JOURNAL OF BACTERIOLOGY, Apr. 2003, p. 2066–2079 Vol. 185, No. 7
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.7.2066–2079.2003

Identification, Timing, and Signal Specificity of *Pseudomonas aeruginosa* Quorum-Controlled Genes: a Transcriptome Analysis†

Martin Schuster,[1] C. Phoebe Lostroh,[1] Tomoo Ogi,[2] and E. P. Greenberg[1]*

Department of Microbiology and W. M. Keck Microbial Communities and Cell Signaling Program, University of Iowa, Iowa City, Iowa 52242,[1] and *Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom*[2]

Received 30 August 2002/Accepted 5 December 2002

There are two interrelated acyl-homoserine lactone quorum-sensing-signaling systems in *Pseudomonas aeruginosa*. These systems, the LasR-LasI system and the RhlR-RhlI system, are global regulators of gene expression. We performed a transcriptome analysis to identify quorum-sensing-controlled genes and to better understand quorum-sensing control of *P. aeruginosa* gene expression. We compared gene expression in a LasI-RhlI signal mutant grown with added signals to gene expression without added signals, and we compared a LasR-RhlR signal receptor mutant to its parent. In all, we identified 315 quorum-induced and 38 quorum-repressed genes, representing about 6% of the *P. aeruginosa* genome. The quorum-repressed genes were activated in the stationary phase in quorum-sensing mutants but were not activated in the parent strain. The analysis of quorum-induced genes suggests that the signal specificities are on a continuum and that the timing of gene expression is on a continuum (some genes are induced early in growth, most genes are induced at the transition from the logarithmic phase to the stationary phase, and some genes are induced during the stationary phase). In general, timing was not related to signal concentration. We suggest that the level of the signal receptor, LasR, is a critical trigger for quorum-activated gene expression. Acyl-homoserine lactone quorum sensing appears to be a system that allows ordered expression of hundreds of genes during *P. aeruginosa* growth in culture.

Pseudomonas aeruginosa is an opportunistic pathogen of humans, other animals, plants, and lower eukaryotes (16). There are two acyl-homoserine lactone (acyl-HSL) quorum-sensing systems in *P. aerugionosa*, LasI-LasR and RhlI-RhlR. LasI is responsible for the synthesis of *N*-3-oxododecanoyl-HSL (3OC12-HSL), and LasR is a 3OC12-HSL-responsive transcription factor. RhlI is responsible for the synthesis of *N*-butanoyl-HSL (C4-HSL), and RhlR is a C4-HSL-responsive transcription factor. Both of the acyl-HSLs can diffuse through the cell envelope, so a critical cell population density is required to produce signals at levels sufficient for quorum-controlled gene regulation. Acyl-HSL signaling is an important virulence factor in *P. aeruginosa* (for recent reviews see references 9, 19, and 31).

Previously, Whiteley et al. identified quorum-controlled genes in *P. aeruginosa* by screening a library of random Tn*5-lacZ* insertions in the genome of an acyl-HSL synthesis mutant for induction of β-galactosidase by signal addition (33); 35 quorum-controlled genes were identified. Based on the number of mutants screened and the number of insertions in putative operons, it was estimated that there were over 200 additional quorum-controlled genes. The *lacZ* induction patterns were grouped into four categories depending on the timing of induction and the signal response specificity. Some genes responded to addition of acyl-HSL signals early in culture growth, and others showed a substantial delay, responding to signals only in the stationary phase. Some genes responded specifically to 3OC12-HSL, and others required both signals for the maximal response. The requirement for both signals to obtain a maximal response is thought to be related to the fact that both *rhlR* and *rhlI* are induced by LasR-LasI (14, 22). We have studied several quorum-controlled promoters. Some of the promoters show a high level of specificity for 3OC12-HSL and LasR, while others show specificity for C4-HSL and RhlR but also show a substantial response to 3OC12-HSL and LasR (32).

Other investigators have presented evidence showing that additional genes are controlled by quorum sensing in *P. aeruginosa* (2, 3, 5, 10–13, 15, 18, 21, 26, 27, 29, 35, 36). Furthermore, several reports have shown that a variety of regulatory proteins can influence the timing of quorum-controlled gene expression (1, 4, 6, 24, 26, 34), but mechanistic details for these proteins are scarce.

Here we describe a transcriptome analysis in which we utilized Affymetrix GeneChip genome arrays to test the hypothesis that there are over 200 quorum-controlled genes in *P. aeruginosa*, to identify as many members of this putative regulon as possible, and to gain insight into the timing and specificity of quorum-controlled gene expression.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *P. aeruginosa* strains used were PAO-MW1 (*rhlI*::Tn*501 lasI*::*tetA*) (33) and PAO *lasR rhlR* (Δ*lasR*::Tc^r Δ*rhlR*::Gm^r), as well as the isogenic PAO1 parent strain (25). Bacteria were grown in buffered Luria-Bertani broth, which contained 10 g of typtone (Difco) per liter, 5 g of yeast extract (Difco) per liter, 5 g of NaCl per liter, and 50 mM 3-(*N*-morpholino)propanesulfonic acid (pH 7.0). Synthetic acyl-HSLs (Aurora Biosciences) were added to PAO-MW1 cultures at final concentrations of 2 μM for 3OC12-HSL and 10 μM for C4-HSL, as indicated below. To inoculate the cultures used for transcript profiling, cells grown to the mid-logarithmic phase were added to 100 ml of prewarmed medium in 500-ml culture flasks. The initial

* Corresponding author. Mailing address: 540 EMRB, Newton Road, Roy and Lucille Carver College of Medicine, University of Iowa, Iowa City, IA 52242. Phone: (319) 335-7775. Fax: (319) 335-7949. E-mail: Everett-greenberg@uiowa.edu.

† For a commentary on this article, see page 2061 in this issue.

optical densities at 600 nm (OD_{600}) were 0.05 for PAO-MW1 and 0.01 for PAO1 and PAO *lasR rhlR*. Cultures were incubated at 37°C in a rotating shaker at 250 rpm. Growth was monitored by determining the OD_{600}.

Expression profiling experiments. For studies with the signal generation mutant we isolated RNA from cultures at the following optical densities: 0.2, 0.4, 0.8, 1.4, 2.0, 3.0, and 4.0. For studies with the signal receptor mutant and its parent we isolated RNA from cultures at optical densities of 0.05, 0.1, 0.2, 0.4, 0.8, 1.4, 2.0, 3.0, and 4.0. Between 1×10^9 and 2×10^9 cells were mixed with RNA Protect Bacteria reagent (Qiagen) and treated as recommended by the manufacturer's mechanical disruption and lysis protocol. RNA was purified by using RNeasy mini columns (Qiagen), including the on-column DNase I digestion described by the manufacturer. In addition, we treated the eluted RNA for 1 h at 37°C with DNase I (0.1 U per μg of RNA). DNase I was removed by using DNA-Free (Ambion) or by RNeasy column purification. RNA integrity was monitored by agarose gel electrophoresis of glyoxylated samples.

Further sample preparation and processing of the *P. aeruginosa* GeneChip genome arrays were done as described by the manufacturer (Affymetrix), with minor modifications suggested by M. Wolfgang (Harvard University). For cDNA synthesis we used 12 μg of purified RNA, semirandom hexamer primers with an average G+C content of 75%, and Superscript II reverse transcriptase (Life Technologies). Control RNAs from yeast, *Arabidopsis*, and *Bacillus subtilis* genes (provided by S. Lory) were added to the reaction mixtures to monitor assay performance. Probes for these transcripts are tiled on the GeneChip arrays. RNA was removed from the PCR mixtures by alkaline hydrolysis. The cDNA synthesis products were purified and fragmented by brief incubation with DNase I, and the 3′ termini of the fragmentation products were labeled with biotin-ddUTP. Fragmented and labeled cDNA was hybridized to an array by overnight incubation at 50°C. Washing, staining, and scanning of microarrays were performed with an Affymetrix fluidic station.

Analysis of expression profiling experiments. We used the Affymetrix Microarray Software suite (MAS) (version 5.0) to determine transcript levels and whether there were differences in transcript levels when different samples were compared. Affymetrix scaling was used to normalize data from different arrays. A scale factor is derived from the mean signal of all of the probe sets on an array and a user-defined target signal. The signal from each individual probe set is multiplied by this scale factor. For any given array between 18 and 28% of the mRNAs were considered absent by MAS, indicating that the corresponding genes were not expressed at levels above background levels. Furthermore, the average changes in control transcript intensities were less than twofold for any comparison of array data. This indicates that the efficiency of cDNA synthesis and labeling was similar from sample to sample.

For comparison analyses, the $\log_2$ ratio for absolute transcript signals obtained from a given pair of arrays was calculated by using MAS. A statistical algorithm of the software also assigned a change call for each transcript pair, which indicated whether the level of a transcript was significantly increased, decreased, or not changed compared to the level for the baseline sample. The baseline samples were those derived from cultures of PAO-MW1 without added acyl-HSL and from cultures of PAO *lasR rhlR*. Graphical analysis of the signal log ratios from each experiment (any pair of arrays) revealed a normal distribution with a mean very close to zero (no change). Among the transcripts with significant increases or decreases compared to the baseline in one or more samples, we subjected those that showed a ≥2.5-fold change to further analysis.

For cluster analyses and transcript pattern analyses we used GeneSpring software (Silicon Genetics, Redwood City, Calif.). The fold change values for each gene were normalized independently by defining the half-maximal value for the gene as 1 and representing all other values as a ratio of that value. This scaling procedure allowed direct visual comparison of gene expression patterns within an experiment, as well as between experiments. GeneSpring was also used to sort genes according to the *P. aeruginosa* genome project (http://www.pseudomonas.com).

Identification of *las-rhl* box-like sequences. A 20-bp consensus sequence (ACCTGCCAGATCTGGCAGGT) was derived from the following previously identified *las-rhl* box-like sequences in quorum-sensing-controlled genes: PA1869 (qsc117), PA1896 (qsc102), *hcnA*, *lasB*, *lasI*, and *phzA* (32), as well as PA2592 (qsc104), PA3327 (qsc126), PA4217 (qsc132), *rhlA*, and *rhlI* (33). To search the entire *P. aeruginosa* genome for sequences similar to this consensus, we developed a computer program based on the program used previously to search for LexA binding sites (8). The scoring matrix of the program is based on a heterology index (HI), which determines the degree of divergence of any 20-nucleotide sequence from the consensus *las-rhl* box sequence. Sequences in a region from 400 bp upstream to 50 bp downstream of annotated translational start sites were considered potential *las-rhl* boxes if they showed an HI of less than 13.

RESULTS

Genes induced by addition of acyl-HSL signals to the *P. aeruginosa* signal generation mutant: validation of the microarray analysis. Previously, the effects of acyl-HSLs on chromosomal *lacZ* insertions in a quorum-sensing signal generation mutant (MW1, a *lasI rhlI* mutant) were studied (33), and 39 loci that responded to the addition of 3OC12-HSL and C4-HSL were identified. These loci correspond to 35 different genes in the previously published annotation of the *P. aeruginosa* genome (28). Two insertions originally thought to reside in two adjacent genes (qsc109 and qsc110) are in a single predicted gene (PA2402), and three insertions (qsc114, qsc127, and qsc136) were oriented in a direction opposite that of the predicted open reading frames.

