Strain-dependent diversity in the *Pseudomonas aeruginosa* quorum-sensing regulon

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AUTHOR SUMMARY

Bacteria use a means of chemical signaling called “quorum sensing” to control gene expression in response to cell density. Many Proteobacteria use acyl-homoserine lactones (AHLs) as signals for quorum-controlled regulation of ecologically and medically important traits. Species-dependent differences in quorum regulons have been well documented (1, 2). *Pseudomonas aeruginosa*, a bacterium occupying a range of environmental and clinical habitats, uses AHL signaling for gene regulation. Little is known about strain-dependent differences in the quorum response of *P. aeruginosa* or any other bacterial species. Recent technology advances in bacterial transcriptomics have made it possible to examine the quorum responses of isolates for which genomic sequence information previously was not available. We found that quorum responses of isolates from diverse habitats show differences that appear to reflect evolution under different ecological circumstances and their adaptation to specific niches.

The *P. aeruginosa* quorum-sensing system employs two distinct AHL-signaling molecules, 3OC12-HSL and C4-HSL, which are produced and sensed by the products of the *lasI-lasR* and *rhlI-rhlR* gene pairs, respectively. We generated draft genomes for each of six strains and annotated each genome. We used a streamlined RNA-seq method to enrich for non-rRNA reads without the need for rRNA depletion to profile the transcriptomes of seven *P. aeruginosa* strains from diverse environmental and clinical habitats. We also created and analyzed quorum-sensing mutants of each strain.

The *lasI-lasR* and *rhlI-rhlR* genes were conserved in each strain; however, our analyses show significant variation in the quorum responses of different *P. aeruginosa* strains. Within the confines of our study, the largest quorum regulon consists of 342 genes and belongs to a soil isolate; the smallest consists of 31 genes and belongs to a clinical isolate from a cystic fibrosis (CF) patient with a chronic lung infection. The quorum regulon of the reference laboratory strain PAO1 comprises 161 genes. Variations among strains are caused by differences in gene expression and differences in gene content.

Our genome-sequence analysis is consistent with previous findings of a core set of genes conserved among the great majority of strains and accessory genes with a limited distribution among strains (3). Quorum-controlled genes were distributed among both the core and accessory genes. For two strains, one isolated from soil and one from a tomato plant, we found examples in which an ecological basis can be inferred for traits regulated by strain-variable genes. Among the core set are genes for the production of public goods, secreted and excreted molecules that can be shared by community members. The strain-variable set includes genes encoding general metabolic enzymes, suggesting a link between quorum sensing and metabolic versatility. The strain-variable regulation of several environmentally responsive genes encoding known and putative transcription factors and two component signal-transduction systems suggests that *P. aeruginosa* coordinates quorum sensing with the differential integration of environmental signals.

The present study underscores the importance of probing multiple strains to understand the population-wide significance of quorum sensing for a species. It also provides a methodology and describes an analysis pipeline that makes possible larger-scale studies involving many more isolates than we have studied here. Specifically, our study of strain-dependent variation in *P. aeruginosa* indicates a broader role for quorum sensing in the ecology of *P. aeruginosa* that extends beyond its role in optimizing expression of public goods and virulence factors. Quorum-sensing circuits may be crucial for the ecological fitness of *P. aeruginosa* in diverse environments.

**References**


The authors declare no conflict of interest.

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Data deposition: The draft genome assemblies and annotations have been deposited in the DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank database (accession nos. AKZD00000000, AKZE00000000, AKZF00000000, AKZG00000000, AKZH00000000, and AKBD00000000).

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SI Materials and Methods
For cDNA synthesis, 1 μL of 100 μM selective primer mix was combined with 1 μL (500 ng) of template and 3 μL of water. The mix was heated at 65 °C for 5 min and chilled at 4 °C before adding 5 μL of reverse transcription master mix (2 μL of 5× first-strand buffer, 0.5 μL of 100 mM DTT, 2 μL of 10 mM dNTP, and 0.5 μL of SuperScript III enzyme). The reaction mixture was incubated at 40 °C for 90 min and cooled to 4 °C. For second-strand synthesis, 10 μL of the first-strand reaction was added to 65 μL of the nick-translation mix (45.5 μL of water, 15 μL of 5× second-strand buffer, 1.5 μL of 10 mM dNTP, 0.5 μL of Escherichia coli DNA Ligase, 2.0 μL of E. coli DNA Pol I, 0.5 μL of E. coli RNase H) (New England Biolabs, Inc.). The reaction mixture was incubated at 16 °C for 2 h and was stopped by adding 25 μL of 0.2 M EDTA.

DNA was purified in 35 μL elution buffer by using a QIAquick PCR Purification kit (Qiagen, Inc.). Ten microliters of the eluate was added to 3 μL of quick-blunting mix (1.25 μL 10× buffer, 1.25 μL 1 mM dNTP, 0.5 μL Blunting enzyme mix) (New England Biolabs, Inc.). The reaction mixture was incubated at 23 °C for 30 min and at 70 °C for 10 min and then was chilled before 1 μL of 12.5 μM forward and reverse adaptor mix was added. Forward adaptors were engineered with three-base barcode tags to allow sample multiplexing. The sample-adaptor mix was added to 16.5 μL of the ligation mix (15 μL of 2× Quick Ligase Buffer and 1.5 μL of Quick ligase) (New England Biolabs, Inc.) and was incubated at 23 °C for 15 min. For PCR amplification, 10 μL of the ligated sample was combined with 90 μL of PCR mix [39 μL of water, 10 μL of 10× reaction buffer (+ Mg), 10 μL of 25 mM MgCl₂, 5 μL of DMSO, 5 μL of 10 mM dNTPs, 10 μL of 10 μM forward primer, 10 μL of 10 μM reverse primer, 1 μL of Taq DNA Polymerase] (New England Biolabs, Inc.). The nick resulting from the previous adaptor ligation step was repaired by incubating the samples at 72 °C for 2 min and at 95 °C for 2 min followed by 20 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and then at 72 °C for 5 min to polish ends before cooling to 4 °C. Double-stranded DNA was purified using AMPure XP magnetic beads (Agencourt).