Electronic Supplementary Information:

Polyunsaturated fatty acid biosynthesis in myxobacteria: Different PUFA synthases and their product diversity†

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EXPERIMENTAL PROCEDURES

Culture conditions

Aetherobacter sp. (SBSr001), *Aetherobacter fasciculatus* (SBSr002), *Aetherobacter rufus* (SBSr003), and *Aetherobacter* sp. (SBSr008) were obtained from Department of Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany. They were grown in liquid MD1G-5gC-medium containing 0.5% casitone, 0.05% $CaCl_2$ x 2 H_2O, 0.2% $MgSO_4$ x 7 H_2O, and 0.35% glucose. The medium was adjusted to pH 7.0 with KOH. The cultures were incubated on a rotary shaker for 5-10 days at 30 °C and 200 rpm.

S. cellulosum So ce56, So ce10, So ce1525, So ce377, So ce38, So ce487, So ce836, and So ceGT47 were generously provided by Department of Microbial Drugs, Helmholtz Centre for Infection Research, Braunschweig, Germany. They were grown in liquid M-medium containing 1% phytone peptone, 1% maltose, 0.1% $CaCl_2$ x 2 H_2O, 0.1% $MgSO_4$ x 7 H_2O, and 50 mM HEPES. The medium was adjusted to pH 7.2 with NaOH and supplemented with 8 mg/l NaFe-EDTA after autoclaving. The cultures were incubated on a rotary shaker for 5-10 days at 30 °C and 200 rpm.

Myxococcus xanthus DK1622 was grown in CTT-medium or on CTT-agar containing 1% casitone, 10 mM Tris, 1 mM KPO_4, 8 mM $MgSO_4$ (and 1.5% agar). The medium was adjusted to pH 7.6. The cultures were incubated for 2-3 days at 30°C (and 200 rpm).

Escherichia coli DH10B was used for cloning experiments. *E. coli* GB05-dir[1] was used for direct cloning experiments. *E. coli* HS996/pSC101-BAD-gbaA[2] was used for modification of a plasmid using Red/ET recombination. The cells were grown in LB-medium or on LB-agar (1% tryptone, 0.5% yeast extract, 0.5% NaCl (1.5% agar)) at 30-37 °C (and 200 rpm) overnight. Antibiotics were used at the following concentrations: 100 µg/ml ampicillin, 80 µg/ml spectinomycin, 50 µg/ml kanamycin, 20 µg/ml zeocin, and 6 µg/ml tetracycline.

Isolation of genomic DNA from myxobacteria

100 ml of a culture were harvested at 8,000 rpm for 10 min at room temperature. After discarding the supernatant completely, the cells were washed once with 15 ml SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris-Cl pH 7.5), pelleted at 8,000 rpm for 10 min at room temperature and resuspended in 5 ml SET buffer afterwards. 50 µl RNase A stock solution

(10 mg/ml in sterile ddH_2O) and 300 µl Proteinase K solution (10 mg/ml in 50 mM Tris-Cl pH 8.0, 1 mM $CaCl_2$) were added, and the tube was inverted several times. Subsequently, 600 µl 10% SDS were added, and the tube was incubated first at 41 °C for 1.5 h and afterwards at 55 °C for 1 h under rotation in a hybridization oven. Thereafter, one volume phenol:chloroform:isoamyl alcohol (25:24:1) was added, and the mixture was incubated at 5 rpm for 1 h on a tube rotator. The tube was centrifuged at 8,000 rpm for 5 min at room temperature, and the upper phase was transferred into a new tube by using an end-cut 1 ml-tip. The extraction step with phenol:chloroform:isoamyl alcohol (25:24:1) was repeated once. Then, one volume chloroform:isoamyl alcohol (24:1) was added to the supernatant, and the mixture was incubated at 5 rpm for 1 h on a tube rotator. The tube was centrifuged at 14,000 rpm for 10 min at room temperature, and 4 ml of the upper phase were transferred into a new tube by using an end-cut 1 ml-tip. 440 µl 3 M sodium acetate pH 7.5 were added, and the tube was inverted several times. After adding 11 ml ice-cold ethanol, the tube was inverted until the appearance of cotton-like DNA. The DNA was collected by wrapping around the tip of a Pasteur pipette. In order to wash the DNA, the tip of the pipette with DNA was immersed into a 2 ml tube containing 70% ethanol. The DNA pellet on the pipette was dried by carefully attaching the inner site of a fresh 2 ml tube to remove the ethanol drops and suspended in 0.5-1 ml of 10 mM Tris-Cl pH 8.0.

