[O-067] Genome-Wide Transcriptional Analysis of Staphylococcus aureus Response to Oxidative Antimicrobials: Hydrogen Peroxide and Peracetic Acid

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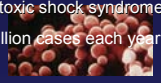
INTRODUCTION

Staphylococcus aureus (S. aureus)

• A Gram-positive human pathogen, causing a variety of diseases, ranging from benign skin infections to life-threatening endocarditis and toxic shock syndrome



• A major cause of hospital-acquired infections (HAI): 2 million cases each year in U.S., which result in 90,000 deaths and $4.5 billion loss

Oxidative antimicrobials against pathogens

• Hydrogen peroxide, peracetic acid, and sodium hypochlorite are active ingredients of EPA-registered disinfectants

• Widely used to prevent HAI in health-care environments

• US Environmental Protection Agency (EPA) has endeavored to determine the mechanism of action of antimicrobials

Microarray technology (GeneChip®)

• Enables a genome-wide analysis of cellular responses to oxidative antimicrobials

How pathogens respond to oxidative antimicrobials?

• Global transcription profiling by microarrays helps understand mechanisms involved in antimicrobial activity and the corresponding cellular response

MATERIALS AND METHODS

Affymetrix S. aureus GeneChip® analysis

• *S. aureus* exposed to each of hydrogen peroxide (HP) and peracetic acid (PA) for 10 and 20 min

• 3 independent microarray experiments in the absence (control) and the presence (experimental) of each of HP and PA upon 10 and 20 min exposures

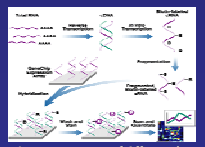


Image courtesy of Affymetrix

• Quantitative real-time PCR used for the validation of the microarray data

Statistical analysis of microarray data

• p-value for the t-test ≤ 0.05

• Fold change in transcript level ≥ 2.0

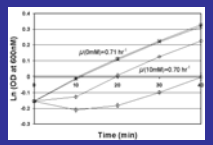
• Presence or marginal calls ≥ 50% replicates on both the experimental and control sets for 10 and 20 min

• The array data accessible through series numbers GSE3415 and GSE4184 at NCBI's Gene Expression Omnibus

RESULTS AND DISCUSSION

1. Hydrogen peroxide-induced transcriptional changes

Growth inhibition by hydrogen peroxide

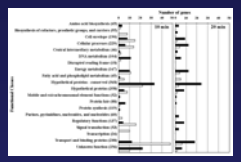


• 10 mM HP triggered a growth inhibition at 10 min. After this adaptation time, cells continued to grow at a same rate as untreated cells

• To better understand how *S. aureus* initially responds to oxidative stress and subsequently, recuperate from the damage, we employed 10 and 20 min exposure times with 10 mM HP

Functional classification of differently expressed genes

• 10 min exposure: 113 up- and 151 down-regulated genes; 20 min exposure: 95 up- and 24 down-regulated genes; a total of 343 differently expressed genes in response to either 10 min or 20 min exposure.



• The transcriptional responses are significantly different between 10 and 20 min exposures to 10 mM hydrogen peroxide; in particular, considerably fewer genes were repressed upon 20 min.

Classification of differently expression genes on the basis of their transcription directions

• Group I: genes induced upon 10 and 20 min exposures (20 genes)

- DNA repair genes (e.g. *uvrBA*, *lexA*): DNA repair system was continuously activated even after the growth of *S. aureus*, which initially had been inhibited by HP, resumed at the same rate as untreated cells

• Group II: genes induced upon 10 min exposure (92 genes)

- DNA repair genes (e.g. *recG*, *recQ*, *nth*): DNA repair mechanisms are selectively induced; this versatile repair capability might be one of the schemes that allow *S. aureus* to resume growing even while part of the damage was apparently still being restored.

- Exotoxin genes: *S. aureus* pathogenesis possibly increased

• Group IV: : genes repressed upon 10 min exposure (132 genes)

- Genes encoding transport and binding proteins; most of these genes exhibited no expression level changes at 20 min, which suggests that the transport system of *S. aureus* was restored, which might be linked to the growth resumption.