To validate the microarray approach, we grew the signal generation mutant with or without 3OC12-HSL and C4-HSL under conditions identical to those used in the previous study (identical medium, growth temperature, acyl-HSL concentrations, etc.) and examined whether the genes identified previously responded to signal addition in a transcriptome analysis. Most genes in the *P. aeruginosa* genome showed no significant response. Among 638 genes that showed a maximal response to acyl-HSL addition of at least 2.5-fold, 29 of the 35 previously described qsc genes (33) were identified (Table 1). The six remaining genes all exhibited relatively low induction levels in the previous study (33). Four of these six genes, PA2385 (qsc112), PA2401 (qsc111), PA2402 (qsc109-110), and PA2426 (qsc108), showed a significant response to signal addition in our analysis, but the response was less than 2.5-fold. Two genes, PA0051 (qsc137) and PA4084 (qsc113), showed no response. Taken together, our results showed quite good agreement with the results of the previous study (33).

Some of the genes identified in the previous study have also been determined by other workers to be quorum controlled (for example, *hcnABC*) (23). There are genes other than those described in the previous study that have been reported to be quorum controlled. Most of these genes (for example, *lasA* [20, 29] and *rsaL* [5]) were confirmed by our transcriptome study. A few were not confirmed, including *toxA* (11) and *sodA* (13). It is not possible to draw conclusions about the genes that were not confirmed by the transcriptome analysis. The experimental conditions and strains that were used previously were different from those that we used.

Quorum-activated regulon. To identify a larger group of genes in the quorum-sensing regulon of *P. aeruginosa*, we used the results of the experiment described above, and we performed an additional independent experiment in which we compared transcripts in a quorum-sensing signal receptor double mutant to transcripts in the parent strain. This was an independent procedure to assess whether genes are controlled by quorum sensing. We reasoned that genes showing differential regulation with both approaches (addition of signals to a signal generation mutant and a parent compared to a signal receptor mutant) were likely influenced by quorum sensing. The wild-type *P. aeruginosa* strain, the signal receptor mutant, and the signal generation mutant grown with and without added acyl-HSL signals showed similar growth patterns under the conditions of our experiments (Fig. 1).

As mentioned above, we identified 638 genes that were in-

TABLE 1. Quorum-activated genes

Gene no.[a]	Description[b]	Maximum change[c]		
		lasI rhlI mutant		Wild type vs *lasR rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA0007	Hypothetical protein	4.4	5.7 (2.0)	14 (1.4)
PA0026	Hypothetical protein[d]	4.4	4.4 (1.4)	5.9 (0.2)
PA0027	Hypothetical protein	3.8	4.9 (0.8)	5.7 (0.2)
PA0028	Hypothetical protein	5.8	7.5 (1.4)	8.2 (1.4)
PA0050	Hypothetical protein	2.8	2.5 (2.0)	3.0 (1.4)
PA0052	Hypothetical protein[d]	4.7	8.3 (1.4)	22 (2.0)
PA0059	*osmC*, osmotically inducible protein OsmC	2.5	6.7 (2.0)	8.9 (2.0)
PA0105	*coxB*, cytochrome *c* oxidase subunit II	3.4	4.0 (2.0)	2.6 (2.0)
PA0106	*coxA*, cytochrome *c* oxidase subunit I	4.2	4.8 (1.4)	3.3 (1.4)
PA0107	Conserved hypothetical protein	4.1	4.8 (2.0)	4.9 (2.0)
PA0108	*coIII*, cytochrome *c* oxidase subunit III	3.0	3.6 (2.0)	2.8 (2.0)
PA0109	**qsc115**, hypothetical protein	2.1	3.5 (1.4)	4.1 (1.4)
PA0122	Conserved hypothetical protein[e]	13	36 (1.4)	51 (1.4)
PA0132	Beta-alanine-pyruvate transaminase	1.6	3.1 (1.4)	4.1 (2.0)
PA0143	Probable nucleoside hydrolase	4.7	4.7 (0.4)	5.4 (0.1)
PA0144	Hypothetical protein	1.5	19 (2.0)	28 (2.0)
PA0158	Probable RND efflux transporter	2.6	2.6 (2.0)	2.6 (2.0)
PA0175	Probable chemotaxis protein methyltransferase	2.0	2.6 (3.0)	4.6 (1.4)
PA0176	Probable chemotaxis transducer	2.1	2.6 (3.0)	3.9 (1.4)
PA0179	Probable two-component response regulator	2.7	2.8 (1.4)	3.7 (1.4)
PA0198	*exbBI*, transport protein ExbB	7.3	10 (4.0)	3.7 (4.0)
PA0263	*hcpC*, secreted protein Hcp	1.7	8.9 (1.4)	9.4 (1.4)
PA0355	*pfpI*, protease PfpI	2.3	4.8 (2.0)	8.1 (2.0)
PA0364	Probable oxidoreductase	2.9	3.1 (2.0)	3.0 (2.0)
PA0365	Hypothetical protein	2.0	2.5 (2.0)	2.7 (2.0)
PA0366	Probable aldehyde dehydrogenase	2.4	2.8 (2.0)	2.5 (3.0)
PA0534	Conserved hypothetical protein	1.5	2.9 (4.0)	9.8 (2.0)
PA0567	Conserved hypothetical protein	6.9	15 (2.0)	11 (2.0)
PA0572	Hypothetical protein	19	22 (0.4)	19 (0.05)
PA0586	Conserved hypothetical protein	2.1	2.6 (1.4)	4.6 (1.4)
PA0852	**qsc129**, *cpbD*, chitin-binding protein CbpD precursor[d]	11	43 (0.4)	94 (0.1)
PA0855	**qsc116**, hypothetical protein	2.4	2.5 (0.8)	3.0 (0.8)
PA0996	Probable coenzyme A ligase[e]	220	90 (0.8)	42 (0.2)
PA0997	Hypothetical protein	110	96 (0.8)	200 (0.05)
PA0998	Hypothetical protein	68	40 (0.4)	200 (0.2)
PA0999	*fabHI*, 3-oxoacyl-(acyl carrier protein) synthase III	37	25 (0.8)	45 (0.2)
PA1000	Hypothetical protein	22	12 (0.8)	44 (0.2)
PA1001	***phnA,*** anthranilate synthase component I	39	23 (0.8)	290 (0.2)
PA1002	***phnB,*** anthranilate synthase component II	17	13 (1.4)	28 (0.8)
PA1003	Probable transcriptional regulator	8.1	6.6 (0.2)	78 (0.05)
PA1130	Hypothetical protein	2.4	9.4 (1.4)	16 (1.4)
PA1131	Probable MFS transporter[d]	1.7	5.0 (2.0)	7.9 (1.4)
PA1173	*napB*, cytochrome *c*-type protein NapB precursor	2.3	2.8 (2.0)	4.1 (1.4)
PA1175	*napD*, NapD protein of periplasmic nitrate reductase	2.6	2.4 (2.0)	3.8 (1.4)
PA1176	*napF*, ferredoxin protein NapF	2.5	2.5 (2.0)	5.8 (1.4)
PA1177	*napE*, periplasmic nitrate reductase protein NapE	2.9	3.6 (1.4)	3.6 (1.4)
PA1215	Hypothetical protein	NC	18 (1.4)	55 (1.4)
PA1216	Hypothetical protein	4.7	15 (0.8)	120 (0.8)
PA1217	Probable 2-isopropylmalate synthase	2.9	41 (1.4)	380 (1.4)
PA1218	Hypothetical protein	NC	6.9 (1.4)	160 (1.4)
PA1221	Hypothetical protein[e]	NC	3.1 (3.0)	11 (1.4)
PA1245	Hypothetical protein[e]	8.6	10 (0.8)	11 (0.2)
PA1246	*aprD*, alkaline protease secretion protein AprD	8.6	9.8 (1.4)	6.6 (0.8)
PA1247	*aprE*, alkaline protease secretion protein AprE	6.2	6.4 (1.4)	9.1 (1.4)
PA1248	*aprF*, alkaline protease secretion protein AprF	7.2	7.6 (1.4)	5.2 (1.4)
PA1249	***aprA,*** alkaline metalloproteinase precursor	25	27 (1.4)	22 (1.4)
PA1250	*aprI*, alkaline proteinase inhibitor AprI[d]	20	20 (0.2)	24 (0.05)
PA1289	Hypothetical protein	2.9	5.7 (1.4)	2.6 (1.4)
PA1317	*cyoA*, cytochrome *o* ubiquinol oxidase subunit II	2.5	4.7 (4.0)	15 (2.0)
PA1318	*cyoB*, cytochrome *o* ubiquinol oxidase subunit I	NC	3.9 (4.0)	16 (2.0)
PA1319	*cyoC*, cytochrome *o* ubiquinol oxidase subunit III	2.0	4.8 (4.0)	7.9 (3.0)
PA1320	*cyoD*, cytochrome *o* ubiquinol oxidase subunit IV	42	71 (4.0)	9.1 (3.0)
PA1323	Hypothetical protein	2.8	6.1 (2.0)	9.6 (2.0)
PA1324	Hypothetical protein	2.4	5.3 (2.0)	8.5 (2.0)
PA1404	Hypothetical protein	2.0	2.7 (2.0)	3.8 (2.0)
PA1431	***rsaL***, regulatory protein RsaL[d]	350	340 (0.2)	39 (0.8)

