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Phosphate Starvation Promotes Swarming Motility and Cytotoxicity of *Pseudomonas aeruginosa*

Manjeet Bains, Lucía Fernández, and Robert E. W. Hancock

Centre for Microbial Diseases and Immunity Research, Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

We investigated the transcriptional responses of *Pseudomonas aeruginosa* under phosphate-deficient (0.2 mM) conditions compared to phosphate sufficiency (1 mM). This elicited enormous transcriptional changes in genes related to phosphate acquisition, quorum sensing, chemotaxis, toxin secretion, and regulation. This dysregulation also led to increased virulence-associated phenotypes, including swarming motility and cytotoxicity.

A characteristic trait of *Pseudomonas aeruginosa* is its high versatility, enabling this Gram-negative microbe to colonize a wide range of habitats such as soil, water, plants, and animals (25). It is the third most common nosocomial pathogen, causing serious opportunistic infections in elderly, immunocompromised, and injured individuals as well as chronic infections in the lungs of cystic fibrosis (CF) patients (18, 26). It has a tremendous capacity to adapt to diverse circumstances, and various conditions, including biofilm, quorum, or swarming lifestyles, exposure to subinhibitory antibiotics, and nutritional deprivation, lead to very large changes in gene expression and altered virulence and/or antibiotic resistance (3, 6, 12, 22, 23). To adapt efficiently to the changes in its surroundings, *Pseudomonas* has evolved sophisticated regulatory networks, and almost 10% of all genes in the *P. aeruginosa* genome encode proteins with a regulatory function (29).

Phosphate is essential for all living organisms, participating in critical biochemical processes, being an essential component of the energy dynamics of cells and a component of nucleic acids, phospholipids in membranes, and other biomolecules. Therefore, the ability to withstand conditions of phosphate starvation, making use of the available phosphate, is of great importance for cell survival. Consequently, microorganisms possess complex regulatory pathways for the control of the mechanisms involved in sensing phosphate availability as well as phosphate uptake and utilization. Furthermore, these networks usually overlap central metabolic routes because of the importance of phosphate in cellular physiology. Among Gram-negative bacteria, the best-characterized phosphate regulon is that of *Escherichia coli*, in which a two-component regulatory system, PhoBR, gets activated under phosphate-limiting conditions and binds to a conserved sequence (Pho-box) in the promoters of its target genes, inducing or repressing their expression (13). These genes include those encoding systems for high-affinity uptake of inorganic phosphate as well as acquisition of phosphate from alternative sources such as phosphonates and organic phosphate. *P. aeruginosa* also possesses homologs to PhoBR (1, 7). Significantly, phosphate limitation in *Pseudomonas* alters the production of quorum-sensing signals (15) and, consequently, it might have an impact on virulence and social behaviors such as biofilm formation and swarming motility. Indeed, Haddad et al. (10) related the expression of the Pho regulon to biofilm formation and the type III secretion system in *P. aeruginosa*.

The phosphate deprivation regulon is involved in influencing virulence traits in various microorganisms (2, 31, 27, 28, 30). Long et al. (17) showed that phosphate depletion is commonly observed after surgery and related this to an increase in the virulence of *P. aeruginosa*. This relationship was confirmed *in vivo*, as the growth of *P. aeruginosa* in a low-phosphate medium resulted in enhanced killing of *Caenorhabditis elegans* due to overexpression of the *Pseudomonas* quinolone signal (PQS) quorum-sensing signal and the iron chelator pyoverdin (33). Recently, two independent studies showed an upregulation of the genes involved in phosphate uptake in *P. aeruginosa* upon contact with differentiated human epithelial cells (4, 8), indicating that environmental cues other than low phosphate might induce expression of these genes inside the host. Thus, a better understanding of the phosphate regulon in *P. aeruginosa* and its correlation with virulence properties would help clarify its potential role in infections.

As a wild-type strain, the sequenced *P. aeruginosa* PAO1 strain H103 was routinely grown in Luria-Bertani (LB) broth or agar. Defined-phosphate, HEPES-based minimal medium was prepared as described previously (11). To identify the genes dysregulated during phosphate starvation, microarray analysis was performed on bacteria grown under phosphate-sufficient (1 mM phosphate) or phosphate-deficient (0.2 mM) conditions at 37°C with shaking (250 rpm) to an optical density at 600 nm (OD_{600}) ~ 0.5. Cells from these cultures were harvested, and total RNA was isolated with an RNeasy Midi RNA isolation kit (Qiagen), processed as described previously (19), and hybridized to *P. aeruginosa* PAO1 DNA microarray epoxy-coated slides from the J. Craig Venter Institute, Pathogen Functional Genomics Resource Center (http://pfgrc.jcvi.org/index.php/microarray.html). Results were analyzed using ArrayPipe (version 1.7). The four biological replicates were averaged to obtain overall changes for the samples grown under low-phosphate compared to phosphate-sufficient conditions. A two-sided one-sample Student's *t* test was used to determine statistical significance, and changes of 2-fold or greater with a *P* value of ≤0.05 were used as the cutoffs for

