Global Transcriptome Analysis of *Pseudomonas aeruginosa Response* to Ortho-phenylphenol

48th Annual ICAAC/ IDSA 46th Annual Meeting

Background

- June 26, 2000 President Clinton met with the Director of the Human Genome Program and the CEO of Celera Genomics announcing the completion of the sequencing of the Human Genome

- August 2000, the complete genome sequence of *Pseudomonas aeruginosa* PA01 published (Cystic Fibrosis Foundation)

- July 2003 the Microarray Research Laboratory (MARL) was established at Fort Meade, MD

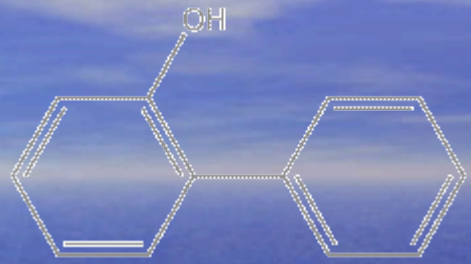
Pseudomonas aeruginosa: Nosocomial infections

- **Nosocomial infections:**
  - **Estimated to occur in 5% of all acute-care hospitalizations.**
  - **More than 2 million cases each year**
  - **Cost of 4.5 billion dollars <u>but most importantly 90,000 die.</u>**

- ***P. aeruginosa***
  - **Gram negative rods**
  - **Most common opportunistic pathogen**
  - **Cystic fibrosis patients: Chronic lung infections**

- **Increasing prevalence of nosocomial infections:**
  - **Linked to increasing antimicrobial and disinfectant resistant pathogens.**

Ortho-phenylphenol and *P. aeruginosa*



- **Ortho-phenylphenol (OPP):**
  - **EPA approved chemical**
  - **Active ingredient in disinfectants.**
  - **Mode of action in bacteria has not been elucidated**

- **Use of OPP as a hospital disinfectant necessitates an understanding of the cellular functions that it affects in different pathogenic bacteria.**
  - **Facilitate determination of mode of action**
  - **Development of antimicrobials which target specific pathogenic bacteria and exert nominal effects on other species**

