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QUALITATIVE AND QUANTITATIVE VARIATIONS OF MEMBRANE LIPID SPECIES IN *ACHOLEPLASMA LAIDLAWII* A

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Summary

In *Acholeplasma laidlawii* A, strain EF 22, the relative amounts of the membrane polar lipids vary as a consequence of different fatty acid supplements to the growth medium. The number of lipid species also varies; a new apolar monoglucolipid containing four fatty acid residues was present only when saturated fatty acids dominated in the growth medium. A new phosphoglucolipid, probably with a glycerophosphoryl-monoglucosyldiglyceride structure, was also found. The most pronounced variations occurred between the two dominating glucolipids, monoglucosyldiglyceride and diglucosyldiglyceride; the former being found in larger amounts when a saturated or a trans-unsaturated fatty acid was present in the medium. The amount of diglucosyldiglyceride decreased accordingly. A qualitative relationship between fatty acid properties and membrane lipid variations was established over a wide fatty acid concentration range. Incorporation of supplied fatty acids reached higher levels than normally found in other acholeplasmas. The ratio between membrane protein and lipids exhibited significant and coherent variations during growth and was to some extent influenced by the fatty acids in the medium. These changes indicate variations in lipid-protein organization in the membranes during growth.

Introduction

The ability of *Acholeplasma* to incorporate various, selectively supplied fatty acids into membrane lipids has contributed to our understanding of membrane function [1]. During ultrastructural studies of membranes and lipids from a strain of *Acholeplasma laidlawii* A [2], we found that this strain incorporated externally supplied fatty acids to a greater extent than other acholeplasmas. Moreover, the relative amounts of membrane lipid species seemed to depend on the fatty acid supplements in the growth medium, and the number of different

polar lipid species varied. Although incorporation of fatty acids into *Acholeplasma* membrane lipids is reported not to affect the relative distribution of membrane polar lipids [3—5], great differences in reported amounts of various polar lipids in *A. laidlawii* B membranes are evident [6—9]. *A. laidlawii* A, the membrane lipids of which have not been examined to the same extent as those from the B strain [6,10], also shows large variations in polar lipid composition [11—15]. To some extent, the fluctuations may be caused by growth conditions, as noted for *Mycoplasma mycoides* [16].

In contrast to the closely related B strain, *A. laidlawii* A has an absolute requirement for an unsaturated fatty acid which it cannot synthesize [17,18]. Thus, the physical milieu created by unsaturated fatty acids in cells of *A. laidlawii* A seems to be of importance for some vital membrane function(s). *A. laidlawii* B can grow without fatty acid supplements, but in spite of the absence of such a requirement, changes in its membrane fatty acid composition have profound effects on its growth ability [19].

Structural differences in lipid polar groups in *A. laidlawii* B give the lipids different physical properties as indicated by their dissimilar gel to liquid crystalline transition enthalpies [20,21]. Our preliminary findings thus promted us to investigate whether *A. laidlawii* A shows variations in relative amounts of membrane lipid species in media containing different fatty acid supplements. Any changes in lipid polar head distributions would, in addition to changing fatty acid properties, probably result in physical alterations in membrane function. The results obtained indicate that, under otherwise identical conditions, this strain varies primarily the relative amounts of its membrane glucolipids as a function of fatty acid content during growth.

Materials and Methods

Organism and growth conditions

A. laidlawii A, strain EF 22, was kindly typed and provided by Prof. E.A. Freundt, FAO/WHO International Mycoplasma Reference Laboratory, Aarhus, Denmark. It was grown in a lipid-depeted bovine serum albumine-tryptose medium (see ref. 22 for composition and extraction procedure) adjusted to pH 8.5. The medium was supplemented with either 0.150 mM oleic, elaidic or palmitoleic acid, or 0.075 mM pamitic plus 0.075 mM oleic acid (Sigma, 99% pure), all added as ethanol solutions. To label the membranes, 10 μCi ^{14}C- or 30 μCi ^{3}H-labelled fatty acid (The Radiochemical Centre, Amersham, U.K., or Applied Science Laboratories, State College, Pa., U.S.A.) was added to 1 l of growth medium. Growth was followed by absorbance measurements at 540 nm. Colony forming units were counted as previously described [23]. Membranes from cells harvested during various growth phases were prepared by lysis, washed in β-buffer diluted 1 : 20 (v/v) and freeze-dried [22].

