[C1-172] Toxicogenomic response of *Staphylococcus aureus* to ortho-phenylphenol

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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

Ortho-phenylphenol (OPP) against pathogens

- OPP is an antimicrobial agent and 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 OPP

How pathogens respond to OPP?

- **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 OPP for 20 and 60 min
- 5 independent microarray experiments in the absence (control) and the presence (experimental) of OPP upon 20 and 60 min exposures
- Quantitative real-time PCR used for the validation of the microarray data

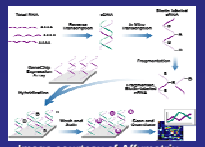


Image courtesy of Affymetrix

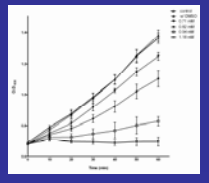
Statistical analysis of microarray data

- *p*-value for the 1-Way ANOVA ≤ 0.05
- Fold change in transcript level ≥ 2.0
- Presence or marginal calls ≥ 50% replicates on both the experimental and control sets for 20 and 60 min
- The array data accessible through series numbers GSE10605 at NCBI's Gene Expression Omnibus

RESULTS AND DISCUSSION

1. OPP-induced transcriptional changes

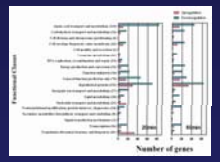
Growth inhibition by OPP



- 0.82 mM OPP triggered a growth inhibition at 20 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 OPP and subsequently, recuperate from the damage, we employed 20 and 60 min exposure times with 0.82 mM OPP

Functional classification of differently expressed genes

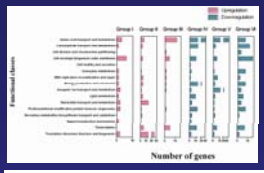
- 20 min exposure: 151 up- and 360 down-regulated genes; 60 min exposure: 101 up- and 317 down-regulated genes; a total of 669 differently expressed genes in response to either 20 min or 60 min exposure.
- The transcriptional responses are significantly different between 20 and 60 min exposures to 0.82 mM OPP.



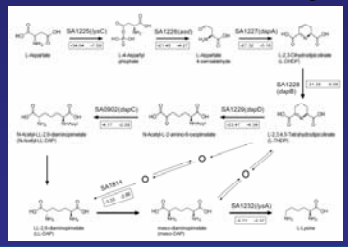
•Classification of differently expression genes on the basis of their transcription directions

- **Group I: genes induced upon 20 and 60 min exposures (18 genes)**
  - Interestingly, five of these genes encode the secretory antigen precursor, SsaA.
  - The production of virulence factors in *S. aureus* may be a secondary effect of OPP and this may provide new insight into the protective response of *S. aureus* to OPP.
- **Group II: genes induced upon 20 min exposure (28 genes)**
  - The gene cluster: SA1041-SA1048 (*pyr*RPBCAAABFE) which is belonged to the functional classes of "purines, pyrimidines, nucleosides, and nucleotides" was upregulated at 20 min.
- **Group III: genes induced upon 60 min exposure (8 genes)**
  - A putative operon containing four open reading frames (ORFs) (*pot*ABCD) was upregulated.
  - Group III contained genes related to integral membrane protein, which belonged to the functional class of "cell division and chromosome partitioning". SA1601 (*crc*B) is a putative integral membrane protein possibly involved in chromosome condensation.
- **Group IV: : genes repressed upon 20 min and 60 min exposure (27 genes)**
  - Among the genes in the class of "amino acid transport and metabolism", SA1225 (*lys*C)-SA1226 (*asd*)-SA1227 (*dap*A)-SA1228 (*dap*B)-SA1229 (*dap*D), and SA1814 (*dap*E) fall within a predicted operon and are all involved in diaminopimelate (DAP) biosynthesis. Decisively SA1225 (*lys*C)-SA1226 (*asd*)-SA1227 (*dap*A)-SA1228 (*dap*B) and SA1229 (*dap*D) show fold highest decreases as -54.6, -21.5, -27.3, -31.4, and -23.5 folds at 20 min and -7.7, -4.3, -5.2, -5.1, and -4.4 folds at 60 min in this experiment.
  - Additional amino acid biosynthesis genes including: SA1164 (*dhoM*)-SA1165 (*thrC*)-SA1166 (*thrB*) involved in threonine biosynthesis were also in this group.
  - cytochrome *bd* complex: SA0937-SA0938 (*cyd*AB) was downregulated upon both 20 min and 60 min exposure.
- **Group V: : genes repressed upon 20 min exposure (35 genes)**
  - The most dominant class was "amino acid transport and metabolism", which contained half of the genes, the *ilv-leu* operon, histidine, methionine and tryptophan biosynthesis, in that group.
- **Group VI: : genes repressed upon 60 min exposure (19 genes)**
  - The genes related to envelope biogenesis were distinctive: SA0144 (*cap*A)-SA0145 (*cap*B)-SA0146 (*cap*C)-SA0147 (*cap*D)-SA0148 (*cap*E)-SA0149 (*cap*F)-SA0150 (*cap*G)-SA0151 (*cap*H)-SA0152 (*cap*I)-SA0154 (*cap*K) were downregulated at 60 min.
  - Intriguingly, the genes were all involved in the riboflavin biosynthesis. SA1586 (*rib*H)-SA1587 (*rib*A)-Sa1588 (*rib*B)-SA1589 (*rib*D) was downregulated at 60 min exposure.
- **Group VII: : genes repressed upon 20 min and imduced upon 60 min exposure (3 genes)**
  - Intriguingly, we observed that SA2459, SA2460 and SA2461 (*ica*ADB) which make up the intercellular adhesion (*ica*) operon and contribute to virulence in *S. aureus* were downregulated after 20 min and upregulated after 60 min of exposure to OPP.

•20 min exposure: 148up- and 359 down-regulated genes; 60 min exposure: 100 up- and 317 down-regulated genes; a total of 669 differently expressed genes in response to either 20 min or 60 min exposure.



- **Functional classification of genes with statistically significant upregulated (*red*) and downregulated (*green*) upon 20 min and 60 min exposures (a total of 431 genes).** Note that the functional classes of "hypothetical genes", "general function prediction only" and "function unknown" are not included in this figure.



- **DAP pathway with transcript level of *S. aureus* genes using real-time PCR.** The real time PCR results are the mean of three biological replicates with three technical replicates for each gene.

CONCLUSIONS

- In this study, we demonstrated how OPP upregulated and downregulated genes in *S. aureus*, for the first time, by utilizing whole-genome microarrays. Moreover, we presented how the transcriptome profile of *S. aureus* was shifted during its cellular response to OPP, which involved the growth inhibition.
- OPP treatment led to the downregulation of several genes involved in amino acid anabolism. The genes involved in the DAP and lysine biosynthetic pathways were most significantly downregulated. Lysine and DAP are essential for building up the peptidoglycan cell wall. This finding proposes that the mode of action of the antimicrobial, OPP in *S. aureus* might be attributed to the inhibition of genes of lysine biosynthesis and subsequently peptidoglycan biosynthesis. We can therefore, conclude, that the mode of action of OPP is similar to the mechanism of action of some antibiotics.
- Further, we showed that the repression of the iron-regulated surface determinant (*isd*) system, hemin and thiamine-related genes accompanied with the growth inhibition.

REFERENCE

- Jang et al. (2008) **BMC Genomics** 15;9(1):411. [Epub ahead of print]
- Jang et al. (2008) **Appl Microbial Biotechnol** 78(4):695-707.
- Chang et al. (2006) **J Bacteriol** 188(4):1648-59

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