

Lipid Tracers of Mycoplasma Phylogeny

THOMAS A. LANGWORTHY, Ph.D.

*Department of Microbiology, School of Medicine, University of South Dakota,
Vermillion, South Dakota*

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Comparison of the lipid composition between members of the *Mycoplasmatales* reveals a striking diversity of lipid structures, not only between the six genera but among species within the same genus. This is in contrast to nearly all other bacterial groups in which members of the same genus possess essentially the same lipids. There are in fact more similarities between lipids of a given species of mycoplasma and a genus of bacterium than there are between mycoplasma species. Mycoplasmal lipids suggest that these organisms do not represent a phylogenetically related group at all, but are probably degenerative forms of bacteria, particularly gram-positive bacteria, which have lost the ability to synthesize a cell wall.

INTRODUCTION

As pointed out by Shaw [1,3], lipid composition is a useful taxonomic method for the classification of bacteria because lipids are present in all bacteria, they are readily extracted and identified, and a great variety of structures exist. The concept has been even further enhanced by the identification of isopranyl glycerol ether lipids, which serve as a distinctive biochemical marker of the thermoacidophilic, halophilic, and methanogenic archaeobacteria [4-6]. The utility of lipids as an aid to classification is even apparent within the grouping of the mycoplasmas themselves, wherein the requirement for and presence of sterols distinguishes the mycoplasmas from the acholeplasmas.

Although the lipids of a vast number of bacteria and many mycoplasmas have certainly not been established, a large enough body of information has accumulated to draw a compelling relationship between mycoplasmas and bacterial counterparts. The following data and discussion, unless noted otherwise, draws largely upon the work and summaries of mycoplasma lipid composition by P.F. Smith [7-10] and the characteristic lipids of bacterial groups summarized by Shaw [1-3].

NEUTRAL LIPIDS

Neutral lipids which include apolar lipid components are perhaps the least useful lipid class for taxonomic purposes and are present in all mycoplasmas and bacteria. Neutral lipid components of the mycoplasmas are shown in Table 1. Although bacteria are incapable of sterol biosynthesis, mycoplasma, spiroplasma, and ureaplasma species contain sterols accumulated from the medium. The acholeplasma species have replaced the sterol requirement by the synthesis of colored or colorless carotenoids which also occur in many bacteria. Likewise,

TABLE I
Neutral Lipids of Mycoplasmas

Compound	Organism	Reference
Sterols	<i>M. arthritis;</i> <i>M. capricolum;</i> <i>M. gallinarum;</i> <i>M. gallisepticum;</i> <i>M. hominis;</i> <i>M. hyorhinis;</i> <i>M. mycoides;</i> <i>M. neurolyticum;</i> <i>M. pneumoniae</i> <i>An. abactoclasticum</i> <i>S. citri</i> <i>Ureaplasma</i> sp.	[10]
Carotenoids	<i>A. axanthum;</i> <i>A. granularum;</i> <i>A. laidlawii;</i> <i>A. modicum</i>	[10]
Glycerides	Ubiquitous	[10]
Diglycerol tetraethers	<i>Th. acidophilum</i>	[4,5]
Free fatty acids	<i>A. axanthum;</i> <i>M. hominis;</i> <i>S. citri;</i> <i>Ureaplasma</i> sp.	[10]
Long chain bases	<i>A. axanthum</i>	[10]
Menaquinones (MK-7)	<i>Th. acidophilum</i>	[17]
Isoprenoid hydrocarbons	<i>Th. acidophilum</i>	[5]

glycerides are ubiquitous in microorganisms and free fatty acids have been detected in the neutral lipids of acholeplasma, mycoplasma, spiroplasma, and ureaplasma species.

The first distinctive component (perhaps too polar to be considered a neutral lipid, but considered here for convenience) are the free long-chain bases of the sphingosine type which occur only in *A. axanthum*. This compound also serves as the hydrophobic backbone of the ceramide-type complex lipids of this organism and can be present with hydroxy fatty acids associated with the molecule. The hydroxy fatty acids, which do not occur in other mycoplasmas, are of course found in a number of bacteria, particularly gram-negatives, in N-acyl linkages in the Lipid A portion of lipopolysaccharides. Long-chain bases are, however, very rare in any prokaryote. Bacteroides, a group which is prone to spontaneous L-form formation [7], contains hydroxy fatty acids and is thus far the only bacterial genus to contain long-chain bases although occurring in the bound form among the complex lipid structures [11,12].