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Address correspondence to Robert E. W. Hancock, bob@hancocklab.com.
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TABLE 1 Selected genes from the microarray comparing the transcriptome of *P. aeruginosa* H103 grown under phosphate-deficient conditions to that grown under phosphate-sufficient conditions

Gene identifier	Gene name	Fold change	*P* value	Description[a]
PA0026	*plcB*	2.4	0.0001	Phospholipase C
PA0083	*tssB1*	4.0	4.1E−05	Type VI secretion system protein
PA0084	*tssC1*	2.7	0.0001	Type VI secretion system protein
PA0085	*htp1*	5.4	0.0004	Type VI secretion system protein
PA0087	*tssE1*	2.1	0.0009	Type VI secretion system protein
PA0088	*tssF1*	3.1	6.0E−05	Type VI secretion system protein
PA0089	*tssG1*	3.9	0.0007	Type VI secretion system protein
PA0090	*clpV1*	5.0	0.0001	Type VI secretion system ATPase
PA0091	*vgrG1*	4.3	0.0001	Type VI secretion system protein
PA0173		5.6	0.0001	Probable chemotaxis-specific methylesterase
PA0174		5.6	0.0003	Conserved hypothetical protein
PA0175		10.6	7.7E−05	Probable chemotaxis protein methyltransferase
PA0176	*aer2*	15.4	0.0001	Aerotaxis transducer methyl-accepting chemotaxis protein
PA0177		9.1	6.7E−05	Probable purine-binding chemotaxis protein
PA0178		4.9	0.0011	Probable two-component sensor
PA0179		4.0	0.0001	Probable two-component response regulator
PA0347	*glpQ*	25.0	1.3E−07	Glycerophosphoryl diester phosphodiesterase, periplasmic
PA0426	*mexB*	2.6	3.2E−05	RND multidrug efflux transporter
PA0450		4.1	0.0002	Probable phosphate transporter
PA0677	*hxcW*	4.6	3.7E−05	HxcW putative pseudopilin
PA0678	*hxcU*	8.2	7.2E−06	HxcU putative pseudopilin
PA0679	*hxcP*	7.3	1.9E−05	Hypothetical protein
PA0680	*hxcV*	2.4	0.0004	HxcV putative pseudopilin
PA0681	*hxcT*	7.6	0.0001	HxcT pseudopilin
PA0682	*hxcX*	13.3	3.7E−05	HxcX atypical pseudopilin
PA0683	*hxcY*	8.8	1.8E−05	Probable type II secretion system protein
PA0684	*hxcZ*	11.7	3.8E−06	Probable type II secretion system protein
PA0685	*hxcQ*	12.3	1.1E−05	Probable type II secretion system protein
PA0686	*hxcR*	13.1	8.4E−06	Probable type II secretion system protein
PA0687	*hxcS*	3.6	5.6E−05	Probable type II secretion system protein
PA0688		308	1.2E−06	Probable binding protein component of ABC transporter
PA0763	*mucA*	2.1	8.7E−05	Anti-sigma factor MucA
PA0764	*mucB*	2.3	0.0074	Negative regulator for alginate biosynthesis MucB
PA0842		33.5	8.3E−07	Probable glycosyl transferase
PA0843	*plcR*	9.7	4.5E−05	Phospholipase accessory protein PlcR precursor
PA0844	*plcH*	25.7	3.2E−05	Hemolytic phospholipase C precursor
PA0996	*pqsA*	4.2	0.019	Probable coenzyme A ligase
PA0997	*pqsB*	5.0	0.0094	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA0998	*pqsC*	4.5	0.0031	Homologous to beta-keto-acyl-acyl-carrier protein synthase
PA0999	*pqsD*	3.2	0.0026	3-Oxoacyl-[acyl-carrier-protein] synthase III
PA1000	*pqsE*	7.8	0.0031	Quinolone signal response protein
PA1001	*phnA*	6.6	0.0033	Anthranilate synthase component I
PA1002	*phnB*	4.7	0.0047	Anthranilate synthase component II
PA1003	*mvfR*	2.4	0.0003	Transcriptional regulator
PA1078	*flgC*	2.1	0.0005	Flagellar basal-body rod protein FlgC
PA1082	*flgG*	2.0	0.0011	Flagellar basal-body rod protein FlgG
PA1086	*flgK*	2.3	0.0003	Flagellar hook-associated protein 1 FlgK
PA1087	*flgL*	2.0	4.9E−05	Flagellar hook-associated protein type 3 FlgL
PA1092	*fliC*	2.3	0.0016	Flagellin type B
PA1130	*rhlC*	2.6	0.0022	Rhamnosyltransferase 2
PA1249	*aprA*	2.1	0.001	Alkaline metalloproteinase precursor
PA1423		2.7	0.0036	Probable chemotaxis transducer
PA1456	*cheY*	2.6	0.0038	Two-component response regulator CheY
PA1561	*aer*	2.2	4.7E−05	Aerotaxis receptor Aer
PA1665		2.0	0.0047	Hypothetical protein
PA1712	*exsB*	−2.1	0.0098	Exoenzyme S synthesis protein B
PA1871	*lasA*	5.5	0.028	LasA protease precursor
PA1900	*phzB2*	11.7	0.025	Probable phenazine biosynthesis protein
PA1901	*phzC2*	6.8	0.0106	Phenazine biosynthesis protein PhzC
PA1902	*phzD2*	11.5	0.0025	Phenazine biosynthesis protein PhzD

