Microarray Analysis of Toxicogenomic Effects of Oxidative Antimicrobials on Staphylococcus aureus

INTRODUCTION

Why Staphylococcus aureus?
- Major cause of hospital acquired (nosocomial) infection
- Causes pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, food poisoning, and toxic shock syndrome
- Many virulence factors: surface proteins, membrane-damaging toxins, exotoxins
- Complex antioxidant strategies that serve to neutralize and repair oxidative damage

Why oxidative antimicrobials?
- Hydrogen peroxide, peracetic acid, sodium hypochlorite
- Widely used in healthcare facility
- A lack of understanding their mode of action and the corresponding defensive mechanisms hinders successful antimicrobial application

Why microarray technology (GeneChip®)?
- Enables a genome-wide analysis of the cellular responses to oxidative stress

How St. aureus responds to oxidative antimicrobials?
- Genome-wide changes in S. aureus transcription
- Reinforce known relationships between genes with previously identified functions
- Reveal new target genes that provide more insight into S. aureus-antimicrobial interactions

MATERIALS AND METHODS

S. aureus growth inhibition by antimicrobials
- Inhibition assessed with various concentrations of the three antimicrobials
- Two exposure times employed to determine transcriptional profile changes

Affymetrix S. aureus GeneChip® arrays
- 3 biological replicates for each sample
- Statistical analysis of microarray data
  - p-value for the test ≤ 0.05
  - Fold change in transcript level ≥ 2.0
  - Presence or marginal calls ≥ 50% replicates on both the experimental and control sets
- Clustering analysis

RESULTS AND DISCUSSION

S. aureus growth with antimicrobials
- We determined concentrations that cause strong growth inhibition but not cell death
  - 10mM hydrogen peroxide (HP, H₂O₂)
  - 1mM peracetic acid (PA, C₂H₄O₂)
  - 2.2mM sodium hypochlorite (SH, NaOCl)
- Early transcriptional selection to investigate early transcriptional changes
- 20min exposure also used to compare transcriptome profiles

Statistical analysis of microarray data
- We identified statistically significant genes that meet the previously mentioned criteria for 10 and 20 min exposures
- More genes showed transcript level changes with PA and SH than with HP

Functional classification
- To classify the statistically significant genes based on their potential functions, we used the gene annotation information at the Institute of Genomic Research.

Clustering analysis
- K-means and hierarchical clustering analyses
  - K-means clustering analysis performed on the statistically significant genes based on the predefined 10 clusters
  - Hierarchical clustering also performed on the same genes

CONCLUSIONS
- Despite the similar inhibitory effects on the growth rate, peracetic acid and sodium hypochlorite caused a larger change in gene expression than hydrogen peroxide.
- In the presence of hydrogen peroxide, 117 upregulated and 175 downregulated genes were found after 10min exposure and 112 upregulated and 28 downregulated genes after 20min exposure.
- The transcriptome profiles provide clues as to the potential involvement of many genes in oxidative stress adaptation and protection.