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Fatty Acid-Related Phylogeny of Myxobacteria as an Approach to Discover Polyunsaturated Omega-3/6 Fatty Acids▽†

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In an analysis of 47 aerobic myxobacterial strains, representing 19 genera in suborders *Cystobacterineae*, *Nannocystineae*, *Sorangiineae*, and a novel isolate, "*Aetherobacter*" SBSr008, an enormously diverse array of fatty acids (FAs) was found. The distribution of straight-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs) supports the reported clustering of strains in the phylogenetic tree based on 16S rRNA genes. This finding additionally allows the prediction and assignment of the novel isolate SBSr008 into its corresponding taxon. *Sorangiineae* predominantly contains larger amounts of SCFA (57 to 84%) than BCFA. On the other hand, *Cystobacterineae* exhibit significant BCFA content (53 to 90%), with the exception of the genus *Stigmatella*. In *Nannocystineae*, the ratio of BCFA and SCFA seems dependent on the taxonomic clade. Myxobacteria could also be identified and classified by using their specific and predominant FAs as biomarkers. *Nannocystineae* is remarkably unique among the suborders for its absence of hydroxy FAs. After the identification of arachidonic (AA) FA in *Phaselicystidaceae*, eight additional polyunsaturated fatty acids (PUFAs) belonging to the omega-6 and omega-3 families were discovered. Here we present a comprehensive report of FAs found in aerobic myxobacteria. Gliding bacteria belonging to *Flexibacter* and *Herpetosiphon* were chosen for comparative analysis to determine their FA profiles in relation to the myxobacteria.

Myxobacteria are one of nature's "talented" and widely distributed microorganisms commonly found in both terrestrial and aquatic ecosystems. They are Gram-negative, rod-shaped eubacteria famous for their unique developmental cycle, culminating in the formation of multicellular fruiting bodies (see Fig. S1 in the supplemental material), which serve as an important basis for myxobacterial classification (39). This group (*Myxococcales*) has also gained attention and fame for production of novel anti-infective drugs and chemotherapeutic agents with uncommon modes of action (53, 54). Surprisingly, myxobacteria were also described for their potential to produce polyunsaturated fatty acids (PUFAs). Although these are rare in bacteria, their production has already been detected in the marine myxobacterial genera *Plesiocystis*, *Enhygromyxa*, and *Haliangium* (8, 19, 20) and recently in the soil isolate *Phaselicystis* (12).

PUFAs are commercially valuable and essential for human health (50, 52). Omega-3 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are of special significance to pharmaceutical and food industry. These fatty acids (FAs) are commonly used as supplements in food and dairy products, and as a drug (e.g., for treatment of hypertriglyceridemia). They play a role in the prevention of many heart-associated diseases (50) and are involved in many immune-inflammatory reactions (9) and brain development (14). Due to their significant benefits to human health, demands are constantly increasing (50). Alternative sources have been explored with various degrees of success, such as the straminopiles *Schizochytrium*, *Thraustochytrium*, and *Saprolegniales*; the fungal *Entomophthorales* (46); the marine piezophilic bacteria *Shewanella*, *Colwellia*, *Moritella*, and *Psychroflexus* (5, 36, 37); and poikilothermic animals, deep-sea fish, and arctic invertebrates (24, 56).

Surprisingly large amounts of EPA and DHA were found in one of our novel *Sorangiineae* isolates, "*Aetherobacter*" SBSr008 (49), described below, prompting us to determine their presence and distribution in aerobic myxobacteria and in morphologically related gliding, nonfruiting bacteria. In order to establish chemo-phylogeny correlations, different aspects of fatty acid content (e.g., FA types, FA ratio, and major markers) were considered in relation to taxonomic clades or taxa. The overall findings update and expand the previous study on FAs of *Nannocystis* (*Nannocystineae*), *Myxococcus*, *Cystobacter*, *Stigmatella* (*Cystobacterineae*), and *Sorangium* (*Sorangiineae*) (7). This study also aims to analyze, in addition to DHA and EPA, other polyunsaturated FAs found in myxobacteria.

MATERIALS AND METHODS

Bacterial strains and cultivation. The type and neotype strains chosen to represent the whole aerobic myxobacteria are listed in Table S1 in the supplemental material. The majority were obtained from our collection at the Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany. *Archangium gephyra* DSM2261^T, *Corallococcus coralloides* DSM2259^T, *Cystobacter* (*Angiococcus*) *disciformis* DSM52716^T, *Enhygromyxa salina* DSM 15217^T, *Haliangium ochraceum* DSM 14365^T, *Haliangium tepidum* DSM 14436^T, proposed neotype *Melittangium lichenicola* DSM14877^T (30), *Myxococcus fulvus*

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DSM16525^T, *Myxococcus virescens* DSM2260^T, *Myxococcus xanthus* DSM16526^T, *Plesiocystis pacifica* DSM14875^T, *Stigmatella aurantiaca* DSM17044^T, and *Stigmatella erecta* DSM16858^T were purchased from the German Culture Collection (DSMZ) in Braunschweig. The novel isolate *Aetherobacter* SBSr008 was obtained from Helmholtz Institute for Pharmaceutical Research (HIPS) in Saarbrücken, Germany. Strains of the gliding bacteria *Herpetosiphon* (Hp g287, Hp g336, Hp g383, and Hp g454) and *Flexibacter* (Flex 23 and Flex 7014) were generously provided by Klaus Gerth, Microbial Drugs, HZI.

Myxobacteria were cultivated in 50 ml of medium in 300-ml flasks shaken at 170 rpm at 30°C (37°C for *Haliangium tepidum*). The media used are as follows: MD1 (45) for *Archangium*, *Corallococcus*, *Hyalangium*, *Melittangium*, *Myxococcus*, *Nannocystis*, *Stigmatella*, *Kofleria*, *Cystobacter disciformis*, and *C. armeniaca*; M medium (35) for all other *Cystobacter* strains; TS6 medium (1% tryptone [Difco], 0.6% soluble starch [Roth], 25 mM HEPES, pH 7.0) for *Pyxidicoccus fallax*; VY/2 (10) for *Byssovorax* (with maltose) (28, 40), *Polyangium*, *Chondromyces*, and *Jahnella*; HS medium (27) for *Sorangium*; VY/4-SWS (with 50% of the concentration of baker's yeast as in VY/2-SWS) (19, 20) for *Enhygromyxa* and *Plesiocystis*; and CY-SWS (18) for *Haliangium.* Strains of *Flexibacter* and *Herpetosiphon* were cultivated in LB broth and VY/2 agar, respectively. *Phaselicystis flava* and *Aetherobacter* SBSr008 were not cultivated in this study; instead, FA data for these species were obtained from earlier studies (11, 49).

Fatty acid extractions, GC-MS, and data analysis. *Cystobacter miniatus*, *M. lichenicola*, and strains of *Herpetosiphon* did not grow well in their corresponding liquid media; hence, only their biomass scraped off on VY/2 agar plates was used for extraction. For other strains, 2-ml aliquots of broth cultures were centrifuged (5,000 rpm, 5 min, 20°C) and cell pellets were dried completely with a speed vacuum for 30 min at 60°C. FA extraction by the fatty acid methyl ester (FAME) method was performed accordingly (3), except that the whole-cell pellet was not resuspended in NaCl solution prior to drying in a vacuum concentrator. Gas chromatography-mass spectrometry (GC-MS) analysis of derivatized samples was also performed according to a previous study (3), with some changes in the parameter settings. The column temperature was kept at 130°C for 2.5 min and then increased to 240°C at 5°C/min. The mass selective detector was operated in scan mode, with a scanning mass range of *m/z* 40 to 500.

16S rRNA gene amplification and phylogenetic tree construction. Extraction and purification of genomic DNA, amplification and sequencing of 16S rRNA genes, sequence alignments, calculations of distance matrices and bootstrap values for 1,000 replicates, and construction of phylogenetic tree were performed as previously described (12). The neighbor-joining trees in Fig. 2 are based on type strains, the holotype strain, and the novel isolate SBSr008. GenBank accession numbers of myxobacterial sequences and outgroup *Desulfovibrio desulfuricans* are listed in Table S1 in the supplemental material. The 11 sequenced strains (GenBank accession no. GU249615, GU207872 to GU207876, and GU207878 to GU207882) were previously described (12). To establish chemophylogeny correlations in the 16S DNA tree, different aspects of FA analysis were considered, which include their ratio (e.g., straight-chain FA [SCFA]/branched-chain FA [BCFA] ratio), types (hydroxy FA and PUFAs), and another determinant marker ($C_{17:1\ 2\text{-}OH}$). In addition, major FAs common to specific genera or clades were assigned based on their highest (2–5) percentage values. The same considerations were applied in some species that do not share common major FAs.