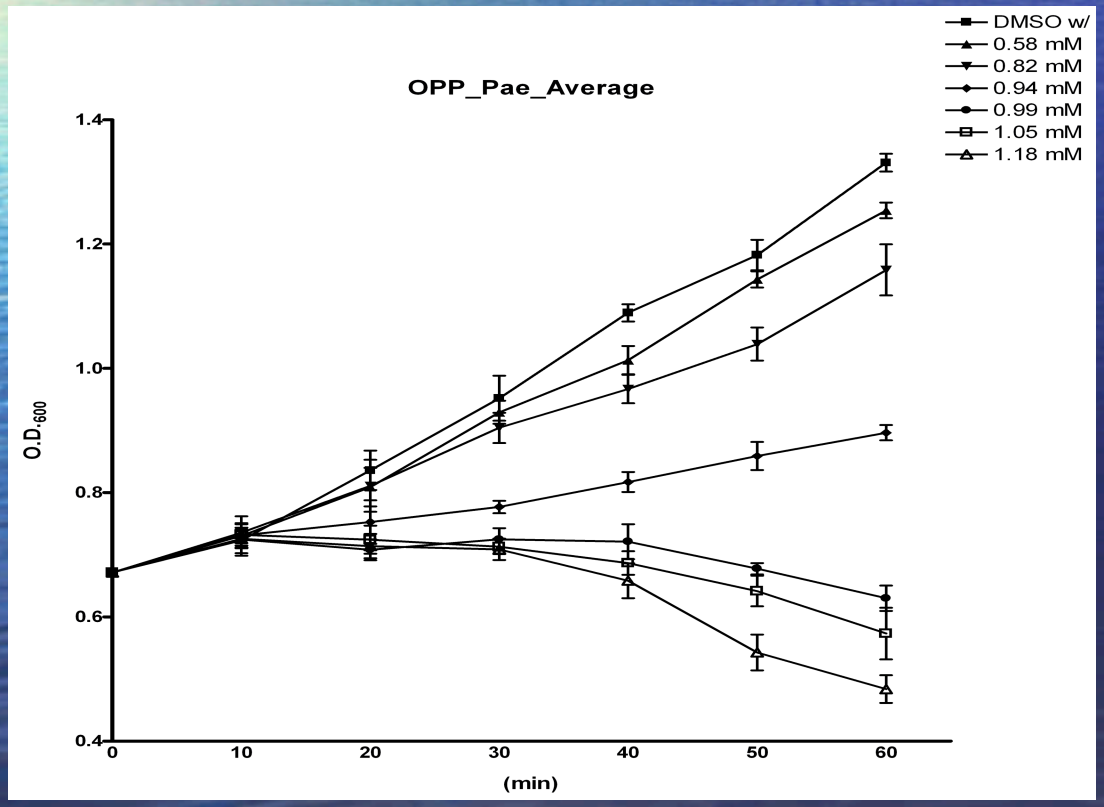
GOALS

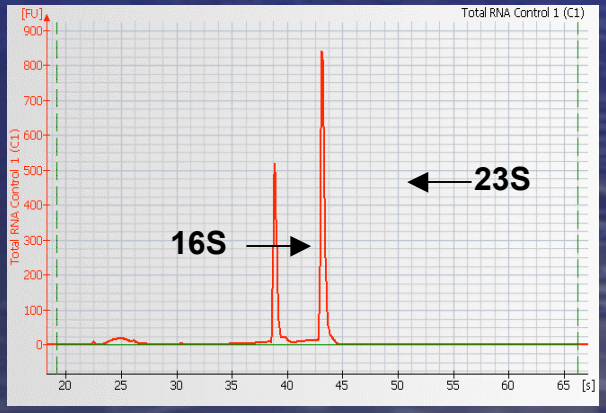
- **What genes, proteins (enzymes), and ultimately metabolic pathways are affected in *P. aeruginosa* as a result of OPP treatment?**

- **What are the potential modes of action by which OPP inhibits *P. aeruginosa* growth?**

