asnani, 1989) and lysophospholipase activity is present in *M. gallisepticum* (Gatt *et al.*, 1982) and *Ac. laidlawii* cell membranes (van Golde *et al.*, 1971), where it may protect cells against lytic concentrations of monoacyl phosphoglycerides. Thus, there may be significant phospholipid catabolism in at least some mollicutes. Extracellular or membrane-bound phospholipase may be of particular significance in pathogenesis and the phospholipase A₂ of *M. mycoides* and *Ac. laidlawii* lysed mammalian erythrocytes (Bhandari & Asnani, 1989). The ability of *M. pulmonis* to produce male infertility in rodents may also be associated with its ability to specifically degrade sulphogalactosylglycerolipid (SGG), the major mammalian male germ cell glycolipid, which is implicated in binding to egg cells (Lingwood *et al.*, 1990); SGG underwent both desulphation and deacylation.

Energy yielding metabolism in ureaplasmas

Urea hydrolysis is a key metabolic function of ureaplasmas. Urea was essential for the growth of *U. urealyticum* in dialysed medium containing horse serum (Kenny & Cartwright, 1977) and growth was prevented by a low concentration (10 µM) of the urease inhibitor fluoroamide, which did not inhibit the growth of other mollicutes (Kenny, 1983). It has been suggested that urea hydrolysis may be coupled to ATP synthesis through the

creation of ionic gradients (Shepard & Masover, 1979). Activity in the cytoplasm of cells (Myles *et al.*, 1991) will lead to the intracellular production of CO₂ and NH₃, and hence, NH₄⁺. The subsequent diffusion of NH₄⁺ from cells may thus generate a transmembrane-proton electrochemical potential, which might be used to synthesize ATP via membrane-bound ATPase activity. Urea increased the membrane potential of *U. urealyticum* cells, and this increase was inhibited by acetohydroxamic acid (urease inhibitor), ionophores (carbonylcyanide *m*-chlorophenylhydrazine and gramicidin) and extracellular NH₄⁺ (Romano *et al.*, 1986*a*). Also, in cells suspended in buffer, ATP synthesis was linearly related to urea concentration in the range 0.125 to 0.5 mM (Romano *et al.*, 1986*b*). However, detailed understanding of the mechanism whereby urea hydrolysis leads to ATP synthesis is lacking. There is no substantial evidence for alternative ATP-generating mechanisms in ureaplasmas. The presence of inorganic pyrophosphatase activity in cell-free extracts of *U. urealyticum* may indicate a role for PP_i metabolism in energetic mechanisms (Davis *et al.*, 1987). Putrescine and other diamines have been reported to replace urea in growth medium (Masover *et al.*, 1974); however, small quantities of urea were detectable in the media used.

Summary

The small genome size of mollicutes, and particularly mycoplasmas and ureaplasmas, precludes their possession of the extensive range of metabolic activities present in most other bacterial groups. Demonstrated catabolic activities appear primarily to be associated with energy generation, rather than the provision of substrates for synthetic pathways, and anabolism is largely dependent upon extracellular sources of amino acids, nucleic acid precursors and lipids. However, the pathways of energy generation in mollicutes are diverse and specialized, and may *in vivo* be dependent upon the presence of a single amino acid (arginine) or urea. Even in those species that utilize carbohydrates the range of substrates is restricted, and while *Ac. laidlawii* has both EMP and PP pathways and is able to oxidize pyruvate to acetate plus CO₂, many mycoplasmas possess only a part of these activities. Such specialization and the infrequent demonstration of inducible enzyme activity in mollicutes implies adaptation to specific habitats in host species, and suggests that differences in the catabolic activities of mollicute strains may be significant in terms of their ecology and pathogenicity.

The demonstrated energy-generating pathways of mollicutes produce low ATP yields. Thus, mollicute growth will generate relatively large quantities of

metabolic end-products and may deplete host tissues of substrates. Arginine depletion may be of particular importance in pathogenesis and the close physical association between mollicutes and host cells will enhance the potential significance of NH_4^+ production from the hydrolysis of arginine and urea, and of H_2O_2 and superoxide formation during carbohydrate metabolism. In addition, lipid and protein catabolism may be associated with virulence where extracellular or membrane-bound enzyme activities exist. Membrane-bound DNAase and RNAase activities have also been demonstrated in mycoplasmas and *Ac. laidlawii* (Pollack *et al.*, 1965) and *U. urealyticum* (Romano & La Licata, 1978).

Many aspects of mollicute catabolism, including energy conservation in some groups, is poorly understood. Also, while substantial catabolic diversity has been demonstrated within mollicutes and new species are continually being isolated, metabolism has been studied in relatively few species, and even in these only single strains or small groups of strains have been used. In this review, therefore, an attempt to avoid generalizations concerning mollicute behaviour has been made. The lack of much basic knowledge concerning mollicute metabolism has also necessitated the widespread use of 'may be' and other equally vague terms. In many species, this reflects the difficulties experienced in growing organisms, rather than their lack of scientific or economic importance; however, fundamental research may be stimulated by the increasing realization of the potential role of mollicutes in human, animal and plant disease. Nevertheless, whilst understanding of the metabolism and genetics of the most widely studied mollicutes (*M. mycoides*, *M. capricolum*) is at an elementary level compared to that of *Escherichia coli*, the small size of the mycoplasma genome might enable the claim that, in terms of the genetic and metabolic functions that we do not understand, mycoplasmologists are ahead.

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