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Research article

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

Shaan L. Gellatly[1], Manjeet Bains[1], Elena B.M. Breidenstein[1], Janine Strehmel[2], Fany Reffuveille[1], Patrick K. Taylor[1], Amy T.Y. Yeung[1], Joerg Overhage[2] and Robert E.W. Hancock[1,*]

[1] Centre for Microbial Diseases and Immunity Research, Department of Microbiology & Immunology, University of British Columbia, Vancouver, BC, Canada
[2] Microbiology of Natural and Technical Interfaces Department, Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

* **Correspondence:** Email: bob@hancocklab.com; Tel: +16048220172; Fax: +16048275566.

Supplementary

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 Fe^{2+}-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 ensor 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		Probably 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*	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	*gltR*	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	*hasS*	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		*rcsB*	Two component response regulator; cup fimbria and biofilm formation	104.0 ± 5.2
PA14_59790		*pvrR*	Two component response regulator; EAL domain, c-di-GMP regulation, cup fimbria and biofilm	103.6 ± 4.7
PA14_59800		*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; PA0929 *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 *mifR*—response regulator with *mifS*, biofilm development; PA14_59780 *rcsC*—sensor with *rcsB*, biofilm formation. [‡]The phenotype for these mutants was different between PA14 and PAO1 as described in the text.