Continued on following page

TABLE 1—*Continued*

Gene no.[a]	Description[b]	Maximum change[c]		
		lasI rhlI mutant		Wild type vs *lasRI rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA1432	***lasI***, autoinducer synthesis protein LasI[d]	NC[f]	NC[f]	7.7 (0.8)
PA1656	Hypothetical protein[a]	2.4	3.7 (1.4)	5.7 (0.8)
PA1657	Conserved hypothetical protein	5.9	15 (0.4)	24 (0.8)
PA1658	Conserved hypothetical protein	3.9	9.3 (0.8)	17 (0.8)
PA1659	Hypothetical protein	4.1	8.5 (0.8)	17 (0.8)
PA1660	Hypothetical protein	2.6	7.9 (0.8)	16 (0.8)
PA1661	Hypothetical protein	2.3	4.4 (1.4)	4.4 (0.8)
PA1662	Probable ClpA/B-type protease	2.9	6.6 (1.4)	7.7 (0.8)
PA1663	Probable transcriptional regulator	2.5	4.5 (0.8)	9.1 (0.8)
PA1664	Hypothetical protein	5.9	16 (0.4)	22 (0.8)
PA1665	Hypothetical protein	21	55 (1.4)	28 (0.8)
PA1666	Hypothetical protein	2.9	12 (0.8)	38 (0.8)
PA1667	Hypothetical protein	3.1	7.6 (0.8)	12 (0.8)
PA1668	Hypothetical protein	2.8	4.6 (0.8)	6.3 (0.8)
PA1669	Hypothetical protein	2.2	3.8 (1.4)	17 (0.8)
PA1670	*stp1*, serine/threonine phosphoprotein phosphatase Stp1	NC	2.8 (1.4)	3.6 (0.8)
PA1745	Hypothetical protein	2.1	2.6 (2.0)	2.8 (1.4)
PA1784	Hypothetical protein[e]	14	15 (1.4)	18 (1.4)
PA1869	**qsc117**, probable acyl carrier protein[e]	7.8	41 (0.2)	340 (0.2)
PA1870	Hypothetical protein	NC	3.2 (2.0)	6.3 (2.0)
PA1871	***lasA***, LasA protease precursor[d]	48	88 (0.8)	130 (1.4)
PA1881	Hypothetical protein	2.4	2.6 (2.0)	2.8 (1.4)
PA1888	Hypothetical protein	2.7	2.3 (2.0)	4.3 (1.4)
PA1891	Hypothetical protein	3.3	4.3 (2.0)	6.5 (0.8)
PA1893	Hypothetical protein	16	13 (0.4)	2.7 (2.0)
PA1894	**qsc101**, hypothetical protein	59	59 (0.8)	5.0 (1.4)
PA1895	Hypothetical protein	36	31 (0.8)	4.2 (1.4)
PA1896	Hypothetical protein	41	49 (1.4)	3.1 (1.4)
PA1897	**qsc102**, hypothetical protein[e]	130	130 (0.4)	8.5 (0.8)
PA1914	Conserved hypothetical protein	42	190 (2.0)	700 (2.0)
PA1921	Hypothetical protein	NC	14 (2.0)	13 (2.0)
PA1930	Probable chemotaxis transducer	2.2	2.9 (2.0)	3.8 (1.4)
PA1939	Hypothetical protein	2.6	3.2 (1.4)	2.9 (2.0)
PA2030	Hypothetical protein	3.3	4.2 (2.0)	14 (2.0)
PA2031	Hypothetical protein	4.5	6.5 (0.4)	12 (1.4)
PA2066	Hypothetical protein	1.9	3.7 (2.0)	12 (1.4)
PA2067	Probable hydrolase	1.8	5.0 (2.0)	19 (1.4)
PA2068	Probable MFS transporter	NC	16 (1.4)	150 (1.4)
PA2069	Probable carbamoyl transferase[e]	NC	45 (1.4)	110 (0.2)
PA2076	Probable transcriptional regulator[d]	3.7	4.3 (0.2)	4.3 (0.2)
PA2080	Hypothetical protein	3.5	4.0 (0.2)	4.0 (0.2)
PA2081	Hypothetical protein	3.3	4.3 (0.2)	3.6 (0.2)
PA2134	Hypothetical protein	3.1	5.4 (3.0)	7.9 (2.0)
PA2142	Probable short-chain dehydrogenase	NC	3.6 (3.0)	19 (2.0)
PA2143	Hypothetical protein	21	39 (2.0)	51 (2.0)
PA2144	*glgP*, glycogen phosphorylase	2.5	4.7 (3.0)	15 (2.0)
PA2146	Conserved hypothetical protein	2.7	4.8 (3.0)	11 (2.0)
PA2147	*katE*, catalase HPII	3.5	7.1 (2.0)	35 (2.0)
PA2148	Conserved hypothetical protein	NC	3.1 (2.0)	3.4 (2.0)
PA2151	Conserved hypothetical protein	2.6	38 (2.0)	34 (2.0)
PA2152	Probable trehalose synthase	2.1	5.3 (2.0)	6.1 (2.0)
PA2153	*glgB*, 1,4-alpha-glucan branching enzyme	2.1	5.6 (2.0)	16 (2.0)
PA2156	Conserved hypothetical protein	2.3	4.8 (3.0)	17 (2.0)
PA2157	Hypothetical protein	2.1	2.8 (3.0)	2.9 (3.0)
PA2158	Probable alcohol dehydrogenase (Zn dependent)	6.7	15 (2.0)	26 (2.0)
PA2159	Conserved hypothetical protein	4.1	5.9 (2.0)	10 (2.0)
PA2160	Probable glycosyl hydrolase[d]	2.3	4.4 (3.0)	5.8 (2.0)
PA2161	Hypothetical protein[d]	4.4	6.3 (2.0)	10 (2.0)
PA2163	Hypothetical protein	2.2	6.9 (2.0)	31 (2.0)
PA2164	Probable glycosyl hydrolase	2.5	4.7 (2.0)	6.5 (2.0)
PA2165	Probable glycogen synthase	3.2	5.7 (2.0)	6.3 (2.0)
PA2166	Hypothetical protein	3.1	9.1 (2.0)	17 (2.0)
PA2167	Hypothetical protein	2.3	2.6 (2.0)	4.7 (2.0)
PA2169	Hypothetical protein	2.8	5.7 (2.0)	5.1 (2.0)
PA2170	Hypothetical protein	3.6	6.9 (2.0)	13 (2.0)
PA2171	Hypothetical protein	5.2	9.1 (2.0)	22 (2.0)
PA2172	Hypothetical protein	3.8	7.7 (2.0)	12 (2.0)

Continued on following page

TABLE 1—*Continued*

Gene no.[a]	Description[b]	Maximum change[c]		
		lasI rhlI mutant		Wild type vs *lasR rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA2173	Hypothetical protein	3.5	6.5 (2.0)	17 (2.0)
PA2176	Hypothetical protein	1.4	5.3 (2.0)	27 (2.0)
PA2180	Hypothetical protein	1.9	2.6 (3.0)	2.7 (3.0)
PA2190	Conserved hypothetical protein	3.4	4.5 (2.0)	7.5 (2.0)
PA2192	Conserved hypothetical protein	NC	10 (2.0)	8.4 (2.0)
PA2193	***hcnA,*** hydrogen cyanide synthase HcnA[e]	140	190 (0.2)	88 (0.2)
PA2194	**qsc128,** *hcnB,* hydrogen cyanide synthase HcnB	37	51 (0.2)	59 (0.8)
PA2195	***hcnC,*** hydrogen cyanide synthase HcnC	16	30 (0.4)	46 (0.8)
PA2274	Hypothetical protein	NC	3.4 (3.0)	11 (2.0)
PA2300	***chiC,*** chitinase[e]	1.7	14 (1.4)	100 (1.4)
PA2302	**qsc100**, probable nonribosomal peptide synthetase	5.2	7.9 (0.8)	130 (1.4)
PA2303	**qsc107**, hypothetical protein	25	28 (0.4)	130 (0.2)
PA2304	Hypothetical protein	8.4	12 (0.8)	29 (0.8)
PA2305	Probable nonribosomal peptide synthetase	52	51 (0.2)	70 (0.2)
PA2327	Probable permease of ABC transporter	5.9	8.9 (4.0)	6.9 (4.0)
PA2328	Hypothetical protein	6.8	9.1 (2.0)	7.5 (3.0)
PA2329	Probable component of ABC transporter	7.8	9.9 (1.4)	18 (3.0)
PA2330	Hypothetical protein	7.9	11 (0.8)	15 (2.0)
PA2331	Hypothetical protein	8.3	19 (1.4)	20 (1.4)
PA2345	Conserved hypothetical protein[e]	2.2	3.2 (2.0)	2.6 (2.0)
PA2365	Conserved hypothetical protein	4.7	5.4 (1.4)	5.9 (1.4)
PA2366	Conserved hypothetical protein	4.3	5.2 (1.4)	6.9 (1.4)
PA2367	Hypothetical protein	4.8	5.1 (1.4)	6.4 (1.4)
PA2368	Hypothetical protein	3.5	3.4 (1.4)	7.5 (1.4)
PA2370	Hypothetical protein	2.9	3.6 (3.0)	3.5 (1.4)
PA2371	Probable ClpA/B-type protease	2.4	2.6 (3.0)	5.0 (1.4)
PA2372	Hypothetical protein	3.2	2.7 (2.0)	3.7 (1.4)
PA2414	L-Sorbosone dehydrogenase	3.1	4.9 (2.0)	21 (0.2)
PA2415	Hypothetical protein	3.5	5.6 (2.0)	14 (2.0)
PA2423	Hypothetical protein	11	11 (0.4)	13 (0.2)
PA2433	Hypothetical protein	2.8	5.9 (2.0)	11 (2.0)
PA2442	*gcvT2*, glycine cleavage system protein T2	2.0	2.6 (3.0)	3.1 (3.0)
PA2444	*glyA2*, serine hydroxymethyltransferase	9.1	12 (3.0)	10 (3.0)
PA2445	*gcvP2*, glycine cleavage system protein P2	6.6	7.5 (4.0)	11 (3.0)
PA2446	*gcvH2*, glycine cleavage system protein H2	12	17 (4.0)	18 (3.0)
PA2448	Hypothetical protein	NC	4.1 (3.0)	12 (1.4)
PA2512	*antA*, anthranilate dioxygenase large subunit	−600	43 (2.0)	27 (3.0)
PA2513	*antB*, anthranilate dioxygenase small subunit	−96	14 (2.0)	13 (3.0)
PA2514	*antC*, anthranilate dioxygenase reductase	−67	9.3 (2.0)	3.8 (4.0)
PA2564	Hypothetical protein	2.9	7.8 (2.0)	21 (1.4)
PA2565	Hypothetical protein	3.1	6.6 (2.0)	14 (2.0)
PA2566	Conserved hypothetical protein[e]	6.5	13 (2.0)	21 (1.4)
PA2570	***pa1L,*** PA-1 galactophilic lectin[d]	NC	26 (1.4)	200 (1.4)
PA2572	Probable two-component response regulator	2.3	2.8 (1.4)	3.3 (1.4)
PA2573	Probable chemotaxis transducer	2.3	4.1 (1.4)	3.9 (1.4)
PA2587	**qsc105**, probable FAD-dependent monooxygenase	12	12 (0.2)	15 (0.1)
PA2588	Probable transcriptional regulator	15	22 (0.2)	46 (0.8)
PA2591	Probable transcriptional regulator[e]	21	25 (0.2)	42 (0.2)
PA2592	**qsc104**, probable spermidine-putrescine-binding protein[e]	5.6	8.7 (0.4)	15 (0.8)
PA2593	Hypothetical protein	NC	4.6 (2.0)	29 (0.8)
PA2717	*cpo*, chloroperoxidase precursor	2.4	2.6 (2.0)	3.4 (1.4)
PA2747	Hypothetical protein	3.6	7.2 (2.0)	11 (2.0)
PA2927	Hypothetical protein	2.6	3.4 (2.0)	14 (1.4)
PA2939	Probable aminopeptidase	38	42 (1.4)	27 (1.4)
PA3022	Hypothetical protein	3.5	4.7 (2.0)	4.3 (2.0)
PA3032	**qsc135**, cytochrome *c*	2.3	3.3 (2.0)	9.3 (2.0)
PA3104	***xcpP,*** secretion protein XcpP	3.1	3.2 (2.0)	4.7 (1.4)
PA3181	2-Keto-3-deoxy-6-phosphogluconate aldolase	1.6	2.9 (3.0)	3.2 (3.0)
PA3182	Conserved hypothetical protein	1.7	3.2 (3.0)	5.0 (3.0)
PA3183	*zwf*, glucose-6-phosphate 1-dehydrogenase	2.0	3.7 (3.0)	4.0 (3.0)
PA3188	Probable permease of ABC sugar transporter	2.9	4.2 (2.0)	6.8 (3.0)
PA3189	Probable permease of ABC sugar transporter	2.0	2.5 (3.0)	3.0 (3.0)
PA3190	Probable component of ABC sugar transporter	2.7	3.4 (2.0)	4.1 (3.0)
PA3194	*edd*, phosphogluconate dehydratase	2.0	3.2 (3.0)	2.9 (3.0)
PA3195	*gapA*, glyceraldehyde-3-phosphate dehydrogenase	3.1	5.0 (3.0)	5.4 (3.0)
PA3274	Hypothetical protein[d]	1.9	4.3 (2.0)	10 (2.0)
PA3311	Conserved hypothetical protein	3.6	3.6 (2.0)	6.0 (1.4)

