Toxicogenomic Effects of Peracetic Acid and Sodium Hypochlorite on *Pseudomonas aeruginosa* [How do antimicrobials work against bacteria?]

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ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is a ubiquitous gram-negative bacterium that grows in water, soil, plants, and animals. As an opportunistic pathogen, *P. aeruginosa* is able to infect humans with defective immune system function such as cystic fibrosis. Disinfectants or antimicrobials have been used in hospitals and other health care facilities for surface sterilization. However, the extent of the resistance of *P. aeruginosa* to disinfectants is still unknown. Although mechanisms have been proposed describing the lethality of disinfectants and development of resistance in bacteria, there is still a lack of understanding of the effects of disinfectants on global gene expression profiles, which determine the biological functions in cells. Recent advances in the field of genomics such as whole-genome DNA microarrays may solve this problem by enabling simultaneous multiple gene analysis. Upregulated and downregulated genes via a complicated network can be universally profiled following exposure to chemicals. The goals of this research are to the analyze genome-wide changes in *P. aeruginosa* in response to exposure to antimicrobials using DNA microarray technology (Affymetrix GeneChip system) and to compare the corresponding genes to those of other bacteria and determine signature genes correlated to a mechanism of action and resistance development. In this study, peracetic acid and sodium hypochlorite were selected as target disinfectants because they supposedly elicit DNA damage and repair. *P. aeruginosa* was incubated with peracetic acid or sodium hypochlorite at a concentration that led to strong growth inhibition but not cellular death. Total RNA was then extracted and reverse-transcribed to cDNA, and labeled cDNA was hybridized onto the Affymetrix *P. aeruginosa* GeneChip array. This information will help us better understand the mechanisms by which disinfectants kill bacteria.

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 system function such as 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 genetic level. The results from this research will provide a better and faster way to test the efficacy of antimicrobial compounds, resulting in increased efficiencies for the OPP Antimicrobial Testing Program. 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 by Affymetrix, 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.

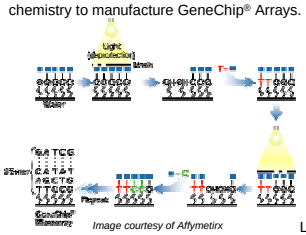

Image courtesy of Affymetrix

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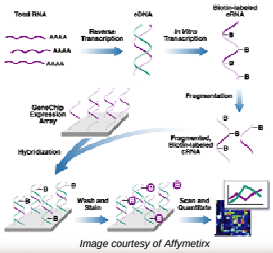


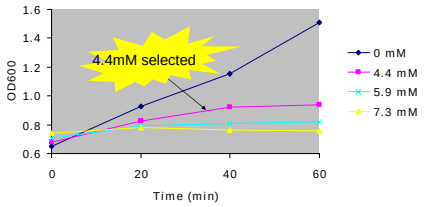
Image courtesy of Affymetrix

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

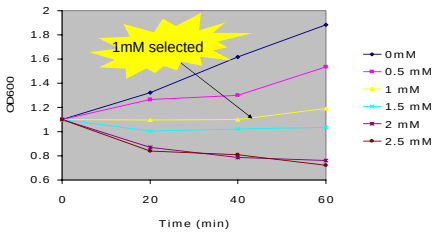
By monitoring the amount of label associated with each DNA location, it is possible to infer the abundance of each mRNA species represented.

RESULTS

Growth Curve After Sodium Hypochlorite Exposure

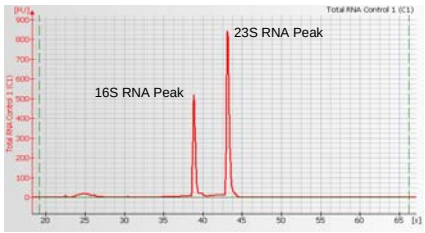


Growth Curve After Peracetic Acid Exposure



The graphs above illustrate the growth of *P. aeruginosa* after exposure to varying concentrations of peracetic acid and 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.