General molecular biology techniques

Routine handling of nucleic acids, such as isolation of genomic and plasmid DNA, restriction endonuclease digestions, DNA ligations, and other DNA manipulations, were performed according to standard protocols[3]. All the enzymes were purchased from Thermo Scientific or New England Biolabs.

Cloning of expression constructs for heterologous PUFA production using linear plus linear homologous recombination and linear plus circular homologous recombination

For direct cloning of the *pfa* gene cluster from *S. cellulosum* So ce56, a vector backbone containing the colE1 origin of replication, an ampicillin resistance gene, a tetracycline promoter plus a gene encoding the TetR transcriptional regulator as well as the appropriate homology arms were amplified from plasmid pGB-amp-P_{tet}[1] using primers So ce-PKS-ET-up and So ce-PKS-ET-dn (Table S1). PCR was performed with *Taq* DNA polymerase (Thermo

Scientific) and standard conditions according to the manufacturer's protocol. The reactions contained 5% DMSO and were carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation for 2 min at 95 °C; 30 cycles consisting of denaturation for 30 s at 95 °C, annealing for 20 s at 60 °C, and extension for 1 min/kb at 72 °C; and a final extension for 10 min at 72 °C. For linear plus linear homologous recombination, 1.4 ml LB-medium in a 1.5 ml tube were inoculated with a GB05-dir overnight culture and incubated in a heating block at 37 °C with shaking at 1,000 rpm until OD_{600} ~0.2. Afterwards, expression of recETgA was induced by adding 20 µl of 20% L-arabinose, and the incubation was continued at 37 °C and 1,000 rpm until OD_{600} ~0.5. The cells were harvested at 9,000 rpm for 30 s at 2 °C. After discarding the supernatant, the cells were resuspended in 1 ml ice-cooled sterile ddH_2O on ice. The cells were spun down at 9,000 rpm for 30 s at 2 °C, and the supernatant was discarded. The washing step was repeated once. The supernatant was discarded leaving around 20-30 µl sterile ddH_2O in the tube, and the cells were resuspended with it. 0.5-1 µg of the PCR product plus 3-6 µg of genomic DNA of So ce56, completely digested with *Nhe*I and *Sca*I, were added. The cells were electroporated in an ice-cooled electroporation cuvette (1 mm) using an Eppendorf electroporator at 1,300 V. 1 ml LB medium was added into the cuvette, and the cell suspension was transferred back into the tube. After incubation in a heating block at 37 °C with shaking at 1,000 rpm for about 70 min, the cells were plated onto LB-agar containing 100 µg/ml ampicillin. The plates were incubated at 37 °C overnight. The colonies that grew under selection for the antibiotic resistance gene were examined for the intended direct cloning product pGB-P_{tet}-Soce-PKS (Table S2) by restriction analysis with a set of different enzymes. Further modification of the vector backbone was performed by Red/ET recombineering using a DNA fragment containing the mariner transposase gene with corresponding inverted repeats, a blasticidin resistance gene, an origin of transfer, the p15A origin of replication, a neomycin resistance gene driven by Tn5 promoter as well as the appropriate homology arms derived from p15A-epo-IR-Tps-bsd-oriT-IR-kan[4] digested with *Bam*HI. For linear plus circular homologous recombination, 1.4 ml LB-medium in a 1.5 ml tube were inoculated with a HS996/pSC101-BAD-gbaA overnight culture harbouring the plasmid to be modified and incubated in a heating block at 30 °C with shaking at 1,000 rpm until OD_{600} ~0.2. Afterwards, expression of the recombinases was induced by adding 14 µl of 20% L-arabinose, and the incubation was continued at 37 °C and 1,000 rpm until OD_{600} ~0.4. The cells were harvested at 11,000 rpm for 30 s at 2 °C. After discarding the supernatant, the cells were resuspended in 1 ml ice-cooled sterile ddH_2O on ice. The cells were spun down at 11,000 rpm for 30 s at 2 °C, and the supernatant was discarded. The washing step was repeated once. The supernatant was

