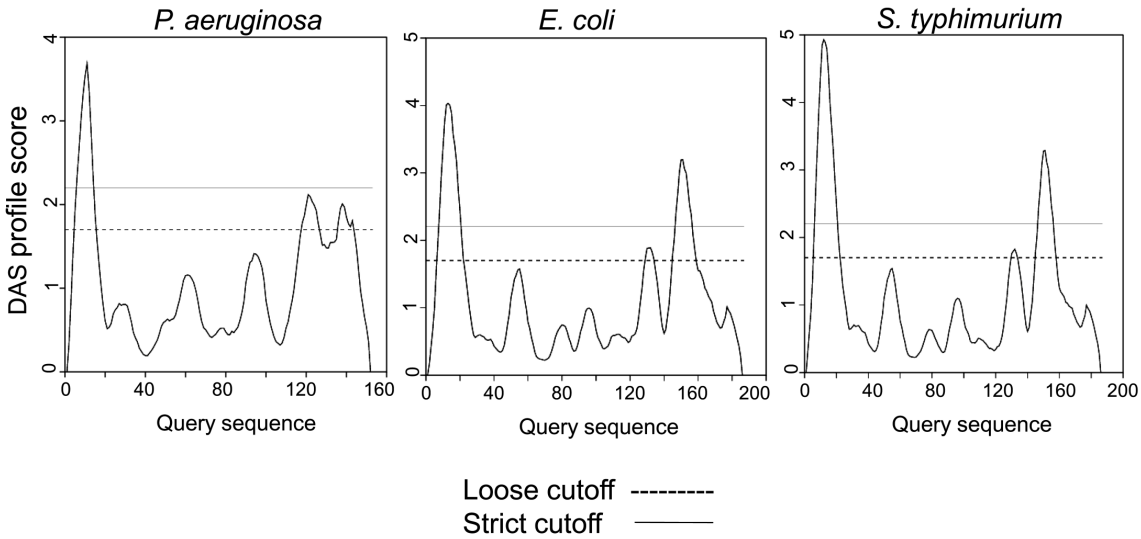
**A divergent *Pseudomonas aeruginosa* palmitoyltransferase essential for cystic fibrosis-specific lipid A**

Iyarit Thaipisuttikul[^1,2], Lauren E. Hittle[^1], Ramesh Chandra[^1], Daniel Zangari[3], Charneal L. Dixon[3], Teresa A. Garrett[4], David A. Rasko[1], Nandini Dasgupta[5], Samuel M. Moskowitz[5,6], Lars Malmström[7], David R. Goodlett[8], Samuel I. Miller[9], Russell E. Bishop[3,*], and Robert K. Ernst[1,*]

[1]Department of Microbial Pathogenesis, University of Maryland School of Dentistry, University of Maryland, Baltimore MD 21201
[2]Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkoknoi, Bangkok 10700, Thailand
[3]Department of Biochemistry and Biomedical Sciences, and the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton ON, Canada L8S 4K1.
[4]Department of Chemistry, Vassar College, Poughkeepsie, New York 12604
[5]Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts
[6]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts
[7]Institute of Molecular Systems Biology, Swiss Federal Institute of Technology, Zurich Switzerland
[8]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, University of Maryland, Baltimore MD 21201
[9]Departments of Microbiology, Immunology, and Genome Sciences, University of Washington, Seattle WA 98115

# Supplementary Information




Supplementary Figure S1. PagP enzyme transmembrane prediction of *P. aeruginosa*, *E. coli,* and *S.* Typhimurium, by using server DAS (http://www.sbc.su.se/~miklos/DAS/). The horizontal scale depicts the relative amino acid number, and vertical scale represents DAS profile score. The horizontal solid line (strict cutoff) and dashed line (loose cutoff) indicates the number of matching segments and actual location of the transmembrane segment respectively.

SUPPLEMENTAL TABLE 1

| Experimenal *m/z* | C-3' | C-2' | C-3 | C-2 | Phosphate configuration |
|---|---|---|---|---|---|
| 1168 | C10(3-OH) | C12(3-OH):C12 | H | C12(3-OH) | Monophosphate |
| 1184 | C10(3-OH) | C12(3-OH) | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1196 | H | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1212 | H | C12(3-OH):C12(2-OH) | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1248 | C10(3-OH) | C12(3-OH):C12 | H | C12(3-OH) | Diphosphate |
| 1264 | C10(3-OH) | C12(3-OH) | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1276 | H | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1366 | C10(3-OH) | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1382 | C10(3-OH) | C12(3-OH):C12(2-OH) | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1446 | C10(3-OH) | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1462 | C10(3-OH) | C12(3-OH):C12(2-OH) | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1536 | C10(3-OH) | C12(3-OH):C12 | C10(3-OH) | C12(3-OH):C12(2-OH) | Monophosphate |
| 1552 | C10(3-OH) | C12(3-OH):C12(2-OH) | C10(3-OH) | C12(3-OH):C12(2-OH) | Monophosphate |
| 1604 | C10(3-OH)C16 | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Monophosphate |
| 1616 | C10(3-OH) | C12(3-OH):C12 | C10(3-OH) | C12(3-OH):C12(2-OH) | Diphosphate |
| 1632 | C10(3-OH) | C12(3-OH):C12(2-OH) | C10(3-OH) | C12(3-OH):C12(2-OH) | Diphosphate |
| 1684 | C10(3-OH)C16 | C12(3-OH):C12 | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1700 | C10(3-OH)C16 | C12(3-OH):C12(2-OH) | H | C12(3-OH):C12(2-OH) | Diphosphate |
| 1854 | C10(3-OH)C16 | C12(3-OH):C12 | C10(3-OH) | C12(3-OH):C12(2-OH) | Diphosphate |

