

Supplementary Tables

Supplementary Table S1. Strains used in this study.

| Strain Name | Genotype/Description | Source |
|-------------------------------------|--|------------|
| <i>E. coli</i> strains | | |
| S17-1 λ pir | <i>thi pro hsdR- hsdM+ ΔrecA</i> RP4-2::TcMu-Km::Tn7 | (1) |
| BTH101 | <i>F⁻, cya⁻⁹⁹, araD139, galE15, galK16, rpsL1 (Str^r), hsdR2, mcrA1, mcrB1</i> | Euromedex |
| <i>P. aeruginosa</i> strains | | |
| SMC 232 | PA14 wild type (WT) | (2) |
| SMC 3351 | Δ <i>bifA</i> | (3) |
| SMC 2893 | Δ <i>pelA</i> | (4) |
| SMC 4233 | Δ <i>bifA</i> Δ <i>pelA</i> | (3) |
| SMC 3577 | Δ <i>flgZ</i> | This study |
| SMC 3578 | Δ <i>bifA</i> Δ <i>flgZ</i> | This study |
| SMC 7310 | Δ <i>bifA</i> Δ <i>flgZ</i> Δ <i>pelA</i> | This study |
| SMC 7311 | Δ <i>flgZ</i> Δ <i>pelA</i> | This study |
| SMC 3662 | Δ <i>bifA</i> Δ PA14_00130 | This study |
| SMC 4747 | Δ <i>bifA</i> Δ PA14_27930 | This study |
| SMC 5570 | Δ <i>bifA</i> Δ <i>pilZ</i> | This study |
| SMC 3663 | Δ <i>bifA</i> Δ PA14_25420 | This study |
| SMC 5572 | Δ <i>bifA</i> Δ <i>alg44</i> | This study |
| SMC 4024 | Δ <i>bifA</i> Δ PA14_56180 | This study |
| SMC 3664 | Δ <i>bifA</i> Δ PA14_60970 | This study |
| SMC 7312 | Δ <i>bifA</i> Δ PA14_00130 Δ <i>pelA</i> | This study |
| SMC 7313 | Δ <i>bifA</i> Δ PA14_27930 Δ <i>pelA</i> | This study |

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| SMC 7314 | <i>ΔbifA ΔpilZ ΔpelA</i> | This study |
| SMC 7315 | <i>ΔbifA ΔPA14_25420 ΔpelA</i> | This study |
| SMC 7316 | <i>ΔbifA Δalg44 ΔpelA</i> | This study |
| SMC 7317 | <i>ΔbifA ΔPA14_56180 ΔpelA</i> | This study |
| SMC 7318 | <i>ΔbifA ΔPA14_60970 ΔpelA</i> | This study |
| SMC 7319 | <i>ΔhptB</i> | This study |
| SMC 7320 | <i>ΔhptB ΔflgZ</i> | This study |
| SMC 7321 | <i>ΔhptB ΔpelA</i> | This study |
| SMC 7322 | <i>ΔhptB ΔpelA ΔflgZ</i> | This study |
| SMC 7323 | <i>ΔbifA ΔpelA ΔflgZ::flgZ</i> -His, allelic replacement of <i>flgZ</i> deletion with 6xHis-tagged wild-type <i>flgZ</i> gene in the <i>ΔbifA ΔpelA ΔflgZ</i> mutant for complementation | This study |
| SMC 7324 | <i>ΔbifA ΔpelA ΔflgZ::flgZ(R140A)</i> -His | This study |
| SMC 7325 | <i>ΔbifA ΔpelA ΔflgZ::flgZ(R144A)</i> -His | This study |
| SMC 7326 | <i>ΔbifA ΔpelA ΔflgZ::flgZ(R144D)</i> -His | This study |
| SMC 7327 | <i>ΔbifA ΔpelA ΔflgZ::flgZ(D172A)</i> -His | This study |
| SMC 7328 | <i>ΔbifA ΔpelA ΔflgZ::flgZ(G177A)</i> -His | This study |
| SMC 4474 | PA14 WT strain carrying pMotA-His plasmid; Gm ^r | This study |
| SMC 7329 | PA14 WT strain carrying pMotC-His plasmid; Gm ^r | This study |
| SMC 7330 | flgZ-HA strain carrying pMQ72 vector control; Gm ^r | This study |
| SMC 7331 | flgZ-HA strain carrying pMotA-His plasmid; Gm ^r | This study |
| SMC 7332 | flgZ-HA strain carrying pMotC-His plasmid; Gm ^r | This study |
| SMC 4196 | <i>ΔbifA</i> strain carrying pMQ72 vector control; Gm ^r | This study |
| SMC 7333 | <i>ΔbifA ΔpelA</i> strain carrying pMQ72 vector control; Gm ^r | This study |