RESULTS AND DISCUSSION

Numerous FAs were detected by GC-MS in all 47 strains of myxobacteria studied. Thirty-two were identified in *Nannocystineae* and 44 in *Cystobacterineae.* In *Myxococcaceae* alone, from the latter suborder, 34 were identified, approximately the same number as reported earlier (1, 2, 6). Most surprising was the discovery of 49 FAs in *Sorangiineae*. To our knowledge, the only previous extensive analytical studies of FA profiles in this suborder were performed on strains of *Sorangium cellulosum* (So ce14 [7] and AJ 13585 [8]), and *Phaselicystis flava* (11). In contrast to the majority of other bacteria, which have only simple (only a few types) FAs (25), myxobacteria appeared far more creative in their biosynthesis of diverse FAs, including those rarely encountered in nature.

Moreover, the occurrence of FA types (see Fig. S2 in the supplemental material) was discovered to be correlated with taxonomic placements of the genera of myxobacteria. SCFAs were dominant in *Sorangiineae* and BCFAs in *Cystobacterineae* (except in *Stigmatella*), and both were found in *Nannocystineae.* Within *Nannocystineae*, the total BCFA content was higher than SCFA in the *Kofleriaceae-Haliangiaceae* clade and vice versa in the *Nannocystaceae* clade.

Suborder *Cystobacterineae*. The suborder *Cystobacterineae* has the highest number of known species (39), all of which are far easier to isolate and maintain in culture than their counterparts in *Nannocystineae* and *Sorangiineae.* It is remarkable for its large amount of BCFAs (53 to 90%), except for *Stigmatella*, whose FA diversity also diverged from most members of the *Cystobacterineae* in the phylogenetic tree. This suggests that it might need to be accommodated in a separate family, as already considered in the preceding phylogenetic paper (12).

***Myxococcus*, *Corallococcus*, and *Pyxidicoccus* clusters.** Certain species of *Corallococcus* and *Myxococcus* have been used as the preferred models in most FA biosynthesis (1, 4, 42) and developmental studies (2, 13, 26, 41). In *Pyxidicoccus*, the FA composition was evaluated here for the first time. These three genera formed a phylogenetically coherent group with 99.5% bootstrap support. The *M. virescens-M. xanthus-M. macrosporus* clade had a much higher ratio of BCFAs to SCFAs than the *M. fulvus-M. stipitatus* clade (Table 1); this was reflected in their phylogenetic divergence (Fig. 1).

iso-$C_{15:0}$ was found to be the major FA (23.1 to 63.5%) in *Myxococcaceae*, as also determined previously (6, 7, 34, 44, 51, 55), and was the FA in the largest amount observed in *Myxococcus xanthus*. This finding was in agreement with those for DK1622 (2) and several other strains (29, 31). The lower percentages in *M. stipitatus-M. fulvus* (23 to 32%) and *Pyxidicoccus* (44%) were reflected in the phylogenetic clustering. *Corallococcus* also had low *iso*-$C_{15:0}$ (34 to 36%), and it is divergent from *Myxococcus*.

Straight-chain $C_{16:1\omega5c}$ ranks as the second-most-abundant FA (7 to 19%) in *Myxococcaceae*, except *Corallococcus*, which contains a maximum of 1% (Table 1). Its low content affirms the previous reports (31, 48). On the other hand, the significant amounts (23.5 to 27.8%) of *iso*-$C_{17:0\ 2\text{-}OH}$ indicate that it could be a determinative marker for *Corallococcus* in *Myxococcaceae* family. *Myxoccoccus* had only 0.5 to 4% of this FA (Table 1), in contrast to the high value found in the *M. fulvus* Mx f2 non-type strain (7). This may be explained by strain-specific differences, as our findings on type strains are in perfect agreement with more recent studies (29, 31). *Corallococcus* also differed from *Myxococcus* in the absence of straight-chain hydroxy and *O*-alkylglycerol (OAG) FAs; however, both genera shared small amounts of *iso*-$C_{17:1\omega11c}$ and diunsaturated *iso*-$C_{17:2\omega5,11,\ all\ cis}$.

C. exiguus differed from *C. coralloides* in the absence of *iso*-$C_{15:0}$ OAG and dimethylacetal (DMA). The latter compound was shown to be derived from an aldehyde by a reduction process from *iso*-$C_{15:0}$ and was found to increase during the first 24 h of development in *Myxococcus xanthus* DK1622 (40). OAG, on the other hand, was determined to be a monoacylglycerol derivative (MAG) compound (12). These *iso*-FAs, OAG and DMA, were shown to be important ether lipid-derived compounds contributing significantly to fruiting body formation in DK1622 (13, 41).

TABLE 1. Fatty acid distribution in *Cytobacterineae* genera *Pyxidicoccus*, *Myxococcus*, and *Corallococcus*

FA type	% of FA in[a]:							
	P. fallax	*M. fulvus*	*M. stipitatus*	*M. virescens*	*M. xanthus*	*M. macrosporus*	*C. coralloides*	*C. exiguus*
SCFAs								
$C_{13:0}$				0.21				
$C_{14:0}$	4.59	2.75	6.36	4.70	4.93	4.60		0.21
$C_{14:1\omega5c}$	1.03	0.51	0.41	1.27	0.75	0.61		
$C_{15:0}$	4.02	0.77	1.59	1.36	0.29	0.26	0.10	0.06
$C_{15:1}$	5.92	0.54	0.35	3.95	0.34			
$C_{16:0}$	4.20	9.31	14.59	1.03	1.44	5.42	1.11	0.91
$C_{16:1\omega5c}$	**19.09**	**16.92**	**14.57**	**7.33**	**8.60**	**11.67**	0.28	1.12
$C_{16:1\omega9c}$						0.11		
$C_{16:1\omega11c}$		2.51	0.56	0.30		1.03		
$C_{17:0}$		0.07						
$C_{18:0}$	1.40	0.56	0.73	0.34	0.74	0.42	1.13	0.79
$C_{18:1\omega9c}$		0.62						
PUFAs								
$C_{16:2}$	2.55	1.86	1.21	3.45	2.00	3.15		
$C_{18:2\omega6,9,}$ all *cis*		2.70						
$C_{18:3\omega6,9,12,}$ all *cis*		1.25						
Hydroxy FAs								
$C_{13:0\ 3\text{-OH}}$								
$C_{14:0\ 3\text{-OH}}$		0.34	0.11	0.27	0.13	0.38		
$C_{15:0\ 3\text{-OH}}$				0.07				
$C_{16:0\ 2\text{-OH}}$		0.16	0.17			0.13		
$C_{16:0\ 3\text{-OH}}$		0.08	0.06	0.17	0.07	0.18		
Total SCFAs	42.80	40.92	40.72	24.45	19.29	27.96	2.62	3.09
BCFAs								
iso-$C_{13:0}$		0.93	1.24	0.58	0.79	0.68	2.76	3.74
iso-$C_{14:0}$							0.70	0.68
iso-$C_{15:0}$	**44.43**	**23.12**	**32.01**	**55.25**	**63.48**	**42.59**	**33.90**	**36.45**
iso-$C_{15:1\omega9c}$				0.95	0.36		0.89	0.38
iso-$C_{16:0}$	1.32	0.25	0.42	0.18	0.19	0.40	2.99	1.76
iso-$C_{17:0}$	2.66	11.70	13.48	4.22	4.15	9.57	**9.35**	**10.69**
iso-$C_{17:1\omega5c}$	1.95	2.95	2.54	2.17	3.26	1.88	**7.99**	**14.72**
iso-$C_{17:1\omega11c}$		0.78	0.45	0.89	0.43	1.25	2.69	1.33
iso-$C_{17:2\omega5,11}$, all *cis*		0.44	0.59	2.32	1.50	1.29	2.79	2.19
anteiso-$C_{15:0}$								0.15
Branched-chain hydroxy FAs								
iso-$C_{15:0\ 3\text{-OH}}$	0.55	2.48	0.81	2.15	1.77	2.30	2.40	1.32
iso-$C_{17:0\ 2\text{-OH}}$		4.07	3.55	1.26	0.47	2.90	**27.83**	**23.50**
iso-$C_{17:0\ 3\text{-OH}}$				0.47	0.13	0.39		
Branched-chain OAG FAs								
iso-$C_{15:0}$		6.32	1.79	3.33	1.95	4.30	1.72	
Branched-chain DMA FA								
iso-$C_{15:0}$	6.29	6.04	2.41	1.79	2.24	4.49	1.37	
Total BCFAs	57.20	59.08	59.28	75.55	80.71	72.04	97.38	96.91

[a] Percentages of major fatty acids are distinguished in boldface type.

