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Phospholipid Fatty Acid Composition of the Syntrophic Anaerobic Bacterium *Syntrophomonas wolfei*†

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The membrane phospholipid fatty acids (PLFAs) from several cocultures and a pure culture of *Syntrophomonas wolfei* were determined by capillary column gas chromatography. Cocultures of *S. wolfei* with a *Desulfovibrio* sp. contained PLFAs from both organisms, whereas PLFAs from a coculture with *Methanospirillum hungatei* contained very little biomass to analyze. The pure culture of *S. wolfei* grown on crotonate provided the best material for analysis of the PLFAs. The predominant PLFAs of *S. wolfei* were the monounsaturated 16:1ω7c and 16:1ω9c and the saturated 16:0 and 14:0. A low concentration of the diunsaturated 18:2ω6 was detected. The PLFA analysis provides additional information for consideration in the determination of the profile of PLFAs obtained from anaerobic environments. In addition, this information may aid in the understanding of the physiology and phylogeny of *S. wolfei* and other syntrophic bacteria.

Bryant et al. (6) discovered in 1967 that "*Methanobacillus omelianskii*" was not an ethanol-utilizing methanogen but was actually a mutualistic coculture of two species of bacteria. This coculture was composed of a nonmethanogenic bacterium that anaerobically metabolized ethanol to acetate and dihydrogen and a methanogenic bacterium that used the dihydrogen to reduce carbon dioxide (6). The suggestion was made by Bryant et al. (6) that the degradation of fatty acids in methane-producing fermentations may require a similar mutualistic association between fatty acid-utilizing bacteria and methanogenic bacteria.

In 1979, McInerney et al. (22) isolated an anaerobic bacterium that metabolized short-chain fatty acids, 4 to 8 carbons in length, with the production of dihydrogen and other fermentation products. This bacterium, subsequently named *Syntrophomonas wolfei* (21), was grown in syntrophic coculture with a dihydrogen-utilizing bacterium. The coculture is a mutualistic association between *S. wolfei*, which requires a low partial pressure of dihydrogen, and the dihydrogen-utilizing bacterium, which requires the dihydrogen as an energy source (21, 22). A second syntrophic bacterium, *Syntrophobacter wolinii*, was isolated by Boone and Bryant (5). *Syntrophobacter wolinii* metabolized only propionate when grown in syntrophic coculture. Acetate was degraded to methane in a nonacetoclastic manner by a syntrophic association isolated from a thermophilic digester (36). Thermophilic digesters were also the source of isolation of butyrate-utilizing syntrophic bacteria by Henson and Smith (15) and more recently by Ahring and Westermann (1).

Compounds other than fatty acids have been shown to be degraded as the result of syntrophic associations. A consortium of bacteria was required for anaerobic degradation of benzoate to methane (11). An anaerobic bacterium capable of benzoate degradation to acetate and, presumably, CO_2 and H_2 or formate was isolated in syntrophic association with a sulfate-reducing or methane-producing bacterium (26). This bacterium was named *Syntrophus buswellii* (25). A complex microbial consortium that degrades 3-chlorobenzoic acid involves seven bacteria, including a syntrophic benzoate-utilizing bacterium, a sporeforming butyrate-utilizing syntrophic bacterium, and a butyrate-utilizing syntrophic bacterium that does not form spores (29). *Syntrophococcus sucromutans*, isolated from a bovine rumen, grew with carbohydrates as the electron donor and a variety of electron acceptors or in coculture with a *Methanobrevibacter* sp. (19).

These syntrophic associations are indicative of the complex and coupled metabolic interactions that occur in anaerobic environments. Syntrophic bacteria have not been well characterized because they are difficult to isolate and slow growing and do not grow to high cell densities in the laboratory. Therefore, this study was undertaken to further the biochemical characterization of these unusual bacteria by determining the lipid characterization of *S. wolfei*. In addition, with the knowledge of the lipid composition of *S. wolfei*, anaerobic environments could be examined for its presence without having to isolate and grow this fastidious organism. A complete discussion of the analytical techniques for lipid characterization and quantitation useful in the study of microbial ecology can be found in reference 32.

MATERIALS AND METHODS

Chemicals. Methanol and chloroform were Baker Resianalyzed and used as purchased (American Scientific Products, McGaw Park, Ill.). Acetone was analytical grade (Fisher Scientific Products, Pittsburgh, Pa.). Poly-β-hydroxybutyrate (PHB) was the kind gift of R. M. Lafferty, Graz, Austria.