Methods

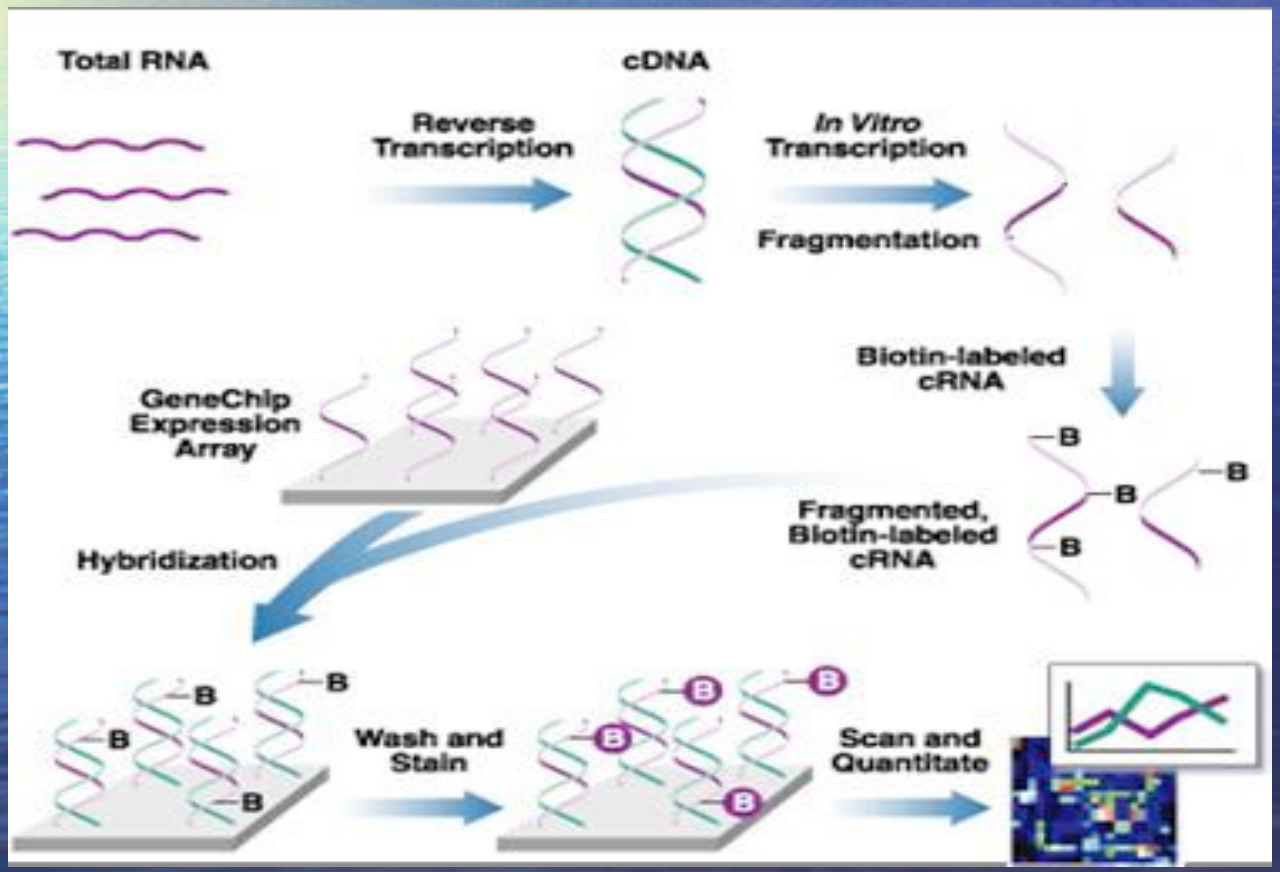
- **Sublethal concentration of OPP that will produce strong growth inhibition: 0.82mM**
- **Early and late transcriptomic response to OPP : RNA extracted after 20 and 60 minutes.**
- **Agilent 2100 Bioanalyzer & RNA LabChip**



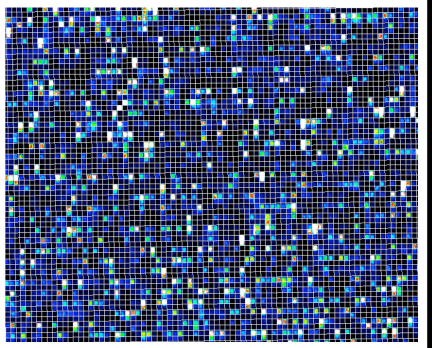


Methods

- 4 replicates each: control, 20 min, 60min
- *P. aeruginosa* GeneChip arrays (Affymetrix)
- Real-time PCR: Validation of microarray results



GeneChip



Analysis and Results

- GeneChip Operating Software (Affymetrix)
- GeneSpring Software(Agilent Technologies)