| | | |
|----------|--|------------|
| SMC 6700 | <i>ΔbifA</i> strain carrying pMotCD-His plasmid; Gm ^r | This study |
| SMC 7334 | <i>ΔbifA ΔpelA</i> strain carrying pMotCD-His plasmid; Gm ^r | This study |
| SMC 7335 | <i>ΔbifA ΔpelA ΔflgZ::gfp-flgZ</i> ; allelic replacement of <i>flgZ</i> deletion with 6xHis-tagged wild-type <i>flgZ</i> gene in the <i>ΔbifA ΔpelA ΔflgZ</i> mutant for complementation | This study |
| SMC 7336 | WT(<i>gfp-flgZ</i>); translational fusion of <i>gfp</i> at N-terminus of <i>flgZ</i> gene on chromosome in wild type | This study |
| SMC 7337 | <i>ΔbifA (gfp-flgZ)</i> ; translational fusion of <i>gfp</i> at N-terminus of <i>flgZ</i> gene on chromosome in <i>ΔbifA</i> strain | This study |
| SMC 7338 | <i>ΔmotCD (gfp-flgZ)</i> ; translational fusion of <i>gfp</i> at N-terminus of <i>flgZ</i> gene on chromosome in <i>ΔmotCD</i> strain | This study |

Supplementary Table S2. Plasmids used in this study.

| Plasmid Name | Description | Source |
|-------------------------------|---|---------------|
| pMQ30 | Shuttle vector for yeast cloning and Gram-negative allelic replacement, Gm ^r | (5) |
| pMQ72 | Shuttle vector for yeast cloning and arabinose-inducible gene expression, Gm ^r | (5) |
| PA14_00130/pMQ30 | Plasmid for deletion of PA14_00130 gene; Gm ^r | This study |
| PA14_27930/pMQ30 | Plasmid for deletion of PA14_27930 gene; Gm ^r | This study |
| <i>pilZ</i> /pMQ30 | Plasmid for deletion of <i>pilZ</i> gene; Gm ^r | This study |
| PA14_25420/pMQ30 | Plasmid for deletion of PA14_25420 gene; Gm ^r | This study |
| <i>flgZ</i> /pMQ30 | Plasmid for deletion of <i>flgZ</i> gene; Gm ^r | This study |
| <i>alg44</i> /pMQ30 | Plasmid for deletion of <i>alg44</i> gene; Gm ^r | This study |
| PA14_56180/pMQ30 | Plasmid for deletion of PA14_56180 gene; Gm ^r | This study |
| PA14_60970/pMQ30 | Plasmid for deletion of PA14_60970 gene; Gm ^r | This study |
| <i>hptB</i> /pMQ30 | Plasmid for deletion of <i>hptB</i> gene; Gm ^r | This study |
| <i>flgZ</i> -His/pMQ30 | Plasmid for allelic replacement of <i>flgZ</i> deletion for complementation | This study |
| <i>flgZ</i> (R140A)-His/pMQ30 | Plasmid for chromosomal R140A substitution in <i>flgZ</i> gene | This study |
| <i>flgZ</i> (R144A)-His/pMQ30 | Plasmid for chromosomal R144A substitution in <i>flgZ</i> gene | This study |
| <i>flgZ</i> (R144D)-His/pMQ30 | Plasmid for chromosomal R144D substitution in <i>flgZ</i> gene | This study |
| <i>flgZ</i> (D172A)-His/pMQ30 | Plasmid for chromosomal D172A substitution in <i>flgZ</i> gene | This study |
| <i>flgZ</i> (G177A)-His/pMQ30 | Plasmid for chromosomal G177A substitution in <i>flgZ</i> gene | This study |