Organisms. *S. wolfei* was cultivated as previously described (22) for growth in coculture with *Methanospirillum hungatei* or *Desulfovibrio* sp. strain G-11. The *S. wolfei-Desulfovibrio* sp. coculture was a gift of Marvin Bryant,

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University of Illinois. Cells of *S. wolfei* grown in coculture with *M. hungatei* were selectively lysed with lysozyme, and *M. hungatei* cells were removed by centrifugation (35). Cell-free extracts of *S. wolfei* prepared by this method have little contamination from cellular components of the methanogen (35). *S. wolfei* was grown in pure culture on crotonate as previously described (2).

Lipid extraction and chemical procedures. Lipids were extracted by a modification (34) of the Bligh and Dyer method (3). Total lipids were separated on a silicic acid column (Unisil, 1 g, 100/200 mesh; Clarkson Chemical Company, Inc., Williamsport, Pa.), in a 0.45-cm (inner diameter) by 29-cm (length) glass buret column, into neutral, glyco-, and phospholipids by elution of 10 ml each of chloroform, acetone, and methanol, respectively. These solvents were removed by a stream of dinitrogen, and the lipids were stored in Teflon-lined, screw-cap tubes at −20°C. The phospholipids were subjected to mild alkaline methanolysis (34) to produce fatty acid methyl esters (FAMEs). The FAMEs were separated from nonsaponifiable lipids on thin-layer chromatography plates (Whatman K6 silica gel, 250-μm thick, 20 by 20 cm) which were developed in nanograde hexane-diethyl ether (1:1, vol/vol) as previously described (13). The solvent was removed by a stream of dinitrogen, and the FAMEs were stored at −20°C.

Gas-liquid chromatography. A Varian 3700 gas chromatograph was used for analysis of FAMEs. The FAMEs were dissolved in hexane for injection in the splitless mode, with the vent opening after 30 s. The FAMEs were separated on a nonpolar cross-linked methyl silicone fused silica capillary column (50-m length by 0.2-mm inner diameter; Hewlett-Packard Co., Palo Alto, Calif.). The oven temperature was programmed from 80 to 140°C at the rate of 20°C per min and then at 4°C per min to 260°C, where it was maintained for 7 min. The injector and detector temperatures were 250 and 290°C, respectively. Hydrogen was the carrier gas. Tentative identification of the peaks was based on coelution of fatty acid standards (Supelco, Inc., Bellefonte, Pa.) or laboratory standards identified previously. The area of each peak was determined with a Hewlett-Packard 3350 series programmable laboratory data system operated with an internal standard program.

Gas chromatography-mass spectrometry. A Hewlett-Packard 5996A gas chromatography-mass spectrometry system was used to verify the identification of the fatty acids. The same capillary column type as described above was used. Samples were injected in the splitless mode with the vent opening after 30 s. The initial oven temperature was 100°C and was programmed to 300°C at 4°C per min. The carrier gas was helium. The following operating conditions of the mass spectrometer were used: electron multiplier voltage, 1,600 V; transfer line, 300°C; source and analyzer, 250°C; automotive file, decafluorotriphenylphosphine (DFTPP) normalized; electron impact energy, 70 eV.

Determination of the position of monounsaturation and geometry by using the Diels-Alder reagent and the ring position in the cyclopropyl fatty acids were as previously described (13).

PHB analysis. The analysis of PHB in the glycolipid fraction of the lipids was as previously described (12).

Fatty acid designation. Fatty acids are designated as (number of carbon atoms:number of double bonds) with the location of double bonds in relation to the aliphatic (ω) end of the fatty acid. The suffixes "c" and "t" refer to the *cis* and *trans* configurations, respectively, for the fatty acids. The prefix "Br" refers to branched fatty acids. The prefix "Cy" refers to cyclopropyl fatty acids with the ring position in parentheses relative to the aliphatic end.

TABLE 1. Composition of ester-linked PLFAs in *S. wolfei* cocultured with *M. hungatei* or as a pure culture grown on crotonate