discarded leaving around 20-30 µl sterile ddH_2O in the tube, and the cells were resuspended with it. 1.5 µl of the linear fragment with homology arms were added. The cells were electroporated in an ice-cooled electroporation cuvette (1 mm) using the Eppendorf electroporator at 1,350 V. 1 ml LB medium was added into the cuvette, and the cell suspension was transferred back into the tube. The tube was incubated in a heating block at 37 °C with shaking at 1,000 rpm for 70 min. Thereafter, the cells were plated onto LB-agar containing 100 µg/ml ampicillin and 50 µg/ml kanamycin. The plates were incubated at 37 °C overnight. The colonies that grew under selection for the antibiotic resistance genes were examined for the intended Red/ET cloning product pTps-p15A-Soce-PKS (Table S2) by restriction analysis and sequencing.

Direct cloning of the *pfa* gene clusters from *Aetherobacter fasciculatus* (SBSr002) and *Aetherobacter* sp. (SBSr008) was carried out via a two-step, double recombination 'fishing' strategy. For the first linear plus linear homologous recombination, a vector backbone containing the p15A origin of replication, an ampicillin resistance gene as well as the appropriate homology arms were amplified from plasmid pACYC177 (New England Biolabs) using primers HA+pACYC177_fwd and HA+pACYC177_rev (Table S1). PCR was performed with *Taq* DNA polymerase (Thermo Scientific) and standard conditions according to the manufacturer's protocol. The reactions contained 8% glycerol and were carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation for 3 min at 95 °C; 30 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 62 °C, and extension for 1 min/kb at 72 °C; and a final extension for 10 min at 72 °C. For the succeeding linear plus circular homologous recombination, a spectinomycin resistance gene with *Pac*I restriction sites as well as the appropriate homology arms were amplified from plasmid pR6K-amp-spec (Gene Bridges) by PCR using primers HA+*Pac*I+spectinomycin_fwd and HA+*Pac*I+spectinomycin_rev (Table S1). PCR was performed with PCR extender system (5 Prime) and standard conditions according to the manufacturer's protocol. The reactions were carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation for 3 min at 95 °C; 33 cycles consisting of denaturation for 1 min at 95 °C, annealing for 1 min at 62 °C, and extension for 1 min/kb at 72 °C; and a final extension for 10 min at 72 °C. Linear plus linear homologous recombination was performed as described previously using 0.5-1 µg of the PCR product plus 3-6 µg of genomic DNA of *Aetherobacter fasciculatus* (SBSr002) or *Aetherobacter sp.* (SBSr008), completely digested with *Sca*I. After electroporation the cells were cultivated in 1 ml LB-medium in a heating block at 37 °C with shaking at 1,000 rpm for 70 min. Thereafter, 100 µg/ml ampicillin were added, and the cells were incubated at 37 °C with shaking at 1,000 rpm overnight. For the succeeding linear plus circular homologous

