Specialized acyl carrier protein used by serine palmitoyltransferase to synthesize sphingolipids in *Rhodobacteria*

Supplementary Tables

**Table S1.** Theoretical and measured masses of acyl carrier proteins AcpR<sub>Cc</sub> and AcpP<sub>Cc</sub>

| Protein species | AcpR<sub>Cc</sub> | AcpP<sub>Cc</sub> |
|-----------------|-----------------|-----------------|
|                 | Detected mass   | Theoret. mass*  | Error (ppm) | Detected mass   | Theoret. mass*  | Error (ppm) |
| Apo             | 9020.3          | 9022.4          | 232         | 8599.1          | 8601.7          | 302         |
| Holo            | 9360.8          | 9361.4          | 64          | 8939.8          | 8940.7          | 101         |
| Palmitoyl       | 9601.2          | 9599.8          | -145        | 9180.3          | 9179.1          | -130        |

*The theoretical masses were determined using [https://www.expasy.org](https://www.expasy.org) and in the case of AcpP<sub>Cc</sub> without the N-terminal methionine.
**Table S2.** Orthologues for sphingolipid biosynthesis proteins in the *Rhodobacteria C. crescentus*, *E. coli* B21(DE3), *S. wittichii*, *S. paucimobilis*, *Z. mobilis*, *G. oxydans*, and *N. eutropha*.

| ORF (Query) | ORF (Subject) | E Value | identity (%) | similarity (%) | coverage (%) |
|-------------|---------------|---------|--------------|---------------|--------------|
| CC_1154Cc (Aca) | ECD_02850Ec | 1e-107  | 44           | 61            | 98           |
| CC_1154Cc (Aca) | Swit_3908Sw  | 1e-104  | 45           | 58            | 97           |
| CC_1154Cc (Aca) | DRN02_007910Sp | 3e-91   | 41           | 59            | 97           |
| CC_1154Cc (Aca) | ZMO1400Zm    | 7e-93   | 40           | 57            | 98           |
| CC_1154Cc (Aca) | GOX2417Go    | 9e-62   | 34           | 50            | 97           |
| CC_1154Cc (Aca) | Neut_1100Ne  | 2e-100  | 41           | 58            | 100          |
| CC_1162Cc (Spt) | ECD_02854Ec  | 4e-144  | 53           | 68            | 96           |
| CC_1162Cc (Spt) | Swit_3900Sw  | 1e-152  | 53           | 73            | 97           |
| CC_1162Cc (Spt) | DRN02_007885Sp | 2e-147 | 53           | 70            | 97           |
| CC_1162Cc (Spt) | ZMO1270Zm    | 3e-151  | 52           | 72            | 97           |
| CC_1162Cc (Spt) | GOX2056Go    | 1e-159  | 55           | 72            | 97           |
| CC_1162Cc (Spt) | Neut_0461Ne  | 1e-133  | 51           | 66            | 97           |
| CC_1163Cc (AcpR) | ECD_02853Ec  | 3e-08   | 41           | 68            | 61           |
| CC_1163Cc (AcpR) | Swit_3899Sw  | 6e-10   | 32           | 54            | 71           |
| CC_1163Cc (AcpR) | DRN02_007880Sp | 1e-09  | 32           | 52            | 71           |
| CC_1163Cc (AcpR) | ZMO2012Zm    | 5e-12   | 38           | 57            | 68           |
| CC_1163Cc (AcpR) | GOX2057Go    | 1e-12   | 46           | 69            | 67           |
| CC_1163Cc (AcpR) | Neut_0460Ne  | 8e-13   | 44           | 71            | 71           |
| CC_1164Cc (Epi) | ECD_02852Ec  | 7e-38   | 34           | 49            | 94           |
| CC_1164Cc (Epi) | Swit_3897Sw  | 2e-47   | 40           | 47            | 95           |
| CC_1164Cc (Epi) | DRN02_007875Sp | 7e-37  | 35           | 48            | 95           |
| CC_1164Cc (Epi) | ZMO0205Zm    | 1e-38   | 31           | 48            | 96           |
| CC_1164Cc (Epi) | GOX1849Go    | 2e-18   | 32           | 47            | 73           |
| CC_1164Cc (Epi) | Neut_0459Ne  | 1e-47   | 38           | 52            | 85           |
| CC_1165Cc (AasR) | ECD_02851Ec  | 1e-155  | 42           | 60            | 97           |
| CC_1165Cc (AasR) | Swit_2559Sw  | 1e-163  | 46           | 63            | 98           |
| CC_1165Cc (AasR) | DRN02_008180Sp | 7e-163 | 46           | 62            | 98           |
| CC_1165Cc (AasR) | ZMO0704Zm    | 3e-164  | 45           | 63            | 98           |
| CC_1165Cc (AasR) | GOX2058Go    | 0        | 54           | 69            | 99           |
| CC_1165Cc (AasR) | Neut_0458Ne  | 6e-152  | 43           | 60            | 99           |