Continued on following page

TABLE 1—*Continued*

Gene no.[a]	Description[b]	Maximum change[c]		
		lasI rhlI mutant		Wild type vs *lasR rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA3326	Probable Clp family ATP-dependent protease[e]	6.6	20 (0.4)	19 (0.8)
PA3327	**qsc126**, probable nonribosomal peptide synthetase[e]	NC	6.8 (0.8)	20 (0.8)
PA3328	**qsc125**, probable FAD-dependent monooxygenase	NC	17 (0.4)	47 (0.8)
PA3329	**qsc124**, hypothetical protein	NC	250 (0.4)	310 (0.8)
PA3330	**qsc123**, probable short-chain dehydrogenase	NC	120 (0.4)	320 (0.8)
PA3331	**qsc122**, cytochrome P450	3.5	39 (0.4)	62 (0.8)
PA3332	Conserved hypothetical protein	2.3	35 (0.8)	41 (1.4)
PA3333	**qsc121**, *fabH2*, 3-oxoacyl-(acyl carrier protein) synthase III	NC	32 (0.4)	64 (0.8)
PA3334	Probable acyl carrier protein	1.8	49 (0.4)	69 (0.8)
PA3335	Hypothetical protein	NC	9.6 (0.4)	29 (1.4)
PA3336	**qsc120**, probable MFS transporter	NC	22 (1.4)	24 (0.8)
PA3346	Probable two-component response regulator	2.7	2.8 (2.0)	4.7 (2.0)
PA3347	Hypothetical protein[c]	2.3	2.8 (2.0)	4.3 (1.4)
PA3361	Hypothetical protein	10	13 (1.4)	68 (1.4)
PA3369	Hypothetical protein	1.9	3.3 (2.0)	4.8 (2.0)
PA3370	Hypothetical protein	1.7	3.5 (2.0)	5.6 (2.0)
PA3371	Hypothetical protein	1.7	3.6 (2.0)	6.0 (2.0)
PA3416	Probable pyruvate dehydrogenase component	2.5	3.2 (2.0)	4.1 (1.4)
PA3418	*ldh*, leucine dehydrogenase	2.6	3.7 (1.4)	5.0 (1.4)
PA3476	**qsc118**, ***rhlI,*** autoinducer synthesis protein RhlI[e]	NC[f]	NC[f]	34 (0.05)
PA3477	***rhlR,*** transcriptional regulator RhlR	8.5	9.6 (0.4)	130 (0.05)
PA3478	**qsc119, *rhlB,*** rhamnosyltransferase chain B	5.3	89 (0.8)	120 (1.4)
PA3479	**qsc119, *rhlA,*** rhamnosyltransferase chain A[e]	10	120 (0.8)	200 (0.8)
PA3520	Hypothetical protein[d]	2.2	13 (1.4)	32 (1.4)
PA3535	Probable serine protease	7.5	8.1 (0.4)	5.9 (0.8)
PA3676	Probable RND efflux transporter	3.9	1.9 (2.0)	5.8 (1.4)
PA3677	Probable RND efflux protein precursor	3.6	3.8 (2.0)	8.3 (1.4)
PA3678	Probable transcriptional regulator	2.9	1.6 (2.0)	3.5 (1.4)
PA3688	Hypothetical protein	3.0	5.4 (0.2)	3.5 (1.4)
PA3691	Hypothetical protein	2.4	4.5 (2.0)	6.3 (2.0)
PA3692	Probable outer membrane protein	3.0	5.8 (2.0)	6.9 (2.0)
PA3724	***lasB,*** elastase LasB[e]	110	180 (0.8)	240 (0.8)
PA3734	Hypothetical protein	NC	4.1 (3.0)	16 (2.0)
PA3888	Probable permease of ABC transporter	NC	3.2 (2.0)	3.9 (2.0)
PA3890	Probable permease of ABC transporter	1.8	4.1 (2.0)	4.7 (2.0)
PA3891	Probable component of ABC transporter	2.1	5.0 (2.0)	8.2 (2.0)
PA3904	Hypothetical protein	49	42 (0.2)	46 (0.05)
PA3905	Hypothetical protein	37	59 (0.2)	87 (0.05)
PA3906	Hypothetical protein	140	130 (0.2)	71 (0.05)
PA3907	**qsc103**, hypothetical protein	20	19 (0.2)	58 (0.05)
PA3908	Hypothetical protein	10	11 (0.2)	55 (0.05)
PA3986	Hypothetical protein	2.7	3.3 (1.4)	2.8 (2.0)
PA4078	**qsc134**, probable nonribosomal peptide synthetase[d]	3.2	4.6 (2.0)	20 (2.0)
PA4117	Probable bacteriophytochrome	5.3	5.6 (1.4)	4.3 (1.4)
PA4129	Hypothetical protein	25	31 (0.8)	15 (0.8)
PA4130	Probable sulfite or nitrite reductase	23	27 (0.8)	1 (0.8)
PA4131	Probable iron-sulfur protein	24	30 (0.8)	21 (0.8)
PA4132	Conserved hypothetical protein	14	15 (0.8)	6.4 (0.8)
PA4133	Cytochrome *c* oxidase subunit (cbb3 type)	100	100 (0.8)	37 (0.8)
PA4134	Hypothetical protein	43	47 (0.8)	21 (0.8)
PA4139	Hypothetical protein	3.1	2.9 (2.0)	3.9 (2.0)
PA4141	Hypothetical protein	2.6	26 (0.4)	73 (1.4)
PA4142	Probable secretion protein	NC	5.2 (2.0)	16 (1.4)
PA4171	Probable protease	3.5	4.6 (2.0)	5.1 (2.0)
PA4172	Probable nuclease	2.0	3.4 (2.0)	14 (2.0)
PA4175	Probable endoproteinase Arg-C precursor	11	15 (2.0)	23 (1.4)
PA4190	Probable FAD-dependent monooxygenase	3.0	2.5 (1.4)	4.0 (0.2)
PA4205	Hypothetical protein	1.9	8.7 (3.0)	56 (2.0)
PA4206	Probable RND efflux protein precursor	1.7	6.3 (3.0)	30 (2.0)
PA4207	**qsc133**, probable RND efflux transporter	NC	2.5 (3.0)	17 (2.0)
PA4208	Probable outer membrane efflux protein	1.6	3.1 (3.0)	19 (2.0)
PA4209	Probable *O*-methyltransferase[e]	4.6	11 (1.4)	27 (1.4)
PA4210	Probable phenazine biosynthesis protein[e,g]	NC	59 (1.4)	71 (1.4)
PA4211	Probable phenazine biosynthesis protein[d]	10	69 (0.8)	220 (0.8)
PA4212	**qsc131**, phenazine biosynthesis protein PhzC[d]	2.2	15 (1.4)	77 (1.4)
PA4213	Phenazine biosynthesis protein PhzD	3.7	36 (1.4)	210 (1.4)
PA4214	Phenazine biosynthesis protein PhzE	2.5	18 (1.4)	59 (1.4)

Continued on following page

TABLE 1—*Continued*

Gene no.[a]	Description[b]	Maximum change[c]		
		lasI rhlI mutant		Wild type vs *lasR rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA4215	Probable phenazine biosynthesis protein	3.1	24 (1.4)	110 (1.4)
PA4216	Probable pyridoxamine 5-phosphate oxidase	3.0	21 (1.4)	56 (1.4)
PA4217	**qsc132**, probable FAD-dependent monooxygenase	4.4	28 (1.4)	41 (1.4)
PA4296	Probable two-component response regulator	2.4	3.6 (1.4)	5.6 (1.4)
PA4297	Hypothetical protein	2.4	3.3 (2.0)	12 (2.0)
PA4298	Hypothetical protein	2.3	4.7 (2.0)	8.7 (2.0)
PA4299	Hypothetical protein	2.1	3.6 (2.0)	7.0 (2.0)
PA4300	Hypothetical protein	2.0	3.5 (2.0)	7.8 (2.0)
PA4302	Probable type II secretion system protein	3.2	6.1 (2.0)	7.4 (2.0)
PA4304	Probable type II secretion system protein	2.2	3.1 (2.0)	6.1 (2.0)
PA4305	Hypothetical protein	2.1	2.7 (2.0)	5.9 (2.0)
PA4306	Hypothetical protein	10	16 (1.4)	38 (1.4)
PA4311	Conserved hypothetical protein	2.5	3.2 (2.0)	2.6 (2.0)
PA4384	Hypothetical protein	NC	2.7 (3.0)	4.0 (3.0)
PA4498	Probable metallopeptidase	1.6	4.5 (3.0)	9.1 (3.0)
PA4590	*pra*, protein activator	9.3	14 (1.4)	13 (0.8)
PA4648	Hypothetical protein	3.4	7.7 (2.0)	17 (1.4)
PA4649	Hypothetical protein	NC	3.2 (2.0)	7.4 (1.4)
PA4650	Hypothetical protein	NC	3.3 (2.0)	8.8 (2.0)
PA4651	Probable pilus assembly chaperone[d]	NC	4.6 (2.0)	15 (2.0)
PA4652	Hypothetical protein	6.0	13 (2.0)	9.6 (2.0)
PA4677	Hypothetical protein	16	13 (0.2)	36 (0.1)
PA4703	Hypothetical protein	3.1	4.3 (2.0)	3.5 (1.4)
PA4738	Conserved hypothetical protein	3.8	9.1 (2.0)	11 (2.0)
PA4739	Conserved hypothetical protein	4.2	9.4 (2.0)	14 (2.0)
PA4778	Probable transcriptional regulator	5.4	4.9 (0.4)	8.6 (0.1)
PA4869	**qsc106**, hypothetical protein[d]	5.0	5.7 (0.4)	3.8 (0.1)
PA4876	*osmE*, osmotically inducible lipoprotein OsmE	2.3	3.6 (2.0)	4.9 (2.0)
PA4880	Probable bacterioferritin	2.2	4.6 (2.0)	5.8 (2.0)
PA4916	Hypothetical protein	1.5	4.3 (4.0)	6.1 (2.0)
PA4917	Hypothetical protein[d]	1.4	5.8 (2.0)	7.7 (2.0)
PA4925	Conserved hypothetical protein	3.8	3.7 (2.0)	5.7 (1.4)
PA5027	Hypothetical protein[e]	1.5	2.8 (2.0)	3.2 (3.0)
PA5058	*phaC2*, poly(3-hydroxyalkanoic acid) synthase 2[e]	4.5	4.7 (1.4)	9.2 (1.4)
PA5059	Probable transcriptional regulator	4.4	5.9 (2.0)	9.3 (1.4)
PA5061	Conserved hypothetical protein	1.7	2.5 (4.0)	2.6 (4.0)
PA5161	*rmlB*, dTDP-D-glucose 4,6-dehydratase	NC	2.9 (4.0)	5.9 (3.0)
PA5162	*rmlD*, dTDP-4-dehydrorhamnose reductase	NC	2.5 (4.0)	4.9 (3.0)
PA5164	*rmlC*, dTDP-4-dehydrorhamnose 3,5-epimerase	NC	2.6 (3.0)	5.6 (2.0)
PA5220	**qsc138**, hypothetical protein	2.8	18 (0.8)	26 (1.4)
PA5352	Conserved hypothetical protein	2.0	2.8 (1.4)	2.9 (1.4)
PA5353	*glcF*, glycolate oxidase subunit GlcF	1.9	3.4 (1.4)	3.5 (1.4)
PA5354	*glcE*, glycolate oxidase subunit GlcE	2.0	2.6 (1.4)	3.2 (1.4)
PA5355	*glcD*, glycolate oxidase subunit GlcD	2.1	3.6 (1.4)	3.8 (1.4)
PA5356	**qsc130**, *glcC*, transcriptional regulator GlcC	2.4	4.1 (1.4)	2.8 (1.4)
PA5415	*glyA1*, serine hydroxymethyltransferase	2.6	2.8 (3.0)	5.0 (3.0)
PA5481	Hypothetical protein	4.1	11 (2.0)	15 (1.4)
PA5482	Hypothetical protein	5.4	15 (2.0)	18 (1.4)

