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Microarray Analysis of Toxicogenomic Effects of Peracetic Acid on *Pseudomonas aeruginosa*

WOOK CHANG,[†] DAVID A. SMALL,[†] FRESHTEH TOGHROL,*[,‡] AND WILLIAM E. BENTLEY[†]

Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, Maryland 20742, and Microarray Research Laboratory, Biological and Economic Analysis Division, Office of Pesticide Programs, United States Environmental Protection Agency, Fort Meade, Maryland 20755

Hospital-acquired (nosocomial) infection (HAI) represents a serious threat to public health, both in terms of human casualty and in terms of economic impact. On an annual basis, 2 million individuals require prolonged hospitalization, and an estimated 90 000 patients die due to HAI. Economic damages are reported to exceed $4.5 billion, annually. While many disinfectants, including peracetic acid, have been employed to eradicate infectious bacteria, a lack of understanding their mode of action and the corresponding defense mechanisms hinders successful antimicrobial application. We report here the first transcriptome analysis of the response of *Pseudomonas aeruginosa*, a pathogen infecting those with cystic fibrosis, upon 20 min exposure to a sublethal concentration (1 mM) of peracetic acid. As a result, we identified that 570 out of a total of 5570 *P. aeruginosa* genes showed statistically significant transcript level changes. Our findings indicate that (i) many genes associated with cellular protective processes were induced, (ii) the transcription of genes involved in primary metabolic pathways was repressed, and (iii) the transcription of genes encoding membrane proteins and small molecule transporters was altered. We also observed that genes within operons were highly cotranscribed in this study. Finally, this global transcriptional profile can help identify signature genes that are also activated with other oxidative disinfectants, which may be used to design new more effective treatments or more efficaciously apply existing compounds.

Introduction

Hospital-acquired infections have drawn much attention over the past decade. Despite improvement in infection control methods, the U.S. Centers for Disease Control and Prevention (CDC) reported that nearly 2 million patients obtain an infection annually during hospital treatment and nearly 90 000 die as a direct or indirect cause of these infections (*1*, *2*). Moreover, the economic cost of these infections mainly resulting from the extended length of stay in the hospital and the additional use of medical and therapeutic resources is $4.5 billion a year (*1*). One of the reasons of this incidence is that an increasing number of pathogenic bacteria has become resistant to antimicrobials including disinfectants, which causes reduced bacterial susceptibility. Certainly, proper use of specific disinfecting agents on hospital surface and patient care items is imperative so as to prevent infection outbreaks in health care environments (*3*−*5*). Hence, the U.S. Environmental Protection Agency (EPA) has focused its efforts on determining the efficacy and the mode of action of antimicrobial products. More than 5000 antimicrobial products have been registered with the EPA, and 800 hospital-level disinfectants are currently being tested against infectious bacteria. Particularly, *Pseudomonas aeruginosa* (*P. aeruginosa*) has been of much concern because of its ability to infect humans, including those with defective immune systems, such as those affected by diseases such as cystic fibrosis (*1*, *2*). Further, its intrinsic resistance to antibiotics and antimicrobials limits disinfection efficacy (*6*−*8*).

Oxidative disinfectants such as hydrogen peroxide (H_2O_2) and peracetic acid (CH_3CO_3H) have been widely used to eradicate pathogens including *P. aeruginosa* in hospital environments. Peracetic acid, a peroxide of acetic acid, has drawn much interest lately since it is a more potent antimicrobial agent than hydrogen peroxide with nontoxic residuals (*9*−*11*). Thus, many antimicrobial products contain peracetic acid as an active ingredient, and its killing activity and its mechanism have been studied by the EPA. However, despite prior speculation about the disinfectant activity (based on the release of active oxygen), the mechanism of action of peracetic acid remains obscure (*11*−*13*). In particular, there are few approaches that enable global analysis of a cells' response to peracetic acid, and to date these have not appeared. That is, advances in the fields of genomics and proteomics, including whole-genome DNA microarrays and peptide mapping of spots excised from 2-D gel electrophoretograms, show great promise to provide a more comprehensive analysis of cellular regulation. The outcomes can help to map affected cell functions and serve to delineate the mechanisms involved in disinfectant activity. For instance, recent transcriptome analyses with antimicrobials such as hydrogen peroxide and paraquat have revealed sets of genes that are related to the protective mechanisms of *Escherichia coli*, *P. aeruginosa*, and yeast (*14*−*17*). On the basis of this information, it is hypothesized that new agents for specific pathogens can be developed, leaving minimal effects on other biospecies, including humans. Moreover, we can identify signature genes that are also activated with other oxidative disinfectants so that we can efficaciously apply existing compounds.

