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NOTES

Transcriptome Analysis of *Pseudomonas aeruginosa* after Interaction with Human Airway Epithelial Cells

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The transcriptional profile of *Pseudomonas aeruginosa* after interactions with primary normal human airway epithelial cells was determined using Affymetrix GeneChip technology. Gene expression profiles indicated that various genes involved in phosphate acquisition and iron scavenging were differentially regulated.

Interaction of *Pseudomonas aeruginosa* with host cells in vitro has typically been studied using a variety of immortalized mammalian cell lines (1, 7, 23). Although several of these studies have suggested that *P. aeruginosa* is capable of attaching and invading epithelial cells, few have examined the interaction by use of primary normal human airway epithelial (PNHAE) cells. These cells have differentiated structures (mucin and cilia) and have tight junctions, unlike their monolayer-grown counterparts (13). Recently, several studies have suggested that cell polarity and the integrity of tight junctions are important in adherence, as disruption of cell polarity increased *P. aeruginosa* adherence and invasion of epithelial cells (6, 16, 22). Host-pathogen interactions have been examined with use of microarrays to determine the transcriptional profiles of *Salmonella enterica* infecting macrophages (4) and human epithelial cells infected with *P. aeruginosa* (12). Recently, *P. aeruginosa* transcriptional profiling data were reviewed for different environmental conditions (8). Nevertheless, a lack of information exists regarding *P. aeruginosa* gene expression when the organism interacts with human host cells.

***P. aeruginosa* localizes to the basolateral surface of PNHAE cells 12 h postinfection.** PNHAE cells grown on transwell inserts were chosen as an in vitro infection model since these cells have their apical surface exposed to air, become fully differentiated, produce extracellular proteins such as mucin and cilia, and form tight junctions (13). PNHAE cells were obtained from healthy organ donors, processed, and seeded at 2.5×10^5 to 5×10^5 cells/cm^2 with use of collagen-coated Millicell-PCF membrane inserts (0.4-μm pore size, 12-mm diameter) in 24-well plates (Millipore) (13). The cells were cultured and maintained in Dulbecco's modified Eagle's medium–Hanks' F-12 supplement (DMEM–F-12) medium (Invitrogen) containing 0.4% glucose, 13 μM $Fe^{2/3+}$, 2 mM L-glutamine, 15 mM HEPES, and 2% Ultroser G supplement, a serum substitute (Crescent Chemicals Co., Inc., Islandia, N.Y.), at 37°C in a 9% CO_2 humidified atmosphere (13). After 7 to 9 days of culture, the cells were stimulated with 100 ng of keratinocyte growth factor/ml to initiate cell replication and to stimulate differentiation. Differentiation was assessed by visual examination of the cells and measurement of the transepithelial electrical resistance across the cell membrane. Transepithelial electrical resistance values of 500 to 1,000 Ω/cm^2 were routinely observed over the membrane for differentiated cells. *P. aeruginosa* strain PAO1 harboring the green fluorescent protein (GFP) expression plasmid pMRP9-1 (25) was used initially for a time course infection study. The bacteria were cultured in 100 ml of Luria-Bertani broth containing 300 μg of carbenicillin/ml at 37°C and grown to an optical density at 600 nm of 0.4. The bacteria were then washed once and resuspended in the described cell culture medium, without serum supplement, and inoculated to the apical cell surface at a multiplicity of infection of 100 (5×10^7 CFU/ml). At 1, 4, 8, 12, and 16 h postinfection, the infected cells were washed once with cell culture medium, fixed, and then stained with DAPI (4′,6′-diamidino-2-phenylindole) and phalloidin as previously described (26). The GFP-expressing *P. aeruginosa* and the stained eukaryotic cells were subsequently visualized by Zeiss Axioplan II deconvolution microscopy.