A. laidlawii B, strain Pg 9, the polar membrane lipids of which have been analyzed in detail [6,10] was used as a reference strain. It was grown under the same conditions, and its membrane lipids were prepared as described below. The lipids were used as chromatografic and chemical standards.

Purification of membrane lipids

Lipids were extracted from the dried membrane preparations by three suc-

cessive extractions with chloroform/methanol (2 : 1, v/v) at room temperature and freed from nonlipid contaminants by passage through a Sephadex-G25 F (Pharmacia) column [24]. The lipid classes were separated on a Bio-Sil HA (minus 325 mesh) silicic acid (Bio-Rad Laboratories) column employing stepwise elution with chloroform, acetone and methanol. Individual lipid species were then isolated by thin-layer chromatography on Silica gel H (Merck) in chloroform/methanol/water (65 : 25 : 4, v/v), solvent A, or petroleum ether (b.p. 40—60°C)/diethylether/acetic acid (80 : 2- : 1, v/v), solvent B. They were located and tentatively identified with Rhodamine 6G, iodine vapour, molybdate reagent for phosphate [25], diphenylamine reagent for lipid sugar [26], ninhydrin (Sigma) for NH_2-groups, periodate-Schiff's reagent for α-glycols [27], and by autoradiography of labelled lipids on Kodirex X-ray film (Kodak). For autoradiography, good separation of all polar lipids could be obtained on the same silica gel H plate in a single lane with one dimensional development in chloroform/methanol/water if the gel was buffered with 1% (w/v) $Na_2B_4O_7 \cdot 10$ H_2O. Areas on chromatography plates which corresponded to the main membrane lipids were scraped into small glass columns and eluted with chloroform/methanol (2 : 1, v/v) (glucolipids) or methanol/formic acid (96 : 4, v/v) (phospholipids and phosphoglucolipids).

Analytical methods

In order to verify the preliminary identifications of the lipids, they were subjected to selective chemical degradations and the obtained products identified. Mild alkaline deacylation with 0.2 M NaOH in methanol was performed as described by Kates [42]. The water-soluble hydrolysis products were separated by chromatography on Whatman no. 1 paper by the use of saturated phenol/water (100 : 38, w/v), solvent C. The obtained fatty acid methyl esters were identified by gas-liquid chromatography (see below). Checks for any alkali-stable lipids were made by thin-layer chromatography on Silica gel H in solvent system A. Identification of spots on the paper chromatograms were achieved by spraying with the periodate-Schiff's reagent [27], acid molybdate for phosphate esters [43], 1% (w/v) *p*-anisidine hydrochloride in ethanol for free sugars [42] and ninhydrin for NH_2-groups.

Sugar content of individual lipids was examined after acid hydrolysis [10,28] by thin-layer chromatography on sodium acetate buffered [29] Silica gel G (Merck) in acetone/water (90 : 10, v/v) and visualized with anisaldehyde-sulphuric acid [30]. Glucose, galactose, mannose, fructose, rhamnose and ribose were used as references. After the hydrolysates were dried over KOH, glucose was analyzed colorimetrically using peroxidase and glucose oxidase enzymes (Sigma). Phosphate was analyzed according to Ames [31] after ashing the samples with ethanolic $Mg(NO_3)_2$. When identified, the water soluble hydrolysis products obtained after the alkaline deacylation were subjected to acid hydrolysis in 2.0 M HCl. Glucolipid derivates were hydrolysed for 3 h at 100°C and phospho- and phosphoglucolipid derivates for 48 h at 100°C. The obtained hydrolysis products were separated by paper chromatography and identified as described above.