Here, we investigated genome-wide changes in *P. aeruginosa* gene transcription upon 20 min exposure to 1 mM peracetic acid using Affymetrix *Pseudomonas* GeneChip arrays, to advance our understanding of peracetic acid-driven gene regulation and cellular response. To corroborate the array results, we then performed real-time PCR analysis on selected genes. In particular, we demonstrated that a number of genes associated with protective and primary metabolic processes was strongly regulated in the presence of peracetic acid. We also found that some of the membrane and/or transport proteins were inactivated. Noticeably, we showed that genes within operons were highly cotranscribed in this study. To our knowledge, the present study is the first microbial microarray analysis of global gene transcription upon exposure to peracetic acid. Consequently, the results

* Corresponding author phone: (410)305-2755; fax: (410)305-3091; e-mail: toghrol.freshteh@epa.gov.
[†] University of Maryland Biotechnology Institute.
[‡] U.S. EPA.

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TABLE 1. Transcript Level Comparison of *P. aeruginosa* Genes between Real-Time PCR Analysis and Microarray Analysis[a]

gene	MRNA level change with real-time PCR	mRNA level change with microarray	sense primer sequence	antisense primer sequence
PA4613 (*katB*)	3.37 ± 0.95	6.70	5′-GAGCAGAACTTCAAGCAGAC-3′	5′-CTCTCGTCGTCGGTGATC-3′
PA2850 (*ohr*)	6.72 ± 2.01	12.42	5′-GAGGTCGAACTGCACATC-3′	5′-GGGTAGCGTTGGAGTAGG-3′
PA4763 (*recN*)	2.99 ± 0.65	2.58	5′-GGAGCAGGAGCAGAAGAC-3′	5′-GTTGAGGCTGGCATTGAG-3′
PA5530	3.52 ± 0.88	6.38	5′-AAGAAGGAAGAGCCGAAGG-3′	5′-ATGTAGGTGGTGTAGGTGTAG-3′
PA0576 (*rpoD*)			5′-CGTCCTCAGCGGCTATATCG-3′	5′-TTCTTCTTCCTCGTCGTCCTTC-3′

[a] The results are the mean of five replicate experiments ± standard error.

presented herein may facilitate the further elucidation of the mechanisms involved in the toxicity of peracetic acid.

Materials and Methods

Bacterial Strains and Growth Conditions. *P. aeruginosa* PA01 was obtained from the laboratory of Dr. E. Peter Greenberg at the University of Iowa. The strain was initially grown in sterilized Luria−Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, and 10 g of sodium chloride per liter) at 37 °C with shaking at 250 rpm for 17 h. Culture volumes for all growth conditions were adjusted to be less than 1/10 the total flask volume to maximize aeration. The overnight cultures were diluted 1:100 in pre-warmed LB broth and incubated at 37 °C with shaking at 250 rpm until an optical density at 600 nm (OD_{600}) reached the early logarithmic phase (∼0.8). Then, the cells were diluted again 1:10 in prewarmed LB broth and incubated at 37 °C with shaking at 250 rpm (*18*). Various concentrations of peracetic acid (Aldrich Chemical Co., St. Louis, MO) were added immediately after the OD_{600} reached 0.8. Cell growth was monitored for 60 min, by measuring OD_{600} with a Lambda 25 spectrophotometer (PerkinElmer, Inc., MA).

RNA Isolation. For microarray analysis, total RNA was isolated from cultures incubated with and without 1 mM peracetic acid for 20 min using RNeasy Mini kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. A total of 2 mL of RNAprotect Bacteria Reagent (Qiagen, Inc., Valencia, CA) was added to 1 mL of cultures before the isolation for the stabilization of RNA. Briefly, cells in the RNAprotect Bacteria Reagent were harvested by centrifugation (5000 rpm, 10 min) and then incubated in TE buffer with 1 mg/mL lysozyme (Roche Applied Science, Indianapolis, IN). Finally, samples were eluted through a silica gel-based membrane with 50 μL of nuclease-free water (Ambion Inc., Austin, TX). Total RNA was treated with DNase I (Qiagen, Inc., Valencia, CA) to eliminate chromosomal DNA contamination. RNA quality was determined using both Lambda 25 spectrophotometer (PerkinElmer, Inc., MA) and RNA 6000 Nano LabChip with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

cDNA Synthesis and Labeling. cDNA was synthesized from 12 μg of total RNA with random primers and SuperScript II (both from Invitrogen Corp., Carlsbad, CA) according to the protocol for the Affymetrix *P. aeruginosa* GeneChip arrays (Affymetrix, Inc., Santa Clara, CA). Control transcripts from *Bacillus subtilis* genes *dap*, *thr*, *phe*, and *lys* (GeneChip Poly-A RNA Control Kit, Affymetrix, Inc., Santa Clara, CA) were spiked into RNA mixtures as internal controls to monitor labeling, hybridization, and staining efficiency. cDNA purified with a QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA) was then fragmented at 37 °C for 10 min by addition of DNase I (0.06 U/μg of cDNA) (Roche Applied Science, Indianapolis, IN) in One Phor-All buffer (Invitrogen Corp., Carlsbad, CA). An Enzo BioArray Terminal Labeling Kit with Biotin-ddUTP (Enzo Life Sciences, Inc., Farmingdale, NY) was utilized to label 3′ termini of fragmented cDNA. Labeling efficiency was assessed using a gel-shift assay with NeutrAvidin (Pierce Biotechnology, Inc., Rockford, IL).

