**Only acyl carrier protein 1 (AcpP1) of *Pseudomonas aeruginosa* functions in fatty acid synthesis**

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**Supplementary Tables**

**Table S1. Bacterial strains and plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| Bacterial strains | Relevant characteristics*a* | Source |
| *E. coli* |  |  |
| DH-5α | F- *deoR* *endA1 gyrA96 hsdR17*(rK-mK+) *recA1* *relA1* *supE*44 *thi-1* Δ(*lacZYA-argF*)*U*169(φ80*lacZ*ΔM15) | Laboratory collection |
| BL21 (DE3) | F- *dcm ompT* *hsdS* (*rB*- *mB*-) *gal* (λDE3) | Laboratory collection |
| S17-1 | F- *thi pro* *hsdR* [RP4-2 Tc::Mu Km::Tn7 (Tp Sm)] | Laboratory collection |
| CY1877 | *E.coli* MG1655 *acpP*::Cmr carrying pBAD24-acpP | Laboratory collection |
| *P. aeruginosa* | |  |
| PAO1 | Wild-type strain | Laboratory collection |
| PA-A1 | PAO1, *acpP1*:: *EcacpP* | This study |
| PA-A2 | PAO1, *acpP2*:: Gmr | This study |
| PA-A3 | PAO1, *acpP3*:: Gmr | This study |
| PA-A12 | PAO1, *acpP1*:: *EcacpP acpP2* :: Gmr | This study |
| PA-A13 | PAO1*, acpP1*::*EcacpP acpP3 ::* Gmr | This study |
| PA-A23 | PAO1, *acpP2* :: Tcr *acpP3 ::* Gmr | This study |
| PA-A123 | PAO1, *acpP1*:: *EcacpP acpP2* :: Tcr *acpP3* :: Gmr | This study |
| PK-Pa | PAO1, *acpP1*:: *EcacpP* carried plasmid pSRKPa | This study |
| Plasmids |  |  |
| pMD19 | Ampr, TA cloning vector | Takara |
| pBAD24m | Ampr; NcoI site of expression vector pBAD24 changed to an NdeI site | (7) |
| pET-28(b) | Kmr; expression vector | Novagen |
| pET-30(a) | Kmr; expression vector | Novagen |
| pBluescript SK(+) | Ampr; clone and expression vector | Laboratory collection |
| pTac85 | Ampr; expression vector | (4) |
| pK18mobscaB | Kmr; *sacB*-based gene replacement vector | (5) |
| p34s-Gm | Ampr; Gm resistance cassette-carrying vector | (1) |
| p34s-Tc | Ampr; Tc resistance cassette-carrying vector | (1) |
| pSRK-Km | Kmr, broad-host-range expression vector containing *lac* promoter and *lacIq*, *lacZα+* | (3) |
| pHSG399 | Cmr; clone and expression vector | (6) |
| pYFJ84 | *V. har*V*eyi aasS* cloned into the NdeI and BamHI sites of pET16(b) | (2) |
| pBAD24m- *acpP1* | Ampr; The PCR fragment of *acpP1* from *P.aeruginosa* genome digested by NdeI and HindIII was clonedinto the same sites of pBAD24m | This study |
| pBAD24m- *acpP2* | Ampr; The PCR fragment of *acpP2* from *P.aeruginosa* genome digested by NdeI and HindIII was clonedinto the same sites of pBAD24m | This study |
| pBAD24m- *acpP3* | Ampr; The PCR fragment of *acpP3* from *P.aeruginosa* genome digested by NdeI and HindIII was clonedinto the same sites of pBAD24m | This study |
| pCD4 | Kmr; The PCR fragment of *acpP1* from pBAD24m-*acpP1* digested by XbaI and HindIII was cloned between XbalI and HindIII sites of pET-28 (b) | This study |
| pCD5 | Kmr; The PCR fragment of *acpP2* from pBAD24m-*acpP2* digested by XbaI and HindIII was cloned between XbalI and HindIII sites of pET-28 (b) | This study |
| pCD6 | Kmr; The *acpP3* fragment of pBAD24m-*acpP3* digested by NdeI and HindIII was cloned between NdeI and HindIII sites of pET-30 (a) | This study |
