**A novel decoy strategy for polymyxin resistance in *Acinetobacter baumannii***

Jaeeun Parka, Misung Kima, Bora Shinb, Mingyeong Kanga, Jihye Yanga, Tae Kwon Leec and Woojun Parka\*

aLaboratory of Molecular Environmental Microbiology, Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

bDepartment of Microbiology and Immunology, School of Medicine, National University of Singapore, Singapore

cDepartment of Environmental Engineering, Yonsei University, Wonju 26493, Korea

**Running title**: OMV production in *Acinetobacter baumannii*

**Keywords**: *Acinetobacter baumannii*; LPS modifications; outer membrane vesicles; two-component regulatory systems; experimental evolution; polymyxin-resistance

**\*Corresponding author:** Dr. Woojun Park, Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

**E-mail:** wpark@korea.ac.kr

**Fax:** +82-2-953-0737

**Phone:** +82-2-3290-3067

**Supplementary File 1a.**

|  |  |  |
| --- | --- | --- |
| **Product name** | **Nucleotide change** | **Amino acid change** |
| **Mutation in PMRLow and PMRHigh** |  |  |
| Two-component sensor histidine kinase (*pmrB*) | A1058T | N353I (PMRLow) |
|  | C704T | T235I (PMRHigh) |
| **Mutation in PMRLow** |  |  |
| GGDEF domain-containing protein (*dgcB*) | ΔA1707 | Internal stop codon |
| DNA translocase (*ftsK*) | ΔG2631 | Internal stop codon |
| **Mutation in PMRHigh** |  |  |
| 5′/3′-nucleotidase (*surE*) | ΔA451 | Internal stop codon |
| Ribosomal-protein-alanine *N*-acetyltransferase (*rimJ*) | ΔA392 | Internal stop codon |
| ABC transporter substrate-binding protein (*mlaC*) | ΔT95 | Internal stop codon |
| Fimbrial biogenesis protein (*fimT*) | ΔA365 | Internal stop codon |
| Cell division protein (*ftsL*) | ΔA47 | Internal stop codon |
| peptidase M23/Uracil-DNA glycosylase (*udg*) | Δ576-580 | Internal stop codon |
| Beta-ketoacyl-[acyl-carrier-protein] synthase I (*fabH*) | ΔC305 | Internal stop codon |
| Site-specific tyrosine recombinase (*xerD*) | ΔT158 | Internal stop codon |
| Ferrous iron transporter B (*feoB*) | ΔG435 | Internal stop codon |
| GTP pyrophosphokinase (*relA*) | ΔC239 | A80V |
| Citrate-proton symporter | ΔT35 | Internal stop codon |
| 16S rRNA (cytidine(1402)-2'-O)-methyltransferase(*rsmI*) | ΔT590 | Internal stop codon |
| Dihydrofolate reductase (*folA*) | ΔT258 | Internal stop codon |
| Glutamine aminotransferase (*guaA*) | ΔT87 | Internal stop codon |

**Supplementary File 1b.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain** | **CDS** | **Sequencing  depth of  coverage** | **Mean  CDS length (bp)** | **Median CDS length (bp)** | **GC ratio (%)** | **Chromosomal DNA (bp)** | **Mean length of intergenic  region (bp)** | **N50 (bp)** | **No. of  rRNA  genes** | **No. of  tRNA  genes** |
| **Lab-WT (ATCC 17978)** | 3803 | 516 | 924.2 | 790 | 38.9 | 3,976,747 | 112.2 | 3,902,113 | 15 | 70 |
| **PMRLow** | 3678 | 299.8 | 947.5 | 816 | 38.9 | 3,971,618 | 132.8 | 3,971,618 | 18 | 72 |
| **PMRHigh** | 3762 | 247.19 | 930.2 | 804 | 38.9 | 3,955,017 | 138.5 | 3,955,017 | 18 | 72 |