recombination the next day, the cells from the first cloning step were used as preculture. Cell cultivation and preparation of electrocompetent cells were performed as described above. 0.5-1 µg of the PCR product was added to the electrocompetent cells. The electroporation conditions were the same as described previously. After incubation in a heating block at 37 °C with shaking at 1,000 rpm for about 70 min, the cells were plated onto LB-agar containing 100 µg/ml ampicillin and 80 µg/ml spectinomycin. The plates were incubated at 37 °C overnight. The colonies that grew under selection for the antibiotic resistance genes were examined for the intended direct cloning products pPfaAf1 and pPfaAs1 (Table S2) by restriction analysis with a set of different enzymes. The spectinomycin resistance gene was subsequently removed via the introduced *Pac*I restriction sites generating plasmids pPfaAf2 and pPfaAs2 (Table S2). In order to introduce a heterologous promoter upstream of the *pfa* gene cluster in the expression construct pPfaAf2 and to exchange the GTG start codon of gene *pfa1* for an ATG start codon, a tetracycline promoter downstream of a neomycin resistance gene driven by Tn5 promoter as well as the appropriate homology arms were amplified from plasmid pTps-p15A-Soce-PKS using primers HA+neo^R_fwd and HA+P_{tet}_rev (Table S1). PCR was performed with PCR extender system (5 Prime) and standard conditions according to the manufacturer's protocol. The reactions were carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation for 3 min at 95 °C; 33 cycles consisting of denaturation for 1 min at 95 °C, annealing for 1 min at 62 °C, and extension for 1 min/kb at 72 °C; and a final extension for 10 min at 72 °C. The linear plus circular homologous recombination was performed as described previously using 1.5 µl of the PCR product resulting in plasmid pPfaAf-P_{tet} (Table S2). Plasmid pPfaAs-P_{tet} (Table S2) was constructed by exchange of the *pfa* gene cluster from *Aetherobacter fasciculatus* (SBSr002) located on plasmid pPfaAf-P_{tet} for the *pfa* gene cluster from *Aetherobacter sp.* (SBSr008) located on plasmid pPfaAs2 via *Eco*91I and *Pac*I restriction sites. Afterwards, a zeocin resistance gene and the gene encoding the Mx9 integrase with *attP* sites both driven by Tn5 promoter derived from pMyx-zeo (S. C. Wenzel, unpublished) were inserted via *Pac*I restriction sites generating plasmids pPfaAf-P_{tet}-mx9.2 and pPfaAs-P_{tet}-mx9.2 (Table S2).

Table S1 Primers used for linear plus linear homologous recombination or linear plus circular homologous recombination.

For each primer, the homology arm is shown in italics and the introduced restriction site is underlined.

Primer	Sequence (5'→3')
So ce-PKS-ET-up	*CCGCTGCGCCAGATGCCGATCGGCTGGAGGCCGGCGCTGCGGAT GACCTGCGGATCCTCAATCAT*GCTGGGTGCCTCCTAGATTATTGT CGAACTATT
So ce-PKS-ET-dn	*TGTAGGCATCAAAATCGTCGAGAAATCCCTGTCCGTCCGCCCGCC TACAGCCGCAGATGGGCCGCAAGTATGGCTGTA*GTAAGCTTGAC CTGTGAAGTGAAAAAT
HA+pACYC177_fwd	*CAATATTTCGACGACCAGCGCTGCGCGGCGAAATATGGCGCGCTC TGGGGC*ATCAGAAGGGCACTGGTGCAACGG
HA+pACYC177_rev	*CGGGCGCCGCGGGATCCCGTACATCTACTTCGACGTGCTCGACGA GGAGA*TCGCTCACTGACTCGCTACGCTCGG
HA+*Pac*I+spectinomycin_fwd	*CTAAGGCCCCTTGTGCCCATTGAGGCACACGTCGAACGATTTCAC CGTCT*TTAATTAAGGGAACAGAAAACGGATACCAAGGCG
HA+*Pac*I+spectinomycin_rev	*ATTTCCGCTCGCCGCAGTCGAACGACCGAGCGTAGCGAGTCAGTG AGCGA*TTAATTAACTGCATCCGATGCAAGTGTGTCGC
HA+neo^R_fwd	*CCCCAGAGCGCGCCATATTTCGCCGCGCAGCGCTGGTCGTCGAAA TATTG*GACAGCAAGCGAACCGGAATTGCC
HA+P_{tet}_rev	*GGCGAGCCCCCTTGAGCGTTCCATCTACCGATTGCAGACA*TGCTG GGTGCCTCCTAGATTATTGTCG