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TABLE 1 (Continued)

Gene identifier	Gene name	Fold change	*P* value	Description[a]
PA1903	*phzE2*	3.0	0.0305	Phenazine biosynthesis protein PhzE
PA1904	*phzF2*	9.0	0.0064	Probable phenazine biosynthesis protein
PA1905	*phzG2*	7.2	0.0055	Probable pyridoxamine 5′-phosphate oxidase
PA1930		3.0	0.0006	Probable chemotaxis transducer
PA1985	*pqqA*	3.2	0.001	Pyrroloquinoline quinone biosynthesis protein A
PA1987	*pqqC*	2.2	0.0005	Pyrroloquinoline quinone biosynthesis protein C
PA2352		5.8	2.4E−05	Probable glycerophosphoryl diester phosphodiesterase
PA2360		3.2	0.003	Hypothetical protein
PA2365		8.7	0.0002	Conserved hypothetical protein
PA2366		7.1	0.0055	Conserved hypothetical protein
PA2367		2.9	0.0025	Hypothetical protein
PA2368		4.5	0.0036	Hypothetical protein
PA2369		3.4	0.0017	Hypothetical protein
PA2370		3.5	0.0016	Hypothetical protein
PA2371		3.0	0.003	Probable ClpA/B-type protease
PA2372		3.2	0.0088	Hypothetical protein
PA2373		3.7	0.0054	Conserved hypothetical protein
PA2374		2.2	0.0089	Hypothetical protein
PA2396	*pvdF*	2.1	0.0449	Pyoverdine synthetase F
PA2426	*pvdS*	4.3	0.0095	Sigma factor PvdS
PA2505		−6.7	9.5E−05	Probable porin
PA2520	*czcA*	−2.9	0.0014	RND divalent metal cation efflux transporter
PA2521	*czcB*	−4.8	0.0015	RND divalent metal cation efflux membrane fusion protein
PA2522	*czcC*	−3.6	0.001	Outer membrane protein precursor CzcC
PA2561	*ctpH*	2.8	0.0011	Probable chemotaxis transducer
PA2573		5.0	0.0022	Probable chemotaxis transducer
PA2788		2.2	0.0019	Probable chemotaxis transducer
PA2803		8.8	6.1E−05	Hypothetical protein
PA2804		14.9	2.9E−06	Hypothetical protein
PA2920		2.2	0.0018	Probable chemotaxis transducer
PA3095	*xcpZ*	2.6	8.0E−05	General secretion pathway protein M
PA3096	*xcpY*	2.8	0.0001	General secretion pathway protein L
PA3097	*xcpX*	2.9	0.0008	General secretion pathway protein K
PA3098	*xcpW*	2.8	9.3E−05	General secretion pathway protein J
PA3099	*xcpV*	2.2	8.2E−05	General secretion pathway protein I
PA3100	*xcpU*	2.9	8.7E−05	General secretion pathway outer membrane protein H
PA3101	*xcpT*	4.9	2.1E−06	General secretion pathway protein G
PA3102	*xcpS*	3.0	5.1E−05	General secretion pathway protein F
PA3103	*xcpR*	2.7	5.1E−05	General secretion pathway protein E
PA3104	*xcpP*	3.9	3.5E−05	Secretion protein XcpP
PA3105	*xcpQ*	4.6	2.4E−05	General secretion pathway protein D
PA3279	*oprP*	250	7.8E−08	Phosphate-specific outer membrane porin OprP
PA3280	*oprO*	16.6	3.3E−06	Pyrophosphate-specific outer membrane porin OprO
PA3296	*phoA*	69.1	2.3E−09	Alkaline phosphatase
PA3319	*plcN*	16.4	1.1E−07	Nonhemolytic phospholipase C precursor
PA3372	*phnP*	5.3	1.8E−05	Conserved hypothetical protein
PA3373	*phnN*	6.3	8.0E−07	Conserved hypothetical protein
PA3374	*phnM*	25.4	1.3E−07	Conserved hypothetical protein
PA3375	*phnL*	18.0	6.5−06	Probable ATP-binding component of ABC transporter
PA3376	*phnK*	18.6	1.3E−06	Probable ATP-binding component of ABC transporter
PA3377	*phnJ*	25.6	4.7E−07	Conserved hypothetical protein
PA3378	*phnI*	20.3	1.4E−06	Conserved hypothetical protein
PA3379	*phnH*	13.8	2.4E−06	Conserved hypothetical protein
PA3380	*phnG*	19.9	5.3E−07	Conserved hypothetical protein
PA3381	*phnF*	6.6	4.7E−06	Probable transcriptional regulator
PA3382	*phnE*	52.6	9.0E−08	Phosphonate transport protein PhnE
PA3383	*phnD*	91.0	2.0E−09	Binding protein component of ABC phosphonate transporter
PA3384	*phnC*	27.2	2.5E−07	ATP-binding component of ABC phosphonate transporter
PA3476	*rhlI*	3.0	0.0002	Autoinducer synthesis protein RhlI
PA3477	*rhlR*	4.7	0.0002	Transcriptional regulator RhlR

