Novel roles for two-component regulatory systems in cytotoxicity and virulence-related properties in *Pseudomonas aeruginosa*

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**Table S1.** Effect of strain PA14 mutations in two-component regulatory system kinases and response regulators on cytotoxicity towards human bronchial epithelial cells. In general 3 or more experiments were performed assessing cytotoxicity (measured as LDH release after 8 hours of infection); results are expressed as the mean ± standard error expressed as a percentage of cytotoxicity caused by WT.

| PA14 locus ID | PAO1 homolog | Gene name | Description | Mean % of WT ± standard error |
|---------------|--------------|-----------|-------------|-----------------------------|
| **Mutants with increased cytotoxicity** | | | | |
| PA14_07840 | PA0601 | agtR | Two-component response regulator; contains a CheY-like receiver domain; amine uptake | 119.9 ± 4.2 |
| PA14_52260 | PA0928 | gacS | Sensor/response regulator hybrid; multi-host virulence through regulation of small regulatory RNAs RsmZ and RsmY | 134.3 ± 4.7 |

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| PA14 locus ID  | PAO1 homolog | Gene name | Description                                                                 | Mean % of WT ± standard error |
|---------------|--------------|-----------|-----------------------------------------------------------------------------|-------------------------------|
| PA14_49170    | PA1180       | phoQ      | Two-component sensor kinase PhoQ                                             | 121.1 ± 5.7‡                 |
| PA14_45590    | PA1458       |           | Probable two-component sensor kinase; putative homolog of E. coli chemotaxis regulator CheA | 144.3 ± 6.3                  |
| PA14_33780    | PA2388       | fpvR      | Probable Fe²⁺-dicitrate sensor kinase                                        | 121.6 ± 7.9                  |
| PA14_30650    | PA2586       | gacA      | Response regulator; multi-host virulence through regulation of small regulatory RNAs RsmZ and RsmY | 144.7 ± 15.8                 |
| PA14_26810    | PA2882       |           | Probable two-component sensor kinase; has binding domain homologous to that found in sensors of C4-dicarboxylates | 139.3 ± 15.7                 |
| PA14_16470    | PA3704       | wspE      | CheA-type sensor kinase; c-di-GMP regulation                                | 134.5 ± 10.4                 |
| PA14_62530    | PA4725       | cbrA      | Two-component sensor kinase; required for carbon-nitrogen balance and control of catabolite repression | 158.8 ± 9.1                  |
| PA14_62540    | PA4726       | cbrB      | Two-component response regulator; required for carbon-nitrogen balance and control of catabolite repression | 140.8 ± 9.7                  |
| PA14_67680    | PA5125       | ntrC      | Two-component response regulator; regulates use of carbon and nitrogen      | 122.6 ± 4.1                  |
| PA14_67670    | PA5124       | ntrB      | Two-component sensor kinase; regulates use of carbon and nitrogen           | 117.1 ± 5.8                  |
| PA14_70790    | PA5364       |           | Probable two-component response regulator                                   | 130.4 ± 9.1                  |
| PA14_72740    | PA5512       | mifS      | Two-component sensor kinase; regulates biofilm development                   | 119.6 ± 13.2                 |
|               |              |           | Mutants with decreased cytotoxicity                                          |                               |
| PA14_05320    | PA0408       | pilG      | Type IV pilus response regulator; required for pilus extension and twitching motility | 28.8 ± 4.4                    |
| PA14_05330    | PA0409       | pilH      | Type IV pilus response regulator; required for pilus retraction             | 25.8 ± 1.5                   |
| PA14_50220    | PA1097       | fleQ      | Flagella major transcriptional regulator; cyclic-di-GMP responsive; potential FleSR modulator | 77.6 ± 12.7                  |
| PA14_50200    | PA1098       | fleS      | Two-component sensor kinase; required for hook and basal body protein biosynthesis for flagellum assembly | 20.6 ± 6.7‡                  |
| PA14_50180    | PA1099       | fleR      | Two-component response regulator; required for hook and basal body protein biosynthesis for flagellum assembly | 12.3 ± 0.8‡                  |
| PA14_16500    | PA3702       | wspR      | Two-component response regulator with GGDEF domain; c-di-GMP regulation    | 16.8 ± 3.7                   |
| PA14_12780    | PA3948       | rocA1     | Two-component response regulator; cyclic-di-GMP regulation                 | 53.2 ± 0.9                   |
| PA14_60260    | PA4547       | pilR      | Two-component response regulator; required for pilus expression and therefore for type IV pilus biosynthesis | 14.6 ± 4.5                   |
| PA14_68230    | PA5165       | dctB      | Two-component sensor kinase; regulates a C4-dicarboxylate transport system with DctD | 14.9 ± 3.5                   |
| PA14_69470    | PA5261       | algR      | Alginate biosynthesis regulatory protein                                    | 33.2 ± 11.7                  |
|               |              |           | No major change in cytotoxicity                                             |                               |
| PA14_00430    | PA0034       |           | Probable two-component response regulator                                  | 108.9 ± 5.1                  |
| PA14_01860    | PA0150       |           | Probable transmembrane sensor kinase                                        | 101.4 ± 3.0                  |
| PA14_02250    | PA0178       | cheA      | Probable two-component sensor kinase                                        | 107.5 ± 4.1                  |
| PA14_02260    | PA0179       |           | Probable two-component response regulator                                  | 103.1 ± 4.1                  |