After transmethylation, the fatty acid composition of individual lipids was determined by gas-liquid chromatography on a Perkin-Elmer model F apparatus

with dual flame ionization detectors and columns containing 5% (w/v) free fatty acid phase on Chromosorb G with N_2 as carrier gas. Reference standard methyl esters were obtained from Supelco Inc., Bellefonte, Pa., U.S.A. In order to detect endogenously produced saturated fatty acids, values for fatty acid composition as revealed by gas-liquid chromatography were compared with those obtained by counting labelled lipids in a Nuclear Chicago Mark II liquid scintillation counter in Omnifluor toluene cocktail (New England Nuclear Co.). Protein content of membranes was estimated by the method of Lowry et al. [32] using bovine serum albumin (Cohn V) as standard. All reagents used were of analytical reagent grade without further purification.

Results

A. laidlawii A (EF 22) contained at the most seven different polar lipid species in its membranes (Fig. 1), whereas the reference strain B contained fewer. Thin-layer chromatography spray reagents (see Materials and Methods) revealed three glucolipids (Fig. 1, spots 4, 6 and 7), two phospholipids (Fig. 1, spots 3 and 5), and two phosphoglucolipids (Fig. 1, spots 1 and 2). The lipids in spots 1, 3, 4, 5 and 6 cochromatographed with reference lipids from *A. laidlawii* B and stained correspondingly. Thin-layer chromatography of acid hydrolysates revealed that the glucolipids contained mainly glucose with only traces of galactose, and that the phospholipids were not contaminated with glucolipids. Chemical analysis of the lipid in spot 1 revealed a phosphate-glucose-fatty acid ratio of 1 : 2 : 2. Deacylation yielded a single water-soluble product on paper chromatography (R_F 0.27 in solvent C) which was phosphate positive and gave a rapid purple colour with the periodate-Schiff's reagent. Further degradation by acid hydrolysis of this water-soluble derivate and separation of products by paper chromatography revealed it to consist of glucose, glycerol and inorganic phosphate. These analytical data are consistent with a glycerophosphoryldiglucosydiglyceride structure [10].

The lipid in spot 2 (Fig. 1), which was absent in the reference strain, had a phosphate-glucose-fatty acid ratio of 1 : 1 : 2, had a R_F value on thin-layer chromatography between those of phosphatidylglycerol and glycerophosphoryldiglucosyldiglyceride and rapidly gave a purple colour with the periodate-Schiff's reagent indicative of the presence of α-glycols. Furthermore, the amount of lipid in spot 2 increased when monoglucosyldiglyceride amounts were large (see below). Its deacylation product had a R_F of 0.39 after paper chromatography in solvent C, but stained correspondingly with the spot 1 lipid (Fig. 1) deacylation product and consisted of the same substances after acid hydrolysis. This may indicate a glycerophosphorylmonoglucosyldiglyceride structure. The lipid in spot 7, glucolipid X, had a glucose-fatty acid ratio of approximately 1 : 4, which is consistent with the high R_F value on thin-layer chromatography indicative of a more apolar structure. The periodate-Schiff's reagent stain was positive but slower than the other glucolipids. Deacylation gave a single product on paper chromatography (R_F 0.57 in solvent C) which rapidly gave a purple colour with the periodate-Schiff's reagent. Acid hydrolysis only revealed glucose and glycerol. This lipid was only detectable when high amounts of saturated fatty acids were present in the growth medium (see