Hybridization and Scanning. A hybridization cocktail was prepared with fragmented and labeled cDNA and B2 control oligonucleotide, which provides alignment signals for image analysis (Affymetrix, Inc., Santa Clara, CA). The cocktail was hybridized onto *P. aeruginosa* GeneChip arrays at 50 °C for 16 h. The arrays were washed and stained with ImmunoPure streptavidin (Pierce Biotechnology, Inc., Rockford, IL), anti-streptavidin goat antibody (Vector Laboratories, Inc., Burlingame, CA), and *R*-phycoerythrin streptavidin (Molecular Probes, Inc., Eugene, OR) using GeneChip Fluidics Station 450. Finally, the arrays were scanned with the Affymetrix GeneChip Scanner 3000.

Data Analysis. Data analysis was performed with the Affymetrix GeneChip Operating Software (GCOS) v. 1.0 and Data Mining Tool (DMT) v. 3.1 (Affymetrix, Inc., Santa Clara, CA). The following parameters were employed for GCOS expression analysis: alpha 1, 0.04; alpha 2, 0.06; tau, 0.015; and target signal, 150. Alpha 1 and 2 are significance levels that define detection calls, while tau determines analysis sensitivity (*19*). Further, the average intensity of arrays was scaled to target signal. The fold change was calculated as the ratio between the signal averages of four untreated (control) and five peracetic acid-treated (experimental) cultures. Note that signal intensity threshold for the fold change calculation in DMT was set to 20; thus, if the signal of a probe set is less than 20, the probe was excluded from the average signal calculation. The GCOS detection calls of present, marginal, and absent are determined based on the Affymetrix detection algorithm (*19*). This call indicates whether a transcript is reliably detected (present) or not detected (absent). Genes that received absent calls from 50% or more of the replicates in GCOS were not used for the analysis. Finally, gene transcription changes with statistical significance were identified by the Mann−Whitney test (cutoff *p*-value, 0.05).

Microarray Data Evaluation Using Quantitative Real-Time PCR Analysis. Transcript level changes obtained with the microarray analysis (four genes) were evaluated by using quantitative real-time PCR. Genes and primer sequences employed for the real-time PCR analysis are listed in Table 1. The real-time PCR was performed by utilizing an iCycler iQ Real-Time PCR Detection System with iScript cDNA Synthesis Kit and One-Step RT-PCR Kit with SYBR Green (BioRad Laboratories, Inc., Hercules, CA). As stated previously, RNA samples were treated with DNase I (Qiagen, Inc., Valencia, CA). DNA contamination was precluded after analysis with Agilent 2100 Bioanalyzer LabChip and gel electrophoresis. In this paper, relative quantification based on the relative expression of a target gene versus a reference gene was utilized to determine transcript level changes (*20*). For each gene, five biological replicates with three technical replicates each were employed. PCR efficiencies were also derived from standard curve slopes in the iCycler software v. 3.1 (BioRad Laboratories, Inc., Hercules, CA). Finally, a melt-curve analysis was performed to evaluate PCR specificity

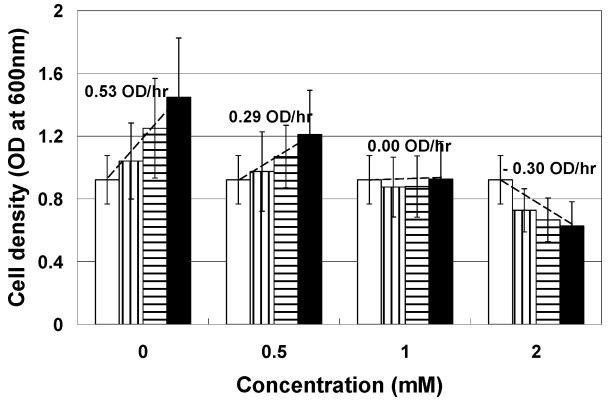

FIGURE 1. ***P. aeruginosa*** **growth after treatment with peracetic acid (bars indicate optical density (*A* at 600 nm)): 0 min (white), 20 min (vertical stripes), 40 min (horizontal stripes), and 60 min (solid) following exposure. The slope of the dotted line represents a growth rate for 60 min (OD/h). The results are the mean of triplicate experiments; the error bars represent standard deviations.**

and resulted in single primer-specific melting temperatures (*21*).