**Supplementary File 1c.**

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Positions in  chromosome | Locus tag | Product |
| PMRLow | Region 1  (680-700 kbp) | A1S\_3508 | Hypothetical protein |
| A1S\_3510 | Hypothetical protein |
| A1S\_3514 | Hypothetical protein |
| A1S\_3515 | Hypothetical protein |
| A1S\_0629 | Hypothetical protein |
| PMRLow | Region 2  (1,830-1,863 kbp) | A1S\_3683 | Hypothetical protein |
| A1S\_1582 | Transcriptional regulator Cro/CI family |
| A1S\_1583 | Hypothetical protein |
| A1S\_1584 | Hypothetical protein |
| A1S\_3698 | Hypothetical protein |
| A1S\_1597 | Phage tail tape measure protein lambda |
| PMRHigh | Region 1  (680-700 kbp) | A1S\_0626 | Hypothetical protein |
| A1S\_3508 | Hypothetical protein |
| A1S\_3509 | Hypothetical protein |
| A1S\_3510 | Hypothetical protein |
| A1S\_3514 | Hypothetical protein |
| A1S\_3515 | Hypothetical protein |
| A1S\_3517 | Hypothetical protein |
| A1S\_3518 | Hypothetical protein |
| A1S\_0629 | Hypothetical protein |
| A1S\_3519 | Hypothetical protein |
| A1S\_3520 | Hypothetical protein |
| PMRHigh |  | A1S\_3683 | Hypothetical protein |
| A1S\_1581 | Methyltransferase putative |
| A1S\_3685 | Hypothetical protein |
| A1S\_3686 | Hypothetical protein |
| A1S\_3687 | Hypothetical protein |
| A1S\_1582 | Transcriptional regulator Cro/CI family |
| A1S\_1583 | Hypothetical protein |
| A1S\_1584 | Hypothetical protein |
| A1S\_1585 | Putative replicative DNA helicase |
| A1S\_3688 | Hypothetical protein |
| A1S\_3689 | Hypothetical protein |
| A1S\_3690 | Hypothetical protein |
| A1S\_3691 | Hypothetical protein |
| A1S\_3692 | Hypothetical protein |
| A1S\_3693 | Hypothetical protein |
| A1S\_3694 | Hypothetical protein |
| Region 2  (1,830-1,863 kbp) | A1S\_3695 | Hypothetical protein |
| A1S\_3696 | Hypothetical protein |
| A1S\_1586 | Ethanol-stimulated virulence protein, EsvK1 |
| A1S\_3697 | Hypothetical protein |
| A1S\_3698 | Hypothetical protein |
| A1S\_1587 | Ethanol-stimulated virulence protein, EsvK2 |
| A1S\_1588 | Phage terminase-like protein large subunit |
| A1S\_1589 | Hypothetical protein |
| A1S\_1590 | Peptidase U35 phage prohead HK97 |
| A1S\_1591 | Phage major capsid protein HK97 |
| A1S\_3699 | Hypothetical protein |
| A1S\_1592 | Putative Phage head-tail adaptor |
| A1S\_1593 | Hypothetical protein |
| A1S\_1594 | Hypothetical protein |
| A1S\_1595 | Hypothetical protein |
| A1S\_3700 | Hypothetical protein |
| A1S\_3701 | Hypothetical protein |
| A1S\_1596 | Hypothetical protein |
| A1S\_1597 | Phage tail tape meausure protein lambda |
| A1S\_3702 | Hypothetical protein |
| A1S\_1598 | Hypothetical protein |
| A1S\_1599 | Hypothetical protein |
| A1S\_3703 | Hypothetical protein |
| A1S\_3704 | Hypothetical protein |

**Supplementary File 1d.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Identity (%)** | **Length** | **Position in chromosome** | **Predicted phenotype** |
| *ADC-79* | 100 | 1152/1152 | 154172-155323 | Determinant of β-lactam resistance |
| 100 | 1152/1152 | 2619269-2620420 |
| *OXA-259* | 100 | 825/825 | 1643403-1644227 | Determinant of β-lactam resistance |
| 100 | 825/825 | 3168483-3169307 |
| *sul2* | 100 | 816/816 | 1272808-1273623 | Antibiotic target replacement protein |
| 100 | 816/816 | 3736874-3737689 |
| *amvA* | 97.50 | 1479 / 1479 | 3766657..3768135 | Efflux pump complex or subunit conferring antibiotic resistance |
| 97.50 | 1479 / 1479 | 2276735..2278213 |
| *abaQ* | 98.77 | 1305 / 1305 | 3507191..3508495 | Efflux pump complex or subunit conferring antibiotic resistance |
| 98.77 | 1305 / 1305 | 2017267..2018571 |
| *abaF* | 100 | 1290 / 1290 | 2959632..2960921 | Efflux pump complex or subunit conferring antibiotic resistance |
| 100 | 1290 / 1290 | 1434550..1435839 |
| *adeL* | 99.31 | 1014 / 1014 | 80072..81085 | Protein(s) and two-component regulatory system modulating antibiotic efflux |
| 99.31 | 1014 / 1014 | 2545169..2546182 |
| *adeN* | 99.69 | 654 / 654 | 3661056..3661709 | Protein(s) and two-component regulatory system modulating antibiotic efflux |
| 99.69 | 654 / 654 | 2171133..2171786 |
| *adeK* | 99.31 | 1455 / 1455 | 591688..593142 | Efflux pump complex or subunit conferring antibiotic resistance |
| 99.31 | 1455 / 1455 | 3056790..3058244 |
| *adeI* | 99.60 | 1251 / 1251 | 587249..588499 | Efflux pump complex or subunit conferring antibiotic resistance |
| *abeM* | 99.18 | 1347 / 1347 | 433763..435109 | Efflux pump complex or subunit conferring antibiotic resistance |
| 99.18 | 1347 / 1347 | 1958865..1960211 |
| *adeA* | 97.98 | 1191 / 1191 | 3407427..3408617 | Efflux pump complex or subunit conferring antibiotic resistance |
| 97.98 | 1191 / 1191 | 1917502..1918692 |
| *adeG* | 97.38 | 1221 / 1221 | 81301..82518 | Efflux pump complex or subunit conferring antibiotic resistance |
| 97.38 | 1221 / 1221 | 2546398..2547615 |
| *adeB* | 98.10 | 3108 / 3108 | 3404320..3407427 | Efflux pump complex or subunit conferring antibiotic resistance |
| 98.10 | 3108 / 3108 | 1914395..1917502 |
| *adeJ* | 99.37 | 3177 / 3177 | 588512..591688 | Efflux pump complex or subunit conferring antibiotic resistance |
| 99.37 | 3177 / 3177 | 3053614..3056790 |
| *abeS* | 88.79 | 330 / 330 | 76433..76762 | Efflux pump complex or subunit conferring antibiotic resistance |
| 88.79 | 330 / 330 | 2541530..2541859 |
| *adeH* | 99.24 | 1452 / 1452 | 85714..87165 | Efflux pump complex or subunit conferring antibiotic resistance |
| 99.24 | 1452 / 1452 | 2550811..2552262 |
| *adeR* | 98.79 | 744 / 744 | 3408763..3409506 | Protein(s) and two-component regulatory system modulating antibiotic efflux |
| 98.79 | 744 / 744 | 1918838..1919581 |
| *adeS* | 96.78 | 1086 / 1086 | 3409538..3410623 | Protein(s) and two-component regulatory system modulating antibiotic efflux |
| 96.78 | 1086 / 1086 | 1919613..1920698 |
| *adeF* | 97.30 | 3180 / 3180 | 82525..85704 | Efflux pump complex or subunit conferring antibiotic resistance |
| 97.30 | 3180 / 3180 | 2547622..2550801 |

