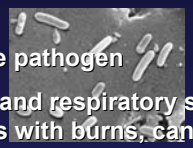
Transcriptome Analysis of *Pseudomonas aeruginosa* Response to Hydrogen Peroxide

Wook Chang [1], David Small [1], Freshteh Toghrol [2], and William E. Bentley [1]

Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742 [1] and
Microarray Research Laboratory, Office of Pesticide Programs, U. S. Environmental Protection Agency, Fort Meade, MD 20755 [2]

INTRODUCTION

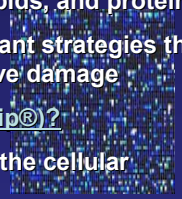


Why *Pseudomonas aeruginosa*?

- 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 (H_2O_2), superoxide (O_2.-), 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



Why microarray technology (GeneChip®)?

- Enables a genome-wide analysis of the cellular responses to oxidative stress

How antioxidant genes are related and regulated?

- Reinforce known relationships between genes with previously identified functions
- Reveal 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 experimentals (w/ hydrogen peroxide) and controls (w/o hydrogen peroxide), respectively
- Quantitative real-time PCR used for the validation of the microarray data

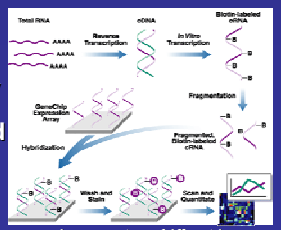


Image courtesy of Affymetrix

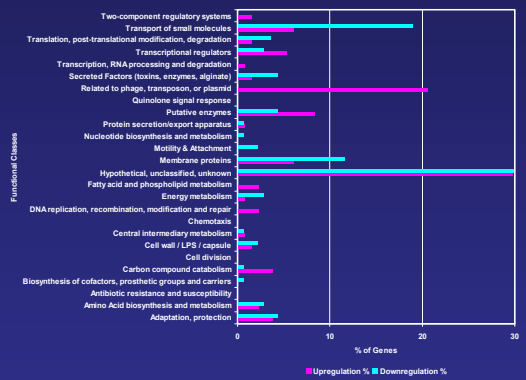
RESULTS AND DISCUSSION

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



- 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

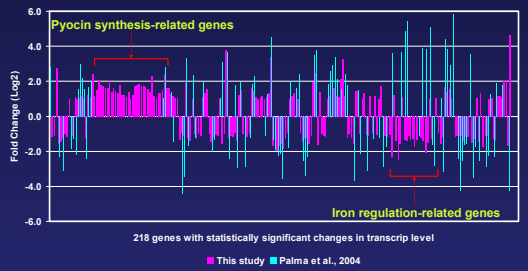
Cellular protective mechanisms

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

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

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)



- However, in this study, genes regulated by Fur (ferric uptake repressor) were repressed (e.g. iron starvation sigma factor, siderophore receptors, siderophore biosynthesis genes)

⇒ Intracellular iron level affected by oxidative stress (e.g. superoxide releases iron from iron-sulfur proteins)

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

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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