Table S2 Plasmids and expression strains constructed in this study.

oriV = origin of replication, *oriT* = origin of transfer, *bla* = ampicillin resistance gene, *BSD* = blasticidin resistance gene, *nptII* = neomycin resistance gene, *aad9* = spectinomycin resistance gene, *ble* = zeocin resistance gene, P_{tet} = tetracycline promoter, *tetR* = gene encoding the tetracycline transcriptional regulator, PTn5 = Tn5 promoter, *tps* = mariner transposase gene, IRs = inverted repeats, Mx9 *int* = Mx9 integrase gene with phage attachment site *attP*.

Plasmid / Strain	Characteristics
pGB-P_{tet}-Soce-PKS	Directly cloned *pfa* gene cluster from *Sorangium cellulosum* So ce56 driven by P_{tet} plus *tetR*. colE1 *oriV*, *bla*
pTps-p15A-Soce-PKS	Derivative of pGB-P_{tet}-Soce-PKS in which colE1 *oriV* was replaced by *tps* plus IRs, *BSD*, *oriT*, p15A *oriV*, and PTn5-*nptII* by Red/ET recombineering
pPfaAf1	Directly cloned *pfa* gene cluster from *Aetherobacter fasciculatus* (SBSr002). p15A *oriV*, *bla*, *aad9*
pPfaAf2	Derivative of pPfaAf1 in which *aad9* was removed via *Pac*I
pPfaAf-P_{tet}	Derivative of pPfaAf2 in which PTn5-*nptII*-P_{tet} was inserted upstream of the *pfa* gene cluster by Red/ET recombineering
pPfaAf-P_{tet}-mx9.2	Derivative of pPfaAf-P_{tet} in which PTn5-*ble*-Mx9 *int* was inserted via *Pac*I
pPfaAs1	Directly cloned *pfa* gene cluster from *Aetherobacter* sp. (SBSr008). p15A *oriV*, *bla, aad9*
pPfaAs2	Derivative of pPfaAf-P_{tet} in which *aad9* was removed via *Pac*I
pPfaAs-P_{tet}	Derivative of pPfaAf-P_{tet} in which the *pfa* gene cluster from *A. fasciculatus* (SBSr002) was replaced by the *pfa* gene cluster from *Aetherobacter sp.* (SBSr008) located on plasmid pPfaAs2 via *Eco*91I and *Pac*I
pPfaAs-P_{tet}-mx9.2	Derivative of pPfaAs-P_{tet} in which PTn5-*ble*-Mx9 *int* was inserted via *Pac*I
M. xanthus DK1622::pTps-p15A-Soce-PKS	*Myxococcus xanthus* DK1622 with pTps-p15A-Soce-PKS randomly integrated into the genome
M. xanthus DK1622::pPfaAf-P_{tet}-mx9.2	*M. xanthus* DK1622 with pPfaAf-P_{tet}-mx9.2 site-specifically integrated at Mx9 *attB2* site of the genome
M. xanthus DK1622::pPfaAs-P_{tet}-mx9.2	*M. xanthus* DK1622 with pPfaAs-P_{tet}-mx9.2 site-specifically integrated at Mx9 *attB2* site of the genome