**Supplementary File 1e**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Chromosomal DNA of the Lab-WT, PMRLow and PMRHigh | | | | |
| Name | IS Family | Group | Origin | E-value |
| IS*Aba18* | IS3 | IS51 | *Acinetobacter baumannii* | 0 |
| IS*Aba1* | IS4 | IS10 | *Acinetobacter baumannii* | 0 |
| IS*Aba11* | IS701 | ISAba11 | *Acinetobacter baumannii* | 0 |
| IS*Aba12* | IS5 | IS903 | *Acinetobacter baumannii* | 0 |
| IS*Aba53* | IS5 | IS903 | *Acinetobacter baumannii* | 0 |
| IS17 | IS5 | IS903 | *Acinetobacter haemolyticus* | 0 |
| IS*Alw23* | IS3 | IS51 | *Acinetobacter lwoffii* | 0 |
| IS*Alw4* | IS3 | IS51 | *Acinetobacter lwoffii* | 0 |
| IS*Aba29* | IS3 | IS51 | *Acinetobacter baumannii* | 0 |
| IS*Alw5* | IS3 | IS51 | *Acinetobacter lwoffii* | 0 |
| IS*Aba19* | IS3 | IS51 | *Acinetobacter baumannii* | 0 |
| IS*Aba2* | IS3 | IS51 | *Acinetobacter baumannii* | 0 |
| IS*Aba33* | IS4 | IS10 | *Acinetobacter baumannii* | 0 |
| IS*Aba34* | IS3 | IS51 | *Acinetobacter baumannii* | 0 |
| IS*Acsp3* | IS3 | IS3 | *Acinetobacter* sp. | 0 |
| IS*Aba59* | IS5 | IS903 | *Acinetobacter baumannii* | 0 |
| IS*1236* | IS3 | IS3 | *Acinetobacter calcoaceticus* | 0 |
| IS*Alw26* | IS3 | IS3 | *Acinetobacter lwoffii* | 0 |
| IS*Aba13* | IS5 | IS903 | *Acinetobacter baumannii* | 0 |
| IS*Aba5* | IS5 | IS903 | *Acinetobacter baumannii* | 0 |
| IS*Aha1* | IS5 | IS903 | *Acinetobacter haemolyticus* | 0 |
| IS*Alw1* | IS5 | IS903 | *Acinetobacter lwoffii* | 0 |
| IS*Aba40* | IS5 | IS903 | *Acinetobacter baumannii* | 1.00E-164 |
| IS*Aha3* | IS5 | IS903 | *Acinetobacter haemolyticus* | 5.00E-155 |
| IS*Aha2* | IS5 | IS903 | *Acinetobacter haemolyticus* | 8.00E-154 |
| IS*Ajo1* | IS5 | IS903 | *Acinetobacter johnsonii* | 7.00E-145 |
| IS*Alw32* | IS5 | ISL2 | *Acinetobacter lwoffii* | 3.00E-141 |
| IS*Aba7* | IS5 | IS903 | *Acinetobacter baumannii* | 5.00E-124 |
| IS*Alw14* | IS5 | ISL2 | *Acinetobacter lwoffii* | 4.00E-103 |
| IS*Abe12* | IS3 | IS3 | *Acinetobacter bereziniae* | 1.00E-13 |
| IS*1301* | IS5 | IS427 | *Neisseria meningitidis* | 3.00E-11 |
| IS*Ppu33* | IS3 | IS51 | *Pseudomonas putida* | 5.00E-04 |
| IS*Stma17* | IS3 | IS51 | *Stenotrophomonas maltophilia* | 5.00E-04 |
| IS*Mca3* | IS3 | IS51 | *Methylococcus capsulatus* | 5.00E-04 |
| IS*1416* | IS3 | IS51 | *Burkholderia glumae* | 5.00E-04 |
| IS*Ate1* | IS5 | IS903 | *Acidovorax temperans* | 0.002 |
| IS*Ali4* | IS3 | IS51 | *Azospirillum lipoferum* | 0.002 |
| IS*Rme15* | IS3 | IS51 | *Ralstonia metallidurans* | 0.002 |
| IS*Pmi1* | IS3 | IS51 | *Proteus mirabilis* | 0.007 |
