Microarray Analysis of Toxicogenomic Effects of Sodium Hypochlorite on *Pseudomonas aeriginosa*

David Small[1], Wook Chang[1], Freshteh Toghrol[2], and William E. Bentley[1]

1. Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, Maryland

2. Microarray Research Laboratory, Biological and Economic Analysis Division , Office of Pesticide Programs, U. S. Environmental Protection Agency

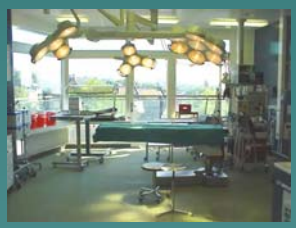
ABSTRACT

Sodium hypochlorite has been utilized as an antimicrobial on pathogenic bacteria such as *Pseudomonas aeruginosa.* Previous studies have not been able to elucidate the mechanisms by which sodium hypochlorite impacts antimicrobial activity, because of the inability to determine the transcriptional responses to the entire genome. The goal of this study was to determine global gene expression changes in *P. aeruginosa* after 20 minute exposure to 4.4 mM sodium hypochlorite by means of Affymetirx Pseudomonas GeneChip arrays. Real-time RT-PCR was used to validate data from the GeneChip arrays. To our knowledge, this is the first microarray analysis of *P. aeruginosa* genome with sodium hypochlorite. The results suggested that sodium hypochlorite induces cellular protective processes related to secreted factors and cellular defenses and represses primary metabolic pathways related to oxidative phosphorylation and electron transport. This global transcriptional profile can be used to further advance the understanding of the mechanisms involved in toxicity of sodium hypochlorite in *P. aeruginosa.*

BACKGROUND

Antimicrobial Pesticides and Pathogens

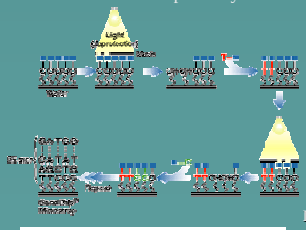
Hospitals are breeding grounds for infectious germs such as *P. aeruginosa*. *P. aeruginosa* is able to infect humans with defective immune systems (such as those suffering from cystic fibrosis). Therefore, chemical disinfectants must be used to cover the large surface areas and sensitive equipment.



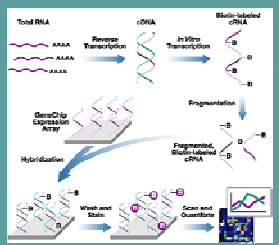
OPP established the Microarray Research Laboratory at Fort Meade, Maryland in July, 2003 to better understand how antimicrobial pesticides work at the genetic level. The results from this research will provide a better and faster method to test the efficacy of antimicrobial compounds, resulting in increased efficiency for the OPP Antimicrobial Testing Program. More importantly, this research work will help the American public and have potential application to health care facilities and patients.

Microarray Technology (GeneChip®)

The Microarray Research Laboratory employs DNA microarray or Gene-chips, a newly-developing technology and method, used to detect genotoxic effects of various disinfectants on bacterial cell response. By using microarrays, the lab produces global gene expression profiles in pathogenic bacteria following exposure to antimicrobial agents. Affymetrix uses a unique combination of photolithography and combinatorial chemistry to manufacture GeneChip® Arrays.



**from Affymetrix web site*

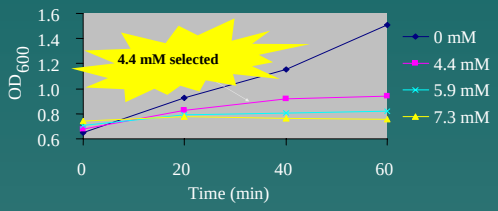


Labeled cDNA or cRNA targets derived from the mRNA of an experimental sample are hybridized to nucleic acid probes attached to the solid support.

By monitoring the amount of label associated with each DNA location, it is possible to infer the abundance of each mRNA species represented.

RESULTS

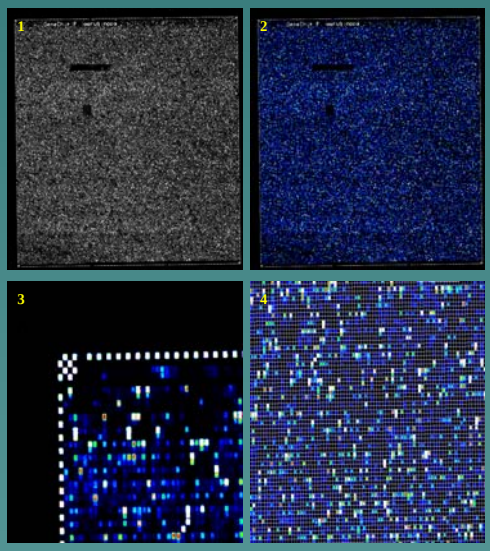
Growth Curve After Sodium Hypochlorite Exposure



Growth of *P. aeruginosa* after exposure to varying concentrations sodium hypochlorite. Sodium hypochlorite below 4 mM did not show significant inhibition. Concentrations are selected that inhibit cell growth, but do not kill the bacteria instantaneously.