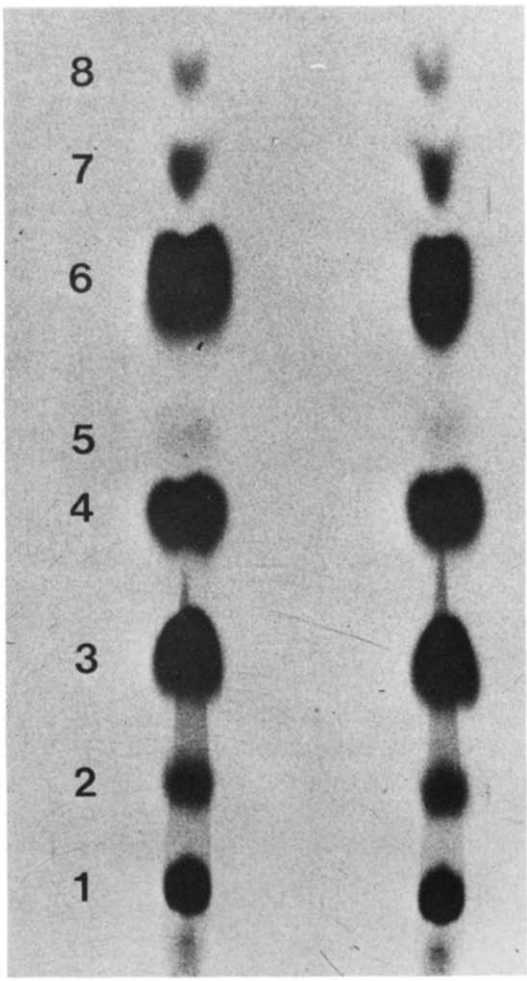

Fig. 1. Autoradiogram of [^{14}C]oleate plus [^{3}H]palmitate labelled membrane lipids of *A. laidlawii* A from two different growth times. The thin-layer chromatogram was performed on $Na_2B_4O_7 \cdot 10\ H_2O$ buffered Silica gel H (1% w/v) developed in chloroform/methanol/water (65 : 25 : 4, v/v). Individual species were identified as described in Materials and Methods. Identification of spots: 1, glycerophosphoryldiglucosyldiglyceride; 2, probably (see text) a glycerophosphorylmonoglucosyldiglyceride; 3, phosphatidylglycerol; 4, diglucosyldiglyceride; 5, traces of diphosphatidylglycerol; 6, monoglucosyldiglyceride; 7, new apolar monoglucolipid (see text); and 8, neutral lipids.

below). By comparison of molar ratios, behaviour and staining of intact lipids, deacylation products and acid hydrolysates on paper and thin-layer chromatography [6,42], spots 3, 4, 5 and 6 lipids (Fig. 1) were confirmed to be identical with the reference strain lipids, i.e.: spot 3, phosphatidylglycerol; spot 4, diglucosyldiglyceride; spot 5, diphosphatidylglycerol and spot 6, monoglucosyldiglyceride. It should be pointed out that the glucolipid X possesed small but distinctive analytical differences when compared to the other glucolipids.

The relative amounts of membrane polar lipids changed during the growth phases and were also influenced by the kind of fatty acid supplied, as shown in Table I. Since glycerophosphorylmonoglucosyldiglyceride and the glucolipid X

TABLE I

INFLUENCE OF FATTY ACIDS ON THE RELATIVE AMOUNTS OF MEMBRANE LIPID SPECIES IN *A. LAIDLAWII* A

Cells were grown in a lipid-depleted bovine serum albumin-tryptose medium with different fatty acids added. The lipids of isolated membranes were separated and quantified by liquid scintillation counting, gas-liquid chromatography and chemical analysis (see Materials and Methods). Standard error ±4%.

Added acid	Growth time (h)	Molar ratio single lipid/total lipids in membranes			
		Monoglucosyl-diglyceride	Digluocsyl-diglyceride	Phosphatidyl-glycerol	Glycerophos-phoryl-diglucos-yldiglyceride
Palmitoleic (16 : 1 *cis*)	8	0.40	0.22	0.20	0.18
	16	0.31	0.31	0.17	0.21
	24	0.22	0.37	0.14	0.23
Elaidic (18 : 1 *trans*)	8	0.44	0.28	0.19	0.09
	16	0.37	0.31	0.21	0.11
	24	0.17	0.50	0.21	0.11
Oleic (18 : 1 *cis*)	8	0.31	0.30	0.26	0.11
	16	0.22	0.35	0.30	0.13
	24	0.05	0.53	0.24	0.19
50% Palmitic (16 : 0) + 50% oleic	8	0.52	0.22	0.16	0.09
	16	0.50	0.20	0.19	0.11
	24	0.31	0.29	0.24	0.15