***Archangium-Cystobacter* cluster.** $C_{16:1\omega5c}$ was the major FA (21 to 27%) in *Archangium*, in agreement with the reported amount found in *A. gephyra* strain 65 (55). It was also the major FA in *Cystobacter*, with the exception of *C. armeniaca*, *C. miniatus*, *C. gracilis*, and *C.* (*Angiococcus*) *disciformis*. These four isolates, as represented by type strains in this study (see Table S1 in the supplemental material), contain higher total BCFAs (70 to 75%), specifically *iso*-$C_{15:0}$ (except *C. gracilis*) and *iso*-$C_{17:0}$, lower $C_{16:0}$, and show the presence of *anteiso*-$C_{17:0}$ (Table 2). The latter FA has been reported in several marine isolates (8, 19), but it was also discovered here in *Cystobacter* spp. *Cystobacter miniatus* also differs from other *Cystobacter* species and even from other members of its suborder through its high (15.3%) $C_{16:1\omega7c}$ content (Tables 1 to 3). The significant differences among FA profiles and polyphyletic position of these strains suggest their assignments to a novel genus, while *C. disciformis* should be reclassified back to its original genus, *Angiococcus* (22).

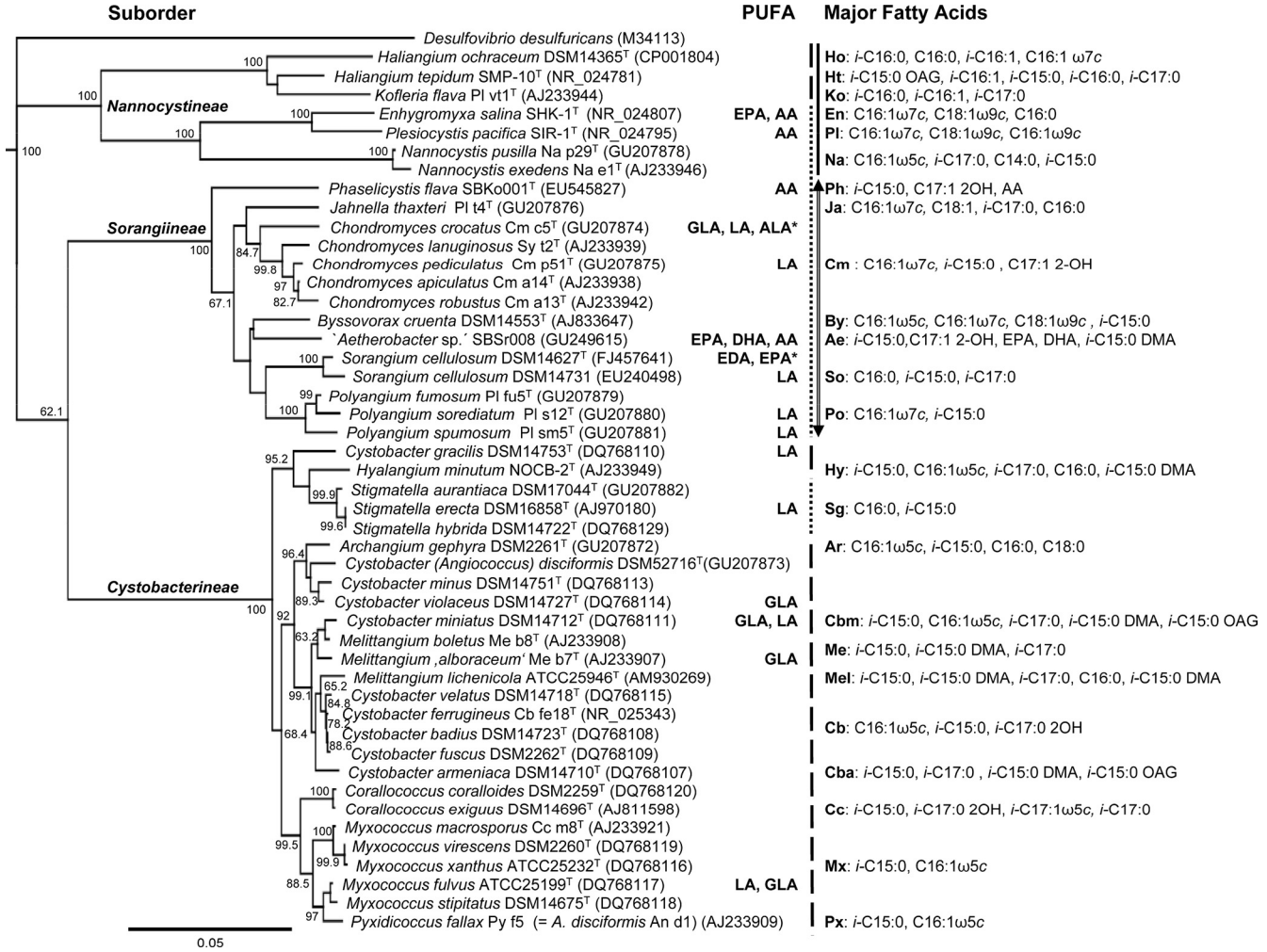

FIG. 1. Chemo-phylogenetic tree of myxobacteria constructed by the neighbor-joining method based on 16S rRNA gene sequences and correlated with the fatty acid profile. The biomarker (major) FAs common for the genus are indicated after each set of bacterial initials on the right side of the tree (Ae, *Aetherobacter*; Ar, *Archangium*; By, *Byssovorax*; Cb, *Cystobacter*; Cc, *Corallococcus*; Cm, *Chondromyces*; En, *Enhygromyxa*; Hy, *Hyalangium*; Ja, *Jahnella*; Ko, *Kofleria*; Me, *Melittangium*; Mx, *Myxococcus*; Na, *Nannocystis*; Ph, *Phaselicystis*; Pl, *Plesiocystis*; Po, *Polyangium*; Px, *Pyxidicoccus*; Sg, *Stigmatella*; So, *Sorangium*) or have been specified for some species that do not agree (Cba, *Cystobacter armeniaca*; Cbm, *Cystobacter miniatus*; Ho, *Haliangium ochraceum*; Ht, *H. tepidum*; Mel, *Melittangium lichenicola*). The tree also highlights the dominant FAs in the three suborders. Fine dotted lines show the predominance of straight-chained fatty acids (SCFAs), while big dotted lines indicate the predominance of the branch-chained fatty acids (BCFAs). The vertical thick line shows the myxobacterial clusters devoid of hydroxy fatty acids, while the arrow line shows clusters with $C_{17:1\ 2OH}$ FA. The tree also localizes the production of omega-3 and omega-6 PUFA-producing strains. The PUFAs were abbreviated as follows: AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid. Asterisks indicate the production of fatty acids in other isolates but only represented here by the type strain. *iso* fatty acids are indicated by "*i.*" The sequence of *Desulfovibrio desulfuricans* roots the tree. The numbers at branch points indicate the percentage of bootstrap support based on 1,000 resamplings. Only values greater than 60 are shown. Bar, 0.05 substitution per nucleotide position.

Archangium is distinct from *Cystobacter* in its higher content of $C_{18:0}$ (7.8%) and absence of straight-chain OAG and hydroxy FAs. However, the 1.2% $C_{18:0}$ and 29.5% $C_{19:0}$ found in *A. gephyra* strain 65 (55) were not detected in the type strain (DSM2261^T), thus suggesting a case of strain variations.

