**SUPPLEMENTAL INFORMATION**

# Robust suppression of lipopolysaccharide deficiency in *Acinetobacter baumannii* by growth in minimal medium

Emma Nagy[1], Richard Losick[1], Daniel Kahne[1,2,3]#

[1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
[2]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA
[3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

#Address correspondence to Daniel Kahne: kahne@chemistry.harvard.edu

## SUPPLEMENTAL INFORMATION

**Figure S1. Passaging results in restoration of wild type growth profiles.** Growth of passaged replicates at the indicated number of days after initiation in LB at 25°C. Panels 1 and 2 are replicates of *ΔlpxC* and panel 3 is a replicate of *ΔmlaAΔlpxC*. Wild type is shown for comparison. The replicates shown are representative of the types of growth improvement seen over the course of the experiment.

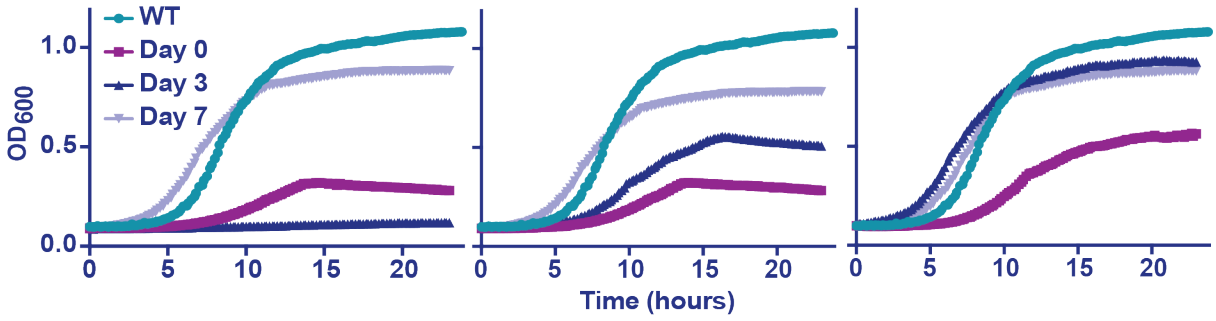

**Figure S2. Mutations in both *mla* and *pldA* are required for growth improvement in an LPS deficient strain.** Strains are grown in LB at 37°C.

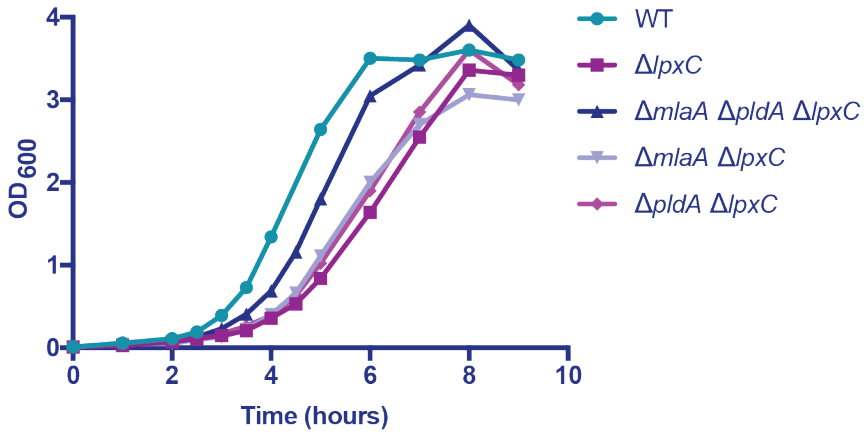

**Table S1. Strains and plasmids used in this study.**

| Strain or Plasmid | Genotype | Reference or Source |
|---|---|---|
| *A. baumannii* ATCC 19606 | Wild type | ATCC |
| *A. baumannii lpxC* mutant | Δ*lpxC*::kan | Moison et al., 2016 (1) |
| *A. baumannii mlaA lpxC* mutant | Δ*lpxC*::apr Δ*mlaA*::kan | This study |
| *A. baumannii pldA lpxC* mutant | Δ*lpxC*::apr Δ*pldA*::kan | This study |
| *A. baumannii mlaA pldA lpxC* mutant | Δ*pldA* Δ*lpxC*::apr Δ*mlaA*::kan | This study |
| pEX18ApGW | Vector for gene replacement | Hoang et. Al, 1998 (2) |
| pEX18ApGW-*pldA* | Deletion vector for *pldA* | This study |

