Transcriptome Analysis of *Pseudomonas aeruginosa* Response to Hydrogen Peroxide

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INTRODUCTION

Why *Pseudomonas aeruginosa*?
- An opportunistic Gram-negative pathogen
- Causes urinary tract infections and respiratory system infections, particularly in patients with burns, cancer, and cystic fibrosis

Why oxidative stress by reactive oxygen species?
- Hydrogen peroxide (H₂O₂), superoxide (O₂⁻⁻⁻⁻), and the hydroxyl radical (OH·) produced by phagocytes during active infection
- Damages cellular materials (DNA, lipids, and proteins)
- *P. aeruginosa* has complex antioxidant strategies that serve to neutralize and repair oxidative damage

How microarray technology (GeneChip®)?
- Enables a genome-wide analysis of the cellular responses to oxidative stress
  - Reinforces known relationships between genes with previously identified functions
  - Reveals new target genes that provide more insight into *P. aeruginosa*-host interactions

MATERIALS AND METHODS

- 1mM Hydrogen peroxide and 20 min exposure
- Affymetrix *P. aeruginosa* GeneChip® arrays
- 5 and 4 biological replicates for experiments (w/ hydrogen peroxide) and controls (w/o hydrogen peroxide), respectively
- Quantitative real-time PCR used for the validation of the microarray data

RESULTS AND DISCUSSION

Statistical analysis of microarray data
- *p*-value for the Mann-Whitney test ≤ 0.05
- Fold change in transcript level ≥ 2.0
- Presence or marginal calls ≥ 50% replicates on both the experimental and control sets

⇒ 115 and 103 out of 5,570 genes had statistically significant increases and decreases in transcript levels.

Functional Classification

### Iron regulation-related genes
- Iron metabolism is coordinately regulated with oxidative stress
- Upregulation of iron starvation-inducible genes reported by Palma et al. (*J Bacteriol*, 2004)

### Pyocin synthesis-related genes
- All types (F, R, and S) of pyocin (bacteriocin) genes induced – New finding!
- Bacteria adapt bacteriocins for the invasion of an ecological population; However, pyocin also toxic to human cancer cells – Cystic fibrosis patients?
- Immunity enzyme repressed – Self-killing activity?

⇒ Pyocin transcription by oxidative stress – New *P. aeruginosa*-host interaction

### Cellular protective mechanisms
- Slowdown of active and/or facilitated transport - transport of small molecules, secreted factors, and membrane proteins
- Repression of primary metabolism functions - cell division inhibitor genes (PA0671 and PA3008) induced

### Catalase (katA and katB) induced
- DNA repair-related genes highly induced – PA3007(lacA), PA3617(recA), PA3008, PA0669, PA3413-3414, and PA4763 (recN)

⇒ DNA repair proteins and catalases among the most central antioxidant mechanisms of *P. aeruginosa*

CONCLUSIONS

- Primary metabolism and membrane transport repressed; DNA repair proteins and catalases induced
- Iron regulation affected by oxidative stress
- Pyocin transcription detected – Another potential defensive mechanism against host cells

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