Results and Discussion

Growth Inhibition by Peracetic Acid. The first step of the study was to determine the concentration that causes strong growth inhibition but not cell death because large transcriptional changes were desired. As a result, *P. aeruginosa* was exposed to four concentrations of peracetic acid (0.0, 0.5, 1.0, and 2.0 mM), and the extent of the growth inhibition was monitored for 60 min. Note that the minimum inhibitory concentration of peracetic acid on *P. aeruginosa* is reportedly 1.3 mM (*10*). As shown in Figure 1, even 0.5 mM peracetic acid exhibited inhibitory effects on the growth. In this study, 1 mM peracetic acid concentration was selected since this concentration did not result in either a large increase or decrease in OD_{600} (Figure 1); thus, a sublethal response was likely induced in *P. aeruginosa*. This is consistent with a previously reported result that a low concentration (<3 mM) of hydrogen peroxide, a decomposition product of peracetic acid, activates a protective response in *E. coli* (*22*, *23*).

Transcriptional Profiles in Response to Peracetic Acid. To investigate early transcriptional changes in response to peracetic acid exposure, we isolated total RNA after 20 min treatment. As stated previously, four and five independent microarray experiments were performed in the absence (control) and the presence (experimental) of 1 mM peracetic acid, respectively. Genome-wide microarray analysis initially suggested that 1926 and 1133 out of a total of 5570 *P. aeruginosa* genes showed transcript level increases and decreases after the peracetic acid treatment, respectively. We refer to statistically significant changes in transcript level for those genes that meet the following criteria: (i) a *p*-value for a Mann−Whitney test should be less than 0.05, (ii) an absolute fold change in transcript level should be equal to or greater than 2, and (iii) a gene should have a presence or marginal call (*19*) from 50% or more replicates on both experimental and control replicate sets. On the basis of these criteria, we pared the set to 387 and 183 genes (a total of 570) that had statistically significant increases and decreases in transcript level, respectively. Interestingly, it was previously demonstrated that a 10 min exposure of 1 mM hydrogen peroxide caused 140 (≥4-fold) and 1854 (≥2-fold) mRNA level changes in *E. coli* and *P. aeruginosa*, respectively (*14*, *17*). Additionally, Salunkhe et al. also found 55 genes from three different *P. aeruginosa* strains, which exhibited 2-fold or more transcription changes by 0.5 mM paraquat (2 h) (*16*). The entire dataset of average signals, *p*-values, and fold changes for all 5790 predicted *P. aeruginosa* open reading frames (ORF) are in the Supporting Information.

To strengthen the validity of the relative transcript levels obtained by the microarray analysis, we employed quantitative real-time PCR analysis on *katB*, *ohr*, *recN*, and PA5530. These genes were selected because they showed a range of mRNA level increases (3−12-fold). Moreover, PA0576 (*rpoD*) was used as a control gene for the relative mRNA level calculation due to the fact that *rpoD* exhibits stable expression level (*24*). As shown in Table 1, the values obtained with real-time PCR have a good correspondence with the results of the arrays.

In our attempt to understand how genes with significant transcription changes were distributed with regard to their functions, we performed functional classification on the 570 genes. For this classification, *P. aeruginosa* gene annotation information was obtained from the *Pseudomonas aeruginosa* Community Annotation Project. As shown in Figure 2, these genes were principally grouped in the hypothetical, unclassified, unknown, transport of small molecules, and membrane proteins classes. It is of particular interest that the membrane proteins class was considerably altered with peracetic acid herein, which is discussed next, but not with hydrogen peroxide in prior research by Palma et al. (*17*). This difference can be probably accounted for by the acidity of peracetic acid, which is known to affect membrane integrity (*13*, *25*).

Genes Induced in Response to Peracetic Acid. In Table 2, we displayed the list of genes whose mRNA levels exhibited the highest fold increases in this study, some of which are discussed next. To further analyze genes with increased transcriptional levels, we examined the results of the functional classification. Figure 2 shows that these genes mainly belonged to the classes of adaptation, protection, chemotaxis, DNA replication, recombination, modification and repair, motility and attachment, protein secretion and export apparatus, and transcriptional regulators. Furthermore, with regard to their functions, genes in these classes are suspected to participate in cellular protective processes against peracetic acid.

Particularly important was the discovery that peracetic acid treatment triggered the transcription of genes encoding superoxide dismutase (SOD), catalase, alkyl hydroperoxide reductase, and glutathione peroxidase/reductase, which comprise the oxidant defense systems of *P. aeruginosa* (*26*, *27)*. Interestingly, it was earlier speculated that peracetic acid combines the active oxygen characteristics of peroxide but from an acetic acid molecule, in that the release of active oxygen is presumably the major bactericidal activity and that peracetic acid might function as much as other peroxides and oxidizing agents (*11*, *13*). Thus, the result here may reinforce the speculation regarding the similarity between peracetic acid and peroxides by demonstrating that peracetic acid also induced antioxidant genes.

