Microarray Analysis of Toxicogenomic Effects of Oxidative Antimicrobials on *Staphylococcus aureus*

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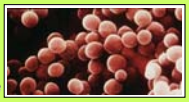
INTRODUCTION

Why *Staphylococcus aureus*?

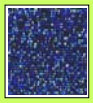


- Major cause of hospital acquired (nosocomial) infection
- Causes pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, food poisoning, and toxic shock syndrome
- Many virulence factors: surface proteins, membrane-damaging toxins, exotoxins
- Complex antioxidant strategies that serve 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 *S. 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 t-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

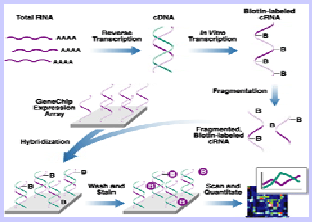
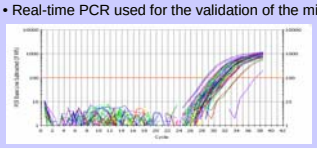


Image courtesy of Affymetrix

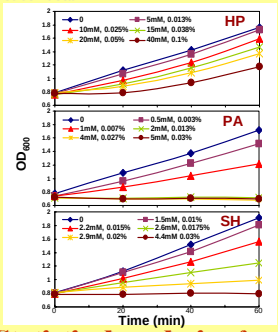
- Real-time PCR used for the validation of the microarray data



RESULTS AND DISCUSSION

S. aureus growth with antimicrobials

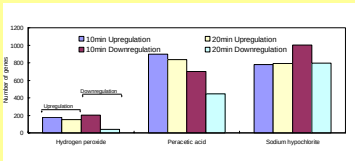
- We determined concentrations that cause strong growth inhibition but not cell death



- 10mM hydrogen peroxide (HP, H_2O_2)
- 1mM peracetic acid (PA, $C_2H_4O_3$)
- 2.2mM sodium hypochlorite (SH, NaOCl)
- 10min exposure selected to investigate early transcriptional changes
- 20min exposure also used to compare transcriptome profiles

Statistical analysis of microarray data

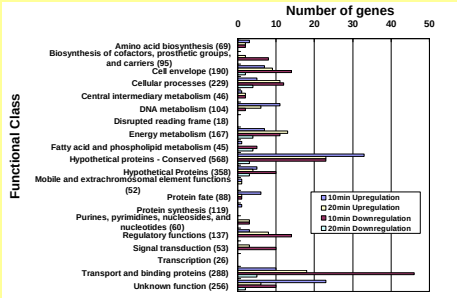
- We identified statistically significant genes that meet the previously mentioned criteria for 10 and 20 min exposures



- 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.

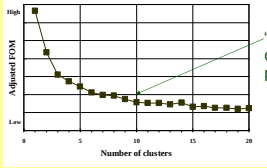


- DNA repair-related genes upregulated and primary metabolism-related genes downregulated upon 10min exposure
- The total number of downregulated genes decreased after 20min exposure (e.g. 173 to 27 genes with HP)

Clustering analysis

K-means and hierarchical clustering analyses

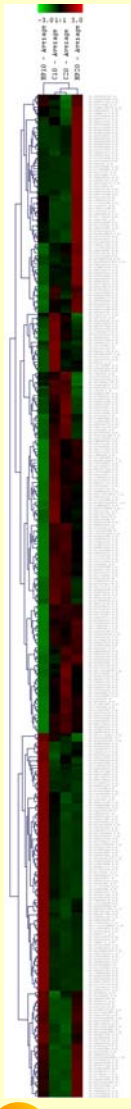
- Figure of merit (FOM): the estimate of the predictive power of a clustering algorithm
- FOM used to determine the optimal number of clusters



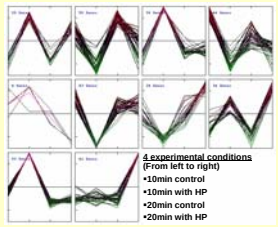
"More than 10 clusters do not provide additional predictive power"

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

Hierarchical clustering



K-means clustering



Clusters with similar gene expression patterns

- Clustering analysis helps understand regulatory relationships between genes
- HP-regulated genes classified according to their expression profiles
  - ➢ Group 1 (Clusters I and VIII)
    - ➢ 54 genes upregulated by HP at 10, 20min
    - ➢ DNA repair (*recA*, *lexA*, *uvrAB*), virulence factor (extoxin 123)
  - ➢ Group 2 (Clusters II and IV)
    - ➢ 103 genes downregulated by HP at 10, 20min
    - ➢ Transport and binding protein (*vraG*, ATP transporter, *glpF*), energy metabolism (*bglA*, *icd*, *gltA*), cell envelope (*femC*, *fmtC*, *icaABCD*)
  - ➢ Group 3 (Clusters III and IX)
    - ➢ 89 genes upregulated by HP only at 10min
    - ➢ Cell envelope (*cap5C*, *murI*, *map*), DNA metabolism (*recG*, *recQ*, *nth*)
  - ➢ Group 4 (Cluster V)
    - ➢ 4 genes upregulated by HP at 10min but downregulated at 20min
  - ➢ Group 5 (Cluster VI)
    - ➢ 43 genes downregulated by HP only at 10min
    - ➢ Transport and binding protein (*nupC*, *gltS*, ABC transports), regulatory functions (*srrAB*, scrR)
  - ➢ Group 6 (Cluster VII)
    - ➢ 24 genes downregulated by HP at 10min and upregulated at 20min
    - ➢ Energy metabolism (*pyc*, *acuAC*, *gntK*, *sdhB*), regulatory functions (*malR*, *gntR*, transcriptional regulators)
  - ➢ Group 7 (Cluster X)
    - ➢ 41 genes upregulated by HP only at 20min
    - ➢ Transport and binding protein (*gntP*, siderophore proteins, ABC transporters, ferritins proteins), energy metabolism (pflAB, arcBC), cellular process (*lytRS*, *hlY*, siderophore proteins)
- The transcription profiles suggest that DNA repair- and virulence factor-related genes be part of cellular protective mechanisms
- Many downregulated genes associated with cellular process, energy metabolism, and transport and binding proteins after 10min showed no significant transcript level changes after 20 min

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 173 downregulated genes were found after 10min exposure and 112 upregulated and 26 downregulated genes after 20min
- The transcriptome profiles provide clues as to the potential involvement of many genes in oxidative stress adaptation and protection