P. aeruginosa was located on the apical surface of the cells 4 h after infection (Fig. 1A). The majority of the bacteria remained on the apical surface up to 8 h postinfection (Fig. 1B). However, after 12 h of infection, *P. aeruginosa* localized to the edges of the airway epithelial cells and the majority of bacteria were observed at the basolateral membrane (Fig. 1C). Our 1- and 4-h observations are in agreement with a study that

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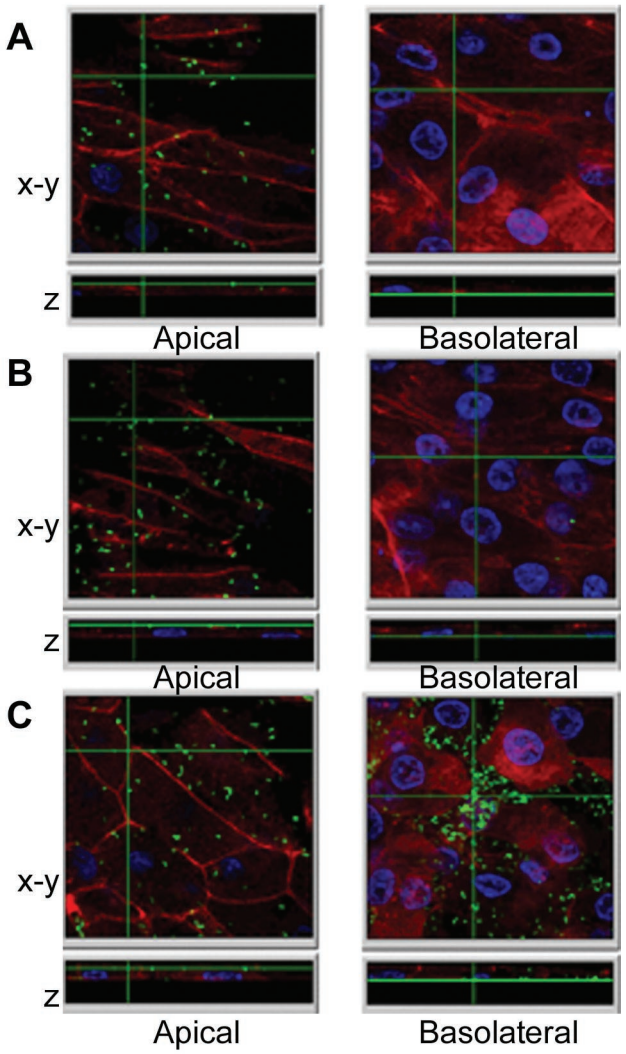

FIG. 1. Interaction of *P. aeruginosa* PAO1-GFP with PNHAE cells for 4, 8, and 12 h (A to C, respectively). Cells were stained with DAPI and phalloidin and visualized using Zeiss Axioplan II deconvolution microscopy.

showed limited adherence and invasion following 3 h of incubation of *P. aeruginosa* with human nasal epithelial cells (6). Plotkowski et al. reported similar results on normal and cystic fibrosis-affected bronchial epithelial cells grown in thick and thin collagen gels (22). Both studies suggested that well-polarized intact epithelia are relatively resistant to *P. aeruginosa* infection. However, our observations at later time points (12 and 16 h) suggest that *P. aeruginosa* was capable of disrupting tight junctions, which may serve as a port of entry for *P. aeruginosa*, facilitating access to the basolateral membrane. The disruption of tight junctions in Calu-3 monolayers and cultured bovine tracheal monolayers has been reported to allow increased *P. aeruginosa* binding and cytotoxicity (16).