PLFA	Amt[a] (mol%) of PLFAs in culture:		
	S. wolfei	Lysozyme-treated *S. wolfei-M. hungatei*	*S. wolfei-M. hungatei*
13:0	ND[b]	3.3	1.0
Br 14:0	0.2	ND	ND
14:1ω7	2.4	ND	ND
14:1ω5	ND	3.1	0.8
14:0	12	2.6	0.2
iso 15:0	0.9	1.6	0.6
anteiso 15:0/15:1ω8	1.8	1.8	1.6
15:1ω6	0.9	ND	ND
15:1	0.3	ND	ND
15:0	2.9	1.2	1.0
iso 16:0	0.3	0.7	1.2
16:1ω9c	16	ND	ND
16:1ω7c	38	6.9	0.6
16:1ω7t	0.7	ND	ND
16:1ω5c	0.1	1.1	0.2
16:0	16	27	36
Br 16:0	ND	2.6	2.1
Br 17:1	1.1	ND	ND
Br 17:1	0.4	ND	ND
iso 17:0	0.04	ND	0.7
anteiso 17:0/17:1ω8	0.4	3.4	3.6
17:1ω6	0.5	ND	ND
Cy 17:0ω7,8	ND	ND	3.7
17:0	0.3	0.8	0.7
18:2ω6	0.6	7	10
iso 18:0	0.1	ND	ND
18:1ω9c	0.4	11	23
18:1ω7c	2.0	4.1	5.1
18:1ω7t	0.2	0.2	0.2
18:0	1.0	4.5	4.5
Cy 19:0	ND	1.1	1.1

[a] The total (dry weight) PLFAs for *S. wolfei*, lysozyme-treated *S. wolfei-M. hungatei*, and untreated *S. wolfei-M. hungatei* were 18.9, 2.6, and 3.9 μmol g^{-1}, respectively.
[b] ND, Not detected.

RESULTS

Phospholipid fatty acids. The phospholipid fatty acids (PLFAs) recovered from *S. wolfei* grown in pure culture or in coculture with *M. hungatei* are presented in Table 1. The PLFAs are in the C_{14} to C_{19} chain length range reported commonly for eubacteria (18, 20, 28). The lipid profile and the amounts of PLFAs varied with the culture conditions. The amounts of PLFAs ranged from 2.6 to 18.9 μmol g^{-1} (dry weight), which were less than the range of 45 to 60 μmol of dry weight g^{-1} reported to be typical for many eubacteria (33). The *S. wolfei-M. hungatei* coculture yielded the lowest amounts of total PLFAs.

The predominant PLFA for *S. wolfei*, grown with *M. hungatei*, was 16:0 (Table 1). When *S. wolfei* was grown as a pure culture on crotonate, the PLFAs 14:0 and 16:0 constituted 28% of the total PLFAs. The even-numbered fatty acids for *S. wolfei* under these conditions constituted a larger percentage of the total PLFAs (see Table 4). Growth on crotonate (*trans*-2-butenoic acid) resulted in the monounsaturated PLFA 16:1ω7c as the predominant PLFA (Table 1). In general, the profiles for the *S. wolfei-M. hungatei*

TABLE 2. Composition of ester-linked PLFAs in the coculture of *Desulfovibrio* sp. strain G-11 and *S. wolfei* grown on butyrate or valerate

PLFA	Amt[a] (mol%) of PLFA in coculture grown on:	
	Butyrate	Valerate
Br 14:0	0.3	0.4
14:1ω7	0.4	0.1
14:0	6.7	1.2
iso 15:0	12	10
anteiso 15:0/15:1ω8	2.3	4.8
15:1ω6	0.1	3.8
iso 15:1	0.8	0.8
15:0	1.2	27
iso 16:0	0.4	0.3
16:1ω9	0.7	0.7
16:1ω7c	4.8	0.2
16:1ω7t	0.2	ND[b]
16:1ω5c	1.1	5.7
16:0	30	5.2
Br 17:1	0.7	ND
Br 17:1	0.2	0.2
iso 17:0	0.5	0.4
anteiso 17:0/17:1ω8	0.4	2.3
17:1ω6	ND	2.0
Cy 17:0ω7,8	17	1.2
iso 17:1ω7	3.0	3.6
17:0	0.5	6.8
iso 18:0	0.2	0.2
18:2ω6	0.5	0.5
18:1ω9c	0.5	0.5
18:1ω7c	0.7	0.4
18:1ω7t	0.8	9.5
18:1ω5c	0.1	0.1
iso Cy 18:0ω8,9	12	10
18:0	1.7	1.3

[a] The total (dry weight) PLFAs for butyrate- and valerate-grown cultures were 69.5 and 66.2 μmol g^{-1}, respectively.
[b] ND, Not detected.

cocultures were similar without regard to lysozyme treatment.