***Stigmatella-Hyalangium* cluster.** The monophyletic position of the *Stigmatella* clade is reflected in its FA pattern. *Stigmatella aurantiaca* had equal amounts (50%) of total SCFAs and BCFAs, while *S. erecta* and *S. hybrida* had much higher total SCFAs (62.9 to 76.5%). This pattern for the latter two species was supported by a high bootstrap value (99.6%) and tree topology (Fig. 1). The three type species shared large amounts of $C_{16:0}$, *iso*-$C_{15:0}$, and *iso*-$C_{17:0}$ FAs. The abundance of the latter two FAs in *S. aurantiaca* Sg a1 was previously reported (7). $C_{16:1\omega5c}$ and *iso*-$C_{17:0}$, both prominent in *Cystobacter*, were also found in considerable amounts in *Stigmatella*, but $C_{16:1\omega7c}$ (32%) was unique to *S. hybrida* (Table 3).

The bifurcation of *Hyalangium minutum* NOCB-2^T to *Stigmatella* was reflected in the ratio of SCFAs to BCFAs. The total level of BCFAs was much higher (65%), as in *Cystobacter*. The major FAs and their corresponding levels (*iso*-$C_{15:0}$, 21%;

TABLE 2. Fatty acid distribution among *Cystobacterineae* genera *Archangium* and *Cystobacter*

FA type	% of FA in[a]:										
	C. (*Angiococcus*) *disciformis*	*A. gephyra*	*C. badius*	*C. ferrugineus*	*C. fuscus*	*C. gracilis*	*C. velatus*	*C. minus*	*C. violaceus*	*C. armeniaca*	*C. miniatus*
SCFAs											
$C_{14:0}$	1.99	1.09	4.60	7.03	5.95	0.26	10.26	5.68	2.91	0.66	
$C_{14:1\omega5c}$	0.29		1.58	0.70	1.53	0.60	0.65	0.07	0.19	0.11	
$C_{15:0}$	0.13		0.30	0.21	0.50	0.23	0.52	0.33	0.36	0.26	3.73
$C_{15:1}$						0.31	0.07	0.10	0.13	0.63	
$C_{16:0}$	4.64	**12.55**	8.10	9.65	13.29	1.44	10.77	10.16	4.59	1.51	2.59
$C_{16:1\omega5c}$	**15.81**	**24.55**	**22.70**	**25.42**	**22.34**	**13.07**	**20.62**	**26.86**	**27.48**	6.12	1.31
$C_{16:1\omega7c}$						0.52					**15.27**
$C_{16:1\omega9c}$				0.11			0.15				
$C_{16:1\omega11c}$	0.61										
$C_{17:0}$						0.07					0.59
$C_{18:0}$	0.75	**7.78**	0.67	0.47	1.24	0.51	0.28	0.30	0.50	0.64	
$C_{18:1}$				0.32		0.21					
$C_{18:1\omega9c}$											1.63
PUFAs											
$C_{16:2}$	0.58										3.95
$C_{18:2\omega6,9,\text{ all } cis}$						0.27					0.55
$C_{18:3\omega6,9,12,\text{ all } cis}$									0.07	0.10	0.99
Hydroxy FAs											
$C_{14:0\ 3\text{-OH}}$	0.23		0.66	0.17	0.77	0.16	0.47	0.37	0.25	0.22	
$C_{16:0\ 2\text{-OH}}$	0.30		0.56	1.10	1.35	1.11	1.66	0.83	1.38		
$C_{16:0\ 3\text{-OH}}$			0.19	0.16	0.11		0.40	0.07		0.22	
OAG FAs											
$C_{16:0}$					0.13	0.51	0.30		0.28		
$C_{16:1}$						6.41					
Total SCFAs	25.34	45.96	39.35	45.34	47.21	25.68	46.15	44.75	38.14	10.44	30.61
BCFAs											
iso-$C_{13:0}$	0.48		0.43	0.27	0.48	0.40	0.41		0.18		0.63
iso-$C_{14:0}$	0.16		0.19			0.43	0.12				
iso-$C_{15:0}$	**28.50**	**21.31**	**19.08**	**14.77**	**15.43**	**11.13**	**15.10**	**17.02**	**16.63**	**41.15**	**33.02**
iso-$C_{16:0}$	2.07	2.13	4.85	2.04	3.92	11.87	4.13	1.89	1.70	1.54	
iso-$C_{17:0}$	11.30	4.99	5.55	4.27	2.81	10.44	3.19	6.51	3.84	**14.84**	**13.65**
iso-$C_{18:0}$						0.09					
iso-$C_{17:1\omega5c}$	2.44		0.82	0.15	0.28	5.10	0.19	0.20	0.48	2.81	
iso-$C_{17:1\omega11c}$	0.27										
iso-$C_{17:2\omega5,11,\text{ all } cis}$	0.23										
anteiso-$C_{15:0}$	0.25									0.20	
anteiso-$C_{17:0}$	0.19			0.05		0.24				0.94	0.79
Branched-chain OH FAs											
iso-$C_{15:0\ 3\text{-OH}}$	1.11	1.59	2.42	0.50	1.81	1.02	1.03	1.19	0.90	2.22	0.71
iso-$C_{17:0\ 2\text{-OH}}$	**17.14**	7.38	**12.07**	**18.80**	**13.17**	**22.99**	**16.00**	**13.80**	**16.43**	3.60	
iso-$C_{17:0\ 3\text{-OH}}$										0.19	
Branched-chain OAG FA											
iso-$C_{15:0}$	2.79	6.04	9.04	9.63	11.47	1.95	13.68	5.74	8.36	**9.35**	**10.36**
Branched-chain DMA FA											
iso-$C_{15:0}$	7.76	10.61	6.21	4.17	3.42	8.66		8.90	13.35	**12.74**	**10.23**
Total BCFAs	74.66	54.04	60.65	54.66	52.79	74.32	53.85	55.25	61.86	89.56	69.39

[a] Percentages of major fatty acids are distinguished in boldface type.

$C_{16:1\omega5c}$, 19%; and *iso*-$C_{17:0}$, 15%) likewise had counterparts in *Cystobacter*. Similarities in FA patterns and morphological characteristics of both genera reaffirm their placement in *Cystobacteraceae*.

***Melittangium* cluster.** The genus *Melittangium* appears polyphyletic (Fig. 1). *Melittangium boletus* strains Me b7 and Me b8^T branch closely with *C. miniatus*, whereas *Melittangium lichenicola* is more closely affiliated with the majority of *Cysto-*

TABLE 3. Fatty acid distribution in *Cystobacterineae* genera *Stigmatella*, *Melittangium*, and *Hyalangium*

FA type	% of FA in[a]:						
	S. aurantiaca	*S. erecta*	*S. hybrida*	*M. boletus* SBMe003	*M. alboraceum*	*M. lichenicola*	*H. minutum*
SCFAs							
$C_{14:0}$	2.89	3.29	1.33		0.56		0.49
$C_{14:1\omega5c}$	0.17	0.29					0.58
$C_{15:0}$	0.24				0.17		0.64
$C_{15:1}$							0.14
$C_{15:1\omega5c}$						10.31	
$C_{16:0}$	**16.27**	**25.17**	**33.32**	3.62	5.99	3.82	**8.33**
$C_{16:1\omega5c}$	11.83	8.14	4.99	3.56	4.66		**18.93**
$C_{16:1\omega7c}$			32.25				
$C_{16:1\omega9c}$	0.19			4.15	0.17	9.71	
$C_{16:1\omega11c}$					0.33		
$C_{17:0}$	0.07						0.30
$C_{18:0}$	0.94	3.94	0.62	1.67	0.54	3.14	0.77
$C_{18:1}$	0.47						
$C_{18:1\omega9c}$	1.53	5.64			1.42	1.48	
PUFAs							
$C_{16:2}$				13.04	0.62		
$C_{18:2}$	11.58				9.22		
$C_{18:2\omega6,9,\ all\ cis}$		14.76					
$C_{18:3\omega6,9,12,\ all\ cis}$					3.96		
$C_{18:3}$						1.75	
Hydroxy FAs							
$C_{14:0\ 3\text{-OH}}$	0.29		0.28	0.13			0.24
$C_{16:0\ 2\text{-OH}}$	0.46	0.28	1.44	0.06			0.31
$C_{16:0\ 3\text{-OH}}$	0.16	0.10		0.20			
OAG FAs							
$C_{15:0}$							0.50
$C_{16:0}$	2.93	1.26	2.30				4.00
Total SCFAs	50.00	62.85	76.52	26.43	27.62	30.21	35.23
BCFAs							
iso-$C_{13:0}$	0.61				1.63		0.31
iso-$C_{14:0}$							0.21
iso-$C_{15:0}$	**16.50**	**13.82**	5.55	**31.59**	**27.51**	**29.62**	**20.98**
iso-$C_{16:0}$	0.76	0.99			0.13	14.67	4.78
iso-$C_{17:0}$	8.90	4.11	9.65	**7.71**	**21.85**	**8.96**	**14.65**
iso-$C_{17:1\omega5c}$					0.33		0.84
iso-$C_{17:1\omega11c}$				1.38	0.67		
anteiso-$C_{17:0}$						2.76	
Branched-chain hydroxy FAs							
iso-$C_{15:0\ 3\text{-OH}}$	2.23	0.97	1.31	3.84	1.60	1.49	1.96
iso-$C_{17:0\ 2\text{-OH}}$	7.17	3.61	6.59	3.73	4.72		6.99
iso-$C_{17:0\ 3\text{-OH}}$				0.86	0.17		
Branched-chain OAG FA							
iso-$C_{15:0}$	5.48	9.79		7.45	6.38		6.02
Branched-chain DMA FA							
iso-$C_{15:0}$	8.36	3.86	0.37	**17.01**	**7.39**	**12.29**	**8.04**
Total BCFAs	50.00	37.15	23.48	73.57	72.38	69.79	64.77