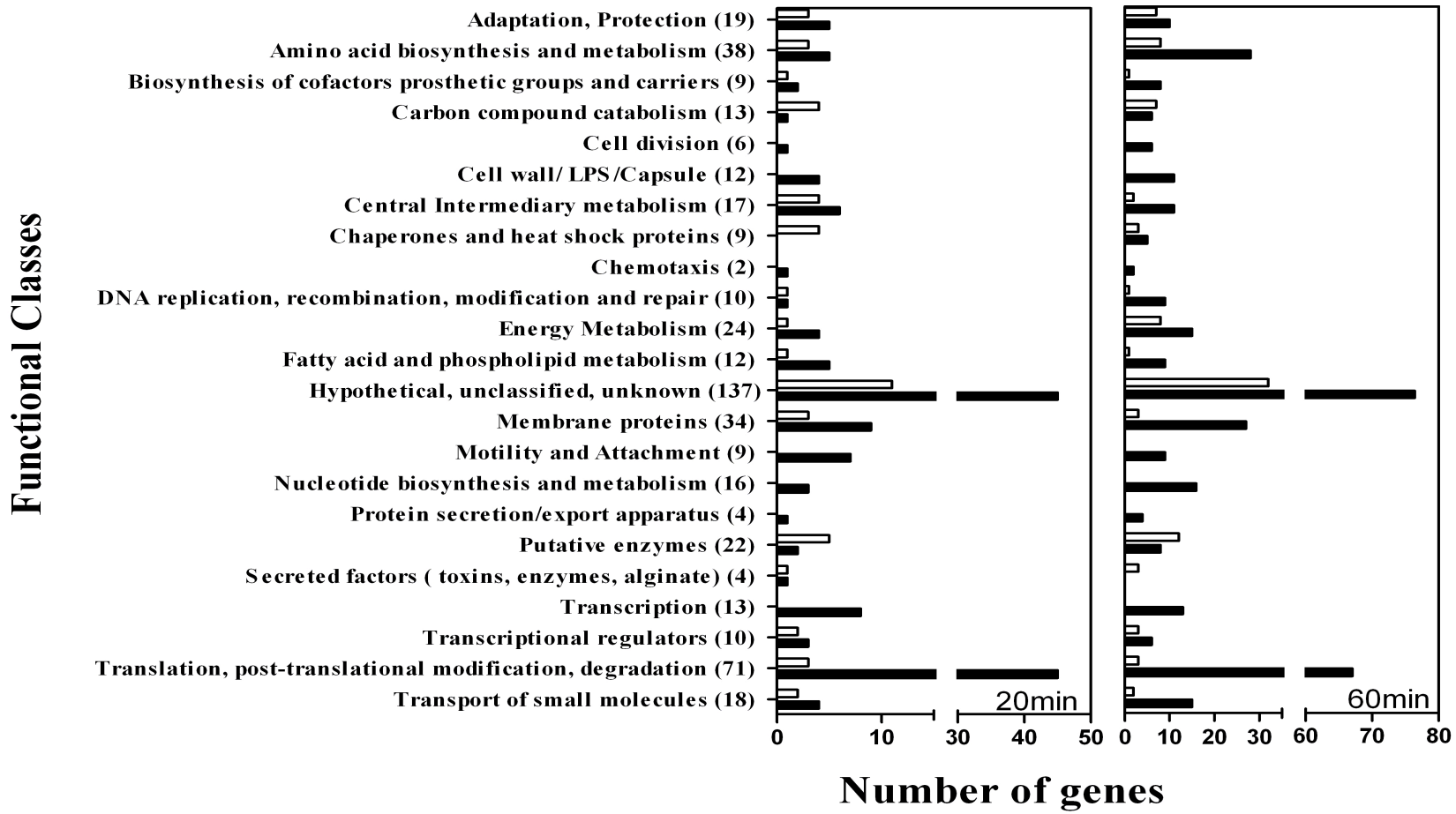
- **One-way ANOVA**: 1012 out of 5900 genes (*P. aeruginosa* genome) were statistically significant ($p \leq 0.05$).

- **Fold Changes**: Calculated as the ratios between the signal averages of four untreated (control) and four OPP-treated (experimental) cultures.