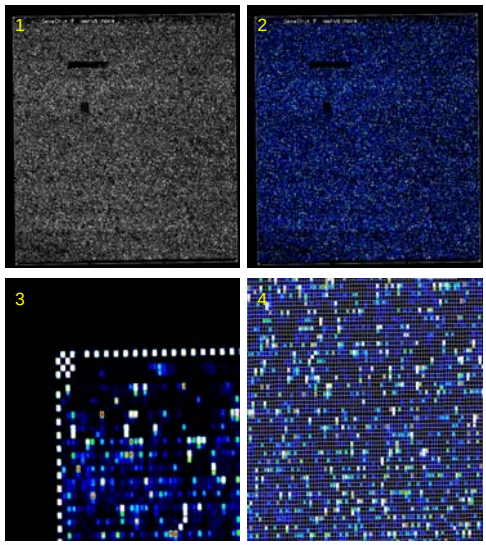
Total RNA Isolation (Extraction)



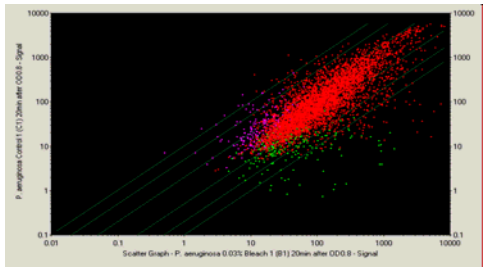
A typical Agilent 2100 Bioanalyzer trace showing RNA quantity and quality. There are two ribosomal RNA (rRNA) peaks at 16S and 23S.

GeneChip® Data

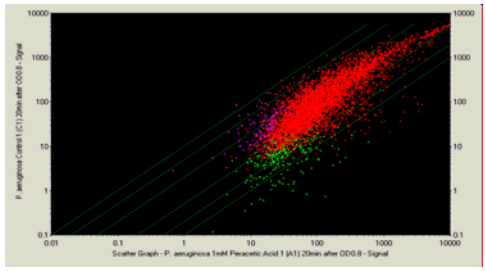
1. A fluorescent intensity scan of a GeneChip®.
2. False-coloring of the scanned GeneChip®.
3. A zoomed image of the corner of the GeneChip®.
4. A zoomed image of the GeneChip® with an overlaid grid.



Analysis of GeneChip® Data Control vs. 4.4 mM Sodium Hypochlorite



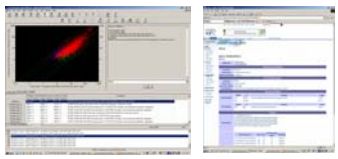
Analysis of GeneChip® Data Control vs. 1 mM Peracetic Acid

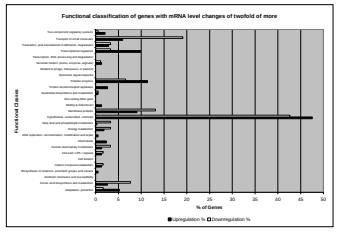


These logarithmic scatter plots show genome-wide changes in gene expression. Gene expression is as follows: pink points are present in the control group and not the experimental groups, green points are present in the experimental groups and not present in the control group, and red points are genes that are present in both groups.

Further Data Analysis

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 exposure to these antimicrobial compounds.





CONCLUSIONS

- *P. aeruginosa* has a total of 5,500 genes. Exposure to sodium hypochlorite affected expression of 711 genes (2 - 175 fold). Exposure to peracetic acid altered expression of 570 genes (2 - 60 fold).
- Genes that show an increase in genetic expression (upregulated) belong to such functional classes as adaptation/protection, protein secretion, and transcriptional regulators.
- Genes that show a decrease in genetic expression (downregulated) belong to such functional classes as transport of small molecules, energy metabolism, central intermediary metabolism, membrane proteins, and amino acid biosynthesis/metabolism.
- The global gene profiles may help us better understand the mechanisms involved in toxicity and resistance.
- Two publications are planned in academic journals.

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