[a] Percentages of major fatty acids are distinguished in boldface type.

bacter species. The third species, *Melittangium alboraceum*, was never cultivated (38). On a morphological basis, strain Me b7 closely matched *M. alboraceum* (47) and was therefore used to represent the taxon in FA analysis. The 16S rRNA gene sequence of the type strain (Me b8^T) of *M. boletus* (39) was used for phylogenetic tree construction. However, strain Me b8^T could not be revived (Klaus Gerth, personal communication) and therefore had to be replaced with strain SBMe003, which

fits the description of *M. boletus* on the basis of its fruiting body structure (see Fig. S1c in the supplemental material) (32, 33) and bright yellow swarm (39).

Melittangium has many similarities to *Cystobacter* in their FA patterns: 70 to 74% of its FAs were BCFAs, of which *iso*-$C_{15:0}$ was the highest (27.5 to 31.6%). *iso*-$C_{15:0}$ DMA and *iso*-$C_{17:0}$ were also present in significant amounts. The low content of $C_{16:1\omega5c}$ (<5%) in *Melittangium* differentiates it from *Cystobacter.*

The similarity between Me b7 and Me b8, as represented by SBMe003, was reflected in tree topology (Fig. 1). Both contained nearly the same ratio of SCFAs and BCFAs (Table 3) and elevated amounts of *iso*-$C_{15:0}$, *iso*-$C_{15:0}$ DMA, and *iso*-$C_{17:0}$. Phylogenetic and FA analyses suggest that Me b7, identified as *M. alboraceum*, (47), appears to be an *M. boletus* strain. We also agree that the described *M. alboraceum* strain (32) was just an immature fruiting stage of *Chondromyces*, as previously suggested (39).

Melittangium lichenicola appears distantly related from Me b7 and Me $b8^T$ in the phylogenetic tree (Fig. 1) and is divergent by the presence of large amounts of $C_{15:1\omega5c}$ (10.3%) and *iso*-$C_{16:0}$ (14.67%) and the absence of $C_{16:1\omega5c}$ and *iso*-$C_{15:0}$ OAG. FA $C_{15:1\omega5c}$ was not detected in Me b7 and SBMe003 and may be considered an important taxonomic marker. Its paraphyletic affiliation with *Cystobacter* in the tree (Fig. 1) is manifested not only in the ratio of SCFAs to BCFAs but also in their similar amounts of unsaturated SCFAs—21.5% in *M. lichenicola* and 21.5 to 26.6% in *Cystobacter* spp. (*C. velatus*, *C. ferrugineus*, *C. badius*, and *C. fuscus*).

Suborder *Nannocystineae*. The suborder *Nannocystineae* is a unique mixture of isolates grouped into two clusters—(i) the marine organisms *Enhygromyxa* and *Plesiocystis*, which are allied to the terrestrial nonhalophilic bacterium *Nannocystis*; and (ii) the *Haliangium-Kofleria* cluster (Fig. 1). Their phylogenetic placement in the same suborder could mean that these myxobacteria originated from a common ancestor but had developed convergent adaptations to different ecological niches in the course of evolution. This is further supported by the FA pattern.

***Haliangium-Kofleria* cluster.** *iso*-$C_{16:0}$ and *iso*-$C_{16:1}$ were the major BCFAs, with the amounts differing between species. Large amounts (5 to 12.6%) of *iso*-$C_{17:0}$ were also found (Table 4). The type strains of *Haliangium tepidum* and *H. ochraceum* differed significantly in the ratios of BCFAs to SCFAs, suggesting that they should be in separate taxa. In *H. tepidum* (SMP-10^T = DSM14436^T), we found 13.7% SCFAs and 86.3% BCFAs, compared to 38.8% SCFAs and 60.4% BCFAs, as previously reported (8). It clusters with sister taxon *Kofleria flava* (Fig. 1), with both having comparable amounts of $C_{16:0}$ (2.9% and 2.8%, respectively), much lower than the 15.1% reported for *H. tepidum* (8). In contrast, *H. ochraceum* had higher (18.4%) $C_{16:0}$ and lower (4.8%) levels of *iso*-$C_{15:0}$ OAG; our data suggest that the $C_{16:0}$ content is less than half of the reported 38.3% (8). Although our study qualitatively reaffirms the presence of these FAs in type strains and supports their position in the phylogenetic tree, their amounts do not agree exactly with previous literature—perhaps a reflection of differences in cultivation media. The detection of 21 different FAs compared with 14 as previously reported in *H. ochraceum* (8) suggests that our medium supports more complex FA formation. Representatives of both studied genera *Haliangium* and *Kofleria* had small amounts (<3%) of *anteiso*-$C_{17:0}$. It was demonstrated earlier that *anteiso*-branched acids serve as chemo-taxonomic markers for marine myxobacteria (8). This raises the question of the ancestor of the terrestrial *Kofleria* (e.g., strain Pl $vt1^T$), which might be similar to the high-salt-tolerant genus *Haliangium*.

***Enhygromyxa-Plesiocystis* cluster.** In agreement with a previous study (20), hydroxy FAs were not detected, suggesting placement of *Enhygromyxa* and *Plesiocystis* in *Nannocystineae* and further justifying their topology and bootstrap support (100%) in the phylogenetic tree (Fig. 1). The predominance of SCFAs in both genera (19, 20) was also confirmed, but much higher values were obtained for the type strains of *Enhygromyxa* (95.9%) and *Plesiocystis* (90.9%), in comparison to 44.4% and 43.4% for *Plesiocystis* SIR-1^T and SHI-1, respectively (20).

The major FAs detected in *Enhygromyxa* were *iso*-$C_{15:0}$, *iso*-$C_{16:0}$, and *iso*-$C_{17:0}$ (20, 43), but here, a predominance of straight-chain $C_{16:1\omega7c}$ (42%), $C_{18:1\omega9c}$ (29%), and $C_{16:0}$ (11%) was found. Although this study reproduced these findings with regard to detection of these FAs, the absence of quantitative data in previous studies prevents a true comparison. This study presents for the first time the complete FA data of *E. salina* DSM15217^T (= SHK-1^T). *Plesiocystis* was also reported to contain significant *iso*-$C_{15:0}$ (32.3 to 35.6%) and *iso*-$C_{16:0}$ (13.5 to 14.6%) (20), but <3% of both FAs were detected (Table 4), which can perhaps also be explained by differences in the cultivation media. Both genera contained $C_{16:1\omega7c}$ (30 to 42%), $C_{18:1\omega9c}$ (24 to 29%), and $C_{16:0}$ (7 to 11%). $C_{16:1\omega9c}$ (22.4%) was found only in *Plesiocystis*, and straight-chain OAG was found only in *Enhygromyxa*. It is possible that the FA $C_{18:1\omega9c}$ is the marker by which these two low-salt-tolerant genera can be distinguished from the high-salt-tolerant genus *Haliangium.*

***Nannocystis* cluster.** *Nannocystis* phylogenetically clusters with "halotolerant" *Enhygromyxa* and *Plesiocystis* in *Nannocystaceae* (Fig. 1), although both differ significantly in cell morphology, source environment, and FA profile. Its predominant FAs were $C_{16:1\omega5c}$ (22 to 27%), *iso*-$C_{17:0}$ (15 to 25%), $C_{14:0}$ (11 to 17%), and *iso*-$C_{15:0}$ (nearly 9%). *Nannocystis* and *Plesiocystis* are distinguished in the suborder by the absence of straight-chain OAG FAs.