Supplementary Table S1. Observed masses and proposed compositions for lipid A, by negative-ion MALDI-MS with an *m/z* scan 1100 to 2200. Only assignable peaks with unambiguous exact masses and charge states are listed.

| SUPPLEMENTAL TABLE 2 | | |
|---|---|---|
| **Strain or plasmid** | **Description** | **Source** |
| **Strains** | | |
| ***Pseudomonas aeruginosa*** | | |
| PAO1 | Wild-type laboratory-adapted strain | S. Lory, Harvard |
| PAO1+PA1343 | Wild-type PAO1 expressing PA1343 in trans | This study |
| Δ*pagP* | PAO1 derivative with PA1343 deleted | This study |
| Δ*pagP*+PA1343 | PAO1 deletion of PA1343, complemented | This study |
| H35F::*pagP* | 35$^{th}$ histidine of PA1343 changed to phenylalanine | This study |
| H35N::*pagP* | 35$^{th}$ histidine of PA1343 changed to asparagine | This study |
| H42F::*pagP* | 42$^{th}$ histidine of PA1343 changed to phenylalanine | This study |
| H42N::*pagP* | 42$^{th}$ histidine of PA1343 changed to asparagine | This study |
| H45F::*pagP* | 45$^{th}$ histidine of PA1343 changed to phenylalanine | This study |
| H45L::*pagP* | 45$^{th}$ histidine of PA1343 changed to leucine | This study |
| H45N::*pagP* | 45$^{th}$ histidine of PA1343 changed to asparagine | This study |
| D84A::*pagP* | 84$^{th}$ aspartic acid of PA1343 changed to alanine | This study |
| D84N::*pagP* | 84$^{th}$ aspartic acid of PA1343 changed to asparagine | This study |
| S85A::*pagP* | 85$^{th}$ serine of PA1343 changed to alanine | This study |
| S85G::*pagP* | 85$^{th}$ serine of PA1343 changed to glycine | This study |
| D86A::*pagP* | 86$^{th}$ aspartic acid of PA1343 changed to alanine | This study |
| D86N::*pagP* | 86$^{th}$ aspartic acid of PA1343 changed to asparagine | This study |
| S87A::*pagP* | 87$^{th}$ serine of PA1343 changed to alanine | This study |
| S87G::*pagP* | 87$^{th}$ serine of PA1343 changed to glycine | This study |
| DSDS-AGNA::*pagP* | 84, 85, 86 and 87 aspartic acid and serine of PA1343 changed to alanine, glycine, asparagine and alanine respectively | This study |
| PAK | Wild-type laboratory-adapted strain | S. Lory, Harvard |
| PAK *pmrB12* | Pm-resistant PAK derivative with *pmrB12* allele | S. M. Moskowitz, Mass General Hospital |
| PA *pmrB12* Δ*pagP* | PAK *pmrB12* derivative with PA1343 deleted | This study |
| PAK Δ*phoQ* | Pm-resistant PAK derivative with *phoQ* deleted | S. I. Miller, University of Washington |
| PAK Δ*phoQ* Δ*pagP* | PAK derivative with *phoQ* and PA1343 deleted | This study |
| *E. coli* BW25113 | Wild-type strain for BKT09 | Pei Zhou, Duke University |
| *E. coli* BKT09 | *E. coli* BW25113ΔpagP, Δ*lpxP*, Δ*lpxM*, Δ*lpxL*::Kan | B. K. Tan,Duke University |
| **Plasmids** | | |
| pUCP19-USER | A hybrid plasmid between pNEB206A (New England Biolab) and pUCP19: Car$^r$ | A. Hinz, University of Washington |
| pPA1343 | pUCP19-USER containing PA1343: Car$^r$ | This study |
| pDONR 201 | Gateway cloning vector Kan$^r$ | Invitrogen |
| pEXGWD | Suicide vector in *P. aeruginosa*; sacB, Gen$^r$ | Invitrogen |
| pET21a-PA1343 | PA1343 expression vector | This study |

Supplementary Table S2: Bacterial strains and plasmids used in this study.