| IS51 | IS3 | IS51 | *Pseudomonas syringae* | 0.007 |
| IS*Cce4* | IS3 | IS3 | *Clostridium cellulolyticum* | 0.028 |
| IS*Rtr2* | IS3 | IS51 | *Rhizobium tropici* | 0.028 |
| IS*Aac2* | IS5 | IS427 | *Actinobacillus actinomycetemcomitans* | 0.028 |
| IS*Bt3* | IS3 | IS51 | *Burkholderia thailandensis* | 0.11 |
| IS*Cpi1* | IS5 | IS903 | *Chromobacterium piscinae* | 0.11 |
| IS*Afe7* | IS3 | IS51 | *Acidithiobacillus ferrooxidans* | 0.11 |
| IS*Jsp1* | IS3 | IS51 | *Janthinobacterium* sp. | 0.11 |
| IS*Pye52* | IS3 | IS51 | *Paracoccus yeei* | 0.44 |
| IS*Pre1* | IS5 | IS5 | *Pseudomonas resinovorans* | 0.44 |
| IS*Teha5* | IS4 | IS4Sa | *Tetragenococcus halophilus* | 1.7 |
| IS*Nov2* | IS5 | IS903 | *Novosphingobium* sp. | 1.7 |
| IS*Mex33* | IS3 | IS51 | *Methylobacterium extorquens* | 1.7 |
| IS*Osp1* | IS4 | IS4Sa | *Oceanobacter* sp. | 1.7 |
| IS*Azsp1* | IS3 | IS51 | *Azotobacter* sp. | 1.7 |
| IS*Pst4* | IS3 | IS51 | *Pseudomonas stutzeri* | 1.7 |
| IS*Alw15* | IS5 | ISL2 | *Acinetobacter lwoffii* | 6.9 |
| IS*Pye26* | IS110 | IS1111 | *Paracoccus yeei* | 6.9 |
| IS*Bth21* | IS5 | IS427 | *Bacillus thuringiensis* | 6.9 |
| IS*Hce1* | IS1595 | ISPna2 | *Helicobacter cetorum* | 6.9 |
| IS*Aur1* | IS1595 | ISPna2 | *Actinobacillus ureae* | 6.9 |
| IS*Cig1* | IS1595 | ISPna2 | *Campylobacter iguaniorum* | 6.9 |
| IS*Aisp8* | IS1595 | ISSod11 | *Acidovorax* sp. | 6.9 |
| IS*Bvi8* | IS5 | IS903 | *Burkholderia vietnamiensis* | 6.9 |
| IS*Asp20* | IS200/IS605 | IS200 | *Anabaena* sp*.* | 6.9 |
| IS*Hli3* | IS200/IS605 | IS1341 | *Halohasta litchfieldiae* | 6.9 |
| IS*Sri1* | ISKra4 | ISKra4 | *Streptomyces rimosus* | 6.9 |
| IS*Cap2* | IS5 | IS903 | *Candidatus Accumulibacter* | 6.9 |
| IS*Wosp5* | IS4 | IS231 | *Wolbachia* sp*.* | 6.9 |
| IS*Spu20* | IS5 | IS903 | *Shewanella putrefaciens* | 6.9 |
| IS*Cpe8* | IS1595 | ISPna2 | *Clostridium perfringens* | 6.9 |
| IS*Acma22* | IS701 | ISAba11 | *Acaryochloris marina* | 6.9 |
| IS*Cba1* | IS1595 | ISPna2 | *Clostridium bartlettii* | 6.9 |
| IS*Kpn4* | IS110 | IS1111 | *Klebsiella pneumoniae* | 6.9 |
| IS*Efa8* | IS3 | IS150 | *Enterococcus faecium* | 6.9 |
| IS*Afe1* | IS110 | IS1111 | *Acidithiobacillus ferroxidans* | 6.9 |
| IS*Sod2* | IS3 | IS407 | *Shewanella oneidensis* | 6.9 |
| IS*Brsp1* | IS3 | IS407 | *Bradyrhizobium* sp. | 6.9 |
| IS1302 | IS3 | IS150 | *Wolinella succinogenes* | 6.9 |
| pAB1 plasmid in Lab-WT | | | | |
| Name | IS Family | Group | Origin | E-value |
| IS*Alw13* | IS5 | IS427 | *Acinetobacter lwoffii* | 4e-50 |