Pairwise protein sequence alignments between *C. crescentus* (Cc) ORFs codifying proteins for dihydroceramide biosynthesis: putative acyl-CoA N-acyltransferase (Aca), serine palmitoyltransferase (Spt), acyl carrier protein (AcpR), predicted dehydrogenase/epimerase (Epi), and acyl-ACP synthetase (AasR); compared to the *E. coli* BL21(DE3) (Ec); *S. wittichii* (Sw), *S. paucimobilis* (Sp), *Z. mobilis* (Zm), *G. oxydans* (Go), and *N. eutropha* (Ne) homologues. Homologous ORFs are shown in equal colors. ORF names/accession numbers are as follows: *Caulobacter crescentus* CB15 (Aca: CC_1154/AAK23138.1; Spt: CC_1162/AAK23146.1; AcpR: CC_1163/AAK23147.1; Epi: CC_1164/AAK23148.1; AasR: CC_1165/AAK23149.1), *Escherichia coli* BL21(DE3) (Aca: ECD_02850/ACT4454.1; Spt: ECD_02854/ACT4458.1; AcpR: ECD_02853/ACT4457.1; Epi: ECD_02852/ACT4456.1; AasR: ECD_02851/ACT4455.1), *Sphingomonas wittichii* RW1 (Aca: Swit_3908/ABQ70253.1; Spt: Swit_3900/ABQ70245.1; AcpR: Swit_3899/ABQ70244.1; Epi: Swit_3897/ABQ70242.1; AasR: Swit_2559/ABQ68917.1), *Sphingomonas paucimobilis* strain AIMST S-2 (Aca: DRN02_007910/QBE91945.1; Spt:...
Padilla-Gómez et al.

DRN02_007885/QBE91942.1; AcpR: DRN02_007880/QBE91994.1; Epi: DRN02_007875/QBE91940.1; AasR: DRN02_008180/QBE91994.1), *Zymomonas mobilis* ZM4 (Aca: ZMO1400/AAV90024.1; Spt: ZMO1270/AAV89894.1; AcpR: ZMO2012/ADK75091.1; Epi: ZMO0205/AAV88829.1; AasR: ZMO0704/AAV89328.2), *Gluconobacter oxydans* 621H (Aca: GOX2417/AAW62148.1; Spt: GOX2056/AAW61792.1; AcpR: GOX2057/AAW61793.1; Epi: GOX1849/AAW61587.1; AasR: GOX2058/AAW61794.1) and *Nitrosomonas eutropha* C91 (Aca: Neut_1100/ABI59355.1; Spt: Neut_0461/ABI58738.1; AcpR: Neut_0460/ABI58737.1; Epi: Neut_0459/ABI58736.1; AasR: Neut_0458/ABI58735.1).

Table S3. Possible orthologues for FadD in *G. oxydans*, *N. eutropha* and *C. crescentus*.