The neutral lipids also distinguish the genus thermoplasma from other mycoplasmas and, in fact, from eubacteria. In addition to the occurrence of menaquinone MK-7 indicative of a respiratory chain, thermoplasma species contain isopranly diglycerol tetraethers and isopranoid hydrocarbons. The major route of lipid biosynthesis in thermoplasmas is the mevalonate pathway for isoprenoid biosynthesis rather than the malonyl-CoA pathway for fatty acid synthesis. These features distinguish thermoplasma species as an archaebacterium and as related to the methanogenic and extremely halophilic bacteria [5].

GLYCOLIPIDS

Glycolipids are perhaps the most useful lipid class for taxonomic purposes. These compounds contain sugar molecules attached to hydrophobic residues, and, because of the variable combination of sugars and their linkages, a wide variety of structures are possible. The most common glycolipids are the glycosyl diglycerides which are usually, although not entirely, found in gram-positive bacteria. In bacteria which

contain glycolipids it is almost always the case that species of the same genus contain the identical glycolipids and, in those cases where there is some exception, there is usually some contention over the taxonomy of the offending organism [3]. As shown in Table 2 and discussed below, the glycolipids of mycoplasmas represent the only glaring exception to this general conclusion and, in fact, the glycolipids show a more harmonious relationship between individual mycoplasma species and bacterial counterparts than between mycoplasmas of the same genus.

First, many mycoplasma species contain no glycolipids at all, which is a familiar case in many gram-negative bacteria (exclusive of macromolecular lipopolysaccharides, of course). Cholesteryl glucosides, which do not occur in bacteria but are constituents of plants, are found in three different mycoplasmas: *A. axanthum*, *M. gallinarum*, and *S. citri*. *A. laidlawii* is the only species shown to contain carotenyl glucosides which occur in some corynebacterium, flavobacterium, and sarcina species.

Glycosyl diglycerides occur in all acholeplasma and the mycoplasma species *M. mycoides* and *M. neurolyticum*. Monoglycosyl diglycerides, containing glucose or galactose sometimes in the furanose form, represent the major glycolipid components in these organisms. These compounds are common in gram-positive bacteria, but in gram-positive cells they are only minor components and the diglycosyl diglycerides are the major compound. The diglycosyl diglycerides of *A. granularum*, *A. laidlawii*, *A. modicum*, and *A. oculi* are distinctive. The glucosyl

TABLE 2
Glycolipids of Mycoplasmas and Bacteria

Glycolipid	Mycoplasma	Bacterium	Reference
None	<i>M. arthritidis</i> ; <i>M. capricolum</i> ; <i>M. gallisepticum</i> ; <i>M. hominus</i> ; <i>M. hyorhinis</i>	Gram-negatives	[1,2,3,10]
Cholesteryl glucosides	<i>A. axanthum</i> ; <i>M. gallinarum</i> ; <i>S. citri</i>	—————	[8,10]
Carotenyl glucosides	<i>A. laidlawii</i>	<i>Corynebacterium</i> <i>Flavobacterium</i> <i>Sarcina</i>	[8,10]
Monoglycosyl diglycerides (glucose, galactose)	<i>A. axanthum</i> ; <i>A. granularum</i> ; <i>A. laidlawii</i> ; <i>A. modicum</i> ; <i>A. mycoides</i> ; <i>M. neurolyticum</i>	Gram-positives	[1,2,3,10]
Diglycosyl diglyceride ($\alpha 1-2$)	<i>A. granularum</i> ; <i>A. laidlawii</i> ; <i>A. modicum</i>	<i>Streptococcus</i>	[1,2,3,10]
Diglycosyl diglyceride ($\alpha 1-6$)	<i>M. neurolyticum</i>	<i>Staphylococcus</i> <i>Bacillus</i>	[1,2,3,10]
Galactosyl glucosyl diglyceride	<i>M. pneumoniae</i>	<i>Pneumococci</i> <i>Lactobacillus</i>	[1,2,3,10]
Triglycosyl diglycerides (glucose, galactose)	<i>M. neurolyticum</i> ; <i>M. pneumoniae</i>	<i>Streptococcus</i> <i>Lactobacillus</i>	[1,2,3,10]
Pentaglycosyl diglyceride (glucose, galactose, heptose)	<i>A. modicum</i>	—————	[18]
Polyglycosyl diglycerides (lipoglycans)	<i>Acholeplasma</i> ; <i>Anaeroplasma</i> ; <i>Thermoplasma</i> ; <i>M. neurolyticum</i>	<i>Mycobacterium</i>	[10]
Acylated glucose	<i>M. gallinarum</i>	<i>Streptococcus</i> <i>Corynebacterium</i> <i>Mycobacterium</i>	[1,2,10]
Glycosylated tetraethers	<i>Th. acidophilum</i>	<i>Methanobacterium</i>	[5,6]