[a] Gene number from the *Pseudomonas* genome project (http://www.pseudomonas.com).

[b] Boldface type indicates genes or gene products previously reported to be controlled by quorum sensing. RND, resistance-nodulation-cell division; FAD, flavin adenine dinucleotide.

[c] Maximum changes (fold) in gene expression (rounded to two significant figures) in the signal generation mutant in the presence of the signal(s) indicated compared with the absence of signal and in the wild-type *P. aeruginosa* strain compared with the receptor mutant. The values in parentheses are the OD_{600} at which the earliest change of ≥2.5-fold was observed (for the signal generation mutant both time courses were considered). NC, no change.

[d] There is a *las-rhl* box-like sequence with an HI of ≥10 and <13.

[e] There is a *las-rhl* box-like sequence with an HI of <10.

[f] The transcript levels for *lasI* and *rhlI* were close to the background level in the signal generation mutant due to the disruption of both loci by insertional mutagenesis.

[g] The GeneChip probes for PA4210 to PA4216 are identical to those for PA1899 to PA1905. Although the sequences for the genes in these two clusters are almost identical, the region upstream of PA4210 contains a *las-rhl* box-like sequence, but the region upstream of PA1899 does not.

duced or repressed by addition of the acyl-HSL signals to the signal generation mutant. We identified 810 genes that were differentially expressed in the parent compared to the signal receptor mutant. In all, there was an overlapping set of 411 genes. Visual inspection of the expression patterns of individual genes led to exclusion of 58 genes. These genes either showed expression levels close to the background level or showed inconsistent regulatory patterns when the two experimental approaches were compared. An interesting example of an inconsistent regulatory pattern was observed with a few genes identified and classified as late 3OC12-HSL-dependent genes in the previous study. These genes, PA2401, PA2402,

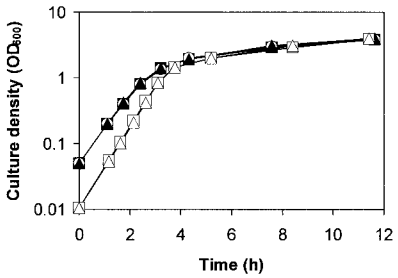

FIG. 1. Growth of wild-type *P. aeruginosa* strain PAO1 (□), growth of the receptor mutant PAO *lasR rhlR* (△), and growth of the signal generation mutant PAO-MW1 without added acyl-HSL (▲), with 3OC12-HSL (○), and with C4-HSL and 3OC12-HSL (■).

and PA2385 (qsc109-110, qsc111, and qsc112) showed the predicted regulatory pattern in our transcriptome analysis of the signal generation mutant (although they showed low response levels of 2.0, 2.1, and 2.3, respectively), but they showed quorum-controlled repression when the parent was compared to the signal receptor mutant. In all then, we identified 315 genes which we believe to be quorum activated. These genes and some information regarding their expression are shown in Table 1.

There is no obvious chromosomal clustering of the genes identified (Fig. 2). The final set of quorum-induced genes represents about 6% of the genome. This is somewhat higher than but not too different from the previous prediction that around 2 to 4% of the genome is quorum induced. However, the genes that we have identified are likely a subset of the quorum regulon. For example, we used one standard set of growth conditions for all of our experiments; it is not unreasonable to believe that other genes in the regulon might be revealed by altering the growth medium or culture conditions. In our experiments about 20 to 30% of the transcripts were undetectable; some of these transcripts might be quorum controlled or expressed at higher levels under different conditions. As discussed above, we also filtered the data set, and we do not believe that the genes that survived the filtering procedure represent an exhaustive compilation of quorum-controlled genes. Rather, the data provide a conservative estimate of quorum-controlled genes. Among the genes listed in Table 1, the most overrepresented categories consist of genes known or predicted to be involved in the production of secreted products. Genes in the adaptation and protection categories and in the central intermediary metabolism categories are also overrepresented.

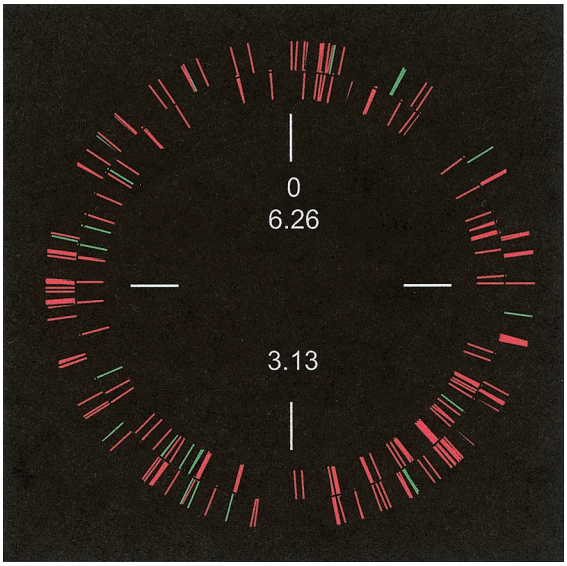

FIG. 2. Map of quorum-regulated genes on the *P. aeruginosa* chromosome. Red, activated genes; green, repressed genes. Genes in the inner circle are transcribed in a counterclockwise direction. The numbers indicate map positions (in megabases).

Quorum-repressed genes. We identified 38 quorum-repressed genes (Table 2). These genes showed lower transcript levels in the late logarithmic and stationary phases in the wild type than in the receptor mutant, as well as in the signal generation mutant in the presence of signals than in the mutant grown without added signal. All of the repressed genes responded as well or nearly as well to 3OC12-HSL alone as they did to 3OC12-HSL and C4-HSL together. The expression patterns of a representative quorum-repressed gene, PA0433, are shown in Fig. 3. A curiousity is that these genes are expressed at low levels throughout growth of the parent strain. They are derepressed only in the mutants and only during the late logarithmic and stationary phases. Among the quorum-repressed genes with known or predicted functions, those involved in carbohydrate utilization or nutrient transport appeared to be the most abundant (Table 2).

Operons and *las-rhl* box-like sequences. One would expect that all of the genes in an operon should show similar quorum control. In fact, we observed that strings of genes appeared (Tables 1 and 2). These strings often represent known or suspected operons, and the genes in a given string show similar quorum responses (signal responses and timing of induction). For example, the *hcn* genes (PA2193 to PA2195) are known to exist in an operon (23). Consistent with this, our transcriptome analysis indicated these genes were coinduced by quorum sensing. PA2365 to PA2372 represent a string of quorum-controlled genes with unknown functions. We suggest that these genes represent an operon. On the other hand, many of the quorum-controlled genes are not adjacent to other quorum-controlled genes listed in Tables 1 and 2. To assess whether these genes may also be organized in operons (neighboring genes would have been eliminated if they showed induction just under the 2.5-fold threshold) and to confirm the hypothesis that strings of adjacent quorum-controlled genes are in operons, we performed a more systematic analysis. Operon organization was allowed only if every gene within a gene cluster was in the same orientation, if every gene was activated or every gene was repressed, if there was less than 250 bp between two adjacent open reading frames, and if the absolute transcript profiles of the candidate genes in the parent *P. aeruginosa* strain showed patterns similar to each other (correlation coefficient of $\geq$0.95 with the GeneSpring standard correlation algorithm). By using these criteria, we identified 87 possible operons, 71 of which were activated and 16 of which were repressed (Fig. 4). More than 60 additional genes showing coregulation with the genes listed in Tables 1 and 2 were identified by this analysis.