- Genes involved in primary metabolic pathways (e.g. energy metabolism and fatty acid and phospholipid metabolism); genes involved in carbohydrate uptake; this might be associated with the growth arrest effect at 10 min

- Intercellular adhesion locus (*icaADBC*)

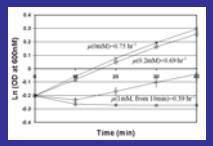
•Group VI: : genes induced upon 20 min exposure (68genes)

- Iron uptake genes: iron metabolism is coordinately regulated with oxidative stress defenses because iron promotes the formation of hydroxyl radicals

- Induction of fermentative metabolism-related genes (*pflBA*, *arcBC*, *ldh*, *nrdGD*) and cytochrome *d* oxidase genes (*cydAB*) while the cells returned to normal growth [real-time PCR-validated] : This result suggests that *S. aureus* might undergo oxygen-limiting state upon exposure to HP. Further, we propose that this phenomenon benefited S. aureus by preventing further cytotoxicity arising from reactive oxygen species produced during oxygen respiration.

2. Peracetic acid-induced transcriptional changes

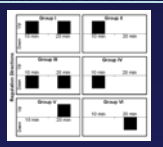
Growth inhibition by peracetic acid



• 1 mM PA showed a growth inhibition at 10 min. At 20 min, cells continued to grow at a same rate as untreated cells

• To better understand how *S. aureus* initially responds to peracetic acid and subsequently, recuperate from the damage, we employed 10 and 20 min exposure times with 1 mM PA

Classification of differently expressed genes



•10 min exposure: 221up- and 232 down-regulated genes; 20 min exposure: 270 up- and 127 down-regulated genes; a total of 648 differently expressed genes in response to either 10 min or 20 min exposure.

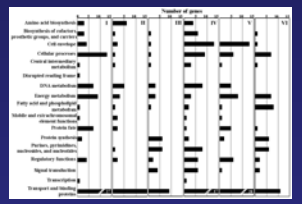
• Group I: genes induced upon 10 and 20 min exposures (147 genes)

- Virulence factor genes (exotoxins, pore-forming hymolytic toxin, clumping factor) [real-time PCR-validated]

- DNA repair genes (e.g. *uvrABC*, *nth*, *sbcC*, *xerD*, *dps*); bacterial competence genes: DNA damage by peracetic acid

• Group II: genes induced upon 10 min exposure (72 genes)

- DNA repair genes (e.g. *recG*, *dnaD*, *radC*, *dinP*): DNA repair mechanisms are selectively induced



• Group IV: : genes repressed upon 10 min exposure (176 genes)

- Genes involved in primary metabolic pathways: this might be associated with the growth inhibition at 10 min
- Intercellular adhesion locus (*icaADBC*)

• Group V: : genes induced upon 20 min exposure (123 genes)

- Iron uptake genes: iron level controlled upon exposure to PA

- DNA repair- and replication-related genes (*recQ1*, *sbcD*, *dnaG*, *holA*): more active DNA replication at 20 min

- Major surface adhesion protein-coding genes (*fnbBA*, *clfB*, *efb*) [real-time PCR-validated] : surface adhesion activity, which enhances *S. aureus* virulence, may be induced while cells partially recovered from the growth arrest.

• Group VI: : genes repressed upon 20 min exposure (71 genes)

- Genes involved in primary metabolism pathway: the profiles of primary metabolism genes that are downregulated are different between 10 and 20 min, which may contribute to the initial growth arrest and the subsequent attenuated growth

CONCLUSIONS

• DNA repair and replication genes, and virulence factor genes were selectively upregulated between initial growth inhibition and recovery in response to HP and PA

• The regulation of membrane transport genes was significantly altered in response to HP and PA

• Primary metabolism-related genes were differently downregulated between the initial growth inhibition and the following recovery in response to HP and PA

• Iron uptake- and storage-related genes were upregulated during the growth resumption in response to HP and PA

• Major surface adhesion protein-coding genes, which enhance *S. aureus* virulence, were upregulated while cells partially recovered from PA-induced growth arrest

• Anaerobic metabolism-related genes were upregulated while the cells returned to normal growth upon exposure to HP

REFERENCE

• Chang et al. (2006) **J Bacteriol** 188:1648-1659
• Chang et al. (2005) **BMC Genomics** 6:115
• Chang et al. (2005) **Environ Sci Technol** 39:5893-5899