Differential *P. aeruginosa* gene expression after 4 h of interaction with PNHAE cells. The 4-h time point was chosen for identification of differentially expressed genes in *P. aeruginosa* PAO1 early in the infection process with use of the Affymetrix GeneChip technology. Three replicates of total RNA were obtained for the control and experimental conditions. Each replicate of the control consisted of total RNA isolated from six wells of *P. aeruginosa* added to the Millicell insert with DMEM–F-12 medium without PNHAE cells. For the experimental condition, each replicate consisted of total RNA pooled from six separate wells of PNHAE cells infected with *P. aeruginosa* PAO1. Since we used total RNA from infected eukaryotic cells, an additional control was employed to eliminate eukaryotic transcripts that hybridize to the *Pseudomonas* GeneChip array. For this control, total RNA was isolated from 18 independent wells of the PNHAE cells alone (RNAs from six wells were pooled for each replicate and hybridized to the *Pseudomonas* Affymetrix GeneChip). For the infection studies, *P. aeruginosa* was grown and added to the PNHAE cells as described above. After washing with DMEM–F-12 medium to remove nonadherent bacteria, total *P. aeruginosa* and/or eukaryotic RNA was isolated by adding lysis buffer (5 mg of lysozyme/ml in Tris-EDTA, 10 mM Tris, pH 8.0) to the Millicell inserts for 5 min and then extracted using the RNeasy Midi kit per the manufacturer's instructions (Qiagen). The RNA was treated with 2 U of DNase I for 15 min at 37°C with the DNase-free kit (Ambion) to remove any contaminating DNA and ethanol precipitated. The quality of the RNA was assessed by size chromatography with an Agilent 2100 Bioanalyzer (Fig. 2). Ten micrograms of total RNA from three replicates of bacteria alone (control 1 [4 h in medium alone], control 2 [12 h in medium alone], and control 3 [cells alone]) and *P. aeruginosa* interacting with eukaryotic cells was used for cDNA synthesis, fragmentation, labeling, and hybridization per the manufacturer's instructions (Affymetrix GeneChip *P. aeruginosa*). cDNA generated from eukaryotic RNA was hybridized to *P. aeruginosa* Genome Arrays in triplicate for identification and subtraction of cross-reacting background signals. Microarray data were generated and analysis was performed using the Affymetrix expression analysis protocol as described previously (17).

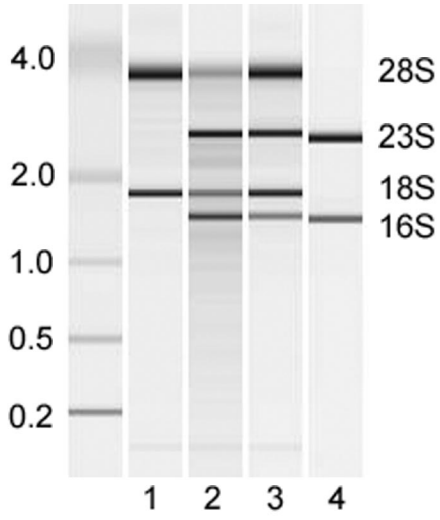

FIG. 2. Agilent chromatogram of RNA used in microarray studies. Representative size chromatographic separation of total RNA from PNHAE cells (lane 1), from *P. aeruginosa* PAO1 and from PNHAE cells (lanes 2 [4 h] and 3 [12 h]), and from *P. aeruginosa* PAO1 (lane 4). The size ladder is shown in kilobases at left.

TABLE 1. Effects of PNHAE cells on *P. aeruginosa* gene expression

Change[a]	No. of genes with changed expression at[b]:					
	4 h			12 h		
	≥4-fold	4- to 10-fold	>10-fold	≥4-fold	4- to 10-fold	>10-fold
Activated	24	24	0	62	40	22
Repressed	17	13	4	59	38	21
Total no.	41	37	4	121	78	43

[a] The direction of gene expression change was determined by comparing transcription in PAO1 (control) with that in PAO1 interacting with PNHAE cells.
[b] Student's *t* test ($P < 0.05$) was used to determine change in expression.

There were 41 differentially expressed *P. aeruginosa* genes after 4 h of infection on PNHAE cells when the transcriptional profiles were compared to those of the bacteria in medium alone (Table 1). Five of the 24 activated genes encode putative proteins involved in membrane transport (PA2019, PA4770 [*lldP*], PA2673, PA1540, and PA2437; Table 2). Additional activated genes encode probable transcriptional regulators (PA0163, PA4203, and PA2877), indicating that they may be important in virulence. The most interesting trend of gene expression observed at the 4-h time point was the repression of eight genes associated with the iron acquisition pyoverdine pathway including *pvdA* (PA2386), *pvdM* (PA2393), *pvdFE-fpvA-pvdD* (PA2396 to PA2399, respectively), *pvdJ* (PA2401), and PA2411 as well as *pcdD* encoding the pyochelin biosynthesis protein PcdD (Table 2). This result may indicate that *P. aeruginosa* is able to acquire ample iron from the eukaryotic cells during the initial stages of infection. Similarly, the *sodM* gene, which is activated only upon iron starvation in *P. aeruginosa* in a quorum-sensing-dependent fashion, was also repressed (11).