**Table S2. Mutations found in suppressor screen.** The first set of replicates were *ΔlpxC* at the start of the screen and the second set listed below were *ΔmlaA ΔlpxC.* Only assigned mutations are listed, with a few exceptions. The references for the genes listed are as follows: *pldA* (DJ41_RS17655), Peptidase M16 (DJ41_RS17650), *mlaA* (DJ41_RS06905), *mlaD* (DJ41_RS11945), *mlaF* (DJ41_RS11935), *adeJ* (DJ41_RS13520), and *adeR* (DJ41_RS18535).

| Replicate | Gene/Reference | Mutation | Position | Result |
|---|---|---|---|---|
| 1 | *pldA* | Insertion with target site duplication (5bp) | 1091 | Disruption |
| | *mlaA* | Deletion (Δ708bp) | * | Disruption |
| 2 | *pldA* | $(A)_{7\rightarrow 6}$ | 238 | Truncation |
| | *mlaA* | Deletion (Δ693bp) | * | Disruption |
| 3 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 2937 | Disruption |
| | DJ41_RS10490 | Insertion with target site duplication (5bp) | 453 | Disruption |
| 4 | DJ41_RS17650 | Insertion with target site duplication (5bp) | 386 | Disruption |
| | *mlaA* | Insertion with target site duplication (4bp) | 448 | Disruption |
| 5 | *pldA* | G→A | 1022 | W341* |
| | *mlaA* | Deletion (Δ709bp) | * | Disruption |
| 6 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 278 | Disruption |
| | *mlaA* | T→G | 457 | N152K |
| 7 | *pldA* | Deletion (Δ3bp) | 1023 | ΔW341 A342C |
| | *mlaA* | Deletion (Δ730bp) | * | Disruption |
| | DJ41_RS17650 | Deletion (Δ92bp) | 5 | Disruption |

| | | | | |
|---|---|---|---|---|
| 8 | *mlaD* | Insertion with target site duplication (5bp) | 296 | Disruption |
| 9 | DJ41_RS17650 | Insertion with target site duplication (5bp) | 1757 | Disruption |
| | *mlaA* | Deletion (Δ1770bp) | * | Disruption |
| 10 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 2932 | Disruption |
| | *mlaA* | Insertion with target site duplication (4bp) | 451 | Disruption |
| 11 | DJ41_RS17650 | Deletion (Δ2193bp) | 548 | Disruption |
| | *mlaA* | Deletion (Δ2820) | * | Disruption |
| 12 | DJ41_RS17650 | Insertion with target site duplication (5bp) | 1730 | Disruption |
| | *mlaA* | Insertion with target site duplication (5bp) | 454 | Disruption |
| 13 | *pldA* | $(A)_{7\rightarrow 6}$ | 238 | Truncation |
| | DJ41_RS10490 | Insertion with target site duplication (4bp) | 452 | Disruption |
| 14 | DJ41_RS17650 | Insertion with target site duplication (4bp) (Unassigned) | 2937 | Disruption |
| | *mlaF* | New junction with repeat region (Unassigned) | 87 | Disruption |
| 15 | *pldA* | Deletion (Δ19bp) | 1078 | Truncation |
| | *mlaA* | Deletion (Δ1529bp) | * | Disruption |

| **Replicate** | **Gene/Reference** | **Mutation** | **Position** | **Result** |
|---|---|---|---|---|
| 1 | *pldA* | Deletion (Δ1bp) | 895 | Truncation |
| 2 | *pldA* | Insertion with target site duplication (4bp) | 340 | Disruption |
| 3 | DJ41_RS17650 | T→A | 140 | L47* |
| 4 | *pldA* | $(A)_{7\rightarrow 6}$ | 238 | Truncation |
| 5 | *pldA* | Deletion (Δ3bp) | 1023 | ΔW341 A342C |
| 6 | *pldA* | Insertion with target site duplication (5bp) | 536 | Disruption |
| 7 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 2815 | DJ41_RS17650 |
| | *adeJ* | Insertion with target site duplication (4bp) | 2385 | Disruption |

| | | | | |
|---|---|---|---|---|
| 8 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 294 | Disruption |
| | DJ41_RS13530 | Insertion with target site duplication (5bp) | 600 | Disruption |
| 9 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 2926 | Disruption |
| 10 | *pldA* | New junction with repeat region (Unassigned) | 400/887 | Disruption |
| | *adeR* | Insertion with target site duplication (5bp) (Unassigned) | 423 | Disruption |
| 11 | *pldA* | Insertion with target site duplication (5bp) | 524 | Disruption |
| | *adeR* | Insertion with target site duplication (4bp) (Unassigned) | 186 | Disruption |
| 12 | DJ41_RS17650 | Insertion with target site duplication (4bp) | 646 | Disruption |
| | *adeJ* | Deletion (Δ13bp) | 1961 | Truncation |