| pCD8 | Ampr; The XbalI-HindIII-digested fragment from pCD04 was cloned between the same sites of pBluescript SK(+) | This study |
| pCD9 | Ampr; The XbalI-HindIII-digested fragment from pCD05 was cloned between the same sites of pBluescript SK(+) | This study |
| pCD10 | Ampr; The XbalI-HindIII-digested fragment from pCD06 was cloned between the same sites of pBluescript SK(+) | This study |
| pCD1 | Ampr; The PCR fragment of *acpP1* from pCD8 digested by BamHI and KpnI was clonedinto the same sites of pTac85 | This study |
| pCD2 | Ampr; The PCR fragment of *acpP2* from pCD9 digested by BamHI and KpnI was clonedinto the same sites of pTac85 | This study |
| pCD3 | Ampr; The PCR fragment of *acpP2* from pCD10 digested by BamHI and SalI was clonedinto the same sites of pTac85 | This study |
| pCD15 | Kmr; The PCR fragment containing *P.aeruginosa pcpS* digested by NdeI and HindIII was between the same sites of pET28(b) | This study |
| pSRKPa | Kmr; The *P.aeruginosa acpP1* was cloned between NdeI and HicdIII sites of pSRK-Km | This study |
| pCD16 | Ampr; The 1.3-kb PCR fragment of *P.aeruginosa* genome containing *acpP1* digested by BamHI and HindIII was cloned between the same sites of pBluescript SK(+) | This study |
| pCD17 | Ampr Gmr; Gm resistant cassette of p34s-Gm digested by EcoRI was cloned into the same site of pCD16 | This study |
| pCD18 | Kmr Gmr; The BamHI-HindIII fragment from pCD17 was cloned between the same sites of pK18mobscaB | This study |
| pCD19 | Cmr; The 1.0-kb PCR fragment of *P.aeruginosa* genome containing *acpP2* digested by EcoRI and HindIII was cloned between the same sites of pHSG399 | This study |
| pCD20 | Cmr Gmr; Gm resistant cassette of p34s-Gm digested by XbaI was cloned into the same site of pCD19 | This study |
| pCD21 | Kmr Gmr; The EcoRI-HindIII fragment from pCD20 between the same sites of pK18mobscaB | This study |
| pCD22 | Kmr Tcr; Tc resistant cassette of p34s-Tc digested by BamHI was cloned into the same site of pCD21 | This study |
| pCD23 | Cmr; The 886-bp PCR fragment of *P.aeruginosa* genome containing *acpP3* digested by EcoRI and HindIII was cloned between the same sites of pHSG399 | This study |
| pCD24 | Cmr Gmr; Gm resistant cassette of p34s-Gm digested by SalI was cloned into the same site of pCD23 | This study |
| pCD25 | Kmr Gmr; The EcoRI-HindIII fragment from pCD24 was cloned between the same sites of pK18mobscaB | This study |
| pCD26 | Ampr; pCD17 was digested with EcoRI and was ligated itself | This study |
| pCD27 | Kmr; The BamHI-HindIII fragment from pCD26 was cloned between the same sites of pK18mobscaB | This study |
| pCD28 | Kmr Gmr; Gm resistant cassette of p34s-Gm digested by BamHI was cloned into the same site of pCD27 | This study |
| pCD29 | Ampr; The PCR fragment of acpP1up-EcacpP-acpP1 down was ligated to pMD19 | This study |
| pCD30 | Kmr; The BamHI-HindIII fragment from pCD29 was cloned between the same sites of pK18mobscaB | This study |
| pCD31 | Kmr Gmr; Gm resistant cassette of p34s-Gm digested by BamHI was cloned into the same site of pCD30 | This study |