To be specific, PA2025 (*gor*) and PA2826, which were induced by 3- and 16-fold after the peracetic acid treatment, are reportedly related to glutathione reductase and glutathione peroxidase, respectively (Table 2). Glutathione peroxidase removes hydrogen peroxide with the aid of glutathione, and glutathione reductase recycles glutathione for further hydrogen peroxide removal (*23*). As mentioned earlier, hydrogen peroxide is one of the decomposition products of peracetic acid along with acetic acid, oxygen, and water (*28*). Thus, it is presumed that PA2025 and PA2826 participated in these roles for the detoxification of hydrogen peroxide in the presence of peracetic acid. Interestingly, as presented in Table 2, the neighboring genes, PA2825 (probable transcriptional regulator) and PA2827, were also among the genes most strongly upregulated by peracetic acid, suggesting that PA2825−PA2827 may be organized in an operon.

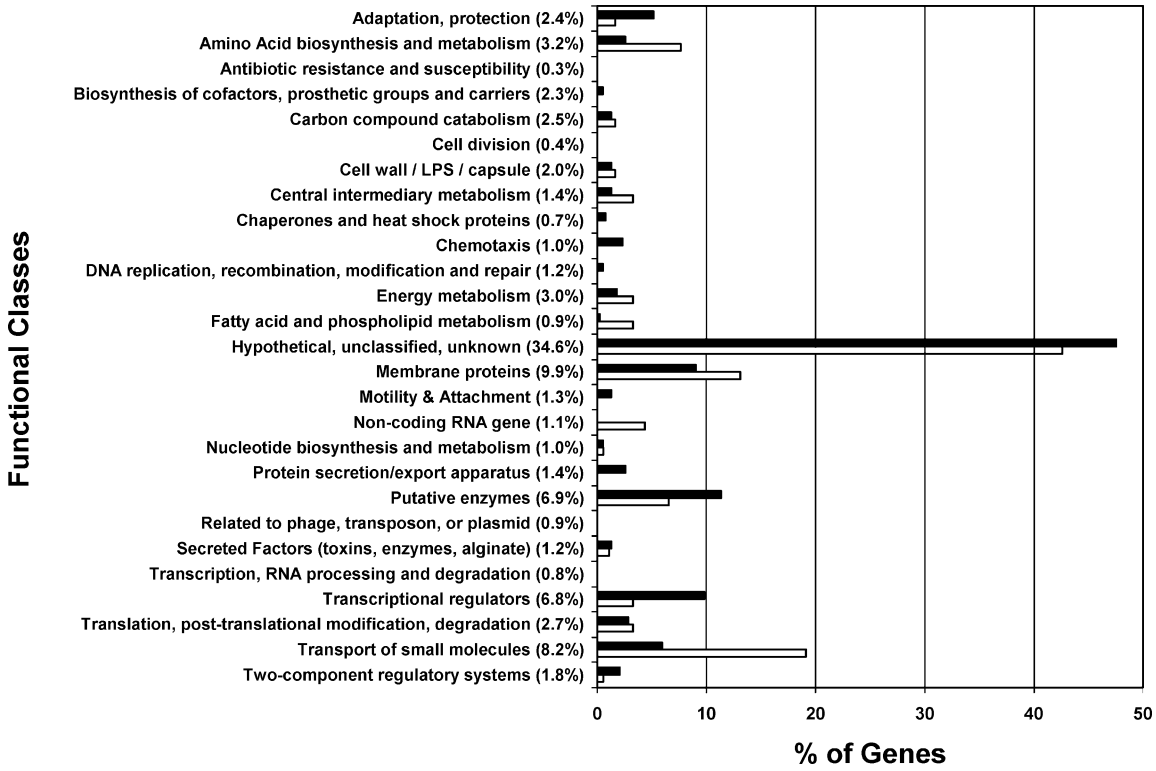

FIGURE 2. Functional classification of genes (consistent with the *P. aeruginosa* Community Annotation having increased (solid) and decreased (white) levels of mRNA (≥2-fold)). The number in parentheses represents the percentage of the total number of genes within the genome in each functional class. The percentages of genes are based on 387 and 183 genes, which showed statistically significant mRNA level increases and decreases, respectively.