We used a computer algorithm to search for *las-rhl* boxes in

TABLE 2. Quorum-repressed genes

Gene no.[a]	Description	Maximum change[b]		
		lasI rhlI mutant		Wild type vs *lasR rhlR* mutant
		3OC12-HSL	C4-HSL + 3OC12-HSL	
PA0165	Hypothetical protein	−2.7	−2.9 (2.0)	−4.8 (2.0)
PA0433	Hypothetical protein	−6.8	−20 (2.0)	−8.9 (1.4)
PA0434	Hypothetical protein	−7.7	−8.5 (2.0)	−5.6 (2.0)
PA0435	Hypothetical protein	−9.4	−26 (2.0)	−34 (2.0)
PA0485	Conserved hypothetical protein[c]	−1.7	−3.4 (1.4)	−3.0 (3.0)
PA0887	*acsA*, acetyl-coenzyme A synthetase	−3.3	−4.2 (2.0)	−3.6 (3.0)
PA1559	Hypothetical protein	−2.4	−3.5 (2.0)	−3.2 (1.4)
PA2007	*maiA*, maleylacetoacetate isomerase	−3.2	−1.4 (4.0)	−3.2 (3.0)
PA2008	*fahA*, fumarylacetoacetase	−3.7	−1.5 (4.0)	−2.6 (3.0)
PA2009	*hmgA*, homogentisate 1,2-dioxygenase	−4.0	−1.5 (4.0)	−2.7 (3.0)
PA2250	*lpdV*, lipoamide dehydrogenase-Val	−3.1	−1.8 (4.0)	−2.6 (3.0)
PA2338	Probable component of ABC maltose transporter	−5.0	−3.2 (3.0)	−4.2 (3.0)
PA2339	Probable maltose-mannitol transport protein	−1.9	−6.8 (3.0)	−4.1 (3.0)
PA2340	Probable maltose-mannitol transport protein	−3.4	−2.0 (3.0)	−3.7 (3.0)
PA2341	Probable component of ABC maltose transporter	−3.1	−2.0 (3.0)	−4.2 (3.0)
PA2343	*mtlY*, xylulose kinase	−1.7	−4.0 (3.0)	−3.2 (4.0)
PA3038	Probable porin	−2.3	−3.5 (2.0)	−4.4 (3.0)
PA3174	Probable transcriptional regulator	−2.1	−3.5 (4.0)	−6.5 (3.0)
PA3205	Hypothetical protein	−1.3	−3.1 (4.0)	−3.1 (4.0)
PA3233	Hypothetical protein	−2.2	−2.7 (3.0)	−5.1 (3.0)
PA3234	Probable sodium-solute symporter	−4.5	−3.4 (2.0)	−7.0 (3.0)
PA3235	Conserved hypothetical protein	−3.9	−4.2 (3.0)	−6.6 (3.0)
PA3281	Hypothetical protein	−5.7	−6.4 (1.4)	−25 (1.4)
PA3282	Hypothetical protein	−8.5	−8.8 (1.4)	−21 (1.4)
PA3283	Conserved hypothetical protein	−9.0	−8.8 (1.4)	−28 (1.4)
PA3284	Hypothetical protein	−7.1	−10 (2.0)	−24 (1.4)
PA3364	*amiC*, aliphatic amidase expression-regulating protein	−2.7	−1.8 (4.0)	−2.7 (1.4)
PA3365	Probable chaperone	−3.0	−1.7 (4.0)	−4.0 (1.4)
PA3575	Hypothetical protein	−1.6	−2.7 (1.4)	−3.3 (2.0)
PA3790	*oprC*, outer membrane protein OprC	−2.7	−3.7 (2.0)	−4.6 (2.0)
PA4359	Conserved hypothetical protein	−1.4	−2.7 (2.0)	−2.8 (1.4)
PA4371	Hypothetical protein	−1.9	−4.1 (2.0)	−2.8 (1.4)
PA4442	*cysN*, ATP sulfurylase GTP-binding subunit	−2.8	−3.4 (3.0)	−7.6 (2.0)
PA4443	*cysD*, ATP sulfurylase small subunit	−3.1	−3.4 (3.0)	−6.5 (2.0)
PA4691	Hypothetical protein	−2.5	−2.8 (2.0)	−2.9 (2.0)
PA4692	Conserved hypothetical protein	−3.8	−3.4 (2.0)	−5.0 (1.4)
PA4770	*lldP*, L-lactate permease	−1.8	−3.7 (2.0)	−5.0 (2.0)
PA5168	Probable dicarboxylate transporter	−2.7	−1.9 (4.0)	−5.8 (2.0)

[a] Gene number from the *Pseudomonas* genome project (http://www.pseudomonas.com).
[b] Maximum changes in gene expression (rounded to two significant figures) in the signal generation mutant in the presence of the signal(s) indicated compared with the absence of signal and in the wild-type *P. aeruginosa* strain compared with the receptor mutant. The values in parentheses are the OD_{600} at which the earliest change of ≥2.5-fold was observed (for the signal generation mutant both time courses were considered).
[c] There is a *las-rhl* box-like sequence with an HI of ≥10 and <13.

regions upstream of quorum-regulated genes. On the basis of a stringent criterion (an HI of <10), 55 of the *P. aeruginosa* genes contain a box in the upstream regulatory region. Twenty-five (45%) of these genes are quorum controlled, and 15 represent the first gene in a predicted operon (Table 1 and Fig. 4). At a lower stringency (an HI of <13), we identified 185 genes with *las-rhl* box-like sequences. Forty-eight (26%) of these genes are quorum controlled, and 19 represent the first gene in a predicted operon. Only one *las-rhl* box-like sequence was found upstream of a quorum-repressed gene. We did not identify potential boxes for all of our quorum-activated genes. This suggests that some of the genes might be controlled indirectly by quorum sensing or that the search criteria were not sufficiently refined. These possibilities are not mutually exclusive. We also found *las-rhl* boxes for genes that did not appear to be quorum controlled. Again, this suggests that these genes might be quorum controlled under other conditions or that the

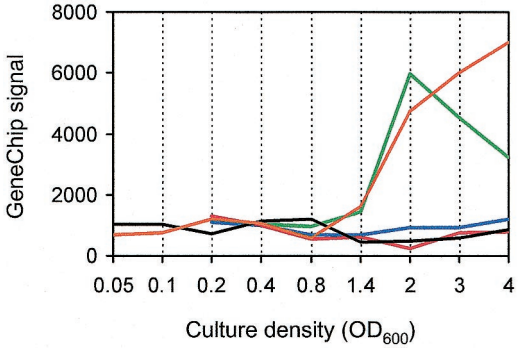

FIG. 3. Transcript abundance of the quorum-repressed gene PA0433 in wild-type *P. aeruginosa* (black line), in the receptor mutant (orange line), and in the signal generation mutant without added acyl-HSL (green line), with 3OC12-HSL (blue line), and with C4-HSL and 3OC12-HSL (red line). The values on the *y* axis represent transcript abundance as determined by the array software.

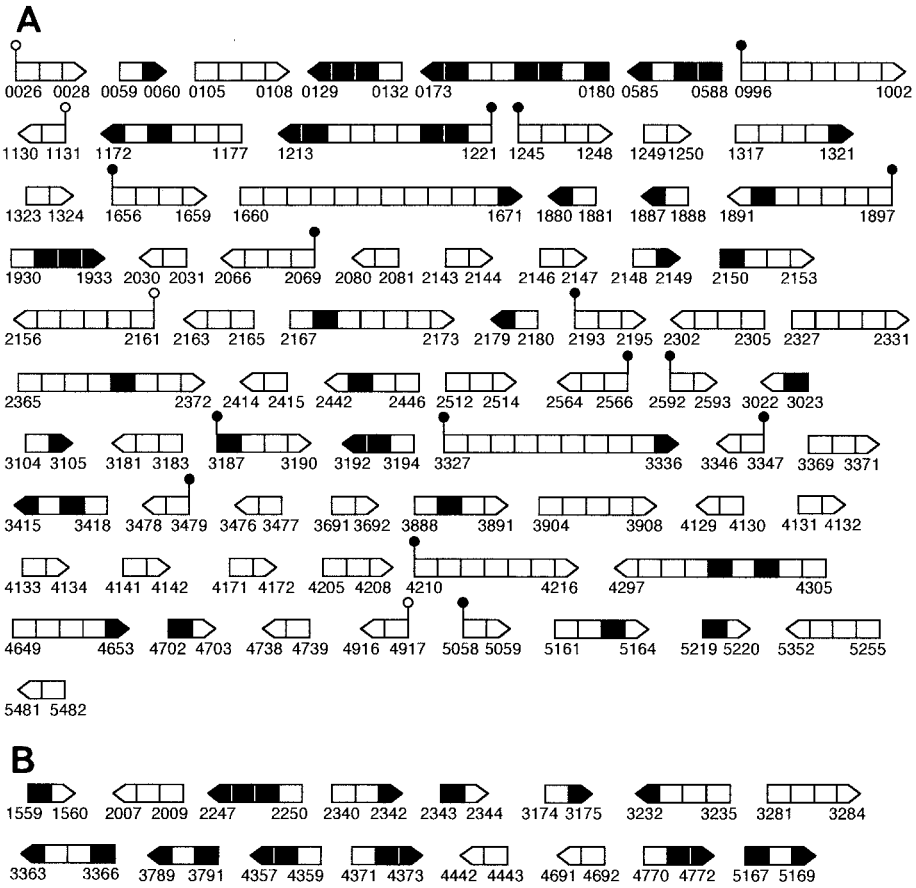

FIG. 4. Predicted quorum-regulated operons. Genes not listed in Tables 1 and 2 are depicted as black boxes. The arrows indicate the directions of transcription. The solid and open circles indicate putative *las-rhl* boxes with an HI of less than 10 and less than 13, respectively. (A) Quorum-activated operons; (B) quorum-repressed operons. Numbers are PA gene numbers from the *Pseudomonas* genome project.

search criteria need further refinement. We believe that the search algorithm was biased because it was based on the relatively small subset of quorum-controlled genes with established *las-rhl* boxes (see Materials and Methods). There was no apparent bias, however, with respect to the timing of induction or signal specificity of the identified genes.

Signal specificity. In a previous limited analysis of quorum-controlled gene expression, genes were classified into the following categories based on their responses to the signals: genes that responded equally well to 3OC12-HSL and to 3OC12-HSL and C4-HSL together and genes that responded best only when both 3OC12-HSL and C4-HSL were present (33). It appears from the microarray data that the responses are on a continuum, with some genes responding no better to both signals than they do to 3OC12-HSL alone and other genes showing progressively greater responses to both signals compared to the responses to 3OC12-HSL alone. For example, PA2423 responded no better to both signals then it did to 3OC12-HSL alone, PA0122 responded well to 3OC12-HSL alone but showed an approximately threefold-greater response with both signals, and PA2069 did not respond at all to 3OC12-HSL alone but showed a large response in the presence of both signals (Table 1). This suggests that some genes respond to 3OC12-HSL specifically, others respond with various specificities to either signal, and others respond to C4-HSL specifically. The genes encoding anthranilate dioxygenase, *antABC* (PA2512 to PA2514), are an exceptional case. These genes were strongly repressed in the presence of 3OC12-HSL alone but were activated in the presence of both signals.

Timing of quorum-controlled gene activation. The previous analysis (33) showed that induction of some genes, early genes, could be triggered early in the logarithmic phase by addition of signals. With other genes, late genes, there was a delay in induction even in the presence of excess added signal (33). By examining the microarray data we were able to learn about the timing of quorum-sensing-controlled gene induction in the wild-type strain, and we were able to obtain a broader understanding of the influence of acyl-HSL signal addition on control of the quorum regulon. The patterns of quorum-controlled gene expression were remarkably similar in the parent and in the signal generation mutant grown in the presence of 3OC12-HSL and C4-HSL (Fig. 5). A small number of transcripts showed the greatest induction early in growth. Other genes exhibited low levels of induction early in growth but did not reach maximum levels of induction until later in growth. Most transcripts were induced at culture optical densities between 0.8 and 2.0. Some transcripts showed increased abundance relative to the baseline level only at culture optical densities greater than 2.0 (stationary phase). Thus, the transcriptome analysis suggests that the timing of quorum-controlled gene