S. wolfei was also examined in coculture with *Desulfovibrio* sp. strain G-11, with the results shown in Table 2. The *S. wolfei* coculture with *Desulfovibrio* sp. strain G-11 contains PLFAs from each member of the coculture. These two cocultures grown on different substrates contain a large contribution of the iso- and anteiso-branched-chain fatty acids. The percentage of branched-chain PLFAs in the coculture with *Desulfovibrio* sp. strain G-11 was 20 to 23%, which was two to four times that found in the coculture with *M. hungatei* or when *S. wolfei* was grown as a pure culture on crotonate (Table 3). Branched-chain PLFAs such as iso 15:0 and iso 17:1ω7c are found in *Desulfovibrio desulfuricans* (4, 30) and were possibly contributed to the coculture by *Desulfovibrio* sp. strain G-11, although the lipid composition of this organism is unknown. Branched-chain PLFAs made minor contributions to the total PLFAs (Table 3) of the coculture with *M. hungatei* or the pure culture of *S. wolfei* grown on crotonate with iso 15:0 and 17:1ω7c contributing less than 1% of the total PLFAs (Table 1).

TABLE 3. Contribution of various types of fatty acids to the ester-linked PLFAs in *S. wolfei* cocultured with *M. hungatei* and as a pure culture growing on crotonate

Type of fatty acid[b]	% of fatty acid type in total PLFAs in culture:		
	S. wolfei	Lysozyme-treated *S. wolfei-M. hungatei*	*S. wolfei-M. hungatei*[a]
Saturated	32.4	45.4	43.0
Monounsaturated	61.7	36.3	29.2
Branched chain	5.3	10.1	9.9
Cyclopropyl	0	7.3	3.7

[a] Growth substrate for the coculture was butyrate.
[b] Each class of PLFAs is exclusive, i.e., the branched-chain saturated and branched-chain monounsaturated PLFAs are included only in the branched-chain category and not in both categories.

The predominant PLFA in each coculture or in a culture of *S. wolfei* alone seemed to be dependent on the carbon source available to the organisms. In the *S. wolfei-Desulfovibrio* sp. strain G-11 coculture grown on butyrate, the PLFA 16:0 was predominant, whereas when the coculture was grown on valerate, the predominant PLFA was 15:0. Growth of the coculture on the 4- or 5-carbon substrates resulted in a PLFA distribution that reflected the original substrate (Table 4). The ratio of even- to odd-numbered fatty acids was about 4 to 1 in the coculture grown on butyrate, whereas this ratio was reversed, about 1 to 3, when the grown substrate was valerate.

The diunsaturated PLFA 18:2ω6 was detected in all samples (Tables 1 and 2). Three of the four samples had about 0.5 mol%, whereas the sample from the *M. hungatei* coculture had 7 to 10 mol%. Another polyunsaturated PLFA (18:3ω3, data not shown) was also detected in the sample from this coculture (1.6 mol%). The PLFAs 18:2ω6 and 18:3ω3 were detected in the medium, which contained 5% ruminal fluid, in concentrations of 2.9 and 0.7 nmol g^{-1}. In addition, the *S. wolfei-M. hungatei* coculture also had the PLFA 18:1ω9c as a predominant component (Table 1). This PLFA was present in the medium (7.1 nmol g^{-1}) as well. The presence of these fatty acids as free fatty acids in the ruminal fluid medium was not determined.

TABLE 4. Comparison of numbers of even- and odd-numbered chain lengths of PLFAs in *S. wolfei* cultures grown in differing electron-donating and -accepting conditions

Culture	Chain length found				Ratio of even- to odd-numbered chains
	Even numbered		Odd numbered		
	pmol	% of total	pmol	% of total	
S. wolfei alone	17,096	90	1,820	10	9.6
S. wolfei-Desulfovibrio sp. with:					
Butyrate	54,800	79	14,724	21	3.7
Valerate	18,349	28	47,812	72	0.38
Lysozyme-treated *S. wolfei-M. hungatei*	2,056	80	527	20	4.0
S. wolfei-M. hungatei with butyrate	3,526	90	369	10	9.4

TABLE 5. Characteristic ion fragments of derivatives formed upon reaction of the monounsaturated PLFA methyl esters with dimethyl disulfide[a]

FAME	Ion fragment[b] (m/z) of dimethyl disulfide adducts			
	M+	ω Fragment	Δ Fragment	Δ-32 Fragment
14:1ω9			161	129
14:1ω7	334	145	189	157
Br 15:1			203	171
15:1ω8	348	159	189	157
15:1ω6	348	131	217	185
16:1ω9	362	173	189	157
16:1ω7c	362	145	217	185
16:1ω7t	362	145		185
17:1ω8	376	159	217	185
17:1ω6	376	131	245	213
18:1ω9	390	173	217	185
18:1ω7	390	145	245	213

[a] The data are from a culture of *S. wolfei* grown on crotonate.
[b] ω, Fragment including the aliphatic end of the molecule; Δ, fragment including the carboxylic end of the molecule.