**Supplementary File 1f**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Lab-WT | Lab-WT + PMB | PMRHigh | PMRHigh + PMB |
| Total reads | 1,387,480 | 1,401,287 | 1,406,593 | 1,418,191 |
| Total mapped reads (%) | 97.6 | 97.7 | 98.6 | 97.8 |
| Average RPKM | 361.6 | 383.4 | 366.5 | 369.6 |
| Median RPKM | 338.8 | 317.2 | 326.0 | 311.1 |

**Supplementary File 1g.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Regulation** | **Venn diagram Part** | **Locus tag** | **Gene** | **Site** | **TM** | **Products** |
| Up | 1 | ATCC1\_00077 | *DnaK* | Cytosol | ND | Chaperone protein DnaK |
| Up | 1 | ATCC1\_00122 | *fkpA* | periplasm | ND | Peptidylprolyl isomerase |
| Up | 1 | ATCC1\_00127 | *wbpB* | Cytosol | ND | UDP-*N*-acetyl-2-amino-2-deoxyglucuronate dehydrogenase |
| Up | 1 | ATCC1\_00225 | *atpH* | Cytosol | ND | ATP synthase subunit delta |
| Up | 1 | ATCC1\_00632 | *rplW* | Cytosol | ND | 50S ribosomal protein L23 |
| Up | 1 | ATCC1\_00637 | *rplP* | Cytosol | ND | 50S ribosomal protein L16 |
| Up | 1 | ATCC1\_00649 | *rplO* | Cytosol | ND | 50S ribosomal protein L15 |
| Up | 1 | ATCC1\_00963 | *pmrC* | Inner membrane | TM | Lipid A phosphoethanolamine transferase |
| Up | 1 | ATCC1\_00964 | *pmrA* | Cytosol | ND | Transcriptional regulatory protein PmrA |
| Up | 1 | ATCC1\_01052 | *groL* | Cytosol | ND | 60 kDa chaperonin |
| Up | 1 | ATCC1\_01563 | *cyoE* | Inner membrane | TM | Protoheme IX farnesyltransferase |
| Up | 1 | ATCC1\_01578 | *yiaD* | Outer membrane | TM | Glycine-zipper family protein |
| Up | 1 | ATCC1\_01620 | *miaA* | Cytosol | ND | tRNA dimethylallyltransferase |
| Up | 1 | ATCC1\_01632 | *aldB* | Cytosol |  | Long-chain-aldehyde dehydrogenase |
| Up | 1 | ATCC1\_01792 | *tusD* | Cytosol | ND | Sulfurtransferase TusD |
| Up | 1 | ATCC1\_01850 | *grxD* | Cytosol | ND | Glutaredoxin |
| Up | 1 | ATCC1\_01865 | *HyP* |  |  | Hypothetical protein |
| Up | 1 | ATCC1\_02021 | *HyP* |  |  | Hypothetical protein |
| Up | 1 | ATCC1\_02064 | *bdh* | Cytosol | ND | 3-hydroxybutyrate dehydrogenase |
| Up | 1 | ATCC1\_02214 | *exbD1* | Inner membrane | TM | Biopolymer transport protein exbD1 |
| Up | 1 | ATCC1\_02290 | *HyP* |  |  | Hypothetical protein |
| Up | 1 | ATCC1\_02340 | *ansB* | Cytosol | ND | Glutamin-(asparagin-)ase |
| Up | 1 | ATCC1\_02512 | *hcp1* | Extracellular |  | Protein hcp1 |
| Up | 1 | ATCC1\_02642 | *loiP* | Cytosol | ND | Belongs to the peptidase M48 family |
| Up | 1 | ATCC1\_02918 | *pgaB* | periplasm | ND | Poly-beta-1,6-*N*-acetyl-d-glucosamine N-deacetylase |
| Up | 1 | ATCC1\_03012 | *coaD* | Cytosol | ND | Pantetheine-phosphate adenylyltransferase |
| Up | 1 | ATCC1\_03514 | *rpoC* | Cytosol | ND | DNA-directed RNA polymerase |
| Up | 1 | ATCC1\_03719 | *pgpA* | Outer membrane | TM | Phosphatidylglycerophosphatase |
| Up | 1 | ATCC1\_03835 | *Hyp* |  |  | Hypothetical protein |
| Down | 1 | ATCC1\_00610 | *mlaC* | Periplasm | ND | Intermembrane phospholipid transport system binding protein MlaC |
| Down | 1 | ATCC1\_00872 | *ompA* | Outer membrane | TM | Outer membrane protein Omp38 |
| Down | 1 | ATCC1\_01297 | *lpp* | Periplasm | ND | Lipoprotein NlpD/LppB like protein |
| Down | 1 | ATCC1\_01335 | *dhbF* | Cytosol | ND | Dimodular nonribosomal peptide synthase |
| Down | 1 | ATCC1\_01342 | *fatA* | Outer membrane | TM | Ferric anguibactin receptor |
| Down | 1 | ATCC1\_01344 | *mbtB* | Cytosol | ND | Phenyloxazoline synthase MbtB |
| Down | 1 | ATCC1\_01348 | *HyP* |  |  | Hypothetical protein |
| Down | 1 | ATCC1\_02159 | *rhbD* | Cytosol | ND | Rhizobactin siderophore biosynthesis protein RhbD |
| Down | 1 | ATCC1\_02622 | *ahpF* | Cytosol | ND | Alkyl hydroperoxide reductase subunit |
| Down | 1 | ATCC1\_02964 | *bamE* | Outer membrane | TM | Outer membrane protein assembly factor BamE |
| Down | 1 | ATCC1\_02991 | *fusA* | Outer membrane | TM | Elongation factor |
| Down | 1 | ATCC1\_03271 | *cusC* | Outer membrane | TM | Cation efflux system protein CusC |