| | | |
|----------------------------|--|------------|
| <i>flgZ</i> -HA/pMQ30 | Plasmid for chromosomal C-terminal insertion of HA epitope tag in <i>flgZ</i> gene | This study |
| pMotA-His | 6xHis epitope-tagged <i>motA</i> gene in pMQ72, Gm ^r | (6) |
| pMotC-His | 6xHis epitope-tagged <i>motC</i> gene in pMQ72, Gm ^r | (6) |
| pMotCD-His | 6xHis epitope-tagged <i>motCD</i> genes in pMQ72, Gm ^r | This study |
| pKT25 | BACTH vector allowing fusion to the C-terminus of the <i>cyaA</i> T25 fragment, Kan ^r | Euromedex |
| pKNT25 | BACTH vector allowing fusion to the N-terminus of the <i>cyaA</i> T25 fragment, Kan ^r | Euromedex |
| pUT18 | BACTH vector allowing fusion to the N-terminus of the <i>cyaA</i> T18 fragment, Amp ^r | Euromedex |
| pUT18C | BACTH vector allowing fusion to the C-terminus of the <i>cyaA</i> T18 fragment, Amp ^r | Euromedex |
| pKT25- <i>zip</i> | Leucine zipper of GCN4 fused to T25 in pKT25, Kan ^r | Euromedex |
| pUT18C- <i>zip</i> | Leucine zipper of GCN4 fused to T18 in pUT18C, Amp ^r | Euromedex |
| pUT18- <i>flgZ</i> | Full length <i>flgZ</i> cloned into pUT18, Amp ^r | This study |
| pUT18- <i>flgZ</i> (R140A) | Full length <i>flgZ</i> (R140A) cloned into pUT18, Amp ^r | This study |
| pKT25- <i>motA</i> | Full length <i>motA</i> with a C-terminal 6xHis tag cloned into pKT25, Kan ^r | This study |
| pKNT25- <i>motA</i> | Full length <i>motA</i> with a C-terminal 6xHis tag cloned into pKNT25, Kan ^r | This study |
| pKT25- <i>motC</i> | Full length <i>motC</i> cloned into pKT25, Kan ^r | This study |
| pKNT25- <i>motC</i> | Full length <i>motC</i> cloned into pKNT25, Kan ^r | This study |
| pKT25- <i>fliG</i> | Full length <i>fliG</i> cloned into pKT25, Kan ^r | This study |
| pKNT25- <i>fliG</i> | Full length <i>fliG</i> cloned into pKNT25, Kan ^r | This study |
| pKT25- <i>fliM</i> | Full length <i>fliM</i> cloned into pKT25, Kan ^r | This study |

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|-----------------------|---|------------|
| <i>pKNT25-fliM</i> | Full length <i>fliM</i> cloned into pKNT25, Kan ^r | This study |
| <i>gfp-flgZ/pMQ30</i> | Plasmid for translational fusion of <i>gfp</i> at N-terminus of <i>flgZ</i> on chromosome | This study |

Supplementary Table S3. Oligonucleotide primers used in this study.