(Continued on following page)

TABLE 1 (Continued)

Gene identifier	Gene name	Fold change	*P* value	Description[a]
PA3478	*rhlB*	9.0	0.001	Rhamnosyltransferase chain B
PA3479	*rhlA*	11.5	0.0005	Rhamnosyltransferase chain A
PA3540	*algD*	2.3	0.0006	GDP-mannose 6-dehydrogenase AlgD
PA3550	*algF*	2.2	0.0099	Alginate *o*-acetyltransferase AlgF
PA3551	*algA*	5.1	0.0002	Alginate biosynthesis enzyme
PA3622	*rpoS*	3.1	0.0005	Sigma factor RpoS
PA3909	*eddB*	144	2.9E−08	Hypothetical protein
PA3910	*eddA*	34.6	3.3E−07	Hypothetical protein
PA4190	*pqsL*	2.6	3.8E−05	Probable FAD-dependent monooxygenase
PA4209	*phzM*	7.2	0.0235	Probable phenazine-specific methyltransferase
PA4210	*phzA1*	2.5	0.0069	Probable phenazine biosynthesis protein
PA4211	*phzB1*	55.1	0.0016	Probable phenazine biosynthesis protein
PA4217	*phzS*	29.6	0.0012	Flavin-containing monooxygenase
PA4290		3.0	0.0094	Probable chemotaxis transducer
PA4292		−3.6	9.7E−05	Probable phosphate transporter
PA4302		2.7	0.0002	Probable type II secretion system protein
PA4350	*olsB*	29.0	2.2E−07	Conserved hypothetical protein
PA4351	*olsA*	18.7	2.2E−08	Probable acyltransferase
PA4551	*pilV*	2.4	0.009	Type 4 fimbrial biogenesis protein PilV
PA4555	*pilY2*	2.0	0.0126	Type 4 fimbrial biogenesis protein PilY2
PA4723	*dksA*	−3.5	2.5E−05	Suppressor protein DksA
PA4844	*ctpL*	7.1	0.0001	Probable chemotaxis transducer
PA4853	*fis*	−3.8	9.8E−05	DNA-binding protein Fis
PA4915		2.1	0.0016	Probable chemotaxis transducer
PA5261	*algR*	2.4	7.9E−05	Alginate biosynthesis regulatory protein AlgR
PA5360	*phoB*	16.1	5.4E−07	Two-component response regulator PhoB
PA5361	*phoR*	9.3	5.4E−05	Two-component sensor PhoR
PA5365	*phoU*	18.7	9.5E−09	Phosphate uptake regulatory protein PhoU
PA5366	*pstB*	19.0	2.2E−08	ATP-binding component of ABC phosphate transporter
PA5367	*pstA*	25.7	7.4E−09	Membrane protein component of ABC phosphate transporter
PA5368	*pstC*	27.7	3.9E−09	Membrane protein component of ABC phosphate transporter
PA5369	*pstS*	223	2.6E−09	Hypothetical protein

[a] RND, resistance-nodulation-cell division.

reporting expression changes. The microarray data are available at MIAMExpress (see below).