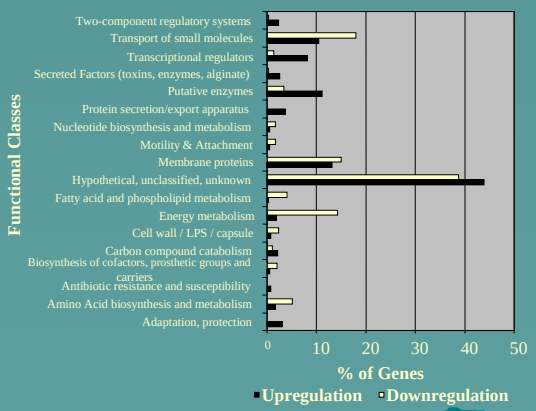
GeneChip® Data

1. A fluorescent intensity scan of a GeneChip®.
2. False-coloring of the scanned GeneChip®.
3. A zoomed image of the corner of the GeneChip®.
4. A zoomed image of the GeneChip® with an overlaid grid.



Function Classification

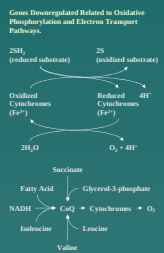
Individual genes can be analyzed on the internet. The gene sequence and gene function may then be determined. This analysis allows us to determine which genes are affected by the exposure to these antimicrobial compounds.



This research is conducted in collaboration with the University of Maryland (Grant identification number: T-83100801-0).

Downregulation of Energy Metabolism Genes

The table below lists a sample of the down regulated genes related to oxidative phosphorylation and electron transport.



Gene (Name)	Fold Change	Protein name
PA1320 (*cyoD*)	-3.47	cytochrome o ubiquinol oxidase subunit IV
PA0517 (*nirC*)	-2.94	probable c-type cytochrome precursor
PA2647 (*nuoL*)	-2.90	NADH dehydrogenase I chain L
PA1319 (*cyoC*)	-2.79	cytochrome o ubiquinol oxidase subunit III
PA2645 (*nuoJ*)	-2.71	NADH dehydrogenase I chain J
PA2638 (*nuoB*)	-2.68	NADH dehydrogenase I chain B
PA2646 (*nuoK*)	-2.59	NADH dehydrogenase I chain K
PA2648 (*nuoM*)	-2.56	NADH dehydrogenase I chain M
PA1317 (*cyoA*)	-2.53	cytochrome o ubiquinol oxidase subunit II
PA2644 (*nuoI*)	-2.48	NADH Dehydrogenase I chain I
PA2649 (*nuoN*)	-2.48	NADH dehydrogenase I chain N
PA1318 (*cyoB*)	-2.45	cytochrome o ubiquinol oxidase subunit I
PA0518 (*nirM*)	-2.44	cytochrome c-551 precursor
PA2639 (*nuoD*)	-2.37	NADH dehydrogenase I chain C,D
PA2643 (*nuoH*)	-2.20	NADH dehydrogenase I chain H
PA2642 (*nuoG*)	-2.19	NADH dehydrogenase I chain G
PA2640 (*nuoE*)	-2.13	NADH dehydrogenase I chain E
PA2641 (*nuoF*)	-2.05	NADH dehydrogenase I chain F

Real-time PCR Validation

Real-time PCR was utilized to validate array date. The values obtained by real-time PCR have a good correspondence to those obtained by the microarray. The variation is most likely simply a result of the different natures of the two methodologies.

Gene	mRNA level change with real-time PCR	mRNA level change with microarray
PA4613 (*katB*)	3.66 (±0.25)	7.50
PA2850 (*ohr*)	23.43 (±1.89)	21.50
PA4763 (*recN*)	1.28 (±0.09)	2.42
PA5530	7.20 (±1.68)	14.95

CONCLUSIONS

P. aeruginosa has a total of 5,500 genes. Exposure to sodium hypochlorite affected expression of 711 genes (2 - 175 fold).

Genes that show an increase in genetic expression (upregulated) were associated with adaptation, protection, and transportation of small molecule processes.

Genes that show a decrease in genetic expression (downregulated) were associated with oxidative phosphorylation and electron transport pathways.