**References**

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**Table S2. Sequences of the PCR primers used**

|  |  |
| --- | --- |
| Name | Sequences (5'→3') |
| AcpP1 NdeI | AAACAAcatatgAGCACCATCGAAGAACGC |
| AcpP1 HindIII | CGACGAaagcttATTGCTGGTGAGCAACG |
| AcpP2 NdeI | CCGCAAcatatgGACGACATCGAGACCAG |
| AcpP2 HindIII | CCAGGGaagcttAGGTCGGCACGGCTTCC |
| AcpP3 NdeI | GGAAGAcatatgCCCAACGACATGGAAG |
| AcpP3 HindIII | TTCGCTaagcttAGGCGGCGCGGTGCTTC |
| AcpPs XbalI | TTTTGGtctagaAGGAGGAATTCCATATG |
| AcpP1 BamHI | ACATATggatccATGAGCACCATCGAAGAACG |
| AcpP2 BamHI | ACATATggatccATGGACGACATCGAGACCAG |
| AcpP3 BamHI | ACATATggatccATGCCTAACGACATGGAAGAC |
| PcpS NdeI | ACCGGCcatatgCGCGCCATGAACGACCGTCTC |
| PcpS HindIII | ATATCTaagcttGATCAGGCGCCGACCGCCAC |
| AcpP1up HindIII | ATAATCaagcttTGCGCATGAAAGACGACGAG |
| AcpP1down BamHI | CGCCGAggatccAACCCATGGATACGCCGATAC |
| AcpP1 up HindIII | CGAGCGaagcttGGGCCTGTCGTTGCGTTTGC |
| AcpP1down EcoRI | GCCTCGgaattcCGCCGCGAGGAAAATTCGGCCTC |
| AcpP1 up Xbal | CTTCCAtctagaTGACCAGTTCGACTACCTCG |
| AcpP1 down Xbal | TCGGCGtctagaATCGCCGATGACGATGCGGAAC |
| Acp3 up HindIIII | CCAGGCaagcttGCAGATGAGCGACTCGGTGC |
| Acp3 down EcoRI | AGGTCGgaattcAGATAGCGGGCATTGAGCAG |
| Gm up EcoRI | GAGCTCgaattcACATAAGCCTGTTCGGTTC |
| Gm down EcoRI | GAGCTCgaattcGCCGCGGCGTTGTGAC |
| Pa acpP down | TAGTGCTCATACCTTGTTTTCACTCCTATGG |
| Ec acpP up | AAAACAAGGTATGAGCACTATCGAAGAACG |
| Ec acpP down | TCTCGAATTCTTACGCCTGGTGGCCGTCGATG |
| Pa acpP up | ACCAGGCGTAAGAATTCGAGACCGAAATCCC |
| AcpP up2 | TGGTCATCGGCACCGCGACCAGCGCGTC |
| AcpP down2 | CGGCCTCGCCATAGGCGATGTTGCGAGC |
| Acp1 up2 | TATCCGGCTCCAGCCCCGGTGCTGATGG |
| Acp1 down2 | ATCACGACGCGCCAGCTCGGCCTCGTCG |
| Acp3 up2 | GGTGGACACTTTCAGCCATACCCTCGAC |
| Acp3 down2 | TCGCGGAAGTTGCCCAGCAGGTTGAACG |

Note: lower case letters show the restriction sites.

**Table S3. Fatty acid composition of total lipid extracts from *P. aeruginosa* *acpPs* mutant strains** *a*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Fatty acid (%) | *P. aeruginosa* strains | | | | |
| PAO1 | PA-A12 | PA-A13 | PA-A23 | PA-A123 |
| *n*-C10:0-3-OH*b* | 3.78 ± 0.69 | 4.80 ± 0.70 | 4.03 ± 0.43 | 4.73 ± 0.42 | 5.47 ± 0.24 |
| *n*-C12:0-3-OH | 6.60 ± 0.76 | 6.71 ± 0.05 | 5.58 ± 0.04 | 6.23 ± 0.87 | 7.40 ± 0.56 |
| n-C16:1 | 11.26 ± 0.50 | 10.42 ± 0.88 | 16.35 ± 0.56 | 12.93 ± 0.37 | 14.49 ± 0.98 |
| n-C16:0 | 34.49 ± 0.36 | 34.80 ± 0.37 | 31.91 ± 0.99 | 33.35 ± 1.16 | 32.40 ± 0.88 |
| n-C18:1 | 35.85 ± 1.91 | 34.02 ± 1.05 | 36.57 ± 0.87 | 38.59 ± 0.74 | 34.09 ± 0.96 |
| n-C18:0 | 8.01 ± 0.35 | 9.24 ± 1.92 | 5.57 ± 0.16 | 4.17 ± 0.41 | 6.15 ± 0.27 |

*a* Cells were grown in LB medium for 12 h at 37°C. Total lipids were extracted and transesterified to fatty acid methyl esters, and products identified by GC-MS. Values are percentages of total fatty acids and are means ± standard deviations of three independent experiments.