These microarrays revealed a highly complex transcriptional response to phosphate-deficient growth conditions, with a total of 842 dysregulated genes, of which 495 were upregulated and 347 were downregulated (Table 1; see also Table S1 in the supplemental material for the full list). Critically, several of these genes were previously identified in our screening for promoters induced under low-phosphate conditions (16). There was a global upregulation of the genes involved in sensing the extracellular concentration of inorganic phosphate as well as in phosphate acquisition and utilization. Among these genes, we found the operon encoding the conserved two-component system PhoRB and the high-affinity phosphate transport system *pstSCAB-phoU* operon as well as gene locus PA0688, which has high homology to *pstS*. Similarly, expression of phosphate-specific porins OprP and OprO was induced under low-phosphate conditions, as was that of the phosphodiesterases *glpQ* and gene locus PA2352, the putative phosphate transporter PA0450, the alkaline phosphatase-encoding *phoA* gene, and the genes involved in the utilization of phosphonates (gene loci PA3372 to PA3384). The microarray data also revealed the upregulation of an operon encoding an extracellular DNase and an alkaline phosphatase (PA3909 and PA3910) that can provide *Pseudomonas* with phosphate and nitrogen from DNA (21). The expression of genes involved in the production of phospholipases, such as *plcB*, *plcH*, and *plcN*, which can lyse the phospholipids in eukaryotic membranes, was also enhanced during phosphate deprivation; presumably to enable phosphate acquisition in the host from organic sources, although phospholipases might additionally play a role in membrane remodeling, as reported for other microorganisms (34).

Another group of gene loci significantly dysregulated in response to phosphate depletion comprised those involved in chemotactic responses (PA0173 to PA0179, PA1423, PA1456, PA1930, PA2561, PA2573, PA2788, PA2920, PA4290, PA4844, and PA4915). Of particular significance were the phosphate-specific chemoreceptors CtpL (PA2561) and CtpH (PA4844) identified by Wu et al. (32) as necessary for chemotaxis with different concentrations of phosphate.

A total of 45 genes, including *rpoS*, *fis*, *gbdR*, and *algR*, that were dysregulated under low-phosphate conditions encoded products with a regulatory function. Other known regulators observed in the microarray were those that participate in quorum-sensing signaling, including *mvfR*, *pvdS*, and *rhlR*, reinforcing the concept that the phosphate regulon and the quorum-sensing network are tightly interconnected. Indeed, we observed dysregulation by

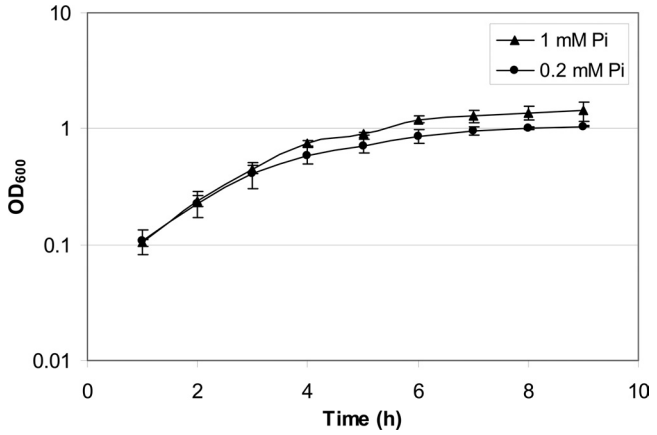

FIG 1 Growth curve of *P. aeruginosa* PAO1 under phosphate (Pi)-deficient (0.2 mM) and phosphate-sufficient (1 mM) conditions. The results shown in this figure represent the averages and standard deviations of the results of three independent experiments.