Table 5 presents the characteristic ion fragments for the monounsaturated PLFA for *S. wolfei* grown on crotonate. These data confirm the position and geometry of unsaturation shown in Table 1.

PHB. The bacterial endogenous storage polymer PHB has been shown to be present in *S. wolfei* (21), and it was quantitated in this study. The *S. wolfei-Desulfovibrio* strain G-11 coculture grown on butyrate had 0.14 μmol mg^{-1}, and the *S. wolfei* pure culture grown of crotonate had concentrations of PHB ranging from 320 to 589 μmol mg^{-1}. The *S. wolfei-Desulfovibrio* strain G-11 coculture value is much lower because the total dry weight of the coculture is composed primarily of the *Desulfovibrio* sp.

DISCUSSION

The use of bacterial membrane phospholipid characterizations as a synecological tool requires a library of signature fatty acids for the interpretation of data from environmental samples. This report is the first collection of data from the group of anaerobic bacteria collectively known as syntrophic bacteria. These organisms play an important role as the intermediate members in the hierarchy that completely degrades organic matter, in the absence of sulfate, nitrate, and light, to methane and carbon dioxide. In addition, the precise determination of the PLFAs of *S. wolfei* may provide a better understanding of the physiology and phylogeny of this bacterium and similar bacteria.

Muramic acid and *meso*-diaminopimelic acid are present in *S. wolfei* (21), indicating the eubacterial nature of the bacterium. The presence of ester-linked fatty acids in the membrane phospholipids is additional evidence that this bacterium is an eubacterium. The overall pattern of PLFAs was typical of other eubacteria (24) in that the fatty acids were predominantly saturated or monounsaturated (Table 3). The branched-chain fatty acids found in the *S. wolfei* cocultures with the *Desulfovibrio* sp. (Table 2) probably reflect the contribution of these fatty acids by the *Desulfovibrio* sp. Iso 17:1ω7 is a major component of the PLFAs in other *Desulfovibrio* spp. (9, 30) and was found in the *S. wolfei-Desulfovibrio* sp. cocultures (Table 2) although not as a major component.

The *S. wolfei-M. hungatei* coculture had very little total biomass to be analyzed (Table 1). The quantification and identification of PLFAs from this sample, therefore, are tentative. The decreased biomass suggests that the growth of *S. wolfei* in concert with *M. hungatei* must be minimal. The membrane phospholipids of *M. hungatei* contributed very little to the biomass reported in Table 1 as they are primarily ether linked (8, 31), and the mild alkaline methanolysis conditions used in this study saponify only ester-linked phospholipids. Also, a similar lipid composition was obtained when cell-free extracts of *S. wolfei*, which had little contamination from the methanogen, were analyzed.

The pure culture of *S. wolfei* grown on crotonate provided more biomass for analysis and is a record of *S. wolfei* only (2). The predominant PLFAs of *S. wolfei* grown under these conditions are the monounsaturated PLFAs 16:1ω7c and 16:1ω9c, with 16:0 and 14:0 as the major saturated PLFAs (Table 1). In addition, the diunsaturated PLFA 18:2ω6 was detected in a low concentration (Table 1). Diunsaturated PLFAs are reported in procaryotes and have been reported in significant quantities in marine vibrios (14, 27). Polyunsaturated PLFAs are not common to procaryotes, with the exception of cyanobacteria (10), deep-sea isolates (7), and a marine *Flexibacter* sp. (17). The polyunsaturated PLFA 18:3ω3 was found consistently when *S. wolfei* was grown in coculture with *M. hungatei*, but it was not found in the pure culture of *S. wolfei*. This PLFA may have been incorporated from the ruminal fluid medium, where it was present in a very low concentration. The polyunsaturated PLFAs 18:3ω3 and 18:3ω6 were observed in the phospholipid fraction of the microbial community from anaerobic digesters (16) that served as a source of isolation for a thermophilic butyrate-utilizing syntroph (15). This environment would be similar to that from which *S. wolfei* was isolated (21–23). At this time, it is not known whether *S. wolfei* can synthesize the polyunsaturated PLFAs de novo or whether this organism is capable of incorporating polyunsaturated fatty acids from its environment.

With additional biochemical and genetic examinations of other syntrophic bacteria as well as of other obligate anaerobes, an understanding of the phylogenetic relationships of these bacteria will be developed. The phospholipid profiles of these bacteria will aid in the strengthening of the understanding of the phylogenetic relationships established by these determinations. In addition, the lipid profiles will add to the list of signature fatty acids that may indicate the presence of or absence of a bacterium, or a similar bacterium, within a microbial community. The present report of the phospholipid profile of *S. wolfei* aids both of these pursuits.

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