Nannocystis exedens differed from its sister taxon *Nanocystis pusilla* by production of less than 19% BCFA and the presence of $C_{16:1\omega9c}$. Both species also differed significantly in *iso*-$C_{17:0}$ and *iso*-$C_{17:1\omega11c}$ contents (Table 4).

Suborder *Sorangiineae*. Of the six genera in the suborder *Sorangiineae*, only *Sorangium*, as represented by *S. cellulosum* strains So ce14 (7) and AJ 13585 (8), had previously been analyzed for FA content. In this study, all 12 species known to date in *Byssovorax*, *Chondromyces*, *Jahnella*, *Phaselicystis*, *Polyangium*, and *Sorangium* were covered. The new representative isolate *Aetherobacter* SBSr008 was also included in the analysis and in the phylogenetic tree (Fig. 1). SCFAs dominated over BCFAs, and out of a total of 49 FAs, 36 were identified as SCFAs. *Sorangiineae* thus appears to be the most complex among the myxobacteria with respect to SCFAs.

***Polyangiaceae-Phaselicystidaceae* cluster.** Straight-chain $C_{16:1\omega7c}$ appears most abundant in *Chondromyces-Jahnella* (14 to 29%) and *Polyangium* (34 to 55%) clades, second to $C_{16:1\omega5c}$ in *Byssovorax* (21%), and was not detectable in *Sorangium* and

TABLE 4. Fatty acid distribution among *Nannocystineae* type strains

FA type	% of FA in[a]:						
	H. ochraceum	*H. tepidum*	*E. salina*	*P. pacifica*	*K. flava*	*N. exedens*	*N. pusilla*
SCFAs							
$C_{13:0}$						0.56	
$C_{14:0}$	0.18		0.79	0.41		**17.33**	**10.59**
$C_{14:1\omega5c}$			0.68	0.64			
$C_{15:0}$	0.63		0.45	0.18	0.35	2.37	
$C_{15:1}$						2.12	
$C_{16:0}$	**18.41**	2.89	**10.69**	**6.67**	2.81	12.01	6.00
$C_{16:1\omega5c}$	3.57		0.96	0.65	0.37	**22.01**	**27.30**
$C_{16:1\omega7c}$	**8.84**		**42.24**	**30.13**			
$C_{16:1\omega9c}$		3.27		**22.41**	0.82	6.68	
$C_{16:1\omega11c}$							4.41
$C_{17:0}$	1.81		0.39	0.20	0.73		
$C_{17:1\omega7c}$	0.52		0.53	0.32			
$C_{18:0}$	4.81	2.23	5.88	3.19	0.76	7.75	3.05
$C_{18:1}$	2.23						
$C_{18:1\omega9c}$	0.72	2.24	**29.09**	**23.59**			
PUFAs							
$C_{20:4\omega6,9,12,15,}$ all *cis*			0.91	2.55			
$C_{20:5\omega3,6,9,12,15,}$ all *cis*			1.44				
OAG FAs							
$C_{14:0}$					0.31		
$C_{15:0}$	0.72		0.33		0.49		
$C_{16:0}$	6.98	0.18	1.48		0.67		
$C_{16:1}$	0.20	2.93			6.55		
Total SCFAs	49.62	13.73	95.87	90.93	13.86	70.84	51.35
BCFAs							
iso-$C_{14:0}$					0.15		
iso-$C_{15:0}$	2.38	**17.86**	0.57	2.76	1.72	**8.69**	**8.53**
iso-$C_{16:0}$	**25.45**	**14.02**	1.20	2.46	**34.14**		
iso-$C_{16:1}$	**8.94**	**18.69**			**27.85**		
iso-$C_{17:0}$	5.00	**8.76**	0.66	0.46	**12.63**	**14.74**	**24.89**
iso-$C_{17:1\omega5c}$		0.76					
iso-$C_{17:1\omega11c}$						3.53	12.67
iso-$C_{18:0}$	0.11				0.45		
anteiso-$C_{17:0}$	2.01	3.14			1.81		
Branched-chain OAG FA							
iso-$C_{15:0}$	4.84	**23.05**	1.69	3.39	4.61		
Branched-chain DMA FA							
iso-$C_{15:0}$	1.65				2.79	2.20	2.56
Total BCFAs	50.38	86.27	4.13	9.07	86.14	29.16	48.65

[a] Percentages of major fatty acids are distinguished in boldface type.

Phaselicystis. FA $C_{16:1\omega5c}$, though extremely rare in nature (26), was also comparatively high in *Archangium*, *Nannocystis*, and many *Cystobacter* strains. Its absence in *Sorangium* was unexpected, as this genus shares many characteristics with *Byssovorax*, like the ability to degrade cellulose. However, in *Sorangiineae*, *Sorangium* has the highest $C_{16:0}$ (palmitic acid) content (20 to 25%).

A previous study showed that *Sorangium* (*S. cellulosum* So ce14) could be differentiated from *Cystobacterineae* through the absence of hydroxy FAs (7); however, we identified trace amounts of $C_{16:0\ 2\text{-OH}}$ and *iso*-$C_{17:0\ 2\text{-OH}}$ and 4.3 to 6.8% $C_{17:1\ 2\text{-OH}}$ in the type and reference strains (Table 5). The lack of detection of hydroxy-type FAs in So ce14 appears to reflect the differences in sensitivity of the analytical methods employed 30 years ago and does not seem to be associated with a particular strain. All other *Sorangium cellulosum* isolates in our collection analyzed for FAs produced hydroxy FAs (data not shown). $C_{17:1\ 2\text{-OH}}$ FA was higher in *Chondromyces* (9 to 15%) and *Phaselicystis* (25%). A total of 37.7% hydroxy FAs were found in the latter genus, while several others in small amounts were detected in other members of the *Sorangiineae* (Table 5).

The characteristic *anteiso* FAs in "marine" myxobacteria (8, 19, 20) were also present in *Chondromyces*, *Polyangium*, and *Jahnella*, although trace amounts (<1%) of only *anteiso*-$C_{17:0}$ were detected.