Differential *P. aeruginosa* gene expression after 12 h of interaction with PNHAE cells. Analysis of the transcriptional profile of *P. aeruginosa* after 12 h of infection on PNHAE cells revealed that 121 genes were differentially expressed (Table 1). Several genes associated with phosphate acquisition were significantly activated (Table 3). One of these genes is also associated with virulence in *P. aeruginosa*, *plcN* (PA3319), encoding a nonhemolytic phospholipase C protein (3, 14, 31). *P. aeruginosa* has been shown to produce and secrete two phospholipase C enzymes, one hemolytic (PlcH) and one nonhemolytic (PlcN), both dependent on the twin-arginine translocation (Tat) system for their transport across the inner membrane (19, 30), and one phospholipase D (32). The enzyme activity of PlcN has been shown to hydrolyze phosphatidylcholine and phosphatidylserine (21), present in both outer and inner leaflets of eukaryotic erythrocytes, respectively. A possible role for PlcN in *P. aeruginosa* lung infection in vivo may be relevant since phosphatidylcholine is also an abundant constituent in lung surfactant and thus may serve as a substrate for the extracellular enzyme PlcN (34).

Other upregulated phosphate genes identified were *phoA* (PA3296), encoding an alkaline phosphatase protein (5); *oprO* (PA3280), encoding a polyphosphate-specific outer membrane porin (24); and *glpQ*, encoding glycerophosphoryl diester phosphodiesterase (30). GlpQ hydrolyzes deacylated phospholipids to glycerol-3-phosphate, which may facilitate the release of inorganic phosphate for alkaline phosphatases, such as PlcN (30), and/or provide a carbon source for the organism. A limited availability of phosphate during the interaction of *P. aeruginosa* with the PNHAE cells is likely an explanation for

TABLE 2. Activated and repressed genes in *P. aeruginosa* following 4 h of interaction with PNHAE cells

PA no.	Fold change[a]	*P* value[b]	Protein description and/or interspecies homology
PA4826	9.4	0.044	Hypothetical
PA1885	8.6	0.019	Conserved hypothetical
PA2019	8.5	0.050	RND[c] multidrug efflux membrane fusion protein precursor
PA4770	8.2	0.027	LldP, L-lactate permease
PA5412	7.5	0.005	Hypothetical
PA0805	7.0	0.035	Hypothetical
PA1540	6.7	0.013	Conserved hypothetical
PA0674	6.3	0.001	Hypothetical
PA0142	6.2	0.024	Hypothetical
PA0163	5.8	0.006	Probable transcriptional regulator
PA2673	5.5	0.010	Probable type II secretion system protein
PA0665	5.3	0.037	Conserved hypothetical
PA1602	5.2	0.047	Probable oxidoreductase
PA2437	4.9	0.044	Hypothetical
PA1360	4.9	0.003	Conserved hypothetical
PA4498	4.8	0.027	Probable metallopeptidase
PA5530	4.5	0.045	Probable MFS[d] dicarboxylate transporter
PA4203	4.4	<0.001	Probable transcriptional regulator
PA2886	4.4	0.005	Hypothetical
PA2278	4.3	0.002	ArsB protein
PA2877	4.3	0.020	Probable transcriptional regulator
PA3375	4.2	0.038	Probable ATP-binding component of ABC transporter
PA1592	4.0	0.003	Hypothetical
PA1237	4.0	0.041	Probable multidrug resistance efflux pump
PA2398	−25.6	0.005	FpvA, ferripyoverdine receptor
PA2397	−24.4	0.004	PvdE, pyoverdine biosynthesis protein
PA2386	−18.0	0.023	PvdA, L-ornithine N_5-oxygenase
PA2393	−14.3	0.049	PvdM, probable dipeptidase precursor
PA2399	−7.5	0.026	PvdD pyoverdine synthetase D
PA0586	−7.2	0.038	Conserved hypothetical
PA4228	−7.1	0.018	PcdD, pyochelin biosynthesis protein
PA0519	−5.1	0.022	NirS, nitrite reductase precursor
PA1245	−5.0	0.002	Hypothetical
PA2411	−4.8	<0.001	Probable thioesterase
PA1244	−4.8	<0.001	Hypothetical
PA2401	−4.6	0.027	PvdJ, probable nonribosomal peptide synthetase
PA2812	−4.6	0.036	Probable ATP-binding component of ABC transporter
PA2396	−4.5	0.023	PvdF, hypothetical
PA3919	−4.2	0.029	Conserved hypothetical
PA0511	−4.2	0.030	NirJ, heme d1 biosynthesis protein
PA5436	−4.0	0.048	Probable biotin carboxylase subunit of a transcarboxylase

