Microarray Research Laboratory: Summary of the Global Toxicogenomic Effects of Disinfectants on Pathogenic Bacteria Impacting Human Health

Establishment of the Microarray Research Laboratory

-June, 2000 President Clinton met with the Director of the Human Genome Program and the CEO of Celera Genomics announcing the completion of the sequencing of the Human Genome.

-August 2000, the complete genome sequence of *Pseudomonas aeruginosa* PA01 published (Cystic Fibrosis Foundation).

-July 2003 the Microarray Research Laboratory (MARL) was established at Fort Meade, MD.

-MARL conducts groundbreaking research using DNA microarrays or Genechips to study the effects of various disinfectants on the entire genome of pathogenic bacteria.

MARL's Research Scope

MARL is currently the only laboratory within the United States government involved in toxicogenomic research in pathogenic bacteria.

-Target bacteria include *Pseudomonas aeruginosa* and *Staphylococcus aureus* which cause hospital-acquired (nosocomial) infections.

-Annually, more than 2 million individuals require prolonged hospitalization and an estimated 90,000 patients die due to hospital acquired infections, causing an ecomomic impact of 4.5 billion dollars.

-MARL is currently also investigating the toxicogenomic response of *Mycobacterium bovis* BCG to disinfectants.

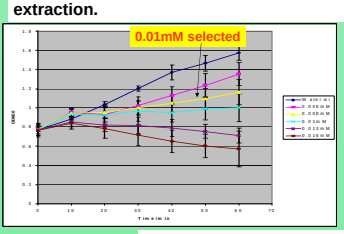
-*M. bovis* BCG is closely related to *Mycobacterium tuberculosis*, a major threat to public health worldwide.

-Results from MARL's research provides information on the mode of action of disinfectants on these pathogenic bacteria.

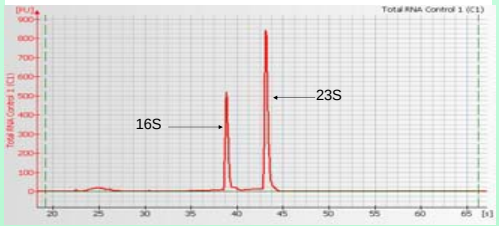
-MARL contributes to the understanding of antimicrobial effectiveness and consequently contributes to protection of the health of the American people, particularly in the hospital setting.

DNA Microarray Procedure

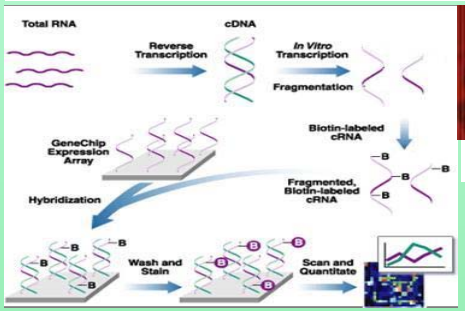
1. Growth Inhibition of bacteria by disinfectant: Time points and concentrations chosen for RNA extraction.



2. Purity of Extracted RNA determined using the 2100 Bioanalyzer

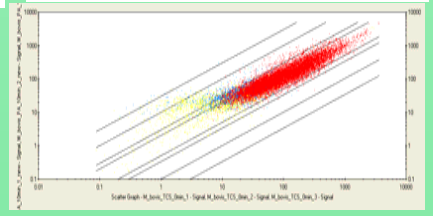


3. Labeled cDNA targets derived from the RNA of an experimental sample are hybridized to nucleic acid probes attached to the GeneChip

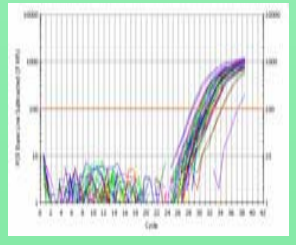


Affymetrix GeneChip

4. Data Analysis: The diagonal lines on the plot represent the fold changes (up or downregulation) in gene expression that occur in the bacteria after disinfectant treatment.



5. Real-time PCR is used to validate the microarray results



Summary of Major Research Findings

Organism / Disinfectant	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Mycobacterium bovis* BCG
Sodium Hypochlorite (Bleach)	-Upregulation of organic sulfur transport and metabolism genes. -Downregulation of genes related to oxidative phosphorylation and electron transport may contribute to cell death.	-Upregulation of staphylococcal virulence factors. -Down regulation of cell wall synthesis, membrane transport and protein synthesis.	-Upregulation of genes belonging to oxidant defense systems. -Bleach inhibits the biosynthesis of outer cell wall mycolic acids.
Peracetic acid	-Upregulation of genes associated with cellular protective processes. -Downregulation of cell membrane protein synthesis and membrane transport.	-Upregulation of virulence/pathogenesis may serve as a protective mechanism. -Downregulation of primary metabolism genes.	-Downregulation of anaerobic metabolism genes. -Upregulation of arginine synthesis which is required for growth and survival of *M. bovis* BCG.
Hydrogen Peroxide	-Upregulation of pyocin genes as protection from oxidative damage. -Downregulation of a cell immunity protein, possibly leading to self-killing activity.	-Upregulation of genes involved in virulence, DNA repair, and notably, anaerobic metabolism. -Downregulation of many membrane function-related genes.	-Upregulation of oxidative stress response genes and genes involved in iron acquisition. -Downregulation of cell cycle control, mitosis and meiosis genes.
Triclosan	Not Applicable	-Upregulation of multidrug resistance genes, coenzyme transport, and metabolism and transcription genes. -Downregulation of lipid metabolism genes may affect cell membrane formation.	In progress
Ortho-phenylphenol	-Upregulation of genes involved in anaerobic respiration, swarming motility. -Downregulation of the ribosome modulation factor (*rmf*) and an RNA alternative sigma factor (*rpoS*) may contribute to reduction in cell viability.	-Downregulation of diaminopimelic acid and lysine biosynthesis which are essential for building up the peptidoglycan cell wall may be related to the mode of action of ortho-phenyl phenol. -Upregulation of virulence factors.	In progress
Chlorhexidine Diacetate	-Upregulation of the *mex*CD multidrug efflux pump may contribute to cell death. -Downregulation of outer membrane protein components.	In progress	In progress

Conclusions

-By using DNA microarrays , the modes of action of different disinfectants on pathogenic bacteria were elucidated.

-This research contributes to the understanding of antimicrobial effectiveness, consequently contributing to protection of the health of the American people, particularly in the hospital setting.

-MARL's research also provides useful novel data for scientists and health-care facilities looking to improve the effectiveness of chemicals based on their effects on different bacterial genomes

For more information on MARL and to access complete copies of MARL's publications in scientific journals, presentations and posters at international conferences, please visit http://www.epa.gov/oppbead1/labs/microarray_lab.htm

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Chantal Note April 8, 2009.