**Supplementary File 1h.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Regulation** | **Venn diagram Part** | **Sample** | **Products** | **Gene** | **MASS (Kda)** | | **Expect** | | **Coverage** |
| Up | 1 | 7411 | Phosphoethanolamine transferase EptA domain protein, partial | *pmrC* | 14.784 | | 7.60E-08 | | 74.8% |
| Up | 1 | 7707 | Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase PgaB | *pgaB* | 75.711 | | 5.01E-09 | | 29.80% |
| Up | 1 | 7808 | Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase PgaB | *pgaB* | 73.429 | | 1.29E-08 | | 30.70% |
| Up | 1 | 7813 | Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase PgaB | *pgaB* | 73.429 | | 6.29E-08 | | 23.50% |
| Up | 1 | 8701 | Serine-type D-Ala-D-Ala carboxypeptidase | *dacC* | 41.742 | | 3.10E-12 | | 11% |
| Up | 1 | 9407 | Alanine racemase | *alr* | 44.972 | | 2.20E-13 | | 45.80% |
| Up | 1 | 9501 | Alanine racemase | *alr* | 47.285 | | 5.04E-13 | | 45.80% |
| Up | 2 | 2506 | Alkyl hydroperoxide reductase C22 subunit | *ahpC* | 18.214 | | 1.60E-10 | | 53% |
| Up | 2 | 3502 | ND | ND | ND | | ND | | ND |
| Up | 2 | 5010 | 50S ribosomal protein L9 | *rplI* | 15.771 | | 1.60E-10 | | 64% |
| Up | 2 | 6006 | ND | ND | ND | | ND | | ND |
| Up | 2 | 6008 | 50S ribosomal protein L9 | *rplI* | 15.771 | | 4.00E-15 | | 68% |
| Up | 2 | 6011 | ND | ND | ND | | ND | | ND |
| Up | 2 | 6021 | ND | ND | ND | | ND | | ND |
| Up | 2 | 7517 | Citrate synthase | [gltA](https://www.uniprot.org/uniprot/B0VSK2) | 47.658 | 2.00E-11 | | 25% | |
| Up | 2 | 8405 | ND | ND | ND | ND | | ND | |
| Up | 2 | 9119 | Chorismate mutase | *pheA* | 19.38 | 3.10E-11 | | 29% | |
| Up | 2 | 9205 | ND | ND | ND | ND | | ND | |
| Up | 3 | 2505 | ND | ND | ND | ND | | ND | |
| Up | 3 | 7410 | N-acetyl-gamma-glutamyl-phosphate reductase | *argC* | 38.212 | 1.60E-08 | | 36% | |
| Up | 3 | 8001 | ND | ND | ND | ND | | ND | |
| Up | 4 | 2711 | ND | ND | ND | ND | | ND | |
| Up | 4 | 4006 | Nucleoside diphosphate kinase | *ndk* | 15,462 | 2.60E-05 | | 49% | |
| Up | 4 | 6104 | ND | ND | ND | ND | | ND | |
| Up | 4 | 6113 | ND | ND | ND | ND | | ND | |
| Up | 4 | 7007 | ND | ND | ND | ND | | ND | |
| Up | 4 | 7106 | Uncharacterized protein | ND | 38.13 | ND | | 23% | |
| Up | 4 | 7418 | ND | ND | ND | ND | | ND | |
| Up | 4 | 7706 | ND | ND | ND | ND | | ND | |
| Up | 4 | 7806 | ND | ND | ND | ND | | ND | |
| Up | 4 | 9206 | ND | ND | ND | ND | | ND | |
| Down | 1 | 2512 | Outer membrane protein OmpA | *ompA* | 38.396 | 5.00E-15 | | 39% | |
| Down | 1 | 3101 | Outer membrane protein W, OmpW | *ompW* | 20.097 | 3.00E-15 | | 54% | |
| Down | 1 | 3405 | Outer membrane protein OmpA | *ompA* | 38.396 | 5.00E-15 | | 39% | |
| Down | 1 | 3511 | Elongation factor Tu | *tufB* | 43.15 | 7.90E-16 | | 61% | |
| Down | 1 | 3514 | Elongation factor Tu | *tufB* | 43.15 | 7.90E-16 | | 61% | |
| Down | 1 | 5423 | TonB-linked outer membrane protein, SusC/RagA family | *fur* | 120.358 | 6.00E-06 | | 17.8 | |
| Down | 1 | 6303 | Bifunctional protein FolD | *folD* | 29.911 | 2.00E-10 | | 44% | |
| Down | 2 | 3303 | Adenylate kinase | *adk* | 24.178 | 2.50E-18 | | 70% | |
| Down | 2 | 5416 | Fructose-1,6-bisphosphate aldolase, class II | *fda* | 37.341 | 6.60E-05 | | 23% | |
| Down | 2 | 5520 | Cysteine desulfurase | [*iscS*](https://www.uniprot.org/uniprot/B0VD51) | 44.996 | 8.60E-08 | | 28% | |
| Down | 2 | 5811 | ND | ND | ND | ND | | ND | |
| Down | 2 | 5815 | Isocitrate dehydrogenase, NADP-dependent | *idh* | 82.626 | 1.06E-08 | | 23.1 | |
| Down | 2 | 6406 | Uncharacterized protein | ND | 10.964 | 1.99E-06 | | 45.5 | |
| Down | 2 | 6521 | Imidazolonepropionase | *hutI* | 44.374 | 3.10E-12 | | 46% | |
| Down | 2 | 6609 | Uncharacterized protein | ND | ND | ND | | ND | |
| Down | 2 | 6701 | Uncharacterized protein | ND | ND | ND | | ND | |
| Down | 2 | 6710 | ND | *ND* | ND | ND | | ND | |
| Down | 2 | 6802 | Isocitrate dehydrogenase, NADP-dependent | *idh* | 82.639 | 3.10E-12 | | 26.40% | |
| Down | 2 | 7509 | Succinyldiaminopimelate transaminase | *dapC* | 43.778 | 3.10E-12 | | 21.30% | |
| Down | 2 | 7602 | Dihydrolipoyl dehydrogenase | F931\_01045 | 51.012 | 7.28E-06 | | 24.30% | |
| Down | 2 | 7607 | Dihydrolipoyl dehydrogenase | F931\_01045 | 51.012 | 7.28E-06 | | 32.10% | |
| Down | 4 | 1513 | Succinate--CoA ligase | *sucC* | 41.675 | 1.60E-20 | | 61% | |
| Down | 4 | 5411 | Succinate-CoA ligase, alpha subunit | *sucD* | 30.594 | 9.60E-04 | | 39.9 | |
| Down | 4 | 6215 | Hypothetical protein | ND | ND | ND | | ND | |
| Down | 4 | 6219 | Hypothetical protein | ND | ND | ND | | ND | |
| Down | 4 | 6405 | Succinate--CoA ligase [ADP-forming] subunit alpha | *sucD* | 30.594 | 4.99E-06 | | 39.9 | |
| Down | 4 | 6511 | Hypothetical protein | ND | ND | ND | | ND | |
| Down | 4 | 6707 | Urocanate hydratase | *hutU* | 61.593 | 1.60E-29 | | 46% | |
| Down | 4 | 7502 | Glutamate dehydrogenase | *gdh* | 46.165 | 1.18E-10 | | 36.60% | |
| Down | 4 | 7510 | Uncharacterized protein | ND | ND | ND | | ND | |