[a] The magnitude of gene expression (fold change) was determined by comparing transcription in three replicates of PAO1 (control) with that in three replicates of PAO1 interacting with PNHAE cells after 4 h. Total RNA from the PNHAE cells alone (three replicates) was isolated, reverse transcribed into cDNA, and hybridized to the *Pseudomonas* GeneChip. The genes present from this experiment were subtracted from the analysis.
[b] *P* values were assessed by Student's *t* test with Data Mining Tools (Affymetrix).
[c] RND, resistance-nodulation-division.
[d] MFS, major facilitator superfamily.

TABLE 3. Activated and repressed genes in *P. aeruginosa* following 12 h of interaction with PNHAE cells

PA no.	Fold change[a]	*P* value[b]	Protein description and/or interspecies homology
PA3910	26.2	0.010	Hypothetical, alkaline phosphatase *tat* signal sequence domain, 41% similar to phosphodiesterase-alkaline phosphatase D (*B. subtilis*)
PA2635	25.5	0.012	Hypothetical, phosphatase
PA3909	24.9	0.004	Hypothetical, extracellular nuclease
PA0347	24.6	0.012	GlpQ, glycerophosphoryl diester phosphodiesterase, periplasmic
PA3280	21.9	0.002	OprO, outer membrane porin precursor
PA0688	21.4	0.003	Hypothetical, 100% identical to PhoA
PA3229	20.5	0.003	Hypothetical
PA0691	19.0	0.023	Hypothetical, transposase
PA4351	18.9	0.006	Probable acyltransferase
PA0693	18.4	0.033	ExbB2, transport protein
PA4350	17.1	0.024	Conserved hypothetical, potential hemolysin
PA3319	16.4	0.023	PlcN, nonhemolytic phospholipase C precursor
PA2494	15.0	0.002	MexF, RND[c] multidrug efflux transporter
PA3296	14.3	0.008	PhoA, alkaline phosphatase
PA3383	13.5	0.014	Binding protein component of ABC phosphonate transporter
PA2491	13.3	0.007	Probable oxidoreductase
PA2493	12.9	0.003	MexE, RND multidrug efflux membrane fusion protein
PA0697	11.8	<0.001	Hypothetical, colicin, adhesion, M protein
PA1195	11.3	0.004	Hypothetical
PA5180	10.7	0.023	Conserved hypothetical
PA2495	10.7	0.004	OprN, outer membrane protein
PA3219	10.6	0.005	Hypothetical
PA4811	9.8	0.001	FdnH, nitrate-inducible formate dehydrogenase
PA4881	9.6	0.004	Hypothetical
PA3382	9.6	0.034	PhnE, phosphonate transport protein
PA0696	9.0	0.030	Hypothetical, outer membrane receptor, Fe
PA2882	8.9	0.018	Probable two-component sensor
PA3368	8.6	0.015	Probable acetyltransferase
PA0674	8.2	0.029	53% similar to ferripyoverdine receptor, PigC
PA4623	7.8	0.015	Hypothetical
PA2881	7.7	0.020	Probable two-component response regulator
PA2331	7.4	0.018	49% similar to macrophage infectivity potentiator
PA4221	−85.6	0.003	FptA, Fe(III)-pyochelin receptor precursor
PA4225	−44.6	0.021	PchF, pyochelin synthetase
PA4231	−39.7	0.006	PchA, salicylate biosynthesis isochorismate synthase
PA4220	−32.0	0.002	Hypothetical
PA4218	−31.3	0.009	Probable transporter
PA4226	−29.2	0.004	PchE, dihydroaeruginoic acid synthetase
PA4230	−29.0	0.003	PchB, salicylate biosynthesis protein
PA4228	−29.0	0.017	PchD, pyochelin biosynthesis protein
PA4156	−28.4	0.002	Probable TonB-dependent receptor
PA4229	−18.6	0.017	PchC, pyochelin biosynthetic protein
PA4224	−18.0	0.003	Hypothetical
PA4219	−16.8	0.005	Hypothetical
PA0929	−14.7	0.017	Two-component response regulator
PA4470	−12.9	0.028	FumC1, fumarate hydratase
PA2034	−12.5	0.026	Hypothetical
PA3600	−12.4	0.001	Conserved hypothetical
PA3899	−11.1	0.022	Probable sigma-70 factor, ECF[d] subfamily
PA4222	−11.0	0.005	Probable ATP-binding component of ABC transporter
PA4155	−10.3	0.003	Hypothetical
PA3900	−10.3	0.003	Probable transmembrane sensor
PA0471	−10.0	0.001	Probable transmembrane sensor
PA4468	−9.7	0.023	SodM, superoxide dismutase
PA2426	−9.1	0.029	PvdS, sigma factor
PA4227	−9.0	0.011	PchR, transcriptional regulator, pyochelin biosynthesis
PA4896	−8.9	0.026	Probable sigma-70 factor, ECF subfamily
PA3432	−8.5	0.013	Hypothetical
PA4469	−8.3	0.024	Hypothetical
PA0672	−8.1	<0.001	Hypothetical
PA4471	−7.8	0.023	Hypothetical
PA4223	−7.7	<0.001	Probable ATP-binding component of ABC transporter