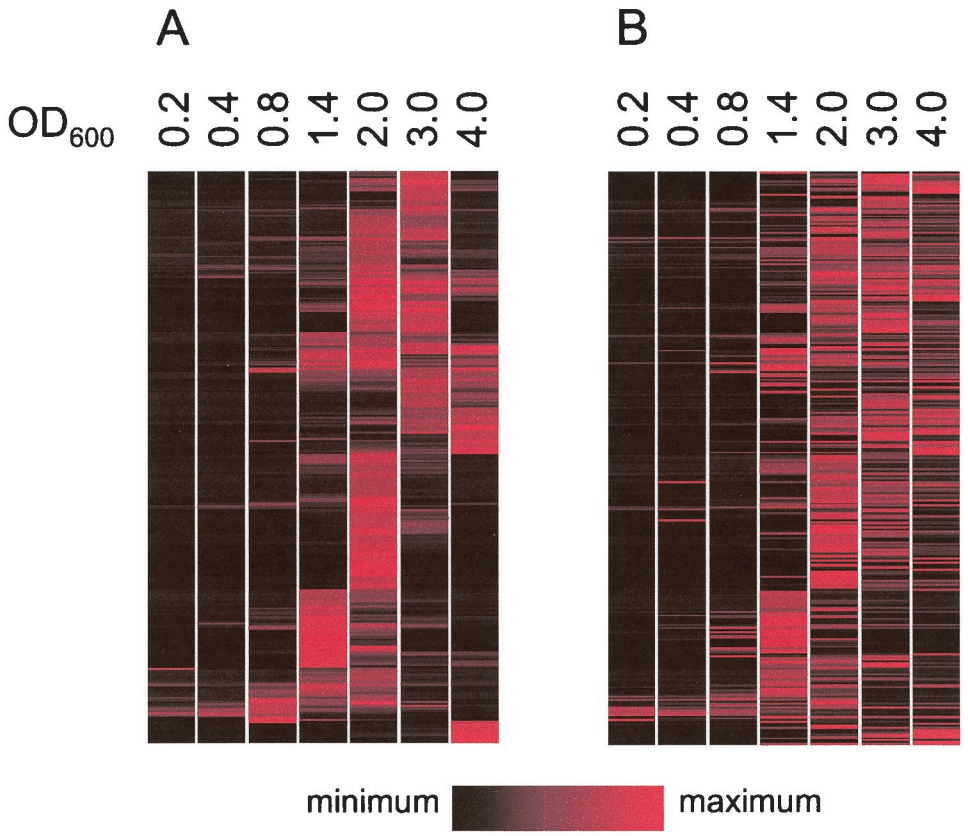

FIG. 5. Relative expression profiles for quorum-activated genes during growth of *P. aeruginosa*. (A) Signal generation mutant with C4-HSL and 3OC12-HSL versus signal generation mutant with no acyl-HSL. (B) Wild-type *P. aeruginosa* versus the receptor mutant. Depicted are fold changes for each gene normalized to the half-maximal level. The genes in panel A are displayed in the order of the hierarchical clustering of their expression profiles, and the genes in panel B are shown in the same order as those in panel A.

induction is on a continuum, although most genes in the regulon appeared to be activated during the transition from the logarithmic phase to the stationary phase (optical densities between 0.8 and 2.0). The timing of induction for most genes was not affected by exogenous addition of 3OC12-HSL and C4-HSL. Examples of each of the gene expression patterns described above are shown in Fig. 6.

We hypothesize that even in the parent strain at the earliest sampling time (optical density, 0.05) there were sufficient acyl-HSL levels for induction of the early genes and that some other factor was limiting expression of transcripts that were triggered to accumulate later in growth (because of the large volume of culture that would be required, it was impractical to examine cultures at lower optical densities). What other factor might account for the acyl-HSL-independent triggering of quorum gene induction? An obvious possibility is that the acyl-HSL receptors are limiting in the early logarithmic phase and that the abundance of these factors increases during culture growth. We hypothesize that quorum-controlled promoters that are active in the early logarithmic phase bind the transcription factors and effectively titrate them away from other quorum-controlled promoters. As the level of LasR increases, additional quorum-controlled genes should show expression. A prediction of this hypothesis is that *lasR*, and consequently *rhlR*, should show increased transcript abundance as a culture grows. Thus, we examined the microarray data with respect to *lasR* and *rhlR* (Fig. 7). Starting at an optical density of around 0.8 the levels of the *lasR* and *rhlR* transcripts increased markedly, which is consistent with previous results obtained with reporter fusions (1, 22). For *lasR*, some increase was observed in the wild-type strain and in the signal generation mutant with or without added signal. Thus, the increase was, at least in part, independent of quorum sensing. This result is consistent with but does not constitute proof of the model for timing of quorum-controlled gene expression described above.

DISCUSSION

We used Affymetrix GeneChip technology to expand the list of genes reported to be controlled by quorum sensing in *P. aeruginosa*. In all, we have identified over 300 genes (about 6% of the *P. aeruginosa* genome) that appear to be part of the quorum regulon (Tables 1 and 2 and Fig. 2 and 4). The fact that these genes were identified in two different types of analyses (one of which involved a comparison of a signal generation mutant with itself in the presence of added signals and the other of which involved a comparison of a signal receptor mutant with its parent) lends confidence to our conclusions. Because timing is important in quorum-controlled gene expression, we examined cultures at several different points dur-

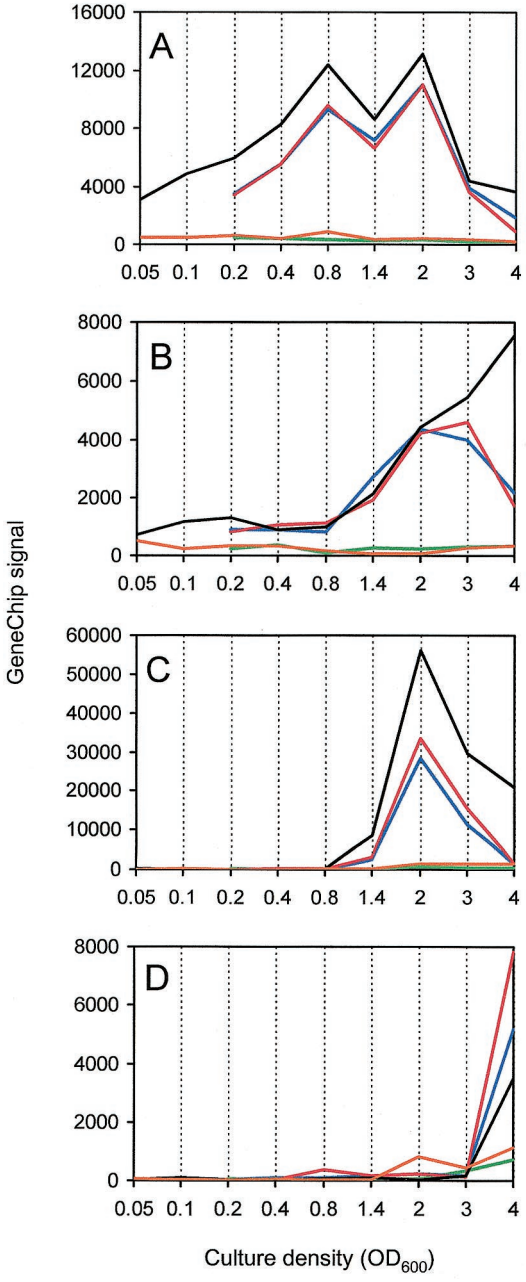

FIG. 6. Transcript levels for selected quorum-activated genes in the wild-type *P. aeruginosa* strain (black lines), in the receptor mutant (orange lines), and in the signal generation mutant without added acyl-HSL (green lines), with 3OC12-HSL (blue lines), and with C4-HSL and 3OC12-HSL (red lines). (A) PA3904; (B) PA4677; (C) PA2939; (D) PA0198. For simplicity, the examples shown are all 3OC12-HSL-specific genes. The values on the *y* axis represent transcript abundance as determined by the array software.

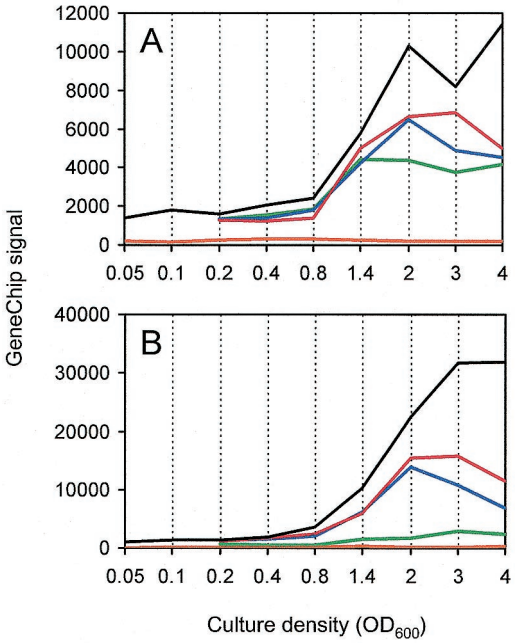

FIG. 7. Expression of *lasR* (A) and *rhlR* (B) in the wild-type *P. aeruginosa* strain (black line), the receptor mutant (orange line), and the signal generation mutant without added acyl-HSL (green line), with 3OC12-HSL (blue line), and with C4-HSL and 3OC12-HSL (red line). The values on the *y* axis represent transcript abundance as determined by the array software.

ing growth. Thus, we minimized problems in identifying genes with relatively unstable transcripts. Nevertheless, the genes that we have identified represent a conservative estimate of the quorum-controlled regulon in *P. aeruginosa*. We filtered the data in several ways, and of course we examined only cultures grown in one medium at one temperature. Additional experiments in which quorum sensing is examined under different conditions are necessary to more fully understand the breadth of quorum sensing as a global regulator of gene expression in *P. aeruginosa*. An accompanying paper (30a) provides further insight in this regard.

Of the genes which we identified, most were induced by quorum sensing, although some were repressed (Tables 1 and 2). Most repressed genes showed a curious response. They showed derepression only in mutant backgrounds (Fig. 3). Perhaps these genes are activated in the wild type under specific conditions other than those which we used. Expression of these genes might require inducers not present in our experiments or might require other environmental conditions.

Although the most strongly activated genes reported by Whiteley et al. (33) were among the most strongly activated genes according to the microarray analysis, we do not want to attach great significance to the levels of induction we observed. For some genes the levels of induction varied substantially between the signal addition analysis and the comparison of the receptor mutant and parent. In the most extreme cases, some genes showed activation by addition of a signal to the signal generation mutant and repression in the comparison of the receptor mutant with the parent. This indicates that there may be other factors in *P. aeruginosa* that contribute to control by acyl-HSLs. One possible factor is the LasR-RhlR signal receptor homolog QscR. We know that this protein somehow influences quorum-controlled gene expression in *P. aeruginosa*, but we do not know how it functions mechanistically (4).

In general, the representation of functional classes in the quorum-controlled regulon was similar to that in the entire *P. aeruginosa* genome. For example, 43% of the genes in Tables

1 and 2 have unknown functions, compared with 46% of the genes in the entire *P. aeruginosa* genome. As expected, many quorum-controlled genes are in the secreted-factor category. There are several genes with adaptation and protection functions, and perhaps more surprising is the finding that there are genes coding for general metabolic functions. Caution should be used when significance is attached to individual genes in a functional group. However, there are genes in the general metabolic function group with well-established functions; for example, PA3183 codes for glucose-6-phosphate dehydrogenase, an enzyme essential for glucose catabolism in *P. aeruginosa*. Why might this gene show quorum control? This is an example of a gene coding for an enzyme with multiple cellular functions. Glucose-6-phosphate dehydrogenase is an NADP-dependent dehydrogenase. As such, it is an NADPH generator, and in fact through this activity it is known to protect *P. aeruginosa* from oxidative stress by enhancing glutathione synthesis (17). We imagine that without quorum sensing glucose-6-phosphate dehydrogenase can be produced in a quantity sufficient for glucose catabolism but that quorum sensing can boost the levels for alternate functions of this enzyme.