Transformation of *M. xanthus* by electroporation and heterologous expression

1.5 x 10^9 cells from an overnight culture of *M. xanthus* DK1622 were harvested at 8,000 rpm for 10 min at 4 °C. After discarding the supernatant, the cells were resuspended in 20 ml ice-cooled sterile ddH_2O on ice. The cells were spun down at 8,000 rpm for 10 min at 4 °C, and the supernatant was discarded. The cells were resuspended in 40 µl ice-cooled sterile ddH_2O on ice and transferred into a 2 ml tube. 2-5 µl of the expression plasmid were added. The cells were electroporated in an ice-cooled electroporation cuvette (1 mm) using a Bio-Rad electroporator at 650 V, 400 Ω, and 25 µF. 1 ml CTT-medium was added into the cuvette, and the cell suspension was transferred back into the tube. The tube was incubated in a heating block at 30 °C with shaking at 1,000 rpm for about 5-8 h. Thereafter, the cells were mixed with 2-3 ml CTT-soft agar (CTT-medium plus 0.75% agar) containing 60 µg/ml kanamycin plus 20 µg/ml zeocin and plated onto two CTT-agar plates containing 60 µg/ml kanamycin plus 20 µg/ml zeocin. The plates were incubated at 30 °C for about 5 days. Thereafter, six colonies were transferred onto new CTT-agar plates containing 60 µg/ml kanamycin and 20 µg/ml zeocin. In order to verify the integration of the expression construct into the genome of *M. xanthus* via Mx9 *attB* sites, DNA from selected clones was isolated and used for PCR analyses[5]. The primers used are listed in Table S3. PCR was performed with *Taq* DNA polymerase (Thermo Scientific) and standard conditions according to the manufacturer's protocol. The reactions contained 8% glycerol and were carried out in an Eppendorf Mastercycler under the following conditions: initial denaturation for 3 min at 95 °C; 30 cycles consisting of denaturation for 30 s at 95 °C, annealing for 30 s at 56 °C, and extension for 1 min/kb at 72 °C; and a final extension for 10 min at 72 °C. Expression was carried out in 50 ml CTT-medium containing 60 µg/ml kanamycin and 20 µg/ml zeocin at 30 °C. The cells were cultivated for 48 h and then harvested at 4,000 rpm for 10 min.

Table S3 Primers used for the verification of the integration of expression constructs into the genome of *M. xanthus* DK1622 via Mx9 *attB* sites.

Primer	Sequence (5'→3')
Mx9*attB1*_up	TGCCAGGGCTTACGGCTTC
Mx9*attB1*_down	CAGCACGGGTGCAGCAAC
Mx9*attB2*_up	TATCCCAGCAACCGCCGGAG
Mx9*attB2*_down	CGAGGTCCGGGACGCGCGCA
Mx9*attP*_up	GCGCCGAACTTAACAAGTTG
Mx9*attP*_down	TCCAGGTCCTCACGCTTGAC

Extraction of cellular fatty acids

The cellular fatty acids were extracted using the FAME method[6]. For this purpose, 50-100 ml of a culture were harvested at 8,000 rpm for 10 min at room temperature. The cell pellet was transferred to a glass vial and dried in a vacuum concentrator. Subsequently, the cell dry weight was determined. 5 µl (50 µg) of *n*-3 DPA (Sigma-Aldrich) and 500 µl of a mixture of methanol, toluene, and sulphuric acid (50:50:2, v/v/v) were added, the vial was capped with a teflon-lined screw cap and incubated at 80 °C for 48 h. After the mixture was cooled to room temperature, 400 µl of an aqueous solution consisting of 0.5 M NH_4HCO_3 and 2 M KCl were added, and the sample was vortexed for 30 s. Phase separation was achieved by centrifugation at 4,000 rpm for 5 min at room temperature. 75 µl of the upper phase were mixed with 25 µl *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and incubated at 37 °C for 30 min. Subsequently, the sample was used for GC-MS analysis.

