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Toxicogenomic Response of *Pseudomonas aeruginosa* to Chlorhexidine Diacetate

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Introduction

-*P. aeruginosa* is commonly implicated in nosocomial infections.

-Annually, more than 2 million individuals require prolonged hospitalization, and an estimated 90,000 patients die due to hospital acquired infections with associated economic costs up to $4.5 billion.

-Chlorhexidine diacetate is an EPA approved cationic biguanide disinfectant that is used in hand washes, dressings and creams, instrument cleaning solutions, mouthwashes and in hospital disinfectant formulations.

-The effect of CHX on the transcriptome of *P. aeruginosa* has not been elucidated.

-Identification of the metabolic processes in *P. aeruginosa* affected by and responsible for resistance to CHX will improve the understanding of its mechanism of action.

Objective of Study

What is the global transcriptomic Response of *P. aeruginosa* to Chlorhexidine diacetate?

Microarray Technology using Affymetrix GeneChips was used to investigate the effects of 0.008mM CHX on *P. aeruginosa.*

Materials and Methods

A: Growth Inhibition of *P. aeruginosa* with CHX

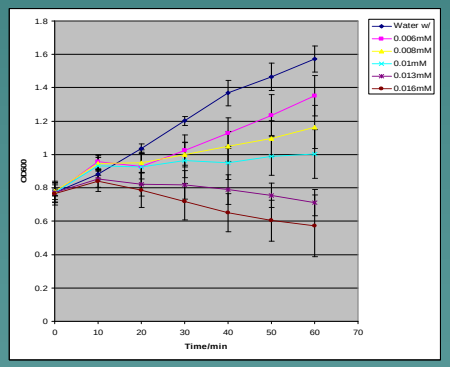


Figure 1: *P. aeruginosa* exposed to 5 concentrations of CHX and OD600 was measured every 10 minutes for one hour

-0.008mM CHX was selected as the test concentration because it caused a non-drastic growth inhibition.

-10 minutes and 60 minutes were chosen as time points for RNA extraction to understand the early and late transcriptomic responses to CHX treatment.

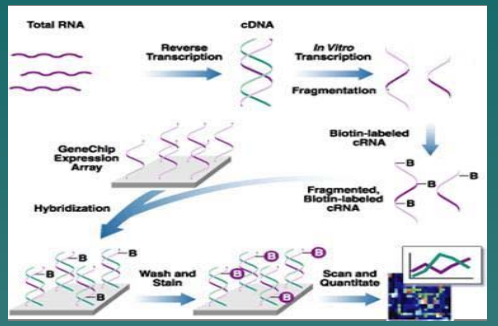
B: DNA Microarray Procedure

-Three separate microarray experiments were performed in the absence (control) and in the presence (experimental) of 0.008mM CHX .

-RNA was isolated after 10 and 60 minutes exposure to 0.008mM CHX.

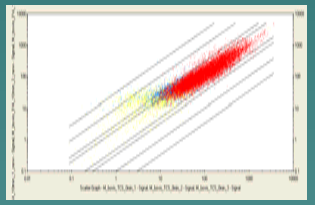
-Microarray results were validated using quantitative real time PCR.

DNA Microarray Procedure Courtesy of Affymetrix



Statistical Analysis of Microarray Data

-**Fold changes** in the expression of the **5,900** genes in the *P. aeruginosa genome* were calculated using the Affymetrix GeneChip Operating and the GeneSpring softwares.



The scatter plot shows the distribution of genes according to fold changes

-After 1-way ANOVA ($p \leq 0.05$), **250** genes showed statistically marked upregulation (≥ 2-fold) or downregulaton (≤ 2-fold).

- Microarray data was validated using quantitative real-time PCR.

Results and Discussion

Functional Classification of Up and Downregulated Genes

-The 250 statistically significant genes were classified according to functional classes of the *P. aerugionsa* Community Annotation Project.