[a] The magnitude of gene expression (fold change) was determined by comparing transcription in three replicates of PAO1 (control) with that in three replicates of PAO1 interacting with PNHAE cells after 12 h. Total RNA from the PNHAE cells alone (three replicates) was isolated, reverse transcribed into cDNA, and hybridized to the *Pseudomonas* GeneChip. The genes present from this experiment were subtracted from the analysis.

[b] *P* values were assessed by Student's *t* test with Data Mining Tools (Affymetrix).

[c] RND, resistance-nodulation-division.

[d] ECF, extracytoplasmic sigma factor.

TABLE 4. Comparison of previously reported iron-regulated genes and genes repressed in *P. aeruginosa* after 4 and 12 h of infection on PNHAE cells

Time of repression and PA no.	Gene	Fold change[a]	Data for known iron-regulated genes[b]	
			Gene or PA no.	Fold change
4 h				
PA1245		−5.0	*aprDEF*	12
PA2386	*pvdA*	−18.0	*pvdA*	216
PA2393	*pvdM*	−14.3	PA2393–PA2395	38
PA2396	*pvdF*	−4.5	*pvdF*	45
PA2397	*pvdE*	−24.4	*pvdE*	33
PA2398	*fpvA*	−25.6	*fpvA*	35
PA2399	*pvdD*	−7.5	*pvdD*	19
PA2401	*pvdJ*	−4.6	*pvdJI*	30
PA2411		−4.8	PA2411–PA2412	126
PA4228	*pchD*	−7.1	*pchDCBA*	124
12 h				
PA0471		−10.0	PA0471	46
PA0472		−5.8	PA0472	46
PA0672	*hemO*	−8.1	*hemO*	138
PA0929	*pirR*	−14.7	*pirR*	6
PA1300		−6.9	PA1300–PA1301	46
PA1317	*cyoA*	−5.3	PA1317–PA1321	4
PA1319	*cyoC*	−4.1	PA1317–PA1321	4
PA2033		−7.7	PA2033–PA2034	95
PA2034		−12.5	PA2033–PA2034	95
PA2426	*pvdS*	−9.1	*pvdS*	177
PA2452		−6.1	PA2452–PA2453	52
PA2468		−7.7	PA2468–PA2467	30
PA3899		−11.1	PA3899–PA3900	13
PA3900		−10.3	PA3899–PA3900	13
PA4218		−31.3	PA4218–PA4219	49
PA4219		−16.8	PA4218–PA4219	49
PA4220		−32.0	*fptA*, PA4220	182
PA4221	*fptA*	−85.6	*fptA*, PA4220	182
PA4222		−11.0	*pchEFG*	55
PA4223		−7.7	*pchEFG*	55
PA4224	*pchG*	−18.0	*pchEFG*	55
PA4225	*pchF*	−44.6	*pchEFG*	55
PA4226	*pchE*	−29.2	*pchEFG*	55
PA4227	*pchR*	−9.0	*pchR*	37
PA4228	*pchD*	−29.0	*pchDCBA*	124
PA4229	*pchC*	−18.6	*pchDCBA*	124
PA4230	*pchB*	−29.0	*pchDCBA*	124
PA4231	*pchA*	−39.7	*pchDCBA*	124
PA4467		−4.8	*fumC-sodA*	119
PA4468	*sodM*	−9.7	*fumC-sodA*	119
PA4469		−8.3	*fumC-sodA*	119
PA4470	*fumC1*	−12.9	*fumC-sodA*	119
PA4471		−7.8	*fumC-sodA*	119
PA4570		−7.2		403
PA4708	*phuS*	−5.0	*phuST*	12
PA4709	*phuT*	−5.8	*phuST*	12
PA4710	*phuR*	−5.2	*phuR*	7
PA4896		−8.9		20