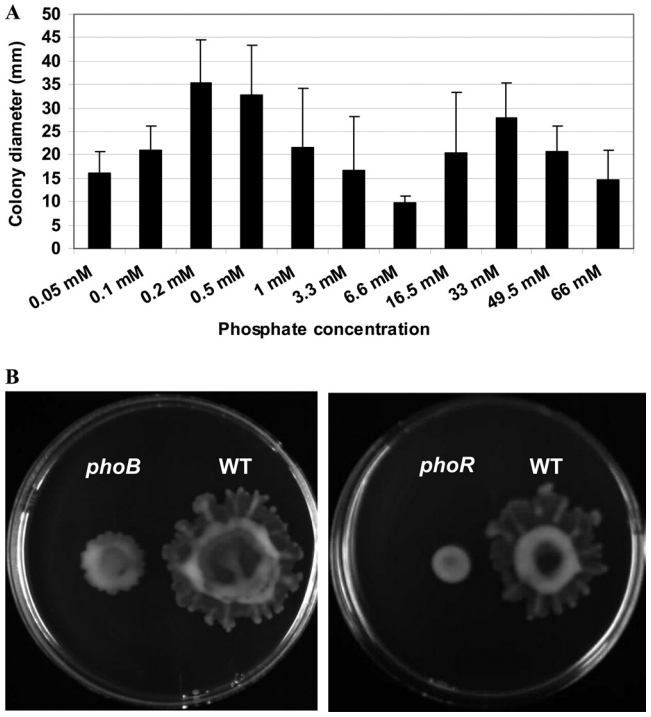

FIG 2 Effect of phosphate availability on swarming motility. (A) Diameters of swarming colonies of wild-type *P. aeruginosa* on plates containing swarming medium with increasing concentrations of phosphate. (B) Swarming motility phenotypes of the wild type (WT) and the *phoB* and *phoR* mutants on plates containing a low (0.2 mM) concentration of phosphate.

phosphate of known quorum-sensing-dependent genes, such as those involved in the biosynthesis of rhamnolipids or phenazines. Jensen et al. (15) previously indicated that the *Pseudomonas* quinolone signal (PQS) can regulate the expression of *rhlRI* partly through PhoB. Additionally, Zaborin et al. (33) observed a clear upregulation of quorum-sensing-regulated genes in the complete absence of phosphate compared to a phosphate-rich medium, which we here confirmed in comparisons of phosphate-sufficient to phosphate-deficient conditions. Furthermore, they linked this induction with the phenomenon of red death in *C. elegans*, which appeared to be mediated by PQS.

The general secretion pathway genes, together with the alternative type II and the type VI secretion systems, were upregulated under low-phosphate conditions, whereas the type III secretion system genes did not show any change or a slight downregulation (*exsB*). Genes representing all three type VI secretion islands of *P. aeruginosa* (HSI-1 [PA0074 to PA0091], HSI-2 [PA1656 to PA1671], and HSI-3 [PA2359 to PA2371]) were upregulated in the microarray analysis. Although our understanding of the mechanisms and roles of type VI secretion systems is still somewhat limited, recent evidence in *Pseudomonas* indicates that they might participate in chronic infection (20, 24). Overall, there was also significant overlap of our results with the microarray analysis of Zaborin et al. (33), who compared cells in the complete absence of phosphate to cells with phosphate.

Before attempting other phenotypical assays, we compared the levels of growth of *P. aeruginosa* in the minimal medium with 0.2 mM and 1 mM phosphate in order to determine if there was a significant growth defect under the former conditions. Our results showed, however, that growth was not significantly affected under phosphate-deficient conditions (Fig. 1).

We observed a significant upregulation of many genes related to quorum sensing and motility and thus tested the *P. aeruginosa* swarming phenotype using various concentrations of phosphate. Swarming motility assays revealed an intriguing bimodal response to the concentration of phosphate in *P. aeruginosa*. As the level of phosphate in the swarming medium decreased from high (33 mM; the normal concentration in BM2 minimal medium) to sufficient (1 mM), there was a clear reduction in the size of the swarming colonies (Fig. 2A), perhaps due in part to the reduction in the growth rate as the availability of phosphate decreased. However, as the concentration dropped further toward deficient levels (0.2 mM), swarming motility gradually increased again (Fig. 2A). Based on the microarray data, it seems likely that the increase in swarming motility under phosphate-deficient conditions was related to the upregulation of quorum-sensing genes and the subsequent increased production of rhamnolipids that is essential for swarming motility (5). In fact, genes involved in synthesis of rhamnolipids, such as *rhlA*, *rhlB*, and *rhlC*, were also significantly induced under these conditions (Table 1).

To show that this was related to regulatory events, we examined the swarming ability of mutants with mutations in the major regulators that respond to low phosphate, namely, *phoB* and *phoR*, which were taken from the University of Washington mutant library (14). Both strains showed a complete loss of swarming motility when grown on plates with a low (0.2 mM) concentration of phosphate (Fig. 2B), indicating that the PhoBR two-component system is necessary to promote swarming under low-phosphate conditions.