| Primer Name | Primer Sequence* |
|--------------------|--|
| PA14_00130 KO P1 | <i>gacggccagtccaagcttgcctgcaggtcgacTCGCCCTTCTTCCTGGCGTAC</i> |
| PA14_00130 KO P2 | <i>CATCACCGTTCAGGCTTCGCTATCCATCGAGTCCCTCGGGAC</i> |
| PA14_00130 KO P3 | <i>GTCCCGAGGGACTCGATGGATAGCGAAGCCTGAACGGTGATG</i> |
| PA14_00130 KO P4 | <i>ccatgattacgaattcgagctcggtaccggggatccATCCGTACAGGAGCGCCTGAG</i> |
| PA14_27930 KO P1 | <i>tgtaaacgacggccagtccaagcttgcctgcctgCCGAGCATGGTCACGTGCTTG</i> |
| PA14_27930 KO P2 | <i>GATGCTCAGCCCCAGGCTATGCATGGGCTGATCCTCATGGTC</i> |
| PA14_27930 KO P3.2 | <i>GACCATGAGGATCAGCCCATGCTCGGCCAGGGCGGCATAGC</i> |
| PA14_27930 KO P4.2 | <i>ccatgattacgaattcgagctcggtaccggggatccCTCGGCAGCGCCTTCCAGGTC</i> |
| pilZ KO P1 | <i>tgtaaacgacggccagtccaagcttgcctgcctgTATCTATCCCTGGCAGCAGG</i> |
| pilZ KO P2 | <i>CCAGGTAGGTTTTCGATCTTGATGGTCAAGGACAGGATG</i> |
| pilZ KO P3 | <i>CATCCTGTCCTTGACCATCAAGATCGAAACCTACCTGG</i> |
| pilZ KO P4 | <i>ccatgattacgaattcgagctcggtaccggggatccGGCGAGGGTATTCCAAATCTG</i> |
| PA14_25420 KO P1 | <i>ccatgattacgaattcgagctcggtaccggggatccGTCGGTGACCACCATCACCAG</i> |
| PA14_25420 KO P2 | <i>CTGCTTCTGGAGGATATGCCGTCATCCGCGTCACGAGTGGAC</i> |
| PA14_25420 KO P3 | <i>GTCCACTCGTGACGCGGATGACGGCATATCCTCCAGAAGCAG</i> |
| PA14_25420 KO P4 | <i>gacggccagtccaagcttgcctgcaggtcgacGACCGTCCACACCGAGACGTG</i> |
| flgZ KO P1 | <i>ctccatacccgttttttgggctagcgaattcgagctcAGCTAGGCAAGCGTCCAATAC</i> |
| flgZ KO P2 | <i>GAAGGTCGCGTCGAGCTTTTCCAGGTTGGCCTGGATTTCCAC</i> |
| flgZ KO P3 | <i>GTGGAAATCCAGGCCAACCTGGAAAAGCTCGACGCGACCTTC</i> |
| flgZ KO P4 | <i>cgccaaaacagccaagcttgcctgcctgcagactagtcATGCGGCTGTTTGGCGCTAAC</i> |
| alg44 KO P1 | <i>tgtaaacgacggccagtccaagcttgcctgcctgATCGACGGCGACACCGTGCTC</i> |

| | |
|------------------|--|
| alg44 KO P2 | CCGATGCTGACCTCGGCCAGCTTGACCCGGGCGAACTG |
| alg44 KO P3 | CAGTTCGCCCCGGTCAAGCTGGGCCGAGGTCAGCATCGG |
| alg44 KO P4 | ccatgattacgaattcgagctcggtaccggggatccGCCTTGAGCAGGTGCCGCTC |
| PA14_56180 KO P1 | tgtaaacgacggccagtccaagcttgcctgAAGCGCATGGCATCTCCTTG |
| PA14_56180 KO P2 | CTCCACCAGTTCGAGGAATCAAGACCCATAATGGCATTTCGC |
| PA14_56180 KO P3 | GCGAAATGCCATTATGGGTCTTGAATTCCTCGAACTGGTGGAG |
| PA14_56180 KO P4 | ccatgattacgaattcgagctcggtaccggggatccAAAGCACCGAGGTCGGCTAC |
| PA14_60970 KO P1 | tgtaaacgacggccagtccaagcttgcctgAGCCAGGCGAGGTGACGTAGCA G |
| PA14_60970 KO P2 | GAGTTCCTCGTCGCCCAGGTTGTCACTCATGTGCGGATCCCTC |
| PA14_60970 KO P3 | GAGGGATCGCGACATGAGTGACAACCTGGGCGACGAGGAACTC |
| PA14_60970 KO P4 | ccatgattacgaattcgagctcggtaccggggatccATCCCAGCAGCAGGTGGCGATG |
| pela KO P1 | tgtaaacgacggccagtccaagcttgcctgACAGCTTCCAGCTACAGGCC |
| pela KO P2 | GAAGTGGTAGTACAGGTGCAGGGGACTCGGCAGCGGCACAAG |
| pela KO P3 | TTGTGCCGCTGCCGAGTCCCCTGCACCTGTACTACCACTTC |
| pela KO P4 | ccatgattacgaattcgagctcggtaccggggatccTGACGGGCAAGTCGTTTCGAG |
| ycgR-His P1 | tgtaaacgacggccagtccaagcttgcctgACGAACTGGGCGAACTGCTG |
| ycgR-His P2 | TGTGAGACTCAATGATGATGATGATGATGGAACAGTTCGTCTTT CTCGAAG |
| ycgR-His P3 | ACGAACTGTTCCATCATCATCATCATTGAGTCTCACAGGAC CACTCCC |
| ycgR-His P4 | ccatgattacgaattcgagctcggtaccggggatccTGCTGGTGACCTTCGGCGTC |
| ycgR-HA P1 | Same as ycgR-His P1 |
| ycgR-HA P2 | TGTGAGACTCATGCATAATCAGGAACATCATAAGGATAGAACA GTTCGTCTTTCTCGAAG |