**Table S3. Primers used in this study.** Underlined regions represent areas of overlap.

| Primer | Sequence (5' - 3') | Application |
|---|---|---|
| **Strain Construction** | | |
| *mlaA*_up_F | GGTGCCAATTCTGGCTCATCAATTAC | Amplification of upstream and downstream regions of *mlaA* for *mlaA*-kanR linear construct |
| *mlaA*_up_R | <u>CGAATTCGCGGCCGCTTCTA</u>TCCGAACTAGCGGCAGATTCTT | |
| *mlaA*_down_F | <u>GAGCTCGCTTGGACTCCTGT</u>GCGTTCCAGATTGCCGAGAA | |
| *mlaA*_down_R | GTACCAGTCGCCTGATAAATAGGCA | |
| *mlaA*_seq_F | GTAGGTCTTTACACCTCAGCCC | Verification of *mlaA* gene knock out |
| *mlaA*_seq_R | CTTCTGGTACATCTTCAGATTCGTCATC | |
| *lpxC*_up_F | CATTACTGGTGGCGATGACATCAC | Amplification of upstream and downstream regions of *lpxC* for *lpxC*-aprR linear construct |
| *lpxC*_up_R | <u>GCGTAATCTGCTGCTTGCAA</u>CTCCATCCACGGTATGTGGAATG | |
| *lpxC*_down_F | <u>GCGGAGAACGAGATGACGTT</u>CAGCTATTACGCAATGTTCAAAGCGA | |
| *lpxC*_down_R | AGGAAACCTTACGTTTCTAACAACGC | |
| *lpxC*_seq_F | CACACTCACGTATGGAATTGGACAG | Verification of *lpxC* gene knock out |
| *lpxC*_seq_R | AGCGAGTGGAATAGGTCTTCATAGC | |
| *pldA*_up_F | GGTATTGGCAGCTTACTTGCGTAATG | Amplification of upstream and downstream regions of *pldA* for *pldA*-kanR linear construct |
| *pldA*_up_R | <u>CGAATTCGCGGCCGCTTCTA</u>GGCTAAGGTGTCGGCATATGC | |
| *pldA*_down_F | <u>GAGCTCGCTTGGACTCCTGT</u>CTGCGCGGACACTTCCAATTAT | |
| *pldA*_down_R | GATGCTACTCATGTTCATCGGTGG | |
| *pldA*_seq_F | TTTACAGCTAAGTATGGGAACCCTG | Verification of *pldA* gene knock out |
| *pldA*_seq_R | CCGGCATAAGTTGCACGATG | |

| | | |
|---|---|---|
| Kan_F | TAGAAGCGGCCGCGAATTCG | Amplification of KanR cassette |
| Kan_R | ACAGGAGTCCAAGCGAGCTC | |
| Apr_F | TTGCAAGCAGCAGATTACGC | Amplification of AprR cassette |
| Apr_R | AACGTCATCTCGTTCTCCGC | |
| *pldA*_up_pEX_F | TTCCCAGTCACGACGTTGTAAAACGACG GCCAGTGCCA GCAGCAACGTAAGACACGCT | Amplification of upstream region of *pldA* with 5' overlap to pEX18ApGW and 3' overlap to the downstream region of *pldA* |
| *pldA*_up_pEX_R | CATAACCATTAAATAATTGGAAGTGTCCG CGCAG GGCTAAGGTGTCGGCATATGC | |
| *pldA*_down_pEX_F | ATGTGCGTCGGTGGCATATGCCGACACC TTAGCC CTGCGCGGACACTTCCAATTAT | Amplification of downstream region of *pldA* with 5' overlap to the upstream region of *pldA* and 3' overlap to pEX18ApGW |
| *pldA*_down_pEX_R | AGGAAACAGCTATGACCATGATTACGAAT TCGAGCTCG TATGGCTTTGCTCGGGGACT | |
| pEX_*pldA*_F | CGAGCTCGAATTCGTAATCATGGTCA | Amplification of pEX18ApGW |
| pEX_*pldA*_R | TGGCACTGGCCGTCGTTTTA | |
| *pldA*_pEX_seq_F | GGGTGCTTCGTTACGCAGTAAG | To verify pEX18ApGW integration at *pldA* site |
| *pldA*_pEX_seq_R | GGCCTTAAACAGCAAATGATGGCT | |

## REFERENCES


1. Moison E, Xie R, Zhang G, Lebar MD, Meredith TC, Kahne D. 2017. A Fluorescent Probe Distinguishes between Inhibition of Early and Late Steps of Lipopolysaccharide Biogenesis in Whole Cells. ACS Chem Biol 12:928-932.

2. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. Gene 212:77-86.