only appeared in the presence of some fatty acids, they have been omitted from the tables. With the growth conditions depicted in Table I, the sum of glycerophosphorylmonoglucosyldiglyceride and glucolipid X never exceeded 10% of the total lipid content (mol/mol). The 24-h sample coincides with the 0-h sample, since fresh cultures were started every 24th hour. The most marked changes occurred with respect to monoglucosyldiglyceride and diglucosyldiglyceride. During the lag phase of growth (0—8 h), monoglucosyldiglyceride synthesis was pronounced, but its rate of synthesis and relation to diglucosyldiglyceride synthesis depended on the kind of fatty acid supplied. These differences led to a different lipid composition for cells entering the exponential phase (8—16 h) which was maintained throughout this phase. In the stationary growth phase (16—24 h), the amount of monoglucosyldiglyceride always declined, but final levels and concomitant increases in the amount of diglucosyldiglyceride were also fatty acid dependent. Monoglucosyldiglyceride is considered to be the biosynthetic precursor to diglucosyldiglyceride [33]. Phosphatidylglycerol and glycerophosphoryl-diglucosyldiglyceride were more stable and showed only small variations. The same was true for the neutral lipid fraction, including free fatty acids. Glycerophosphoryl-monoglucosyldiglyceride levels (not shown in Table I) rose with increased saturated fatty acid concentrations and thus increased monoglucosyldiglyceride levels, but it could be detected with most supplements tested. The glucolipid X was only synthesized with comparable high amounts of saturated fatty acids present and was not found with the other supplements. In all cases the total amount of glucolipids was greater than that of phospho- and phosphoglucolipids.

TABLE II

INCORPORATION OF FATTY ACIDS INTO DIFFERENT MEMBRANE LIPID SPECIES

Fatty acid composition of individual lipids were estimated by gas-liquid chromatography after transmethylation and by liquid scintillation counting of labelled lipids. The results of the two methods were compared in order to detect endogenously produced fatty acids.

Added acid	Growth time (h)	Molar ratio added fatty acid/total fatty acids in membrane lipids			
		Monoglucosyl-diglyceride	Diglucosyl-diglyceride	Phosphatidyl-glycerol	Glycerophosphoryl-diglucosyldiglyceride
Palmitoleid	8	0.69 *	0.72	0.75	0.62
	16	0.92	0.92	0.96	0.85
	24	0.91	0.93	0.91	0.93
Elaidic	8	0.79 *	0.79	0.89	0.74
	16	0.95	0.94	0.99	0.95
	24	0.91	0.94	0.95	0.90
Oleic	8	0.70 *	0.68	0.75	0.66
	16	0.93	0.93	0.98	0.84
	24	0.86	0.95	0.99	0.90
50% palmitic + 50% oleic	8	0.67 + 0.25 **	0.58 + 0.37	0.50 + 0.45	0.57 + 0.43
	16	0.66 + 0.33	0.61 + 0.38	0.56 + 0.42	0.58 + 0.40
	24	0.53 + 0.45	0.44 + 0.54	0.37 + 0.61	0.53 + 0.46

* The remaining fatty acids were lauric (12 : 0), myristic (14 : 0), and palmitic (16 : 0).
** The remaining fatty acids were lauric (12 : 0) and myristic (14 : 0).

Of several saturated fatty acids tested at various concentrations, none could support growth when supplied alone. As little as 6% (mol/mol) unsaturated fatty acid in a saturated plus unsaturated fatty acid supplement yielded a good growth response. Various unsaturated fatty acids supported growth in the concentration range 0.015—0.200 mM. Optimum conditions were around 0.150 mM. The unsaturated fatty acids supplied were incorporated to a very high degree (Table II) when given separately. After 16 h of growth the lipids contained almost exclusively the fatty acid supplied. Table II also shows that the organism had a more vigorous endogenous synthesis of saturated fatty acid during the early growth phase. When oleic acid was given together with palmitic acid, the incorporation of these compounds was dissimilar in the different lipids during all growth phases. On an average, incorporation of palmitic acid was higher in the beginning and stopped after 16 h of growth (Tables II and III). The reverse held for oleic acid which yielded increased amounts of this acid during later growth.