SUPPLEMENTAL TABLE 3

| Primer name | Sequence |
|---|---|
| **For cloning PA1343** | |
| PA1343_USER_fp | GGAGACAUGTCACGTCTTGCCTTCCTTC |
| PA1343_USER_rp | GGGAAAGUGGGAGTCTCCTGTCGAGTGA |
| pUCP19-PA1343_fp | TATACATATGGCCGACGGCGACTT |
| pUCP19-PA1343_rp | TATACTCGAGTCAGAGACGCAGGCCGA |
| **For deleting PA1343** | |
| PA1343-1 | GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGGAGATGATGTTCATGCC |
| PA1343-2 | CCCGTTCCTGGCCTCAGAGACGCAGGGATCCGAGATAGCGCATGGGGACTCCAGGC |
| PA1343-3 | GCCTGGAGTCCCCATGCGCTATCTCGGATCCCTGCGTCTCTGAGGCCAGGAACGGG |
| PA1343-4 | GGGGACCACTTTGTACAAGAAAGCTGGGTGGTATCCTCTGAATGACGG |
| **For point-mutagenesis** | |
| H35F_fp | AGCGTCTACACCCGGTTTTTCAACCCGGACCCT |
| H35F_rv | AGGGTCCGGGTTGAAAAACCGGGTGTAGACGCT |
| H35N_fp | AGCGTCTACACCCGGAATTTCAACCCGGACCCT |
| H35N_rv | AGGGTCCGGGTTGAAATTCCGGGTGTAGACGCT |
| H42F_fp | AACCCGGACCCTGAATTCAACAATCACCAGGAC |
| H42F_rv | GTCCTGGTGATTGTTGAATTCAGGGTCCGGGTT |
| H42N_fp | AACCCGGACCCTGAAAACAACAATCACCAGGAC |
| H42N_rv | GTCCTGGTGATTGTTGTTTTCAGGGTCCGGGTT |
| H45F_fp | CCTGAACACAACAATTTCCAGGACCTGCTCGGC |
| H45F_rv | GCCGAGCAGGTCCTGGAAATTGTTGTGTTCAGG |
| H45L_fp | CCTGAACACAACAATCTCCAGGACCTGCTCGGC |
| H45L_rv | GCCGAGCAGGTCCTGGAGATTGTTGTGTTCAGG |
| H45N_fp | CCTGAACACAACAATAACCAGGACCTGCTCGGC |
| H45N_rv | GCCGAGCAGGTCCTGGTTATTGTTGTGTTCAGG |
| D84A_fp | CCTGGGCAAGCGTTTCGCCAGTGACAGCTACCCGG |
| D84A_rv | CCGGGTAGCTGTCACTGGCGAAACGCTTGCCCAGG |
| D84N_fp | CCTGGGCAAGCGTTTCAACAGTGACAGCTACCCGG |
| D84N_rv | CCGGGTAGCTGTCACTGTTGAAACGCTTGCCCAGG |
| S85A_fp | GGGCAAGCGTTTCGACGCTGACAGCTACCCGGTCT |
| S85A_rv | AGACCGGGTAGCTGTCAGCGTCGAAACGCTTGCCC |
| S85G_fp | GGGCAAGCGTTTCGACGGTGACAGCTACCCGGTCT |
| S85G_rv | AGACCGGGTAGCTGTCACCGTCGAAACGCTTGCCC |
| D86A_fp | CAAGCGTTTCGACAGTGCCAGCTACCCGGTCTACC |
| D86A_rv | GGTAGACCGGGTAGCTGGCACTGTCGAAACGCTTG |
| D86N_fp | CAAGCGTTTCGACAGTAACAGCTACCCGGTCTACC |
| D86N_rv | GGTAGACCGGGTAGCTGTTACTGTCGAAACGCTTG |
| S87A_fp | CGTTTCGACAGTGACGCCTACCCGGTCTACCTG |
| S87A_rv | CAGGTAGACCGGGTAGGCGTCACTGTCGAAACG |
| S87G_fp | CGTTTCGACAGTGACGGCTACCCGGTCTACCTG |
| S87G_rv | CAGGTAGACCGGGTAGCCGTCACTGTCGAAACG |
| D84A-S85G_fp | CCTGGGCAAGCGTTTCGCCGGTGACAGCTACCCGGTC |
| D84A-S85G_rv | GACCGGGTAGCTGTCACCGGCGAAACGCTTGCCCAGG |
| D86N-S87A_fp | CAAGCGTTTCGACAGTAACGCCTACCCGGTCTACCTG |
| D86N-S87A_rv | CAGGTAGACCGGGTAGGCGTTACTGTCGAAACGCTTG |
| D84A-S85G-D86N-S87A_fp | CCTGGGCAAGCGTTTCGCCGGTAACGCCTACCCGGTCTACCTG |
| D84A-S85G-D86N-S87A_rv | CAGGTAGACCGGGTAGGCGTTACCGGCGAAACGCTTGCCCAGG |
| **For sequencing** | |
| PA1343 seq-1 | GAGCTCGTCAGCGACGAC |
| PA1343 seq-2 | GGTTTTCTGACGGTTCGTTC |
| PA1343 seq-3 | GTGGAACCGCTGACGATT |

Supplementary Table S3: List of the primers used for the PCR and mutagenesis.