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| PA14 locus ID | PAO1 homolog | Gene name | Description | Mean % of WT ± standard error |
|---------------|--------------|-----------|-------------|------------------------------|
| PA14_06060    | PA0463       | creB      | Two-component response regulator; catabolism and motility | 94.2 ± 3.3 |
| PA14_06070    | PA0464       | creC      | Two-component sensor kinase; catabolism and motility | 109.9 ± 5.4 |
| PA14_07820    | PA0600       | agtS      | Two-component sensor kinase; amine uptake | 95.9 ± 7.0 |
| PA14_54510    | PA0756       |          | Probable two-component response regulator | 103.3 ± 1.3 |
| PA14_54500    | PA0757       |          | Probable two-component sensor kinase | 109.4 ± 4.9 |
| PA14_52240    | PA0930       | pirS      | Two-component sensor kinase; iron acquisition | 109.9 ± 3.5 |
| PA14_49440    | PA1157       |          | Probable two-component response regulator | 107.5 ± 3.8 |
| PA14_49420    | PA1158       |          | Probable two-component sensor kinase | 113.8 ± 5.7 |
| PA14_49180    | PA1179       | phoP      | Two-component response regulator; regulates cytotoxicity and resistance to cationic peptides | 104.4 ± 1.8 |
| PA14_49160    | PA1243       |          | Probable sensor kinase /regulator hybrid | 94.0 ± 8.5 |
| PA14_47390    | PA1301       |          | Probable transmembrane sensor kinase | 111.2 ± 8.2 |
| PA14_46980    | PA1336       | aauS      | Two-component sensor kinase; amino acid uptake | 110.1 ± 0.3 |
| PA14_46850    | PA1347       |          | Probable transcriptional regulator | 106.6 ± 1.3 |
| PA14_46370    | PA1396       |          | Probable two-component sensor kinase | 98.5 ± 3.5 |
| PA14_46060    | PA1422       | gbuR      | Transcriptional regulator | 95.5 ± 4.5 |
| PA14_45880    | PA1437       |          | Probable two-component response regulator | 106.7 ± 3.1 |
| PA14_45870    | PA1438       |          | Probable two-component sensor kinase | 111.6 ± 4.5 |
| PA14_43350    | PA1636       | kpdD      | Two-component sensor kinase; potassium transport regulation | 109.8 ± 4.5 |
| PA14_42220    | PA1727       | mucR      | Two component sensor kinase; alginate regulation | 113.0 ± 4.5 |
| PA14_41490    | PA1785       | nasT      | Response regulator; nitrate/nitrite assimilation | 103.2 ± 2.6 |
| PA14_41270    | PA1798       | parS      | Two-component sensor kinase; resistance to cationic peptides | 93.9 ± 8.5 |
| PA14_41260    | PA1799       | parR      | Probable two-component response regulator; resistance to cationic peptides | 107.7 ± 4.3 |
| PA14_40570    | PA1851       |          | Probable two-component response regulator | 104.4 ± 1.9 |
| PA14_38970    | PA1976       | ercS'     | Probable two-component sensor kinase; ethanol oxidation | 112.7 ± 5.1 |
| PA14_38900    | PA1980       | eraR      | Probable two-component response regulator; ethanol oxidation | 98.7 ± 5.0 |
| PA14_37980    | PA2051       |          | Probable transmembrane sensor kinase | 103.9 ± 5.7 |
| PA14_36420    | PA2177       |          | Probable histidine sensor kinase | 100.6 ± 4.6 |
| PA14_32580    | PA2479       |          | Probable two-component response regulator | 107.3 ± 4.7 |
| PA14_31960    | PA2523       | czcR      | Two-component response regulator; metal and imipenem resistance | 107.4 ± 4.1 |
| PA14_31950    | PA2524       | czcS      | Two-component sensor kinase; metal and imipenem resistance | 107.5 ± 1.0 |
| PA14_30830    | PA2572       |          | Probable two-component response regulator | 111.9 ± 5.2 |
| PA14_30700    | PA2583       |          | Probable sensor kinase/response regulator hybrid | 100.3 ± 6.5 |
| PA14_29740    | PA2656       |          | Probable two-component sensor kinase | 101.6 ± 6.5 |
| PA14_29360    | PA2687       | pfeS      | Two-component sensor kinase; iron acquisition | 88.0 ± 4.8 |
| PA14_27940    | PA2798       |          | Probable two-component response regulator | 108.6 ± 3.4 |
| PA14_27810    | PA2809       | copR      | Probable two-component response regulator; copper resistance | 106.4 ± 5.3 |
| PA14_27800    | PA2810       | copS      | Two-component sensor kinase; copper resistance | 93.7 ± 2.3 |