The results of the experiments dealing with fatty acid influence on variation in lipid species indicated effects of the qualitative composition of the fatty acid supplement. In order to investigate the concentration dependence of these effects, the acids tested in Tables I and II were added to the growth medium at various concentrations. In the interval 0.050—0.180 mM the lipid patterns for the different fatty acids were approximately the same as in Table I. Incorporation of the fatty acids into lipids reached the same high values as those in Table II. The same constancy held for viable counts and growth rates. Average size of

cell aggregates and thus cell yields and incorporated amounts of fatty acids increased with higher fatty acid concentrations, reaching a maximum around 0.150 mM. Absorbances of the cultures parallelled the cell yield values. Although some physiological cell changes thus occurred with altered acid concentrations, the variations in lipid species only followed the physical properties of the fatty acids. These studies further strengthen the idea of a relationship between fatty acid structure and membrane glucolipid synthesis.

In order to study the relationship between endogenous production of saturated fatty acids and lipid species variations, 0.5% (w/v) sodium acetate was added to the growth medium. This compound is known to be an efficient precursor for both *A. laidlawii* A and B fatty acid synthesis. However, the lipid patterns remained he same, and no response could be detected from sodium acetate in combination with various concentrations of different fatty acids.

Table III shows that the fatty acids influenced the total amount of membrane produced since the viable count curves were very similar (not shown). Thus elaidic acid promoted membrane protein synthesis more than the other acids tested. In the late lag phase (8 h) all cultures exhibited a similar lipid-protein ratio, but during the exponential phase this ratio differed. During the stationary growth phase the diverging trends continued. The main feature in common was the strong relative increase in lipid to protein ratio during the log phase. The same trend is also evident in the column headed "% added fatty acid incorporated into lipids" (Table III).

TABLE III

MEMBRANE YIELDS AND CHANGES IN MEMBRANE COMPOSITION

Membrane protein content was estimated from 100 ml of culture volume due to the aggregate nature of cell growth. In "glycerolipids" all polar membrane lipids were included.

Added acid	Growth time (h)	mg membrane protein/100 ml culture	μmol glycero-lipids/mg membrane protein	% total lipids in membranes (w/w) *	% of added fatty acids incorporated into membrane lipids
Palmitoleic	8	0.43	0.76	44	2.1
	16	2.78	1.11	52	38
	24	3.08	1.31	59	52
Elaidic	8	0.78	0.71	41	6.0
	16	3.12	1.01	51	40
	24	7.15	0.58	41	57
Oleic	8	0.56	0.81	43	4.2
	16	2.99	1.35	57	49
	24	5.80	0.82	46	60
50% palmitic	8	0.51	0.73	41	6.2 + 3.3
+ 50% oleic	16	2.28	1.33	56	50 + 29
	24	4.38	0.94	50	51 + 57

* Neutral lipids included.