Extraction and fractionation of lipids

Extraction of lipids from microbial cells was carried out using the method of Bligh and Dyer[7], modified by Lewis *et al.*[8] on a small scale. In the first step, the cell pellet from a 100 ml culture was transferred into a polypropylene tube. Successively, 4 ml chloroform, 8 ml methanol, and 3.2 ml 1% NaCl were added, and the tube was vortexed at high speed for 15 s after every addition. The sample was agitated on a tube rotator at 30 rpm overnight. 4 ml chloroform and 4 ml 1% NaCl were then added, and the tube was inverted 30 times. Phase separation was achieved by centrifugation at 4,000 rpm for 5 min at room temperature. The bottom layer containing the lipid extract was evaporated to dryness under a gentle stream of nitrogen and dissolved in 1 ml of a chloroform:methanol mixture (2:1, v/v). The lipid solution was fractionated by solid-phase extraction on a Strata SI-1 silica column with 100 mg sorbent mass (Phenomenex) equilibrated with chloroform + 1% acetic acid. Neutral lipids and free fatty acids were eluted from the column with 1 ml of chloroform + 1% acetic acid, glycolipids were eluted with 1.5 ml of an acetone:methanol mixture (9:1, v/v), and phospholipids were eluted with 1 ml methanol. For GC-MS analysis, the fractions were dried in a vacuum concentrator and further processed according to the FAME method described previously.

Analysis of fatty acid methyl esters by GC-MS

GC-MS was carried out on an Agilent 6890N gas chromatograph (Agilent Technologies) equipped with a 7683B split/splitless injector with autosampler (Agilent Technologies) and coupled to a 5973 electron impact mass selective detector (Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1 ml/min. 1 µl of the sample was injected in split mode (split ratio, 10:1). The analytical column was a (5% phenyl)-methylpolysiloxane capillary column (Agilent J&W DB-5ht; 30 m × 0.25 mm i.d. × 0.1 µm film thickness, maximum temperature 400 °C; Agilent Technologies). The column temperature was kept at 130 °C for 2.5 min, increased to 240 °C at a rate of 5 °C/min, then ramped to 300 °C at 30 °C/min, and held at 300 °C for 5 min. Other temperatures were as follows: inlet, 275 °C; GC-MS transfer line, 280 °C; ion source, 230 °C; and quadrupole, 150 °C. The mass selective detector was operated in scan mode, scanning the mass range from *m*/*z* 40 to 700. Scan control, data acquisition, and processing were performed by MSD ChemStation and AMDIS software, version 2.68, based on the fragmentation patterns and retention time, in comparison with Supelco 37 Component FAME Mix and LC-PUFAs (all Sigma-Aldrich) as reference standards, and NIST 08 library. Absolute amounts of PUFAs were quantified by integration of the peaks using MSD ChemStation and by subsequent calculation in relation to the integral of *n*-3 DPA and to cell dry weight. In case *n*-3 DPA was produced by the culture itself, the sample was spiked with *n*-3 DPA for the calculation of the absolute amounts of PUFAs.

Bioinformatic analysis of DNA and protein sequences

Prediction of open reading frames was performed with FramePlot 4.0[9], and functional annotation of proteins was based on BlastP[10] and Pfam searches[11]. The Geneious software suite (Biomatters)[12] was used for *in silico* analyses of DNA and protein sequences. All sequence alignments were performed using Geneious and ClustalW[13]. For the construction of the phylogenetic tree by the neighbour-joining method, the following DNA sequences of the 16S ribosomal RNA from the myxobacterial strains which are not deposited in any database were used:

>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce10

AGAGTTTGATCCTGGCTCAGAACGAACGTTAGCGGCGCGCTTAACACATGCAAGTCGAGCGAGAA
AGGGCTTCGGCCCCGGTAAAGCGGCGCACGGGTGAGTAACACGTAGGTAATCTGCCCCCAGGTGG
TGGATAACGTTCCGAAAGGAGCGCTAATACAGCATGAGACCACGTCCTCGAAAGGGGATGAGGTC
AAAGCCGGCCTCTTCATGAAAGCTGGCGCCAGGGGATGAGCCTGCGGCCCATCAGCTAGTTGGTAG