($\alpha 1 \rightarrow 2$) glucosyl ($\alpha 1 \rightarrow 1$)-diglyceride is the same as in the streptococci. The glucosyl ($\alpha 1 \rightarrow 6$)-glucosyl ($\alpha 1 \rightarrow 1$) diglyceride of *M. neurolyticum*, on the other hand, is identical to that of *Staphylococcus aureus*. The galactosyl glucosyl diglyceride of *M. pneumoniae* and *M. neurolyticum* are like those of the pneumococci, lactobacilli, and streptococci. Perhaps the largest extractable (by organic solvents) glycosyl diglyceride known is the pentaglycosyl diglyceride of *A. modicum*; galactosyl ($\alpha 1 \rightarrow 2$)-galactosyl-($\alpha 1 \rightarrow 3$)-heptosyl($\beta 1 \rightarrow 3$)-glucosyl($\alpha 1 \rightarrow 2$)-glucosyl($\alpha 1 \rightarrow 1$) diglyceride. *A. modicum* is the only organism known to contain this compound. It is notable that heptose is present in this glycolipid. Heptose occurs in gram-negative lipopolysaccharides, and the only other known heptose-containing glycolipid is a monoheptosyl diglyceride reported in *Pseudomonas vesicularis* [13].

Polyglycosyl diglycerides, which can be considered as extended glycosyl diglycerides or lipoglycans, occur in all acholeplasma, anaeroplasm, and thermoplasm species examined but are absent from the genus mycoplasma with the exception of *M. neurolyticum*. These compounds really have no bacterial counterpart, but are perhaps reminiscent of the acylated polysaccharides of mycobacteria.

The simplest glycolipid structure, a triacylglucose molecule, is found only in *M. gallinarum* among the mycoplasmas, but similar compounds are characteristic of *Streptococcus faecalis* and species of the corynebacterium and mycobacterium. The glycolipids of thermoplasm are glycosylated diglycerol tetraethers which are similar to the archaebacterial glycolipids of the methanogenic bacteria and *Sulfolobus* [5].

ACIDIC LIPIDS

The acidic lipids of mycoplasmas can be found as two basic types: glycerophospholipids and phosphoglycolipids. The latter type can be considered a glycolipid to which a glycerolphosphate radical is attached to the sugar residue.

The glycerophospholipids are composed of the common phosphatides, diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidic acid, the lyso-forms, and, in several instances, aminoacylated phosphatidyl glycerol. These phospholipids are ubiquitous in mycoplasmas as well as bacteria and of little use taxonomically. A possible exception is the presence of phosphatidyl ethanolamine in ureaplasma species, the only mycoplasma known to contain this phospholipid. Phosphatidyl ethanolamine is generally absent from gram-positive cells, with the exception being the genus *Bacillus* [2]. Ureaplasma also contains an O- and N-acylated diamino-hydroxy compound perhaps similar to the ornathine lipids of thiobacilli. And, as noted

TABLE 3
Acidic Lipids of Mycoplasmas and Bacteria

Compound	Mycoplasma	Bacterium	Reference
Glycerophospholipids	Ubiquitous	Ubiquitous	[2,3,10]
O-acyl, N-acyl diaminiolipid	<i>Ureaplasma</i>		[10]
Ceramide phosphoryl glycerols	<i>A. axanthum</i>	<i>Bacteriodes</i>	[2,10,11,12]
Glycerolphosphoryl diglucosyl diglyceride	<i>A. laidlawii</i>	<i>Streptococcus</i>	[1,2,3,8,10]
Phosphatidyl diglucosyl diglyceride	<i>A. laidlawii</i>	<i>Streptococcus</i>	[2,3,10]
Glucosyl glycerolphosphoryl diglucosyl diglyceride	<i>A. granularum</i>	<i>Streptococcus</i>	[14,15,16]
Glycerolphosphoryl, glycosyl diglycerol tetraether	<i>Th. acidophilum</i>	<i>Methanobacterium Sulfolobus</i>	[5,17]

earlier, *A. axanthum* contains, in addition to the common phospholipids, ceramide phosphoryl glycerols known only in *Bacteroides* (Table 3).