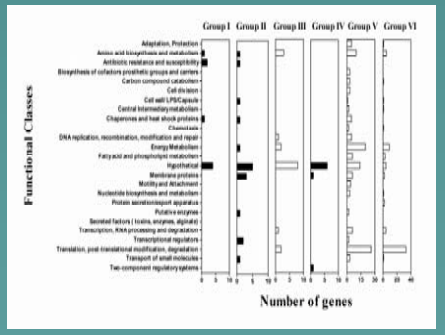


Figure 2: Classification of differentially regulated genes into six groups based on their transcription directions after 10 and 60 minutes exposure to 0.008mM CHX.

Group I: Upregulation 10 min, Upregulation 60 min

Group II: Upregulation 10 min, No change 60 min

Group III: Downregulation 10 min, No change 60 min

Group IV: No change 10 min, Upregulation 60 min

Group V: No change 10 min, Downregulation 60 min

Group VI: Upregulation 10 min, Upregulation 60 min

Group I: Genes Upregulated after both 10 and 60 minutes

-The most upregulated genes were *mexC* and *mexD*.
-MexC and MexD are components of the the **MexCD-OprJ multi-drug efflux pump** whose transcription is reported to be upregulated in *P. aeruginosa* in response to treatment with CHX and other antimicrobials.
-The upregulation of these genes corroborates the function of the MexCD-OprJ pump in the intrinsic resistance of *P. aeruginosa* to CHX.

Group II: Genes Upregulated at 10 minutes Only

-The *opr*H gene was upregulated approximately 3-fold after 10 minutes.

-Overexpression of outer membrane protein H1 precursor (OprH) blocks the self-promoted uptake pathway of polycationic antimicrobials such as CHX .

-Upregulation of the oprH gene possibly contributes to an early protective response by reducing the transportation of CHX across the outer membrane.

-Upregulation of the *nar*G gene which is involved in anaerobic respiration suggests that *P. aeruginosa* may switch to anaerobic metabolism under CHX-induced stress.

Group III: Genes Downregulated after 10 minutes Only

-Genes of respiratory Complex I of the oxidative phosphorylation pathway were downregulated implying that aerobic cellular respiration is suppressed.

-The downregulation of the DNA repair gene, recQ, suggests that inhibition of DNA repair by CHX may contribute to early growth inhibition.

Group IV: Genes Upregulated after 60 minutes Only

-One gene in this group, PA2825 encodes a probable transcriptional regulator with unknown function.

-All other genes encode hypothetical proteins with no known functions.

Group V: Genes Downregulated after 60 minutes only

-Increased evidence of the suppression of aerobic cellular respiration was seen in the downregulation of genes of the oxidative phosphorylation pathway: respiratory complexes I, II, III, IV and the ATP synthase enzyme (complex VI).

-Outer membrane proteins responsible for pore formation and maintaining the integrity of the outer membrane were downregulated, validating other studies that indicate that the outer membrane permeability is affected by CHX.

-Type IV pili and flagella genes which mediate virulence and environmental adaptation processes such as flagella-mediated motility, twitching motility and pili-mediated biofilm formation were downregulated.

Group VI: Genes Downregulated after both 10 and 60 minutes

-Several genes involved in glycolysis, the TCA cycle, respiratory complexes I, IV and V were downregulated, further supporting the fact energy production through aerobic respiration was repressed.

Conclusions

The mechanism of action of CHX in *P. aeruginosa* may be a multifaceted process involving:

-Changes in outer membrane permeability

-The attenuation of virulence and environmental adaptation processes (such as flagella-mediated motility, twitching motility and pili-mediated biofilm formation)

-Energy deprivation through the repression of genes involved in aerobic cellular respiration.

The protective response of *P. aeruginosa* to CHX treatment may involve a switch to anaerobic metabolism after 10 minutes and the reduction of CHX transport into the cell through the blockage of the self-promoted uptake pathway.