We found that the specificities of responses to 3OC12-HSL versus the specificities of responses to the two signals together showed great variability (although genes in apparent operons showed responses similar to each other). Some of the genes shown in Table 1 appeared to respond specifically to 3OC12-HSL; other genes seemed to respond to 3OC12-HSL, but activation was boosted by addition of C4-HSL; and still other genes seemed to respond to C4-HSL, showing no response to 3OC12-HSL alone. A previous view was that some genes show specificity for 3OC12-HSL and others show specificity for C4-HSL (9, 31). Some of the C4-HSL-dependent genes show some relaxation of specificity (32). The array data suggest that there is a continuum of specificity responses.

The idea of a continuum of responses extends to the timing of quorum-controlled gene induction. We were more interested in the timing of induction or repression than the maximal transcript levels because the maximal levels result from a complex set of factors that presumably include rates of transcription and transcript stability. When we examined timing we saw that there was great variability (Table 1 and Fig. 5 and 6). Some genes showed increased expression early in growth; for most genes the onset of induction was in the late logarithmic to early stationary phase; and some genes were not induced until the stationary phase. In general, timing was similar in the wild type and in the signal generation mutant grown in the presence of saturating levels of added signals. This indicates that over the range of culture densities in our experiments, the trigger for quorum-controlled gene activation was not signal accumulation. The *P. aeruginosa* quorum-sensing elements are the two signals synthesized by the signal generators LasI and RhlI and the two signal receptors, LasR and RhlR. If the levels of the signals do not govern the onset of induction, then one might hypothesize that receptor levels govern the onset of induction. In fact, we observed that *lasR* and *rhlR* transcript levels increased during the late logarithmic and early stationary phases (Fig. 7), which coincided with the induction of most quorum-activated genes. This finding is consistent with the hypothesis, but more evidence is required to determine its validity. The *lasR* transcript levels were largely independent of quorum sensing. There are other factors in *P. aeruginosa* that have been reported to control *lasR* transcription. These factors include the regulators Vfr (1) and GacA (26), as well as the stringent response (30).

Our results for the timing of gene expression raise an interesting question. Are genes whose expression is not directly triggered by exogenous signals really quorum controlled? We believe that there is no evidence to the contrary. Activation of any of the genes which we identified requires sufficient signal. Signal can accumulate only when a critical population density has been reached. The fact that under at least some conditions additional criteria must be met for transcriptional activation of many genes in the regulon does not alter the fact that a quorum is nevertheless required. In fact, a culture medium-dependent delay in the induction of *Vibrio fischeri* luminescence, even with ample signal, was observed by Eberhard in 1972 (7).

The finding that signal specificity is on a continuum and the hypothesis that levels of LasR and RhlR might control the precise timing of quorum-controlled gene expression lead to the idea that this regulatory system consisting of two signal generators and two signal receptors can allow for an elaborate pattern of expression of hundreds of genes in *P. aeruginosa*. Genes can be triggered at different times during culture growth, and they can respond to one or both of the signals to various degrees. The simplistic view that quorum sensing leads to the coordinate expression of genes in the quorum-controlled regulon at a critical population density at which the signals have accumulated to a requisite concentration underestimates the complexity of this regulatory circuitry.

ACKNOWLEDGMENTS

We thank Matt Wolfgang and Steve Lory for sharing information and tools for GeneChip use and analysis, we thank Lora Huang from the University of Iowa DNA Facility for array processing, and we thank Niels Bagge for helpful discussions.

This study was supported by grant GM59026 from the National Institute of General Medicine. Support was also provided through a therapeutic development grant from the Cystic Fibrosis Foundation and by a grant from The Procter & Gamble Company, Cincinnati, Ohio. C.P.L. was supported by U.S. Public Health Service training grant T32-AI 07511.

The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of General Medicine.

REFERENCES

1. **Albus, A. M., E. C. Pesci, L. J. Runyen-Janecky, S. E. West, and B. H. Iglewski.** 1997. Vfr controls quorum sensing in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**3928–3935.
2. **Brint, J. M., and D. E. Ohman.** 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. J. Bacteriol. **177:**7155–7163.
3. **Chapon-Herve, V., M. Akrim, A. Latifi, P. Williams, A. Lazdunski, and M. Bally.** 1997. Regulation of the *xcp* secretion pathway by multiple quorum-sensing modulons in *Pseudomonas aeruginosa*. Mol. Microbiol. **24:**1169–1178.
4. **Chugani, S. A., M. Whiteley, K. M. Lee, D. D. Argenio, C. Manoil, and E. P. Greenberg.** 2001. QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **98:**2752–2757.
5. **de Kievit, T., P. C. Seed, J. Nezezon, L. Passador, and B. H. Iglewski.** 1999. RsaL, a novel repressor of virulence gene expression in *Pseudomonas aeruginosa*. J. Bacteriol. **181:**2175–2184.
6. **Diggle, S. P., K. Winzer, A. Lazdunski, P. Williams, and M. Camara.** 2002. Advancing the quorum in *Pseudomonas aeruginosa*: MvaT and the regulation of *N*-acylhomoserine lactone production and virulence gene expression. J. Bacteriol. **184:**2576–2586.

7. **Eberhard, A.** 1972. Inhibition and activation of bacterial luciferase synthesis. J. Bacteriol. **109:**1101–1105.
8. **Fernandez De Henestrosa, A. R., T. Ogi, S. Aoyagi, D. Chafin, J. J. Hayes, H. Ohmori, and R. Woodgate.** 2000. Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. Mol. Microbiol. **35:**1560–1572.
9. **Fuqua, C., M. R. Parsek, and E. P. Greenberg.** 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu. Rev. Genet. **35:**439–468.
10. **Gambello, M. J., and B. H. Iglewski.** 1991. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. J. Bacteriol. **173:**3000–3009.
11. **Gambello, M. J., S. Kaye, and B. H. Iglewski.** 1993. LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (*apr*) and an enhancer of exotoxin A expression. Infect. Immun. **61:**1180–1184.
12. **Glessner, A., R. S. Smith, B. H. Iglewski, and J. B. Robinson.** 1999. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of twitching motility. J. Bacteriol. **181:**1623–1629.
13. **Hassett, D. J., J. F. Ma, J. G. Elkins, T. R. McDermott, U. A. Ochsner, S. E. West, C. T. Huang, J. Fredericks, S. Burnett, P. S. Stewart, G. McFeters, L. Passador, and B. H. Iglewski.** 1999. Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. Mol. Microbiol. **34:** 1082–1093.
14. **Latifi, A., M. Foglino, K. Tanaka, P. Williams, and A. Lazdunski.** 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. Mol. Microbiol. **21:**1137–1146.
15. **Latifi, A., M. K. Winson, M. Foglino, B. W. Bycroft, G. S. Stewart, A. Lazdunski, and P. Williams.** 1995. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. Mol. Microbiol. **17:**333–343.
16. **Lyczak, J. B., C. L. Cannon, and G. B. Pier.** 2000. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microbes Infect. **2:**1051–1060.
17. **Ma, J. F., P. W. Hager, M. L. Howell, P. V. Phibbs, and D. J. Hassett.** 1998. Cloning and characterization of the *Pseudomonas aeruginosa zwf* gene encoding glucose-6-phosphate dehydrogenase, an enzyme important in resistance to methyl viologen (paraquat). J. Bacteriol. **180:**1741–1749.
18. **Ochsner, U. A., and J. Reiser.** 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **92:**6424–6428.
19. **Parsek, M. R., and E. P. Greenberg.** 2000. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. Proc. Natl. Acad. Sci. USA **97:**8789–8793.
20. **Passador, L., J. M. Cook, M. J. Gambello, L. Rust, and B. H. Iglewski.** 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science **260:**1127–1130.
21. **Pearson, J. P., E. C. Pesci, and B. H. Iglewski.** 1997. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J. Bacteriol. **179:**5756–5767.
22. **Pesci, E. C., J. P. Pearson, P. C. Seed, and B. H. Iglewski.** 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**3127–3132.
23. **Pessi, G., and D. Haas.** 2000. Transcriptional control of the hydrogen cyanide biosynthetic genes *hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. J. Bacteriol. **182:**6940–6949.
24. **Pessi, G., F. Williams, Z. Hindle, K. Heurlier, M. T. Holden, M. Camara, D. Haas, and P. Williams.** 2001. The global posttranscriptional regulator RsmA modulates production of virulence determinants and *N*-acylhomoserine lactones in *Pseudomonas aeruginosa*. J. Bacteriol. **183:**6676–6683.
25. **Rahim, R., U. A. Ochsner, C. Olvera, M. Graninger, P. Messner, J. S. Lam, and G. Soberon-Chavez.** 2001. Cloning and functional characterization of the *Pseudomonas aeruginosa rhlC* gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. Mol. Microbiol. **40:**708–718.
26. **Reimmann, C., M. Beyeler, A. Latifi, H. Winteler, M. Foglino, A. Lazdunski, and D. Haas.** 1997. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer *N*-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. Mol. Microbiol. **24:**309–319.
27. **Stintzi, A., K. Evans, J. M. Meyer, and K. Poole.** 1998. Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: *lasR*/*lasI* mutants exhibit reduced pyoverdine biosynthesis. FEMS Microbiol. Lett. **166:**341–345.
28. **Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. Wong, Z. Wu, and I. T. Paulsen.** 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature **406:**947–948.
29. **Toder, D. S., M. J. Gambello, and B. H. Iglewski.** 1991. *Pseudomonas aeruginosa* LasA: a second elastase under the transcriptional control of *lasR*. Mol. Microbiol. **5:**2003–2010.
30. **van Delden, C., R. Comte, and A. M. Bally.** 2001. Stringent response activates quorum sensing and modulates cell density-dependent gene expression in *Pseudomonas aeruginosa*. J. Bacteriol. **183:**5376–5384.

30a. **Wagner, V. E., D. Bushnell, L. Passador, A. I. Brooks, and B. H. Iglewski.** 2003. Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. J. Bacteriol. **185:**2080–2095.

31. **Whitehead, N. A., A. M. Barnard, H. Slater, N. J. Simpson, and G. P. Salmond.** 2001. Quorum-sensing in Gram-negative bacteria. FEMS Microbiol. Rev. **25:**365–404.
32. **Whiteley, M., and E. P. Greenberg.** 2001. Promoter specificity elements in *Pseudomonas aeruginosa* quorum-sensing-controlled genes. J. Bacteriol. **183:** 5529–5534.
33. **Whiteley, M., K. M. Lee, and E. P. Greenberg.** 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **96:**13904–13909.
34. **Whiteley, M., M. R. Parsek, and E. P. Greenberg.** 2000. Regulation of quorum sensing by RpoS in *Pseudomonas aeruginosa*. J. Bacteriol. **182:**4356–4360.
35. **Winson, M. K., M. Camara, A. Latifi, M. Foglino, S. R. Chhabra, M. Daykin, M. Bally, V. Chapon, G. P. Salmond, B. W. Bycroft, et al.** 1995. Multiple *N*-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **92:**9427–9431.
36. **Winzer, K., C. Falconer, N. C. Garber, S. P. Diggle, M. Camara, and P. Williams.** 2000. The *Pseudomonas aeruginosa* lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. J. Bacteriol. **182:**6401–6411.