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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 triclosan as antimicrobials?

- Triclosan is one of the most common antimicrobial agents on the market today.

- 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



- responds to oxidative stress

How *S. aureus* responds to Triclosan?

- 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

- 5 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 $\geq 50\%$ replicates on both the experimental and control sets
- Clustering analysis

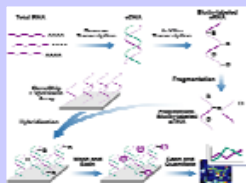
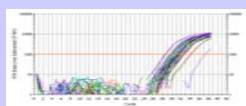


Image courtesy of Affymetrix

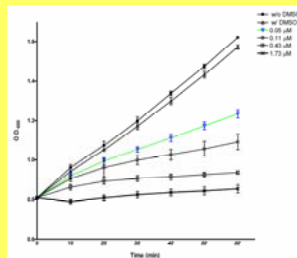
- Real-time PCR used for the validation of the microarray data



RESULTS AND DISCUSSION

S. aureus growth with antimicrobials

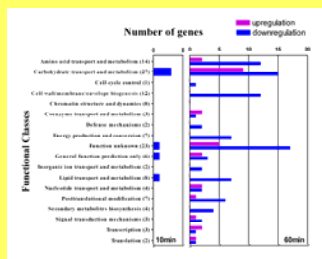
- We determined concentrations that cause strong growth inhibition but not cell death



- 0.05μM triclosan
- 10min exposure selected to investigate early transcriptional changes
- 60min 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 60 min exposures

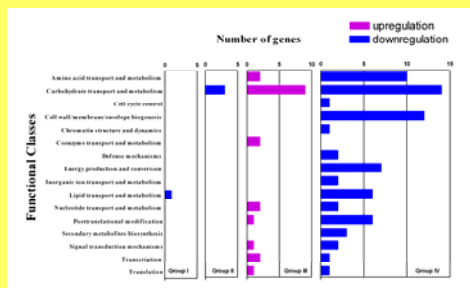


- Lipid transport and metabolism related-gene (acyl-CoA dehydrogenase) downregulated and primary metabolism-related genes upregulated upon 10min exposure

- The total number of downregulated genes increased after 60min exposure

Functional classification

- The transcriptional responses are significantly different between 10 and 60 min exposures to 0.05 μM triclosan; in particular, considerably more genes were repressed upon 60 min.



Clustering analysis

- 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
- Hierarchical clustering analysis performed on the statistically significant genes based on the predefined 10 clusters

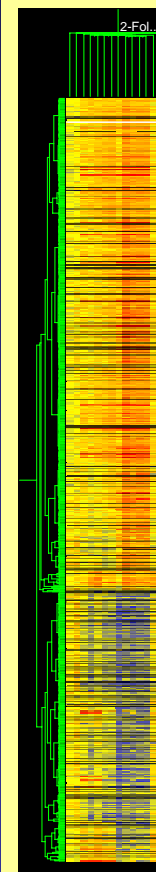


Table 1. Transcript level comparison of virulence factor genes of *S. aureus* between real-time PCR and microarray analyses.

Gene	real-time change with 0.05μM TC	real-time change with 0.175μM TC	Gene name	Antimicrob. gene response
SA0223	-1.79 (1.0-3.0)	-3.84	E-ACG GAG GCG ACC ACC ACC ACC ACC	E-TTT GAA GCG ACC ACC ACC TTT TGG T
SA0224	-1.79 (1.0-3.0)	-12.76	E-TTT GAA GCG ACC ACC ACC ACC ACC	E-TTT GCG TGG CTT TGT TGG AAA TGG T
SA0225	1.69 (1.1-2.6)	2.018	E-TGT GCG TGG CTT TGT TGG AAA TGG T	E-ATG CTT AAC TTT TGT TGG TGG T
SA0227	-1.46 (1.0-2.0)	-2.36	E-AGC CTT ACC TTT TGT TGG TGG T	E-TTT GAA ACA ACC ACC ACC ACC ACC
SA0228	-1.079 (0.679)	-1.18	E-TTT GAA ACA ACC ACC ACC ACC ACC	E-ATG GCG ACC TTT TTT TGG TGG T
SA0229	-1.74 (1.0-3.0)	-3.00	E-TTT GCG CTT ACC ACC TTT TGT TGG T	E-ATG GAA CAA TTA TAC ACC ACC ACC
SA0230	-1.05 (0.6-1.6)	-1.00	E-ATG GAA CAA TTA TAC ACC ACC ACC	E-TGG GAG CAA ACC ACC ACC ACC ACC
SA0235	-1.14 (0.6-2.0)	-2.36	E-TGG GAG CAA ACC ACC ACC ACC ACC	E-TGG GAG CAA ACC ACC ACC ACC ACC
SA0237	-1.68 (1.0-2.6)	-1.04	E-AGC CTS TCA CTT TACT CTS GTS TGG T	E-AGC CTS TCA CTT TACT CTS GTS TGG T
SA0238	1.00	1.00	E-GGA AAC TGT CAG CAG ACC T	E-GGA AAC TGT CAG CAG ACC T
SA0239	1.00 (0.6-1.6)	1.00	E-ACA TCA ACC CTT ACC ACC TCA T	E-ACC CTT TACT CTT ACC ACC CTT

Clusters with similar gene expression patterns

- Clustering analysis helps understand regulatory relationships between genes
- Triclosan-regulated genes classified according to their expression profiles
 - Group I
 - Acyl-CoA dehydrogenase downregulated by triclosan at 10, 60min
 - 13.31 fold decrease ($P=0.00277$)
 - Group II
 - 5 genes downregulated by triclosan only at 10min
 - fructose transport and metabolism (*fruA* and *B*), murein hydrolase (*lrgA* and *B*)
 - Group III
 - 20 genes upregulated by triclosan only at 60min
 - carbohydrate transport and metabolism (*gntK*, *P* and *R*, *scrA*, *bgjA*), Nucleotide transport and metabolism (*xprT* and *pbuX*)
 - Group IV
 - 70 genes downregulated by triclosan only at 60min
 - Cell wall/membrane/envelope biogenesis (*capABCDEFHJ*), carbohydrate transport and metabolism (*scrA* and *glvC*), glycine betaine/carnitine/choline ABC transporter (*opuCA*, *CB*, and *CC*), Urease (*ureABC*), lantibiotic epidermin biosynthesis proteins (*epiP*, *epiB*, and *epiA*), two-component regulatory system (*kdpD/kdpE*), ClpB chaperone

- Our result shows that triclosan downregulates two genes, *accC* and *accB* (SA1434 and SA1435), which are acetyl-CoA carboxylase homologs on fatty acid biosynthesis. SA0223, SA0224, SA0225, and SA0227 encode acetyl-CoA acetyltransferase homologue, 3-hydroxyacyl-CoA dehydrogenase, and acyl-CoA dehydrogenase, which are involved in fatty acid oxidation. In addition, acyl-CoA dehydrogenase gene is also downregulated in 10 min in Group I. Interestingly, enoyl-ACP reductase gene, SA0869, was not changed after treatment of triclosan. Our microarray results were corroborated with real-time PCR analysis.

CONCLUSIONS

- The first genome-wide transcriptional analysis of *S. aureus* response to triclosan
- In the presence of triclosan, acyl-CoA dehydrogenase was downregulated on 10min exposure and 60 upregulated and 70 downregulated genes after 60min exposure
- Triclosan may kill *S. aureus*, by interfering with the ability to form cell membranes. Another important implication of our result is that *S. aureus* may generate resistance factors under triclosan stress.