This study also revealed that peracetic acid induced several alkyl hydroperoxide reductase genes such as PA2850 (*ohr*), PA0848 (*ahpB*), and PA0140 (*ahpF*), which are essential for optimal resistance to oxidative stress compounds (*27*, *29*). In particular, PA2850 (*ohr*), PA0140 (*ahpF*), PA0849 (*trxB2*), and PA0848 (probable alkyl hydroperoxide reductase gene, *ahpB*) exhibited mRNA level increases by 2−12-fold. On the other hand, PA0139 (*ahpC*) showed an insignificant increase (<2-fold). This low increase could be explained by the fact that *ahpC* is already normally expressed at a high level during aerobic growth (*27*), so that significant induction might be difficult to track. Consistent with this hypothesis, our data showed that *ahpC* had considerably high average signal intensities in both control and peracetic acid-treated replicates. Finally, it should be mentioned that although *ahpB*, *ahpC*, and *ahpF* encode proteins for organic hydroperoxide detoxification, they are also important for resistance to hydrogen peroxide (*30*).

As expected, a catalase gene, PA4613 (*katB*), was strongly induced (7-fold) with the peracetic acid treatment (Table 2). The *katB* gene is mainly inducible upon exposure to hydrogen peroxide, leaving oxygen and water as byproducts (*31*, *32*). It is notable that PA4612, adjacent to *katB*, also showed a significant mRNA level increase (2-fold), coincident with the prior report that PA4612 expression is required for optimal KatB activity (*33*). In addition, our data indicated that the mRNA level of PA4236 (*katA*) was not dramatically increased (<2-fold) with peracetic acid. Interestingly, other studies reported a similar outcome that *katA*, also highly induced in all phases of growth like *ahpC*, exhibits only a slight increase in expression upon exposure to oxidants (*16*, *18*, *27*, *34*).

Especially noteworthy was the observation that two SOD genes, PA4468 (*sodM*) and PA4366 (*sodB*), showed no significant transcriptional changes with peracetic acid despite their role as superoxide (O_2^-) scavengers in *P. aeruginosa* (*35*, *36*). This result is comparable to that of recent similar reports, which demonstrated a high mRNA level increase in PA4468 (*sodM*) but not in PA4366 (*sodB*) with paraquat and hydrogen peroxide (*16*, *17*). Although the reason is unclear, the difference could be attributed to the significant response of PA4468 (*sodM*) under iron limitation (*16*, *34*, *37*, *38*). This hypothesis is intriguing since our study also found that none of iron starvation-inducible genes that were previously induced in response to hydrogen peroxide (*pchR*, *pchB*, *phcD*, *fptA*, *pvdS*, *pfeR*, *tonB*, and PA0471) (*17*) was identified with peracetic acid. Besides, whereas the whole PA4468−PA4471 operon, also inducible by iron starvation, was significantly expressed in both peroxide and superoxide reports (*16*, *17*), only PA4469 and PA4470 (*fumC*, a fumarate hydratase gene) were upregulated in this work. Further, the high transcript level increase of an iron storage protein gene here (PA4880, probable bacterioferritin gene) suggests that this gene might be involved in the regulation of iron to impede the limitation. At this point, it is unclear why neither hydrogen peroxide (*17*) nor peracetic acid (in this study) has caused a significant transcriptional change to *sodB*, an iron-cofactored superoxide dismutase. However, it should be pointed out that in our work, the transcript level of *sodB* was high both with and without peracetic acid, which is consistent with the hypothesis that this mutase may be highly expressed to counter superoxide production even during normal aerobic respiration.

Of interest was that a number of genes inducible by DNA damage displayed transcript level increases upon exposure to peracetic acid. Earlier, it was disclosed that hydrogen peroxide causes oxidative DNA damage by generating hydroxyl or ferryl radicals (*23*, *39*−*41*). Moreover, the exposure of hydrogen peroxide results in the induction of SOS regulon genes, such as *lexA* and *recA* (*17*). In this study, it was surprising that *lexA* and *recA* were not considerably induced by peracetic acid; yet, PA0610 (*prtN*), PA0611 (*prtR*), PA0923 (*dinP*, DNA damage inducible protein), and PA4763 (*recN*,

TABLE 2. List of the 50 *P. aeruginosa* Genes Most Strongly Induced and Repressed with Peracetic Acid

