

Comparative Global Transcriptome Analysis of Sodium Hypochlorite, Peracetic Acid, and Hydrogen Peroxide on *Pseudomonas aeruginosa*

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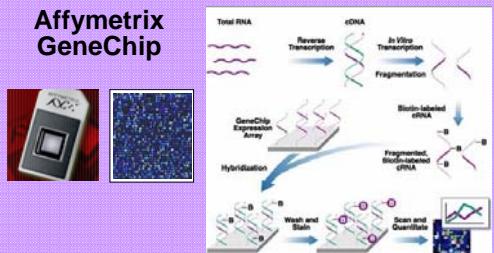
Background

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen capable of infecting almost any immunocompromised tissue. Disinfectants are routinely used in hospitals and healthcare facilities for surface sterilization; however, the extent of the resistance of *P. aeruginosa* to disinfectants remains unknown. Consequently, nosocomial infections from *P. aeruginosa* result in considerable casualties and economic hardship. The goal of this research is to analyze genome-wide changes in *P. aeruginosa* in response to oxidative antimicrobial exposure using DNA microarrays.

Methodology

P. aeruginosa was incubated for 20 min with sodium hypochlorite, peracetic acid, or hydrogen peroxide concentrations causing a strong growth inhibition, but not cellular death, along with a set of water-diluted control samples. Total RNA was then extracted and reverse-transcribed to cDNA, and labeled cDNA was hybridized onto the Affymetrix *P. aeruginosa* GeneChip array. The antimicrobial samples were then normalized to the control samples and compared using GeneSpring GX gene-expression software. Real-time quantitative polymerase chain reaction was used to validate the microarray data.

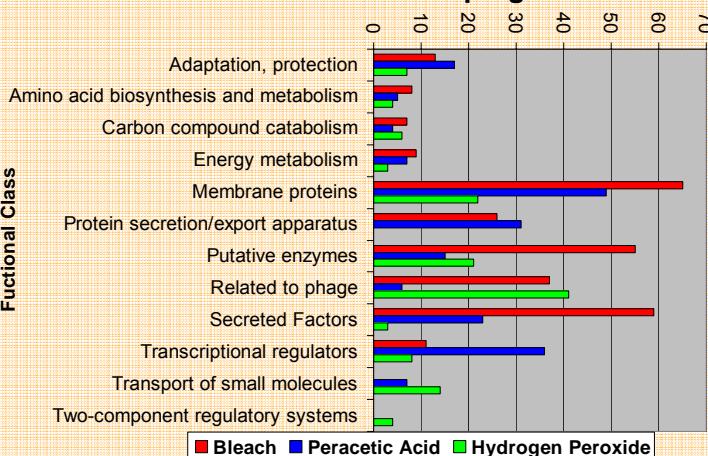
Affymetrix GeneChip



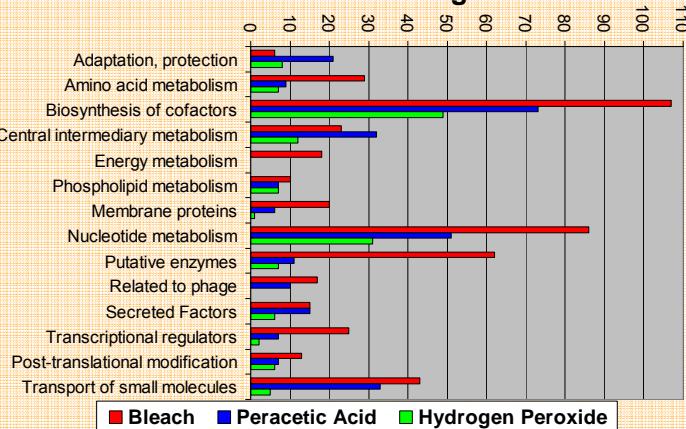
Function classification of the number of genes with statistically significant increased and decreased mRNA levels

The number of genes are shown for each functional classification based on a 2-fold or more increase (upregulated) or a 2-fold or more decrease (downregulated) in mRNA transcript level for 4.4 mM sodium hypochlorite (■), 1 mM peracetic acid (□), and 1 mM hydrogen peroxide (◆) after a 20 min exposure to each corresponding disinfectant.

Number of Genes Upregulated

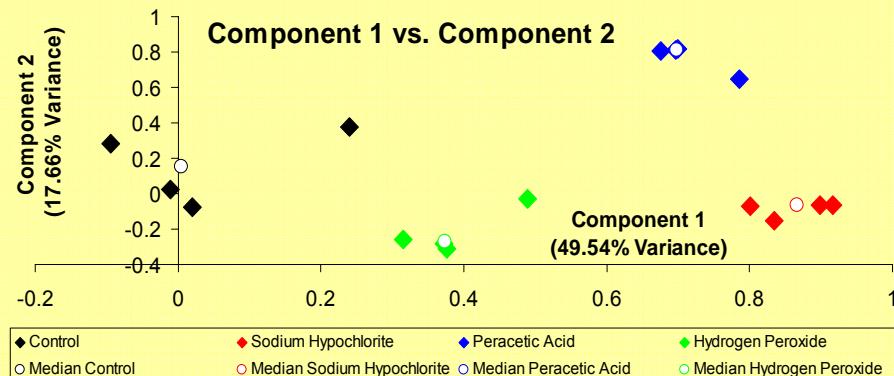


Number of Genes Downregulated



Principal Component Analysis (PCA) of Variance on Conditions

A PCA on conditions reveals that the control samples (■), 4.4 mM sodium hypochlorite-exposed samples (◆), 1 mM peracetic acid-exposed samples (□), and 1 mM hydrogen peroxide-exposed samples (◆) group into distinct regions indicating that all three oxidative disinfectants did not affect the cells in the same way.



2-Fold or More Change summary of the Statistically Significant Genes

From the 5,670 genes in the *P. aeruginosa* genome, 3,197 genes showed statistical significance based on a 1-way ANOVA (95% confidence level).

Disinfectant	Genes Upregulated	Genes Downregulated
NaOCl	457	625
CH ₃ CO ₂ H	362	413
H ₂ O ₂	187	204

Conclusions

- Sodium hypochlorite-exposed cells had downregulations of the genes involved in oxidative phosphorylation, electron transport, and glucose metabolism, and upregulation of organic sulfur transport genes.
- Hydrogen peroxide-exposed cells had an upregulation of DNA protection genes that translated into an upregulation of pycocins (bacteriocins) synthesis genes.
- Peracetic acid-exposed cells had a downregulation of glucose transport genes, but an upregulation of glycerol transport and metabolism genes.
- All three disinfectants resulted in an increase in the genes encoding adaptive and protective process.