GGTAATGGCCTACCAAGGCGAAGACGGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTG
ACGCAGCGACGCCGCGTGAGTGATGAAGGCCTTCGGGTTGTAAAGCTCTGTGGAGGGGGACGAAT
AAGGGTTGGCTAACATCCAGCTCGATGACGGTACCCCTTTAGCAAGCACCGGCTAACTCTGTGCCA
GCAGCCGCGGTAAGACAGAGGGTGCAAACGTTGTTCGGAATTACTGGGCGTAAAGCGCATGTAGG
CGGTTCGTAAAGTCAGATGTGAAAGCCCTGGGCTTAACCCAGGAAGTGCATTTGAAACTCACGAAC
TTGAGTCCCGGAGAGGAAGGCGGAATTCTCGGTGTAGAGGTGAAATTCGTAGATATCGAGAGGAA
CATCGGTGGCGAAGGCGGCCTTCTGGACGGTGACTGACGCTGAGATGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGTGCTAGGTGTCGCGGGCTTTGACT
CCTGCGGTGCCGTAGCTAACGCATTAAGCACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTCAATTCGACGCAACGCGCAGA
ACCTTACCTGGGCTAGAAAATGCAGGGACCTGGTTGAAAGATCGGGGTGCTCTTCGGAGAACCTGT
AGTTAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCCTATCGTTAGTTGCCAGCGGTTCGGCCGGGCACTCTAGCGAGACTGCCGATATTTAAAT
CGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGTCCAGGGCTACACACGTGCTACA
ATGGGCGGTACAAACGGTTGCGAACTCGCGAGGGGAAGCCAATCCGAAAAAACCGTCCTCAGTAC
GGATAAGAGTCTGCAACTCGACTCTTTGAAGTTGGAATCGCTAGTAATCCCTGATCAGCAGGCAGG
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTCGATTGCTCCAGAAG
TGGCTACGCCAACCCGCAAGGGAGGCAGGCCCCCAAGGAGTGGTTGGTAACTGGGGTGAAGTCGT
AACAAGGTAGCCGTAGGGGAACCTGCGGCTGGATCACCTCCTTT

>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce1525

AGAGTTTGATCCTGGCTCAGAACGAACGTTAGCGGCGCGCTTAACACATGCAAGTCGAGCGAGAA
AGGGCTTCGGCCCCGGTAAAGCGGCGCACGGGTGAGTAACACGTAGGTAATCTGCCCCCAGGTGG
TGGATAACGTTCCGAAAGGAGCGCTAATACAGCATGAGACCACGTCCTCGAGAGGGGATGAGGTC
AAAGCCGGCCTCTTCACGAAAGCTGGCGCCAGGGGATGAGCCTGCGGCCCATCAGCTAGTTGGTA
GGGTAATGGCCTACCAAGGCGAAGACGGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAC
TGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCT
GACGCAGCGACGCCGCGTGAGTGATGAAGGCCTTCGGGTTGTAAAGCTCTGTGGAGGGGGACGAA
TAAGGGTTGGCTAACATCCAGCTCGATGACGGTACCCCTTTAGCAAGCACCGGCTAACTCTGTGCC
AGCAGCCGCGGTAAGACAGAGGGTGCAAACGTTGTTCGGAATTACTGGGCGTAAAGCGCATGTAG
GCGGTTCGTAAAGTCAGATGTGAAAGCCCTGGGCTTAACCCAGGAAGTGCATTTGAAACTCACGAA
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AATGGGCGGTACAAACGGTCGCGAACCCGCGAGGGGAAGCCAATCCGAAAAAACCGTCCTCAGTA
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GTGGCTGCGCCAACCCGCAAGGGAGGCAGGCCCCCAAGGAGTGGTTGGTAACTGGGGTGAAGTCG
TAACAAGGTAGCCGTAGGGGAACCTGCGGCTGGATCACCTCCTTT

>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce377

AGAGTTTGATCCTGGCTCAGAACGAACGTTAGCGGCGCGCTTAACACATGCAAGTCGAGCGAGAA
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>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce38

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>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce836

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>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ceGT47

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>16S ribosomal RNA gene sequence from *Sorangium cellulosum* So ce487

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