gene (name)	fold change	*p*-value	protein name
			Induction
PA0565	58.14	0.008	conserved hypothetical protein
PA3281	48.58	0.008	hypothetical protein
PA3283	41.46	0.008	conserved hypothetical protein
PA3282	40.71	0.008	hypothetical protein
PA4692	31.26	0.008	conserved hypothetical protein
PA3284	29.19	0.008	hypothetical protein
PA2827	27.71	0.008	conserved hypothetical protein
PA2691	22.68	0.008	conserved hypothetical protein
PA4691	17.62	0.008	hypothetical protein
PA2826	15.77	0.008	probable glutathione peroxidase
PA2825	14.64	0.008	probable transcriptional regulator
PA3459	13.35	0.008	probable glutamine amidotransferase
PA2850 (*ohr*)	12.42	0.008	organic hydroperoxide resistance protein
PA5470	11.92	0.008	probable peptide chain release factor
PA5471	11.40	0.008	hypothetical protein
PA4154	9.26	0.008	conserved hypothetical protein
PA2331	8.24	0.024	hypothetical protein
PA2485	8.11	0.008	hypothetical protein
PA3690	8.03	0.008	probable metal-transporting P-type ATPase
PA3720	7.65	0.008	hypothetical protein
PA3937	7.48	0.048	probable ATP-binding component of ABC taurine transporter
PA0805	7.48	0.008	hypothetical protein
PA3280 (*oprO*)	7.23	0.008	outer membrane porin OprO precursor
PA0603	6.84	0.008	probable ATP-binding component of ABC transporter
PA4613 (*katB*)	6.71	0.008	catalase
			Repression
PA4131	8.33	0.008	probable iron−sulfur protein
PA1869	5.69	0.008	probable acyl carrier protein
PA2114	5.57	0.008	probable MFS transporter
PA2113	5.53	0.008	probable porin
PA2111	4.89	0.008	hypothetical protein
PA3905	4.34	0.008	hypothetical protein
PA4024 (*eutB*)	4.14	0.008	ethanolamine ammonia−lyase large subunit
PA3189	3.98	0.008	probable permease of ABC sugar transporter
PA2194 (*hcnB*)	3.96	0.008	hydrogen cyanide synthase
PA4133	3.83	0.008	cytochrome *c* oxidase subunit
PA2193 (*hcnA*)	3.76	0.008	hydrogen cyanide synthase
PA1659	3.72	0.008	hypothetical protein
PA1073 (*braD*)	3.71	0.008	branched-chain amino acid transport protein *BraD*
PA4514	3.62	0.008	probable outer membrane receptor for iron transport
PA3190	3.62	0.008	probable binding protein component of ABC sugar transporter
PA2110	3.52	0.008	hypothetical protein
PA1657	3.50	0.008	conserved hypothetical protein
PA3904	3.48	0.008	hypothetical protein
PA3038	3.48	0.024	probable porin
PA0094	3.45	0.008	hypothetical protein
PA2112	3.44	0.008	conserved hypothetical protein
PA1658	3.36	0.008	conserved hypothetical protein
PA0087	3.30	0.008	hypothetical protein
PA1845	3.29	0.008	hypothetical protein
PA0866 (*aroP2*)	3.26	0.024	aromatic amino acid transport protein

DNA repair protein gene) showed increased transcription (2−5-fold), suggesting that peracetic acid might produce oxidative agents such as superoxide and hydroxyl radicals that attack DNA (*39*). Besides, *prtN* and *prtR* (transcriptional regulator genes) were also found to have increased transcript levels; these regulate the production of DNA damage-inducible pyocin (*42*).

Other noticeable results included mRNA level increases of genes reportedly inducible by heat shock and weak acid. Several genes probably encoding heat shock proteins exhibited higher (2−4-fold) transcription levels after the treatment: PA3126 (*ibpA*), PA3881 (*hscB*), and PA4542 (*clpB*). This result is interesting because it was previously described that heat shock proteins may play a role in the regulation of antioxidant enzyme production in *E. coli* (*23*). Further, a mRNA level increase (5-fold) of weak-acid-inducible PA4378 (*inaA*) might be due to the acidity of peracetic acid.

Last, some of the virulence-related genes exhibited transcript level increases upon exposure to peracetic acid. For instance, PA3841 (*exoS*) and PA0044 (*exoT*), which encode exoenzymes S and T, respectively, showed significant mRNA level increases (3-fold). Exoenzyme S is associated with the ability of *P. aeruginosa* to cross epithelial barriers and cause a blood-borne infection (*43*). Recently, it was demonstrated that hydrogen peroxide induces several virulence factor-related genes in *P. aeruginosa* (*17*). Further, virulence-related enzymes are reportedly involved in microbial defense systems against oxidants; that is, microorganisms may secrete these enzymes to damage phagocytes and/or impair oxidants (*23*). For instance, prior studies showed that virulence factors scavenge reactive oxygen species (*44*−*46*). Therefore, this outcome may indicate that *P. aeruginosa* employs virulence-related enzymes as one of the cellular protective mechanisms against oxidative stress.

TABLE 3. Descriptive Statistics for the Cotranscription Level of Adjacent Gene Pairs

	no. of gene pairs[a]	mean $R \pm$ standard error	median R
pairs within operons	136	0.6729 ± 0.0352	0.8622
random pairs	1400	0.3609 ± 0.0126	0.4592

[a] Each gene pair has nine microarray datasets.