Phosphoglycolipids occur in two acholeplasmas, *A. laidlawii* and *A. granularum*, and in the genus thermoplasma. These are perhaps the most important lipid components which indicate a relationship of mycoplasmas to the cell-walled bacteria. Phosphoglycolipids are present in small quantities in all gram-positive bacteria that produce lipoteichoic acids (LTA). As shown by Fischer and associates [14,15] phosphoglycolipids are not accumulated in the membrane but are biosynthetic intermediates of LTA and, usually in a more fully acylated form, provide the lipid "anchor" of the LTA molecule for insertion into the membrane.

A. laidlawii contains two phosphoglycolipids. The first is the diglucosyl diglyceride of the organism to which *sn*-3-glycerolphosphate is attached to the C-6 position of the terminal glucose, resulting in a glycerolphosphoryl diglucosyl diglyceride. The same compound, with the exception that the glycerol phosphate has the *sn*-1-glycerol stereo-configuration, is present as an initial precursor of LTA biosynthesis in streptococcus [15]. The second component, which is found only in older cultures of *A. laidlawii*, is a phosphatidyl diglucosyl diglyceride in which the glycerolphosphate radical of the above compound is fully acylated. This component is also present as the free compound and not bound to LTA in streptococci, the exception being that the phosphatidyl radical is attached to the internal glucose. *A. granularum* contains a third type of phosphoglycolipid [16], extending the analogy to LTA intermediates even further. It is the same glycerolphosphoryl diglucosyl diglyceride found in *A. laidlawii* but contains a glucose molecule attached to the terminal glycerol phosphate residue, e.g., glucosyl glycerolphosphoryl diglucosyl diglyceride. This compound is analogous to the second intermediate of LTA biosynthesis in streptococcus proposed by Fischer et al. [15]. It differs, however, in the additional feature that it contains a glyceraldehyde attached to the phosphate molecule, but this may lend even more credence to the idea that the phosphoglycolipids of the acholeplasmas represent a pile-up of LTA precursors. The high concentrations of such compounds in *A. laidlawii* and *A. granularum*, as opposed to minimal concentrations in gram-positive cells, strongly suggests that these mycoplasmas have arisen from streptococci which have lost some terminal steps in LTA biosynthesis.

Thermoplasma is the other genus to contain phosphoglycolipids which account for almost 80 percent of the acidic lipids. They are of the archaebacterial type found in *Sulfolobus* and methanogenic bacteria.

CONCLUSION

Clearly, even from this short synopsis, mycoplasmas contain a heterogeneous diversity of lipid structures, even between members of the same genera. In a large number of instances, particularly the acholeplasmas, the lipids are almost indistinguishable from bacterial counterparts. In the best case, the lipids of thermoplasma establish it as an archaebacterium. In the worst case, most of the *Mycoplasma* species, with the exception of *M. mycoides*, *M. neurolyticum*, and *M. pneumoniae*, lack glycolipids and have similar lipid compositions perhaps suggesting a close phyletic relationship to each other, but this is negative evidence.

Based upon the lipid composition of mycoplasmas, it is this author's opinion that the mycoplasmas do not represent a distinct phylogenetic group that has evolved from a common ancestor. Rather they represent a conglomeration of different bacterial species which somewhere during the course of time have lost portions of their genomes and the ability to synthesize cell walls. This is not to say that grouping

these organisms as a unit based upon the absence of cell walls is not a useful operational definition as long as it is remembered that these organisms reflect a phylogenetic relationship to bacterial counterparts rather than to each other. However, tracing the true phyletic relationships of all mycoplasmas to their correct original derivatives could be a Sisyphian if not impossible task.

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