| | |
|---|---|
| ycgR-HA P3 | ACGAACTGTTCTATCCTTATGATGTTCTGATTATGCATGAGTC TCACAGGACCACTCCC |
| ycgR-HA P4 | Same as ycgR-His P4 |
| ycgR R140A For | GAAGTGCTCTACCACCAGCGCGCCAACGCCTACCGCGCCAG |
| ycgR R140A Rev | CTGGCGCGGTAGGCGTTGGCGCGCTGGTGGTAGAGCACTTC |
| ycgR R-D144 F | AGCGCCGCAACGCCTACGACGCCAGCATCAAGCAGAGCCAGC |
| ycgR R-D144 R | GCTGGCTCTGCTTGATGCTGGCGTCGTAGGCGTTGCGGGCGCT |
| ycgR R144A F | CAGCGCCGCAACGCCTACGCCGCCAGCATCAAGCAGAGC |
| ycgR R144A R | GCTCTGCTTGATGCTGGCGCGGTAGGCGTTGCGGGCGCTG |
| ycgR D172A F | GCTGAGCGGACAACACTGCTGGCTATCTCCGCCACCGGCG |
| ycgR D172A R | CGCCGGTGGCGGAGATAGCCAGCAGTTGTCCGCTCAGC |
| ycgR G177A F | GCTGGATATCTCCGCCACCGCCGCAAGCTGCGCTTCG |
| ycgR G177A R | CGAAGCGCAGCTTGGCGCGGTGGCGGAGATATCCAGC |
| ycgR BTH F | GGCG TCTAGA G GTGCTATCATTGAGG |
| ycgR BTH R | GGCG GATCC TC GAACAGTTCGTCTTT |
| fliM BTH F | GGCG TCTAGA G ATGGCCGTGCAAGAT |
| fliM BTH R | GGCG GGATCC T CGCGCGAGCGCTCGA |
| B2H-FliG For | GGCGTCTAGA G ATGAGTGAGAATCGTCTCG |
| B2H-FliG Rev | GGCGGGATCC TC GATCATCTCCTCGCCACC |
| B2H-MotA For | GGCGTCTAGA G ATGTCAAAAATCATCGGCATCATCG |
| B2H-MotA Rev (used a His tagged template for this amplification) | GGCG GGATCC TC GTGGTGATGGTGGTGGTG |
| B2H-MotC For | GGCGTCTAGA G ATGGATGTGCTCAGCCTGGTC |

| | |
|-----------------|---|
| B2H-MotC Rev | GGCGGGATCC TC GTCCATGAAGCCTTGCAGC |
| GFP-ycgR P1 | tgtaaacgacggccagtgccaaagctgcatgcctgGTCGAGGTGAATATGTAGAG |
| GFP-ycgR P2 | agttcttctcctttactcat TGTGCCTTGATACGTGTGCT |
| GFP-ycgR P3 | AGCACACGTATCAAGGCACA atgagtaaaggagaagaact |
| GFP-ycgR P4 new | CGAATGCCTCAATGATAGCAC tttgtatagttcatccatgcc |
| GFP-ycgR P5 new | ggcatggatgaactatacaaa GTGCTATCATTGAGGCATTCG |
| GFP-ycgR P6 | ccatgattacgaattcgagctcggtacccggggatccTGCTGGACGGCGACTACCTG |

* In primer sequences, lowercase letters indicate sequences that are complementary to the cloning vector pMQ30, uppercase boldface letters indicate a 6xHis or HA tag, and lowercase boldface letters indicate sequences of *gfp*. Uppercase italicized letters indicate a 5' sequence flanking a deletion. Underlined letters indicate point mutations.

References

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