**Supplementary File 1i.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics (µg/ml)/**  **strain** | **Polymyxin B** | **Colistin** | **Ampicillin** | **Gentamicin** | **Spectinomycin** | **Kanamycin** | **Chloramphenicol** | **Clindamycin** | **Rifampicin** |
| Lab-WT | 2 | 1 | 150 | 8 | 128 | 1 | 512 | 32 | 12 |
| PMRLow | 4 | 1 | 150 | 8 | 128 | 1 | 512 | 32 | 12 |
| PMRHigh | 128 | 32 | 150 | 8 | 128 | 1 | 512 | 32 | 12 |
| F-1025 | 4 | 2 | >150 | >16 | >128 | >1 | >512 | >64 | 12 |
| F-1208 | 2 | >16 | >150 | >16 | >128 | >1 | >512 | >64 | >48 |
| F-1379 | 2 | 1 | >150 | >16 | >128 | >1 | >512 | >64 | 12 |
| F-1410 | 8 | 2 | >150 | >16 | >128 | >1 | >512 | >64 | 12 |
| F-1629 | 128 | >64 | >150 | >16 | >128 | >1 | >512 | >64 | >48 |

**Supplementary File 1j.**

|  |  |  |
| --- | --- | --- |
| Strain | Description | Reference |
| Lab-WT | Wild-type, laboratory-cultured *A. baumannii* ATCC 17978 | Laboratory stock |
| PMRLow | Isolated from 2 μg/mL PMB containing agar plate | This study |
| PMRHigh | Isolated from 16 μg/mL PMB containing agar plate | This study |
| Δ*pmrBw* (Δ*pmrB*) | *pmrB* (encoded by ATCC1\_00956) mutant; insertion of pVIK112-*pmrB* into Lab-WT | This study |
| Δ*pmrBH* | *pmrB* (encoded by ABA17978\_00959) mutant; insertion of pVIK112-*pmrB* into PMRHigh | This study |
| Lab-WT*+* pRK415 | Complementation strain; insertion of empty vector, pRK415, into Lab-WT | This study |
| Lab-WT*+* pRK-*pmrB* | Complementation strain; insertion of empty vector, pRK415, into Lab-WT | This study |
| Δ*surE* | *surE* (encoded by ATCC1\_01296) mutant; insertion of pVIK112-*surE* into Lab-WT | This study |
| Δ*lpp* | *lpp* (encoded by ATCC1\_01297) mutant; insertion of pVIK112-*nlpD* into Lab-WT | This study |
| Δ*fabH* | *fabH* (encoded by ATCC1\_03686) mutant; insertion of pVIK112-*fabH* into Lab-WT | This study |
| Δ*ftsL* | *ftsL* (encoded by ATCC1\_00505) mutant; insertion of pVIK112-*ftsL* into Lab-WT | This study |
| Δ*udg* | *udg* (encoded by ATCC1\_00328) mutant; insertion of pVIK112-*udg* into Lab-WT | This study |
| Plasmids | **Description** | **Reference** |
| pVIK112 | Kmr, R6K, *oriV*, suicide vector, *lacZ* fusion |  |
| pRK415 | Tcr, *oriV*, broad-host-range vector |  |
| pCasAb-apr | Aprr, *oriV*, broad-host-range vector | Wang et al., 2019 |
| pSGAb-km | Kmr*, ColE1, WH1266* | Wang et al., 2019 |
| pVIK112-*pmrBW* | Kmr, internal *pmrB* fragment (encoded by ATCC1\_00956) in pVIK112 | This study |
| pVIK112-*surE* | Kmr, internal *surE* fragment (encoded by ATCC1\_01296) in pVIK112 | This study |
| pVIK112-*lpp* | Kmr, internal *nlpD* fragment (encoded by ATCC1\_01297) in pVIK112 | This study |
| pVIK112-*fabH* | Kmr, internal *fabH* fragment (encoded by ATCC1\_03686) in pVIK112 | This study |
| pVIK112-*ftsL* | Kmr, internal *ftsL* fragment (encoded by ATCC1\_00505) in pVIK112 | This study |
| pVIK112-*udg* | Kmr, internal *udg* fragment (encoded by ATCC1\_00956) in pVIK112 | This study |
| Knockout primers | **Sequence (5′-3′)** | **Reference** |
| *pmrB*\_KO\_F (*KpnI*) | CCGGAATTCGAACCGTTCTTGGTGACTGCAC | This study |
| *pmrB*\_KO\_R (*EcoRI*) | TCCCCCGGGCGCCAAATGAACAACACGCA | This study |
| *surE*\_KO\_F (*KpnI*) | CCGGAATTCTAATGGCACACCAGCCGATT | This study |
| *surE*\_KO\_R (*EcoRI*) | TCCCCCGGGACGCTGTGGATCAGTTACCG | This study |
| *nlpD*\_KO\_F (*KpnI*) | CCGGAATTCGAACCGTTCTTGGTGACTGCAC | This study |
| *nlpD*\_KO\_R (*EcoRI*) | TCCCCCGGGCGCCAAATGAACAACACGCA | This study |
| *fabH*\_KO\_F (*KpnI*) | CCGGAATTCTCTGCAACAAGCCCAGATGA | This study |
| *fabH*\_KO\_R (*EcoRI*) | TCCCCCGGGCCTGAGCATCTTCACTGCCA | This study |
| *ftsL*\_KO\_F (*KpnI*) | CCGGAATTCGAACCGTTCTTGGTGACTGCAC | This study |
| *ftsL*\_KO\_R (*EcoRI*) | TCCCCCGGGCGCCAAATGAACAACACGCA | This study |
| *udg*\_KO\_F (*KpnI*) | CCGGAATTCGAACCGTTCTTGGTGACTGCAC | This study |
| *udg*\_KO\_R (*EcoRI*) | TCCCCCGGGCGCCAAATGAACAACACGCA | This study |
| qRT-PCR primers | **Sequence (5′-3′)** | **Reference** |
| *mlaD*\_qRT PCR\_F | ACGAGCAGGTTCTGCATTGA | This study |
| *mlaD*\_qRT PCR\_R | ATACCAGCCCAAAGAGGTGG | This study |
| *lpp*\_qRT PCR\_F | AATGGACAACTCGCAGCCAT | This study |
| *lpp*\_qRT PCR\_R | CCCGCTTTGGCAATACGAAG | This study |
| Wang, Yu., Wang, Z., Chen,Y., Hua ,X., Yu, Y. and Ji, Q. 2019. A highly efficient CRISP Cas9-based genome engineering platform in *Acinetobacter baumannii* to understand the H2O2-sensing mechanism of OxyR. *Cell Chem Biol.* 26,1732-1742. | | |