In addition to the effects on motility, we evaluated the impact of phosphate concentration on the cytotoxicity of *P. aeruginosa*. Cells from the wild-type strain or *phoB* or *phoR* mutants were grown in medium with low (0.2 mM) or sufficient (1 mM) phosphate prior to interaction with the human bronchial epithelial cell line 16HBE14o- at a multiplicity of infection of 50, as described previously (9). At 6 h postinfection, the percentage of cell lysis was determined. Two-fold-greater cytotoxicity, determined by increased release of lactate dehydrogenase, was

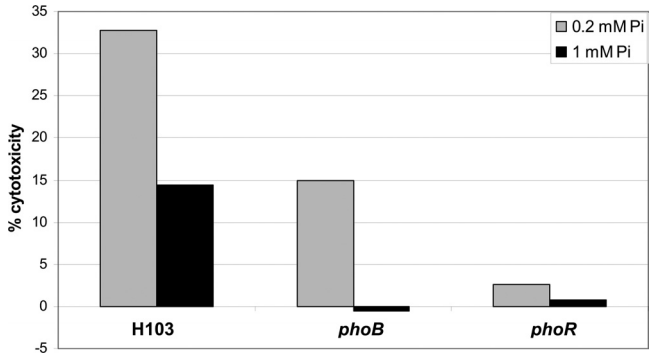

FIG 3 Analysis of the effect on cytotoxicity of phosphate deficiency prior to infection of HBE cells. The graph shows percent cytotoxicity compared to total cell lysis inflicted by treatment with Triton X-100 of the wild-type H103 strain and *phoB* and *phoR* mutant strains after growth in a sufficient (1 mM) or deficient (0.2 mM) concentration of phosphate. Data represent the results of a single experiment representative of three with the same trends.

observed for strain H103 grown in a low-phosphate medium before the infection compared to that of cells grown under phosphate-sufficient conditions (Fig. 3). Comparison of the cytotoxicities of the *phoB* and *phoR* regulatory mutants showed a clear reduction under both phosphate-sufficient and -deficient conditions. The *phoR* mutant had the greatest effect on cytotoxicity under phosphate-limiting conditions, but under phosphate-sufficient conditions, both regulatory mutants demonstrated no cytotoxicity at 6 h. A major candidate for the proteins responsible for altered cytotoxicity would be the phosphate-inducible hemolysins.

In conclusion, these results demonstrate how the opportunistic pathogen *P. aeruginosa* adapts to even moderate reductions in inorganic phosphate supply by modulating global gene expression. Indeed, phosphate deprivation led to a remarkably intricate transcriptional response that affected genes involved in a wide range of cellular functions, including virulence properties such as swarming and cytotoxicity. The induction of cytotoxicity under adverse conditions would appear to be directed at the acquisition of phosphate from affected cells.

Microarray data accession number. The microarray data determined in this work are available under MIAMExpress accession number E-MTAB-1170.