Although the proposed novel isolate *Aetherobacter* SBSr008

TABLE 5. Fatty acid distribution among *Sorangiineae* type strains

FA type	% of FA in[a]:													
	C. apiculatus	*C. robustus*	*C. lanuginosus*	*C. pediculatus*	*C. robustus*	*P. fumosum*	*P. sorediatum*	*P. spumosum*	*S. cellulosum*	*S. cellulosum* ("*nigrum*")[b]	*B. cruenta*	*Aetherobacter*[c]	*J. thaxteri*	*P. flava*[d]
SCFAs														
$C_{13:0}$			0.11								0.06	0.08		
$C_{14:0}$	0.83	0.89	0.64	0.79	0.36		0.59	0.57	2.60	1.25	1.79	0.20	0.44	0.73
$C_{14:1\omega5c}$	0.26			0.40	0.22		0.98	0.95			1.05		0.69	
$C_{15:0}$	0.29	0.45	0.92	0.61			0.14	0.09	0.48		1.44	1.86	0.17	
$C_{15:1}$			0.31								0.41	0.06		
$C_{15:1\omega5c}$													0.12	
$C_{16:0}$	9.46	16.42	4.51	11.72	3.39	5.65	5.96	4.17	**19.60**	**24.85**	4.98	2.14	**8.29**	6.01
$C_{16:0,9,10}CH_2$				5.25								0.42		
$C_{16:1\omega5c}$	3.30	1.68	2.11	1.38	1.71	1.27	0.46	0.71	7.79	6.82	**21.09**	2.82	0.87	
$C_{16:1\omega7c}$	**26.54**	**17.52**	**14.12**	**29.43**	**25.84**	**55.25**	**33.68**	**37.27**			**17.50**		**28.18**	
$C_{16:1\omega9c}$									0.12	0.59				
$C_{16:1\omega11c}$			0.31	0.26										
$C_{17:0}$	1.99	4.47	3.98	2.18	0.66		1.19	0.26	1.58	0.24	1.59	1.31	0.87	5.53
$C_{17:1\omega7c}$					0.31		0.50	0.51					0.45	
$C_{18:0}$	2.94	2.53	2.11	4.84	4.82	1.75	2.67	3.18	4.61	8.18	1.97	1.43	7.05	3.22
$C_{18:1}$													**25.80**	
$C_{18:1\omega9c}$	3.02	0.33	7.47	1.85	20.23	5.40	1.29	25.53	2.59	1.45	**13.90**			
PUFAs														
$C_{16:2}$										0.74				
$C_{18:2}$									27.26					
$C_{18:2\omega6,9c}$	2.06	0.33	0.86	1.93	9.30		1.26	1.53		18.17			0.89	
$C_{18:3}$												0.03		
$C_{18:3\omega6,9,12,}$ all *cis*		1.08												
$C_{20:2\omega6,9,}$ all *cis*									1.09					
$C_{20:4\omega6,9,12,15,}$ all *cis*												1.47		**12.44**
$C_{20:5\omega3,6,9,12,15,}$ all *cis*												**10.90**		
$C_{22:6\omega3,6,9,12,15,18,}$ all *cis*												**9.49**		
Hydroxy FAs														
$C_{16:0\ 2\text{-OH}}$	1.45	1.44					0.17		0.84			0.50	0.56	0.08
$C_{16:1\ 2\text{-OH}}$														1.69
$C_{17:0\ 2\text{-OH}}$														6.57
$C_{17:1\ 2\text{-OH}}$	**8.87**	**14.88**	**11.27**	**10.98**	**11.38**	0.62	2.86	5.94	6.77	4.31	5.24	**13.50**	2.09	**25.19**
$C_{18:0\ 2\text{-OH}}$														0.32
$C_{18:1\ 2\text{-OH}}$														1.81
OAG FAs														
$C_{14:0}$			0.47											0.79
$C_{15:0}$	1.27	1.45	1.58	0.61	0.66		0.37				0.77	10.21	0.16	1.43
$C_{16:0}$	9.13	17.23	1.12	5.59	4.89	1.44	7.44		2.84	5.67	0.87	1.85	3.73	2.92
$C_{16:1}$	11.86	0.38	5.33				1.42				0.61	4.86	0.52	
Total SCFAs	83.26	81.05	57.22	77.81	83.77	71.38	60.97	80.72	78.17	72.28	73.25	63.13	80.86	68.73

BCFAs								0.94	0.45	0.31	0.06			
iso-$C_{13:0}$		0.29								0.20				
iso-$C_{14:0}$		0.83												
iso-$C_{15:0}$	**7.70**	**8.47**	**17.30**	**11.32**	**10.94**	**14.41**	**23.41**	**9.96**	**9.78**	**7.97**	**11.11**	**23.17**	3.52	**25.52**
iso-$C_{16:0}$	0.76	0.18	8.98	1.78	0.10	1.44	0.82		0.58	0.41	2.25		0.52	
iso-$C_{17:0}$	4.90	7.21	8.49	5.68	2.39	9.45	8.06	2.77	**7.61**	**16.27**	7.15	2.05	**8.56**	3.70
iso-$C_{17:1\omega5c}$											0.29	0.03		
iso-$C_{18:0}$			1.64	0.95							0.50		1.13	
anteiso-$C_{17:0}$			0.50				0.21						0.20	
Branched-chain OH FAs														
iso-$C_{17:0\ 2\text{-}OH}$	0.15						0.05	0.06	1.22			0.35	4.44	0.16
iso-$C_{17:1\ 2\text{-}OH}$														1.89
iso-$C_{17:0\ 3\text{-}OH}$	0.18	0.23		0.11										
Branched-chain OAG FA														
iso-$C_{15:0}$	2.50	2.38	4.33	1.72	1.23		0.64	0.84	1.70	2.63	4.94	1.81	0.61	
Branched-chain DMA FA														
iso-$C_{15:0}$	0.55	0.48	0.43	0.63	1.57	3.32	5.85	5.65				**9.40**	0.15	
Total BCFAs	16.74	18.95	42.78	22.19	16.23	28.62	39.03	19.28	21.83	27.72	26.75	36.87	19.14	31.27

[a] Percentages of major fatty acids are distinguished in boldface type.
[b] *S. cellulosum* ("*nigrum*") DSM14731 (=So ce 1654).
[c] *Aetherobacter* SBSr008 (49).
[d] *Phaselicystis flava* (11).

contained *iso*-$C_{15:0}$ as the major FA, it lacked $C_{18:1\omega9c}$, and, unlike *Chondromyces*, *Polyangium*, *Jahnella*, and *Byssovorax*, it also lacked $C_{16:1\omega7c}$. In contrast to other members of *Polyangiaceae*, PUFAs constituted more than 20% of its total FAs and even higher levels in some strains within this cluster (49).

Gliding, nonfruiting bacteria: *Herpetosiphon* and *Flexibacter*. Strains of *Flexibacter* (Flex 23 and Flex 7014) and *Herpetosiphon* (Hpg 287, Hpg 336, Hpg 383, and Hpg 454) have much simpler FA patterns (see Table S2 in the supplemental material) than myxobacteria. In *Flexibacter*, 14 FAs were identified, with roughly equal amounts of total SCFAs and BCFAs found in strains Flex 23 and Flex 7014. $C_{16:1\omega5c}$ (34 to 40%) and *iso*-$C_{15:0}$ (41 to 51%) were dominant in both strains. In a similar study, large amounts of $C_{16:1\omega5c}$ were also detected in *Flexibacter* sp. strain Inp (21). PUFA production in the genus appears to be both species and strain specific. Neither EPA, known in one strain (17) and *Flexibacter polymorphus* (23), nor linoleic and linolenic acids, in strain Inp (21), were found in Flex 23 and Flex 7014. Arachidonic acid was reported recently in the gliding bacteria *Aureispira marina* and *Aureispira maritima* (15, 16).

The four strains of *Herpetosiphon* analyzed contained 4 to 10 straight-chain and even-numbered FAs, in agreement with a previous study (6). $C_{18:1\omega9c}$, $C_{16:0}$, and $C_{18:0}$ were dominant in all strains, except for $C_{18:1\omega9c}$ in Hpg 383 (<3%). One PUFA, $C_{18:2\omega6,9,\ all\ cis}$, was also found in strains Hpg 287 and Hpg 336.

Hydroxy fatty acids. The total hydroxy FAs were, on average, high in *Cystobacterineae* in comparison with *Sorangiineae* and absent in *Nannocystineae*. So far, only 2-OH and 3-OH hydroxy FAs were found in myxobacteria, agreeing with an earlier study (55). In *Cystobacterineae*, hydroxy BCFAs dominate over the hydroxy SCFAs. The straight-chain $C_{17:1\ 2\text{-}OH}$ was only found in *Sorangiineae* and appears to be an FA marker for the suborder. The presence of both the *iso*-branched and straight-chained $C_{17:0\ 2\text{-}OH}$ in *Phaselicystis flava* supports its current placement as a separate family (11).

***iso*-even and *iso*-odd BCFAs.** *iso*-odd BCFAs, though not necessarily found in larger total amounts, were more diversified than *iso*-even BCFAs. *iso*-$C_{15:0}$ and *iso*-$C_{17:0}$ serve as the major *iso*-odd FAs. The presence of *iso*-$C_{15:0}$ has been shown to be crucial in fruiting body development in *Myxococcus xanthus* (41). Low levels of this FA (1.7 to 2.8%) in marine myxobacteria may account for their reported inability to form "true" fruiting bodies in aquatic environments (18, 19, 20). The formation of sporangioles containing ovoid spores in *Haliangium tepidum* SMP-10^T (8), also reproducible in our study, may be associated with its high *iso*-$C_{15:0}$ content (18%).

Of the 15 identified *iso*-FAs in the suborders, only 4 were *iso*-even (Table 4). Among those, the most common and abundant was *iso*-$C_{16:0}$, while *iso*-$C_{16:1}$ was determined to be exclusive to members of the *Kofleria*-"*Haliangiaceae*" cluster (Fig. 1) and therefore may be regarded as an important marker for the *Kofleriaceae*.