**Supplementary File 1k.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Product size (bp)** | **Sequence primers** | **Sequence (5′-3′)** | **Reference** |
| *pmrB*  (1,484) | *pmrB*\_F | GAGGACTGGGCTACCGTTTG | This study |
| *pmrB*\_R | GCTGGGTCGTTTGGGCAATA | This study |
| *pmrB\_*Intermidiate | ACGCGATTCCGAAGAACTCA | This study |
| *dgcB*  (189) | *dgcB*\_F | AGATGCACGTCTCTTAGCCG | This study |
| *dgcB* \_R | ACATTTCGTCCCCGAGCTTT | This study |
| *ftsK*  (303) | *ftsK*\_F | GCAGCCGTTTATTTGCGAGA | This study |
| *ftsK*\_R | GTGGTAAACTAGGCCCGACC | This study |
| *surE*  (582) | *surE­*\_F | GCGTTAAAACCACTAGGCCG | This study |
| *surE­*\_R | ACGCTGTGGATCAGTTACCG | This study |
| *rimJ*  (136) | *rimJ* \_F | ATGCATGGGGTGTGGGTTAT | This study |
| *rimJ* \_R | ACTTGTTGTGACCTCACGTT | This study |
| *mlaC*  (579) | *mlaC*\_F | GTGAGCTGGCCGTAGGTATC | This study |
| *mlaC*\_R | CTGCGCCACCACCAGTAATA | This study |
| *fimT*  (464) | *fimT*\_F | AGTTGAGCTCACTATAACACTCGT | This study |
| *fimT*\_R | GCACATGGCCCATTTTGCTA | This study |
| *ftsL*  (319) | *ftsL*\_F | AGCAGTGATGAAATCGAAACCAC | This study |
| *ftsL*\_R | TTTGCTCTGAGGTCATCGGT | This study |
| *udg*  (493) | *udg*\_F | TGAAATGGGAGCCGATTACCC | This study |
| *udg*\_R | GGAGCTACTGTAACCGCAGG | This study |
| *fabH*  (481) | *fabH*\_F | TTAAAAGCAGGCCGCTCTGG | This study |
| *fabH*\_R | CTGCCAGCATGAGATCCTGT | This study |
| *xerD*  (335) | *xerD*\_F | CGTATTCCGAGCCCTGTTCA | This study |
| *xerD*\_R | GCTCGGCCAATTTTTGGTGA | This study |
| *feoB*  (534) | *feoB*\_F | ACACAAGTCGGTTTAGCCTCA | This study |
| *feoB*\_R | TACCAAGGCGATGCGCTAAT | This study |
| *relA*  (562) | *relA*\_F | ACACAAGTCGGTTTAGCCTCA | This study |
| *relA*\_R | TACCAAGGCGATGCGCTAAT | This study |
| Citrate-proton symporter  (221) | Citrate-proton symporter\_F | TGGATGTCGTTAATAACCGTAGG | This study |
| Citrate-proton symporter\_R | ACCCAAGAAAATAGCGCCGA | This study |
| *rsmI*  (402) | *rsmI*\_\_F | GGCCTACCAAGTGACCGTTT | This study |
| *rsmI*\_R | AGCCAGTTGAGATGCTGCTT | This study |
| *folA*  (423) | *folA*\_F | TGTCGAAGGTCCAAATGGGG | This study |
| *folA* \_R | CTACCAATCAGCACAGGCGA | This study |
| *guaA*  (439) | *guaA\_*F | AACACAACAAACGCCTTCCC | This study |
| *guaA* \_R | TGTCGCCATGCCAGTGTAAA | This study |