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REFERENCES

1. **Anba J, Bidaud M, Vasil ML, Lazdunski A.** 1990. Nucleotide sequence of the *Pseudomonas aeruginosa phoB* gene, the regulatory gene for the phosphate regulon. J. Bacteriol. **172**:4685–4689.
2. **Aoyama T, Takanami M, Makino K, Oka A.** 1991. Cross-talk between the virulence and phosphate regulons of *Agrobacterium tumefaciens* caused by an unusual interaction of the transcriptional activator with a regulatory DNA element. Mol. Gen. Genet. **227**:385–390.
3. **Breidenstein EBM, de la Fuente-Núñez C, Hancock REW.** 2011. *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol. **19**: 419–426.
4. **Chugani S, Greenberg EP.** 2007. The influence of human respiratory epithelia on *Pseudomonas aeruginosa* gene expression. Microb. Pathog. **42**:29–35.
5. **Déziel E, Lépine F, Milot S, Villemur R.** 2003. *rhlA* is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology **149**:2005–2013.
6. **Fernández L, Breidenstein EBM, Hancock REW.** 2011. Creeping baselines and adaptive resistance to antibiotics. Drug Resist. Updat. **14**:1–21.
7. **Filloux A, Bally M, Soscia C, Murgier M, Lazdunski A.** 1988. Phosphate regulation in *Pseudomonas aeruginosa*: cloning of the alkaline phosphatase gene and identification of *phoB*- and *phoR*-like genes. Mol. Gen. Genet. **212**:510–513.
8. **Frisk A, et al.** 2004. Transcriptome analysis of *Pseudomonas aeruginosa* after interaction with human airway epithelial cells. Infect. Immun. **72**: 5433–5438.
9. **Gooderham WJ, et al.** 2009. The sensor kinase PhoQ mediates virulence in *Pseudomonas aeruginosa*. Microbiology **155**:699–711.
10. **Haddad A, Jensen V, Becker T, Häussler S.** 2009. The Pho regulon influences biofilm formation and type three secretion in *Pseudomonas aeruginosa*. Environ. Microbiol. Rep. **1**:488–494.
11. **Hancock REW, Poole K, Benz R.** 1982. Outer membrane protein P of *Pseudomonas aeruginosa*: regulation by phosphate deficiency and formation of small anion-specific channels in lipid bilayer membranes. J. Bacteriol. **150**:730–738.
12. **Høiby N, Ciofu O, Bjarnsholt T.** 2010. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future Microbiol. **5**:1663–1674.
13. **Hsieh YJ, Wanner BL.** 2010. Global regulation by the seven-component Pi signaling system. Curr. Opin. Microbiol. **13**:198–203.
14. **Jacobs MA, et al.** 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A. **100**:14339–14344.
15. **Jensen V, et al.** 2006. RhlR expression in *Pseudomonas aeruginosa* is modulated by the *Pseudomonas* quinolone signal via PhoB-dependent and -independent pathways. J. Bacteriol. **188**:8601–8606.
16. **Lewenza S, et al.** 2005. Construction of a mini-Tn5-luxCDABE mutant library in *Pseudomonas aeruginosa* PAO1: a tool for identifying differentially regulated genes. Genome Res. **15**:583–589.
17. **Long J, Zaborina O, Holbrook C, Zaborin A, Alverdy J.** 2008. Depletion of intestinal phosphate after operative injury activates the virulence of *Pseudomonas aeruginosa* causing lethal gut-derived sepsis. Surgery **144**: 189–197.
18. **Lyczak JB, Cannon CL, Pier GB.** 2000. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microbes Infect. **2**:1051–1060.
19. **McPhee JB, et al.** 2006. Contribution of the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems to Mg^{2+}-induced gene regulation in *Pseudomonas aeruginosa*. J. Bacteriol. **188**:3995–4006.
20. **Mougous JD, et al.** 2006. A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. Science **312**:1526–1530.
21. **Mulcahy H, Charron-Mazenod L, Lewenza S.** 2010. *Pseudomonas aeruginosa* produces an extracellular deoxyribonuclease that is required for utilization of DNA as a nutrient source. Environ. Microbiol. **12**:1621–1629.
22. **Ochsner UA, Wilderman PJ, Vasil AI, Vasil ML.** 2002. GeneChip expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: identification of novel pyoverdine biosynthesis genes. Mol. Microbiol. **45**:1277–1287.
23. **Overhage J, Bains M, Brazas MD, Hancock REW.** 2008. Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. J. Bacteriol. **190**: 2671–2679.
24. **Potvin E, et al.** 2003. In vivo functional genomics of *Pseudomonas aeruginosa* for high-throughput screening of new virulence factors and antibacterial targets. Environ. Microbiol. **5**:1294–1308.
25. **Rahme LG, et al.** 1995. Common virulence factors for bacterial pathogenicity in plants and animals. Science **268**:1899–1902.
26. **Rowe SM, Miller S, Sorscher EJ.** 2005. Cystic fibrosis. N. Engl. J. Med. **352**:1992–2001.
27. **Rüberg S, Pühler A, Becker A.** 1999. Biosynthesis of the exopolysaccharide galactoglucan in *Sinorhizobium meliloti* is subject to a complex control by the phosphate-dependent regulator PhoB and the proteins ExpG and MucR. Microbiology **145**:603–611.
28. **Slater H, Crow M, Everson L, Salmond GP.** 2003. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in

Serratia via both quorum-sensing-dependent and -independent pathways. Mol. Microbiol. **47**:303–320.
29. **Stover CK, et al.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature **406**:959–964.
30. **von Krüger WM, et al.** 2006. The phosphate-starvation response in *Vibrio cholerae* O1 and *phoB* mutant under proteomic analysis: disclosing functions involved in adaptation, survival and virulence. Proteomics **6**:1495–1511.
31. **Winans SC.** 1990. Transcriptional induction of an *Agrobacterium* regulatory gene at tandem promoters by plant-released phenolic compounds, phosphate starvation, and acidic growth media. J. Bacteriol. **172**:2433–2438.
32. **Wu H, et al.** 2000. Identification and characterization of two chemotactic transducers for inorganic phosphate in *Pseudomonas aeruginosa*. J. Bacteriol. **182**:3400–3404.
33. **Zaborin A, et al.** 2009. Red death in *Caenorhabditis elegans* caused by *Pseudomonas aeruginosa* PAO1. Proc. Natl. Acad. Sci. U. S. A. **106**:6327–6332.
34. **Zavaleta-Pastor M, et al.** 2010. *Sinorhizobium meliloti* phospholipase C required for lipid remodeling during phosphorus limitation. Proc. Natl. Acad. Sci. U. S. A. **107**:302–307.