***b*** n-C10:0 3-OH, 3-hydroxyldecanoic acid; n-C10:0 3-OH, 3-hydroxyldodecanoic acid; n-C16:1, *cis*-9-hexadecenoic acid; n-C16:0, hexadecanoic acid;n-C18:1, *cis*-11-octadecenoic acid; n-C18:0, octadecanoic acid.

**Supplementary Figures**

**Figure S1**



**Fig. S1** A. Strategy for isolation of *P. aeruginosa* *acpPs*mutant strains.B. Genetic organization of the *acpPs* region inPAO1 (a) or *acpPs* strains (b). C. Colony PCR analysis of mutant strains. Lane 1 and 3, PCR products of strain PAO1; lane 2, PCR products of PA-A2; lane 4, PCR products of PA-A3. Abbreviations: CH, chromosome; Up, upstream fragment of *acpPs*; Dn, downstream fragment of *acpPs*, Gm, gentamicin resistant cassette; pCDs, suicide plasmids used for disruption of *P. aeruginosa acpPs*.

**Figure S2**

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**Fig. S2** Strategy for deletion of *P. aeruginosa acpP1*. Abbreviations: CH, chromosome; Up, upstream fragment of *acpP1*; Dn, downstream fragment of *acpP1*, Gm, gentamicin resistant cassette; pCD28, suicide plasmids used for disruption of *P. aeruginosa acpP1*.

**Figure S3**

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**Fig. S3** A.Strategy for replacement of *P. aeruginosa acpP1* with *E.coli acpP*. B. Genetic organization of the *acpP1* region inPAO1 (a) or *acpP1*:: *EcacP* (b). C. Colony PCR analysis of *P. aeruginosa* strains in (B).Abbreviations: CH, chromosome; Up, upstream fragment of *acpP1*; Dn, downstream fragment of *acpP1*, Gm, gentamicin resistant cassette; pCD31, suicide plasmids used for replacement of *P. aeruginosa acpP1* with *E.coli acpP*; *EcacpP*, *E.coli acpP*.

**Figure S4**

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**Fig. S4** Colony PCR analysis of *P. aeruginosa acpPs* mutantstrains. A. Colony PCR analysis of *P. aeruginosa acpP1::EcacpP acpP2:: Gm* mutantstrain. B. A. Colony PCR analysis of *P. aeruginosa acpP1*:: *EcacpP acpP3:: Gm* mutantstrain. C. A. Colony PCR analysis of *P. aeruginosa acpP2*::Tc *acpP3*::Gmmutantstrain. D. A. Colony PCR analysis of *P. aeruginosa acpP1*::*EcacpP acpP2*::Tc *acpP3*::Gmmutantstrain. Abbreviations: Gm, gentamicin resistant cassette; Tc, tetracycline resistant cassette; *EcacpP*, *E.coli acpP*.

**Figure S5**

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**Fig. S5 MALDI-TOF-MS of *P. aeruginosa* three ACPs.** A. Mass spectrum of *P. aeruginosa* AcpP1 purified from *E.coli* cell. B. Mass spectrum of *P. aeruginosa* AcpP1 purified after Phosphopantetheinylatation with *P. aeruginosa* PcpS. C. Mass spectrum of *P. aeruginosa* AcpP2 purified from *E.coli* cell. D. Mass spectrum of *P. aeruginosa* AcpP2 purified after Phosphopantetheinylatation with *P. aeruginosa* PcpS. E. Mass spectrum of *P. aeruginosa* AcpP3 purified from *E.coli* cell. F. Mass spectrum of *P. aeruginosa* AcpP3 purified after Phosphopantetheinylatation with *P. aeruginosa* PcpS.

**Figure S6**

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**Fig. S6 Analysis production of rhamnolipids in *P. aeruginosa acpP1* mutant strains by colorimetric detection of rhamnose.** PK-Pa -, *acpP1* mutant PA-A1 carried plasmid pSRKPa grown under no IPTG induction. PK-Pa +, *acpP1* mutant PA-A1 carried plasmid pSRKPa grown under IPTG induction.