Genes Repressed in Response to Peracetic Acid. As shown in Figure 2, exposure to peracetic acid repressed a variety of genes involved in primary metabolic processes including fatty acid biosynthesis and metabolism and energy metabolism. Table 2 displays the list of genes with the highest transcript level decreases in our study. In Table 2, PA1869, PA2193 (*hcnA*), PA2194 (*hcnB*), PA4024 (*eutB*), and PA4133 likely participated in those processes. Notably, our data indicated that part of amino acid or fatty acid biosynthesis could possibly be inhibited by repression (2−3-fold) of such genes as PA0865 (*hpd*), PA1337 (*ansB*), PA1338 (*ggt*), PA2247 (*bkdA1*), PA2248 (*bkdA2*), PA2249 (*bkdB*), PA2250 (*lpdV*), PA2444 (*glyA2*), PA3333 (*fabH2*), PA4758 (*carA*), and PA5112 (*estA*). Next, several genes associated with carbon compound catabolism, central intermediary metabolism, or energy metabolism showed transcription decreases by 2−4-fold (e.g., PA0927 (*ldhA*), PA2003 (*bdhA*), PA2193 (*hcnA*), PA2194 (*hcnB*), PA2195 (*hcnC*), PA2638 (*nuoB*), PA3636 (*kdsA*), and PA4024 (*eutB*)). Evidently, this result suggests that peracetic acid might interfere with proteins, enzymes, and/or intermediary metabolites, ultimately leading to metabolic inhibition and thus lethal damage for *P. aeruginosa*. Alternatively, this phenomenon can probably be described as one of enzyme regulatory activities. That is, since many metabolic enzymes are regulated at the transcriptional level by the end products of their respective metabolic pathways, it is also possible that an increased level of metabolic end products contributed to the repression of these genes.

As briefly mentioned previously, we observed that a large number of genes in the membrane proteins and transport of small molecule classes showed distinct mRNA level decreases with peracetic acid. Among the 25 most down-regulated genes presented in Table 2, more than one-fourth appeared to belong to the class of transport of small molecule (PA2114, PA2113, PA3189, PA4514, PA3190, PA3038, and PA0866 (*aroP2*)). Additionally, peracetic acid suppressed the transcription of many ATP-binding cassette (ABC) transporter and/or membrane protein genes (e.g., PA1339−PA1340−PA1341, PA3187−PA3189−PA3190, PA4496, PA4500, PA4502, PA4503, PA4504, PA5155, and PA5230). As discussed before, this finding possibly implies that membrane components of *P. aeruginosa* were altered and that further, active and/or facilitated transport through the cell membrane was attenuated upon exposure to peracetic acid. This outcome may be utilized to verify earlier speculation that peracetic acid disrupts the lipoprotein cytoplasmic membrane and corresponding transport (*4*, *5*).

Cotranscription of Genes in Operons. Genes within operons are largely regulated together; thus, we expected that these genes would exhibit significant cotranscription throughout nine (four control and five experimental) microarray datasets used in our study. To test this, we compared the cotranscription level of adjacent genes within operons with that of randomly selected adjacent genes across the genome by calculating the Pearson's correlation coefficient (R). We first examined the predicted operons of genes described in this paper, using the predicting operons in microbial genome resource of the Institute for Genomic Research. Accordingly, as shown in Table 3, we analyzed the nine array datasets of 136 adjacent gene pairs in 20 putative operons and of 1400 pairs of randomly selected adjacent genes. Table 3 clearly shows that the mean and median R values were higher for the genes sharing the same operon, indicating that these genes were strongly cotranscribed in this study. This outcome also provides more confidence in the transcript level changes of the genes that we discussed in this paper.

The present study is the first microarray analysis of global gene transcription of *P. aeruginosa* upon exposure to peracetic acid. The overall results herein support the following conclusions: (i) peracetic acid induced genes associated with cellular protective processes such as adaptation, DNA repair, and transcriptional regulation, (ii) peracetic acid repressed the transcription of genes whose products have functions in primary metabolic processes such as amino acid, fatty acid, central intermediary, and energy metabolism, (iii) peracetic acid altered the transcription of genes encoding membrane proteins and/or proteins involved in the transport of small molecules, and (iv) genes within operons were highly cotranscribed in this study. Furthermore, these data may strengthen the findings of prior studies on the function and characteristics of *P. aeruginosa* genes (e.g., *katA*, PA4612-*katB*, *ahpC*, and *sodM*) and the bactericidal action of peracetic acid (e.g., DNA damage and membrane protein disruption). This transcriptome profile can be utilized to better elucidate the mechanisms involved in the toxicity of peracetic acid in *P. aeruginosa*. Finally, based on this information, the identification of signature genes that are also activated with other oxidative disinfectants such as hydrogen peroxide and sodium hypochlorite may reveal general organizational and regulatory information regarding these disinfectants that can be used to design new more effective treatments or more efficaciously apply existing compounds (currently under investigation).

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Supporting Information Available

Probe set signal data for experimental and control samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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