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| PA14 locus ID | PAO1 homolog | Gene name | Description | Mean % of WT ± standard error |
|---------------|---------------|-----------|-------------|------------------------------|
| PA14_27550    | PA2824        | sagS      | Two-component sensor/regulator hybrid; motile/sessile switch, activates Gac/Hpt/Rsm system | 88.9 ± 8.6 |
| PA14_24720    | PA3044        | rocS2     | Two-component sensor kinase; c-di-GMP regulation | 96.9 ± 4.5 |
| PA14_24710    | PA3045        | rocA2     | Two-component response regulator; c-di-GMP regulation | 103.2 ± 4.0 |
| PA14_24350    | PA3077        | cprR      | Probable two-component system regulatory protein; cationic peptide resistance | 112.8 ± 9.5 |
| PA14_24340    | PA3078        | cprS      | Probable two-component sensor kinase; cationic peptide resistance | 98.4 ± 6.2 |
| PA14_22960    | PA3191        | gtrS      | Probable two-component sensor kinase; virulence | 107.3 ± 4.4 |
| PA14_22940    | PA3192        | gtrR      | Probable two-component response regulator; virulence | 98.6 ± 2.6 |
| PA14_22730    | PA3206        | Probable two-component sensor kinase | 102.5 ± 6.3 |
| PA14_21700    | PA3271        | Probable two-component sensor kinase | 105.9 ± 11.3 |
| PA14_20820    | PA3343        | Probable two-component response regulator | 108.8 ± 7.1 |
| PA14_20780    | PA3346        | Probable two-component response regulator | 105.7 ± 3.9 |
| PA14_20000    | PA3409        | hanS      | Probable Fe^{2+}-dicitrate sensor kinase; diguanylate cyclase | 102.5 ± 5.7 |
| PA14_17670    | PA3604        | erdR      | Two-component response regulator; ethanol oxidation | 99.4 ± 10.1 |
| PA14_16350    | PA3714        | Probable two-component response regulator | 110.9 ± 5.3 |
| PA14_13740    | PA3878        | narX      | Two-component sensor kinase; nitrate respiration, biofilm, motility | 100.5 ± 10.9 |
| PA14_12820    | PA3900        | fecR      | Probable transmembrane sensor protein | 110.9 ± 5.6 |
| PA14_12820    | PA3946        | rocS1     | Two-component sensor kinase; c-di-GMP regulation | 103.4 ± 7.4 |
| PA14_12810    | PA3947        | rocR      | Antagonist of rocA1 response regulator; c-di-GMP regulation with EAL domain | 93.5 ± 1.9 |
| PA14_11680    | PA4032        | Probable two-component response regulator | 106.0 ± 2.6 |
| PA14_11630    | PA4036        | Probable two-component sensor kinase | 110.9 ± 3.8 |
| PA14_10770    | PA4112        | Probable sensor/regulator hybrid | 97.4 ± 9.4 |
| PA14_09690    | PA4196        | bfiR      | Probable two-component response regulator; biofilm formation | 90.8 ± 9.9 |
| PA14_09680    | PA4197        | bfiS      | Two-component sensor kinase; biofilm formation | 97.5 ± 2.9 |
| PA14_55780    | PA4293        | pprA      | Two-component sensor kinase; outer membrane permeability and resistance | 108.8 ± 4.1 |
| PA14_55810    | PA4296        | pprB      | Probable two-component response regulator; outer membrane permeability and resistance | 100.5 ± 6.8 |
| PA14_56950    | PA4381        | colR      | Probable DNA-binding response regulator; polymyxin resistance | 103.8 ± 2.5 |
| PA14_57140    | PA4396        | Probable two-component response regulator | 103.0 ± 10.0 |
| PA14_57170    | PA4398        | Probable two-component response regulator | 105.5 ± 12.7 |
| PA14_58300    | PA4493        | roxR      | Two-component response regulator; regulates production of cyanide insensitive oxidase | 107.0 ± 3.8 |
| PA14_58320    | PA4494        | roxS      | Two-component sensor kinase; regulates production of cyanide insensitive oxidase | 108.1 ± 6.4 |
| PA14_60250    | PA4546        | pilS      | Two-component sensor kinase; type IV pili production | 113.5 ± 5.3 |