Saturated and unsaturated SCFAs. Saturated SCFAs were, in general, more abundant than unsaturated SCFAs in *Myxococcales*. The larger amount of saturated SCFAs in *Stigmatella* (up to 37% in *S. aurantiaca*), marks its divergence from *Cystobacter.* In *Nannocystineae*, the *Haliangium-Kofleria* cluster had higher saturated than unsaturated SCFAs; this was the reverse in the *Nannocystis-Enhygromyxa-Plesiocystis* clade. The *Nanno-*

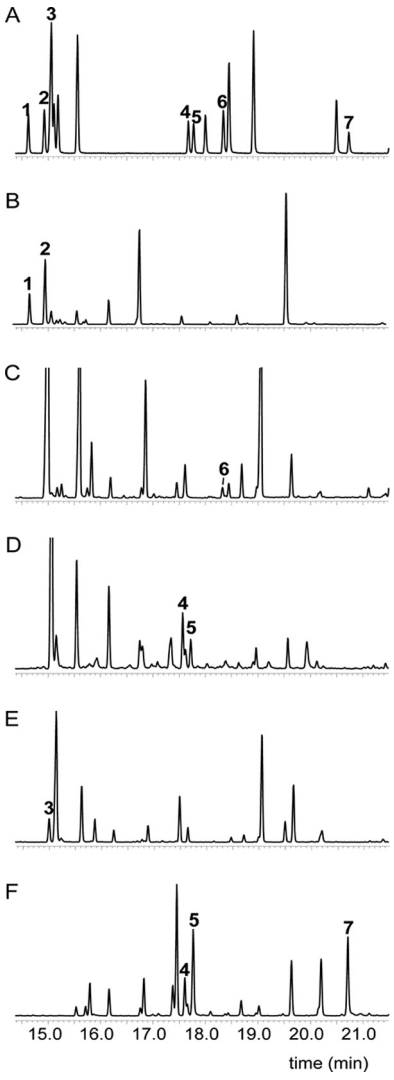

FIG. 2. GC-MS chromatograms of PUFA-producing myxobacteria. (A) FAME reference standard mixture; (B) *Myxococcus fulvus* (ATCC 25946^T) DSM1625^T; (C) *Sorangium cellulosum* So ce1871^T (DSM14627^T); (D) *Enhygromyxa salina* (SHK-1^T) DSM15217^T; (E) *Chondromyces crocatus* SBCm010; (F) *Aetherobacter* SBSr008. PUFAs identified in myxobacteria: 1, $C_{18:3\omega6,9,12,\ all\ cis}$, γ-linolenic acid (GLA); 2, $C_{18:2\omega6,9,\ all\ cis}$, linoleic acid; 3, $C_{18:3\omega3,6,9,\ all\ cis}$, α-linolenic acid (ALA); 4, $C_{20:4\omega6,9,12,15,\ all\ cis}$, arachidonic acid (AA); 5, $C_{20:5\omega3,6,9,12,15,\ all\ cis}$, eicosapentaenoic acid (EPA); 6, $C_{20:2\omega6,9,\ all\ cis}$, eicosadienoic acid (EDA); and 7, $C_{22:6\omega3,6,9,12,15,18,\ all\ cis}$, docosahexaenoic acid (DHA).

cystis and *Enhygromyxa-Plesiocystis* clusters were differentiated by unsaturated SCFAs, of which the latter cluster contained as much as twice the amount (73 to 77%). The *Sorangiineae* generally contained larger amounts of unsaturated SCFAs (24 to 65%), except in *Sorangium* and the novel strain *Aetherobacter* SBSr008. Furthermore, *Phaselicystidaceae* differ from *Polyangiaceae* by the absence of unsaturated SCFA (Table 5), supporting their divergence as a family (Fig. 1).

Omega-6 PUFAs. Using a FAME reference mixture (Fig. 2A), PUFAs were identified in myxobacteria. Linoleic acid (LA; $C_{18:2\omega6,9,\ all\ cis}$) and γ-linolenic acid (GLA; $C_{18:3\omega6,9,12,\ all\ cis}$) (Fig. 2B) appear to be distributed among the *Cystobacterineae* and *Sorangiineae* suborders but were not found in *Nannocystineae* (Fig. 1).

PUFA $C_{20:2\omega6,9,\ all\ cis}$ (eicosadienoic acid; EDA) (Fig. 2C) was also detected in myxobacteria, but only in *Sorangium cellulosum* So ce1851^T (= DSM 14627^T). We found later that almost half (45%) of the *Sorangium* strains in our collection were positive for EDA (R. Garcia et al., unpublished data); its absence in the reference isolate (So ce1654) suggests that EDA production is strain specific.

PUFA $C_{20:4\omega6,9,12,15,\ all\ cis}$ (arachidonic acid; AA) was observed exclusively in *Sorangiineae* and *Nannocystineae.* Earlier, we reported its abundance in *Phaselicystis flava* (11) and have since detected it in small amounts in other strains belonging to the two suborders. The previously detected but unidentified $C_{20:4}$ FA (19, 20) was confirmed and identified here as AA in the type strains of *Plesiocystis pacifica* and *Enhygromyxa salina* (Fig. 2D). However, the amount found in *P. pacifica* SIR-1^T (DSM14875^T) was low (2.6%) in comparison to the reported 14.1 to 17.5% (20). As pointed out in earlier sections, changes in the percentages of FA production may be attributed to differences in media and cultivation conditions.

Omega-3 PUFA. Unlike omega-6 PUFAs, the omega-3 FAs were only discovered exclusively in *Sorangiineae* and *Nannocystineae*. PUFA $C_{18:3\omega3,6,9,12,\ all\ cis}$ (α-linolenic acid; ALA) was present in some *Chondromyces* isolates (e.g., SBCm010) (Fig. 2E), but not in the five type strains studied. $C_{20:5\omega3,6,9,12,15,\ all\ cis}$ (eicosapentaenoic acid; EPA), previously unknown in myxobacteria, was found initially in novel *Sorangiineae* isolates, such as SBSr008, SBSr002, and SBSr003 (49). Strain SBSr008 represents this cluster, with a significant level (10.9%) of EPA (Table 5 and Fig. 2F) compared with some *Sorangium* isolates (Garcia et al., unpublished). In *Nannocystineae*, only the *Enhygromyxa salina* type strain was found to produce EPA (Table 4). $C_{22:6\omega3,6,9,12,15,18,\ all\ cis}$ (docosahexaenoic acid; DHA), also previously unknown in myxobacteria, was detected only in the novel *Sorangiineae*, such as SBSr008. We plan to propose the assignment of this unique strain, along with SBSr002 and SBSr003, which appear to be phylogenetically and morphologically closely related, to the novel genus, *Aetherobacter.* In a later screen with other novel isolates, we also discovered two additional PUFAs, identified as docosapentaenoic acid [$C_{22:5(n\text{-}3)}$] and omega-6 homo-γ-linolenic acid [$C_{20:3(n\text{-}6)}$] (data not shown).

Conclusions. A comprehensive report of the cellular FA content in the order *Myxococcales* (myxobacteria) is presented here for the first time, covering most type strains and a representative novel isolate, to allow deduction of various FA correlations which might become useful for further follow-up work. Our study highlights the expanded FA profile of *Sorangiineae* and the discovery of PUFAs, particularly the omega-3 family.

Eight PUFAs, identified as linoleic acid, γ-linolenic acid, homo-γ-linolenic acid, eicosadienoic acid (all ω6), α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid (all ω3), were discovered for the first time in myxobacteria. Production of EPA appears restricted to certain genera in *Sorangiineae* and *Nannocystineae*. We described the extensive FA profile of *Enhygromyxa salina* and documented the production of EPA. Additionally, the discovery of DHA in the novel isolate *Aetherobacter* SBSr008 appears to be a unique

characteristic exclusive to that genus. In our analysis, *Herpetosiphon* and *Flexibacter* (gliding, nonfruiting bacteria) show not only completely different FA profiles in comparison to myxobacteria, but also an absence of PUFAs, with the exception of linoleic acid in *Herpetosiphon*.

We have shown that myxobacteria could be potential sources of valuable omega-3 FAs for biotechnological and biopharmaceutical applications. Our overall study of their FA profiles shows complementarily with phylogeny findings and therefore might be regarded as a significant tool for the chemo-taxonomic classification of myxobactetria, especially for the discovery of novel PUFA producer strains.

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