[a] The magnitude of gene expression (fold change) of iron-regulated genes after interaction of *P. aeruginosa* PAO1 with PNHAE cells after 4 and 12 h.
[b] Iron-regulated genes as reported by Ochsner et al. (20).

the increased transcription of these genes. In addition, *phoA*, *oprO*, *plcN*, and *glpQ* are induced by phosphate starvation (5, 21, 24, 30). Our study also identified uncharacterized genes that may be associated with phosphate acquisition as determined by homology to genes in other organisms and in *P. aeruginosa*. These include PA3910 (41% homology to phosphodiesterase-alkaline phosphatase), PA2635 (probable phosphatase), PA0688 (100% identical to PhoA), PA3909 (probable extracellular nuclease), PA4350 (potential hemolysin), and PA2331 (49% homology to macrophage infectivity protein). These data indicate that genes involved in acquisition and metabolism of phosphate may be important for *P. aeruginosa* infection of host cells and survival.

After 12 h of infection on the PNHAE cells, *P. aeruginosa* repressed the transcription of 30 genes more than sevenfold (Table 3). Surprisingly, 14 of these genes (PA4218 to PA4231) are associated with the siderophore-mediated iron acquisition pyochelin pathway. Moreover, 24 other genes that are responsive to iron (20) were repressed at this time point (Table 4). In fact, the number of repressed iron-regulated genes increased over time (10 genes at 4 h and 38 genes at 12 h [with use of fourfold change as a cutoff]), correlating with probable increased airway epithelial cell damage (15). One explanation for the observed repression of these iron-regulated genes may be that iron is made available during interaction with the PNHAE cells. The increase in the number of repressed iron-associated genes supports this possibility. The iron concentration of the medium used in this study was fairly low, approximately 13 μM. However, the effects of these iron levels were likely not observed, as the medium was identical in the control and experimental conditions. In addition, this concentration of iron in minimal medium has been shown to induce, not repress, iron-responsive genes (20). It is well documented that the pyoverdine, pyochelin, and iron-regulated genes are important during *P. aeruginosa* murine lung infection, and this is in stark contrast to our observations of repression of these genes (2, 9, 10, 15, 18, 27–29, 33). *P. aeruginosa* during in vivo infection is likely subjected to the normal clearance mechanisms of cells such as macrophages and polymorphonuclear leukocytes, cells that are absent in our epithelial cell model. However, in the study conducted by Takase et al. (27), the siderophores were not required for lung infection of immunosuppressed mice. These authors suggest that non-siderophore-mediated iron acquisition, such as heme uptake, may play an important role in *P. aeruginosa* infections. We did not observe a change in expression of non-siderophore-mediated iron acquisition genes in our infection model.

The present work evaluated the transcriptional profiles of *P. aeruginosa* after 4- and 12-h interactions with PNHAE cells in vitro. Global expression analysis revealed activation of phosphate and repression of iron acquisition genes. The number of genes showing these trends increased over time, suggesting that *P. aeruginosa* may be able to acquire ample iron but not phosphate for growth from the epithelial cells during infection. Further studies are warranted to explore the role of the genes involved in phosphate acquisition during epithelial cell interaction.

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