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| A14 locus ID | PAO1 homolog | Gene name | Description | Mean % of WT ± standard error |
|-------------|--------------|-----------|-------------|-----------------------------|
| PA14_63150  | PA4776       | pmrA      | Two-component response regulator; cationic peptide resistance | 104.3 ± 2.3 |
| PA14_63160  | PA4777       | pmrB      | Two-component sensor kinase; cationic peptide resistance | 92.0 ± 10.8 |
| PA14_64050  | PA4843       | gcbA      | Probable two-component response regulator; diguanylate cyclase | 108.8 ± 3.0 |
| PA14_64880  | PA4983       |           | Probable two-component response regulator | 108.5 ± 5.4 |
| PA14_68250  | PA5166       | dctD      | Two-component response regulator; C4-dicarboxylate metabolism | 97.1 ± 3.6 |
| PA14_68680  | PA5199       | amgS      | Two-component sensor kinase; membrane stress, aminoglycoside resistance | 91.5 ± 3.3 |
| PA14_70750  | PA5360       | phoB      | Two component response regulator; phosphate regulation and quorum sensing | 105.9 ± 3.5 |
| PA14_70760  | PA5361       | phoR      | Two-component sensor kinase; phosphate regulation and quorum sensing | 108.8 ± 3.3 |
| PA14_72380  | PA5483       | algB      | Two-component response regulator; alginate production | 96.1 ± 3.2 |
| PA14_72390  | PA5484       | kinB      | Two-component sensor kinase; alginate production | 93.6 ± 5.6 |
| PA14_59770  | PA5511       | rcsB      | Two component response regulator; cup fimbria and biofilm formation | 104.0 ± 5.2 |
| PA14_59790  | PA5511       | pvrR      | Two component response regulator; EAL domain, c-di-GMP regulation, cup fimbria and biofilm | 103.6 ± 4.7 |
| PA14_59800  | PA5511       | pvrS      | Two component sensor kinase; c-di-GMP regulation, cup fimbria and biofilm | 110.0 ± 4.2 |

†Mutants are listed in order of the PAO1 homolog. Mutants not in the screen included PA1637 kpdE—response regulator with KpdD, potassium transport; PA1786 nasS—sensor with nasT, nitrate/nitrite assimilation; PA2686 pfeR—response regulator with pfeS, iron acquisition; PA9299 pirR—response regulator with pirS, iron acquisition; PA3345 htpB—response regulator with sagS, activates Gac/Htp/Rsm system; PA3879 narL—response regulator with narX, nitrate respiration, biofilm, motility; PA3974 ladS—sensor, virulence, biofilm formation, cytotoxicity; PA4380 colS—sensor with colR, polymyxin resistance; PA4101 bfmR—response regulator with bfmS, biofilm development; PA4102 bfmS—sensor with bfmR, biofilm development; PA4856 retS—sensor, virulence, biofilm formation, cytotoxicity; PA4959 fimX—sensor (orphan?); contains GGDEF and EAL domains; cytotoxicity; PA5200 amgR—response regulator with amgS, membrane stress and aminoglycoside resistance; PA5262 fimS—sensor with algR, virulence, alginate biosynthesis, motility, biofilm; PA5511 mjrR—response regulator with mjrS, biofilm development; PA14_59780 rcsC—sensor with rcsR, biofilm formation. **The phenotype for these mutants was different between PA14 and PAO1 as described in the text.