| ORF (Query) | ORF (Subject) | E Value  | identity (%) | similarity (%) | coverage (%) |
|-------------|---------------|----------|--------------|----------------|--------------|
| ECD_01775Ec (FadD) | AD932_04675Go | $1 \times 10^{-44}$ | 28 | 45 | 85 |
| ECD_01775Ec (FadD) | Neut_1417Ne | $5 \times 10^{-60}$ | 29 | 46 | 93 |
| ECD_01775Ec (FadD) | CC_1321Cc | $1 \times 10^{-65}$ | 31 | 48 | 91 |
| ECD_01775Ec (FadD) | CC_0966Cc | $3 \times 10^{-58}$ | 28 | 45 | 95 |
| SMc02162Sm (FadD) | AD932_04675Go | $3 \times 10^{-42}$ | 28 | 44 | 93 |
| SMc02162Sm (FadD) | Neut_1417Ne | $5 \times 10^{-38}$ | 27 | 42 | 89 |
| SMc02162Sm (FadD) | CC_0966Cc | $3 \times 10^{-60}$ | 29 | 48 | 90 |
| SMc02162Sm (FadD) | CC_1321Cc | $8 \times 10^{-56}$ | 31 | 49 | 87 |

Pairwise protein sequence alignments between ORFs codifying for acyl-CoA synthetase (FadD) from *E. coli* (Ec) and *S. meliloti* (Sm); compared to *G. oxydans* (Go), *N. eutropha* (Ne) and *C. crescentus* (Cc) proteins. Possible homologous ORFs are shown in equal colors. ORF names/accession numbers are as follows: *Caulobacter crescentus* CB15 (CC_1321/AAK23302.1; CC_0966/AAK22950.1), *Escherichia coli* BL21(DE3) (ECD_01775/ACT43629.1), *Gluconobacter oxydans* 621H (AD932_04675/KXV13177), *Nitrosomonas eutropha* C91 (Neut_1417/ABI59664.1) and *Sinorhizobium meliloti* 1021 (SMc02162/CAC41921.1).
**Table S4.** Possible orthologues for AcpR, AcpP, AasR, FadD and Aas proteins in *B. thetaiotaomicron, B. stolpii, M. xanthus, P. gingivalis, S. multivorum, S. aurantiaca* and *S. cellulosum.*