Discussion

The conditional ratios between membrane lipid species suggest that *A. laidlawii* A strain EF 22, in addition to the capability of incorporating various fatty acids, can adjust the relative distribution of polar lipid species. The smaller variations associated with different stages of growth are most readily explained by a qualitative effect of the different fatty acids, since growth measured by absorbance and viable counts proceeds similarly with different fatty acids. The synthesis of two lipids not reported earlier, glycerophosporyl-monoglucosyldiglyceride and glucolipid X, seems to be stimulated by the presence of saturated fatty acids. These lipids are not identical with the phosphatidyldiglucosyldiglyceride which may appear in late stationary cultures of *A. laidlawii* B [40], although structural similarities probably exist. The observed relationship between monoglucosyldiglyceride and diglucosyldiglyceride may be interpreted in terms of differences in the effects of fatty acids, i.e. high levels of saturated fatty acids endogenously produced early during growth or externally supplied stimulated monoglucosyldiglyceride synthesis at the expense of diglucosyldiglyceride. Elaidic acid (18 : 1 *trans*), being more viscous because of the *trans* bond allowing a denser packing of the hydrocarbon chains, gave higher levels of monoglucosyldiglyceride than the corresponding *cis*-isomer (oleic acid). When exposed to both a saturated and an unsaturated fatty acid, the cells preferably incorporated the saturated fatty acid during the active growth phases. This yielded a larger monoglucosyldiglyceride/diglucosyldiglyceride ratio than found with the other acids tested. In the same experiment, incorporation of saturated fatty acids stopped after the exponential phase, and the high selective incorporation of unsaturated fatty acid that followed did not increase the amount of diglucosyldiglyceride significantly, probably because the saturated fatty acid content was high enough to affect the synthesis. Furthermore, membrane lipids have slower turn-over rates than proteins [11]. This fact will give a slow metabolic dilution of the preexisting saturated lipids. In a study of the metabolic pathways for glucolipid biosynthesis, Smith [33] found that the membrane-bound enzymes behaved differently upon treatment with salt and detergent. It is thus possible that the fatty acids have an allosteric influence on lipid synthesizing enzymes. A main physical feature of fatty acids is their impact, by way of additive London-van der Waals forces, on membrane viscosity. This phenomenon is also temperature dependent. Studies of lipid metabolism in our strain after sudden temperature shifts during growth (from 37 to 17°C) did reveal higher monoglucosyldiglyceride levels at lower temperatures, consistent with a viscosity influence (Christiansson, A. and Wieslander, Å., unpublished). A striking difference in phase structure between monoglucosyl- and diglucosyldiglyceride as revealed by X-ray and nuclear magnetic reconance studies [44] support the different physiological behaviour of these lipids. A significant impact of the variations between the structurally similar monoglucosyldiglyceride and diglycosyldiglyceride on the packing of fatty acids in the membrane bilayer may not be the case. The variations among lipid polar heads in our strain might instead be a mechanism to adapt or compensate for changes in permeability caused by viscosity changes. In *Staphylococcus aureus* pH-induced lipid polar moiety variations are known to affect membrane barrier properties [36,37].

Like other A strains, our strain has an absolute requirement for an unsaturated fatty acid. The incorporation of these acids however, reaches an extent seldom found in other acholeplasmas and bacteria [1]. Even with cerulenin, a potent inhibitor of endogenous fatty acid synthesis, it was not possible to obtain such high incorporation of externally supplied unsaturated fatty acids in an oral *A. laidlawii* A strain [34]. Our strain obviously lacks the biosynthetic background of endogenously produced saturated fatty acids in the lipids, upon which incorporation of externally supplied unsaturated fatty acids is added [1]. Since the growth medium is very rich, and even addition of a proper precursor failed to give a response, a block or a deficiency in the saturated fatty acid synthesis of this strain is probably responsible. In *Escherichia coli* fatty acid auxotrophs, either a too low or a too high content of unsaturated fatty acids in membrane lipids (the balance being saturated fatty acids) yields disturbances in membrane barrier properties [35]. For *Acholeplasma* this is obviously not the case, or at least the cells can live with such malfunctions in the membranes.

The systematic differences in fatty acid composition of the various lipids (Table II) perhaps mirrors a regulatory function, since the fatty acid patterns probably are introduced in lipid precursors [4].

Although the fatty acids have different effects on membrane protein synthesis (Table III), the changes in total amount of lipid per mg membrane protein indicate that, in spite of fatty acid influence, there is no strict regulation in the balance between membrane proteins and lipids. Increase in membrane protein content tends to increase membrane viscosity [41]. Furthermore, because the different membrane lipids have relatively similar packing properties [2], such a regulation would probably produce differences in the physical properties of the membrane surface if one assumes a bilayer configuration. If a constant bilayer surface is to be maintained [38], and asymmetric protein packing is possible as recently suggested for *Mycoplasma hominis* [39].

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