| ORF (Query) | ORF (Subject) | E Value | identity (%) | similarity (%) | coverage (%) |
|-------------|---------------|---------|--------------|---------------|--------------|
| CC_1163Ec (AcpR) | BT_3359Bs | 9e⁻¹¹ | 41 | 51 | 65 |
| CC_1163Ec (AcpR) | COV70_14770Bs | 7e⁻⁹⁷ | 29 | 60 | 57 |
| CC_1163Ec (AcpR) | MXAN_6637Ms | 1e⁻⁹³ | 42 | 60 | 51 |
| CC_1163Ec (AcpR) | PGN_1705Pg | 2e⁻¹⁰ | 38 | 56 | 60 |
| CC_1163Ec (AcpR) | NCTC11343_01004Sm | 5e⁻¹⁰ | 39 | 58 | 55 |
| CC_1163Ec (AcpR) | STAUR_1257Sa | 7e⁻⁰² | 33 | 60 | 51 |
| CC_1163Ec (AcpR) | scc7052Sc | 1e⁻⁰⁹ | 44 | 66 | 65 |
| CC_1677Ec (AcpP) | BT_3359Bs | 5e⁻²⁶ | 59 | 74 | 96 |
| CC_1677Ec (AcpP) | COV70_14770Bs | 3e⁻¹⁴ | 42 | 67 | 93 |
| CC_1677Ec (AcpP) | MXAN_4769Ms | 4e⁻²¹ | 53 | 75 | 93 |
| CC_1677Ec (AcpP) | PGN_1705Pg | 2e⁻²³ | 52 | 79 | 93 |
| CC_1677Ec (AcpP) | NCTC11343_01004Sm | 2e⁻²⁵ | 58 | 75 | 93 |
| CC_1677Ec (AcpP) | STAUR_5619Sa | 8e⁻²¹ | 51 | 75 | 93 |
| CC_1677Ec (AcpP) | scc3814Sc | 2e⁻²⁰ | 56 | 74 | 91 |
| EC01090Ec (AcpP) | BT_3359Bs | 6e⁻²⁷ | 62 | 73 | 97 |
| EC01090Ec (AcpP) | COV70_14770Bs | 2e⁻¹⁴ | 40 | 65 | 96 |
| EC01090Ec (AcpP) | MXAN_4769Ms | 6e⁻³¹ | 66 | 80 | 97 |
| EC01090Ec (AcpP) | PGN_1705Pg | 1e⁻²² | 55 | 75 | 93 |
| EC01090Ec (AcpP) | NCTC11343_01004Sm | 3e⁻²⁴ | 62 | 72 | 93 |
| EC01090Ec (AcpP) | STAUR_5619Sa | 5e⁻²⁹ | 63 | 78 | 97 |
| EC01090Ec (AcpP) | scc3814Sc | 1e⁻²² | 62 | 78 | 84 |
| CC_1165Ec (AasR) | BT_2782Bs | 1e⁻³³ | 27 | 45 | 68 |
| CC_1165Ec (AasR) | COV70_02865Bs | 8e⁻³⁰ | 24 | 40 | 68 |
| CC_1165Ec (AasR) | MXAN_6636Ms | 1e⁻⁸⁷ | 36 | 52 | 91 |
| CC_1165Ec (AasR) | CF001_1738Pg | 4e⁻¹⁷ | 24 | 40 | 79 |
| CC_1165Ec (AasR) | NCTC11343_01971Sm | 1e⁻¹³ | 24 | 38 | 73 |
| CC_1165Ec (AasR) | STAUR_1258Sa | 3e⁻⁹² | 36 | 52 | 97 |
| CC_1165Ec (AasR) | scc7053Sc | 6e⁻¹²² | 39 | 56 | 96 |
| EC01775Ec (FadD) | BT_2782Bs | 4e⁻⁶³ | 30 | 50 | 89 |
| EC01775Ec (FadD) | COV70_02865Bs | 0 | 55 | 74 | 98 |
| EC01775Ec (FadD) | MXAN_7148Ms | 6e⁻⁷⁹ | 33 | 50 | 93 |
| EC01775Ec (FadD) | CF001_1738Pg | 2e⁻²⁹ | 25 | 42 | 80 |
| EC01775Ec (FadD) | NCTC11343_01971Sm | 8e⁻³⁷ | 28 | 45 | 74 |
| EC01775Ec (FadD) | STAUR_3279Sa | 0 | 58 | 73 | 98 |
| EC01775Ec (FadD) | scc3825Sc | 5e⁻⁵⁴ | 32 | 47 | 94 |
| LA59_RS23465Yh (Aas) | BT_2782Bs | 7e⁻³⁸ | 25 | 42 | 95 |
| LA59_RS23465Yh (Aas) | COV70_10230Bs | 2e⁻³⁴ | 27 | 45 | 93 |
| LA59_RS23465Yh (Aas) | MXAN_6374Ms | 4e⁻¹¹⁵ | 36 | 55 | 97 |
| LA59_RS23465Yh (Aas) | CF001_1738Pg | 1e⁻¹⁵ | 22 | 40 | 75 |
| LA59_RS23465Yh (Aas) | NCTC11034_01400Sm | 3e⁻⁸ | 23 | 42 | 79 |
| LA59_RS23465Yh (Aas) | STAUR_2914Sa | 2e⁻¹¹² | 37 | 55 | 97 |
| LA59_RS23465Yh (Aas) | scc5736Sc | 1e⁻¹⁰⁷ | 36 | 52 | 97 |

Pairwise protein sequence alignments between ORFs codifying for: acyl carrier protein (AcpR) and acyl-ACP synthetase (AasR) from *C. crescentus* CB15 (Cc), acyl-CoA synthetase (FadD) from *E. coli* BL21(DE3) (Ec), and acyl-ACP synthetase (Aas) from *V. harveyi* (Vh); compared to the *B. thetaiotaomicron* (Bs), *B. stolpii* (Bs), *M. xanthus* (Ms), *P. gingivalis* (Pg), *S. multivorum* (Sm), *S. aurantiaca* (Sa), and *S. cellulosum*. 

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"Padilla-Gómez et al."
aurantiaca (Sa) and S. cellulosum (Sc) proteins. Possible homologous ORFs are shown in equal colors. ORF names/accession numbers are as follows: Bacteroides thetaiotaomicron VPI-5482 (BT_3359/Q8A2E6.1; BT_2782/AAO77888), Bacteriovorax stolpii (C0V70_14770/AUN99345.1; C0V70_02865/AUN97064; C0V70_10230/AUN98475), Myxococcus xanthus DK 1622 (MXAN_6637/ABF91660.1; MXAN_4769/ABF9032.1; MXAN_6636/ABF92798; MXAN_7148/ABF89596; MXAN_6374/ABF88458), Porphyromonas gingivalis ATCC 33277 (PGN_1705/B2RLH9.1; CF001_1738/AUR49213), Sphingobacterium multivorum (NCTC11343_01004/SPZ84464.1; NCTC11343_01971/SPZ85409; NCTC11034_01400/SUJ04263), Stigmatella aurantiaca DW4/3-1 (STAUR_1257/ADO69061.1; STAUR_5619/ADO73384.1; STAUR_1258/ADO69062; STAUR_3279/ADO70706) and Sorangium cellulosum So ce56 (sce7052/ CAN97221.1; sce3814/CAN93974.1; sce7053/CAN97222.1; sce3825/CAN93985.1; sce5736/CAN95899.1)

Table S5. Oligonucleotides used for amplification of different sphingolipid biosynthesis genes. Sites for recognition by restriction enzymes are underlined.

| Primers for expression plasmids | Sequence (5’-3’) |
|---------------------------------|------------------|
| oLOP227                         | AGGAATACATATGATCAGCCACGCGGTC |
| oLOP228                         | AAAGGTACCTAGTCCGACATTCTCGAGCG |
| oLOP264                         | AGGAATACATATGATCAGCCACGCGGTC |
| oLOP265                         | AAAGGTACCTAGTCCGACATTCTCGAGCG |
| oLOP423                         | ACTGGGTACCATTGAGCTATTTGATAAGCACC |
| oLOP424                         | ACTGCTAGAGTCCTAGCGCGCGCGCGCG |
| oLOP432                         | ACTGCAATGTGAAATCTGGAATAGTAATG |
| oLOP433                         | ACTGGATCCTATTATATTTCTCAGGCGT |
| oLOP434                         | ACTGACTCCATGCGATGCGTATAGGATAATGCGC |
| oLOP435                         | ACTGACTCCATGCGATGCGTATAGGATAATGCGC |
| oLOP436                         | ACTGACTCCATGCGATGCGTATAGGATAATGCGC |
| oLOP437                         | ACTGACTCCATGCGATGCGTATAGGATAATGCGC |
| oLOP438                         | ACTGACTCCATGCGATGCGTATAGGATAATGCGC |
| oLOP439                         | ACTGGGGCCGGCCCTAGGCGCGCGCGCGCG |
| oLOP440                         | ACTGCAATGTGATATGTCTATATAAATC |
| oLOP443                         | ACTGCTAGTCCGAGGATTCC |

Table S6. Construction of different expression plasmids

For details see Materials and methods in main text.

| Plasmid | ORFs cloned | oligonucleotides used | restricted with | cloned into plasmid restricted with () |
|---------|-------------|-----------------------|-----------------|----------------------------------------|
| pDG04   | CC_1165     | oLOP227/oLOP228       | Ndel/KpnI       | pET17b (Ndel/KpnI)                      |
| pPEG01  | CC_1677     | oLOP264/oLOP265       | Ndel/BamHI      | pET9a (Ndel/BamHI)                      |
| pJPG05  | CC_1162     | oLOP243/oLOP242       | KpnI/XbaI       | pBAD24 (KpnI/XbaI)                      |
| pJPG06  | ECD_02854   | oLOP434/oLOP435       | Ncol/XbaI       | pBAD24 (Ncol/XbaI)                      |
| pJPG07  | Swit_3900   | oLOP436/oLOP437       | Ncol/XbaI       | pBAD24 (Ncol/XbaI)                      |
| pJPG12  | ECD_02853   | oLOP432/oLOP433       | Ndel/BamHI      | pET9a (Ndel/BamHI)                      |
| pJPG15  | CC_1162     | oLOP438/oLOP439       | EcoRV/FseI      | pJPG13 (MCS-2) (EcoRV/FseI)             |
| pJPG17  | ECD_02851   | oLOP440/oLOP443       | Ndel/XhoI       | pET17b (Ndel/XhoI)                      |
| pJPG20  | ECD_02853/  | oLOP432/oLOP435       | Ndel/BamHI      | pCDFDuet-1 (MCS-2) (Ndel/BglII)         |