  – 509 genes: Upregulated (≥ 2-fold) and downregulated (≤ 2-fold) after 20 and 60 minutes exposure to 0.82mMOPP

Results and Discussion

- Functional classes: *P. aeruginosa* Community Annotation Project
- Upregulation: Filled bars
- Downregulation: Empty bars



Results and Discussion

Group I: Up 20, Up 60

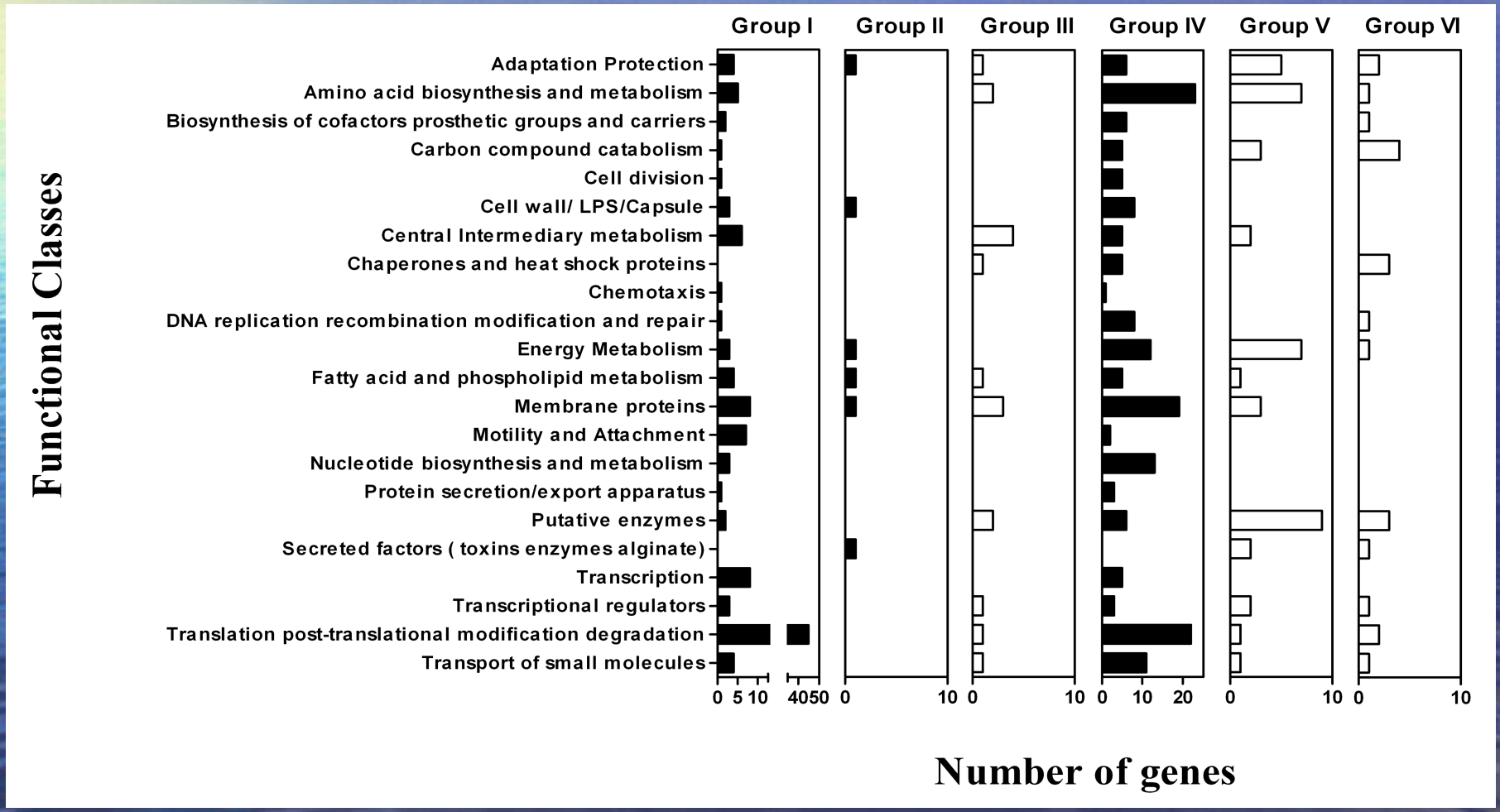
Group II: Up 20, No change 60

Group III: Down 20, No change 60

Group IV: No change 20, Up 60

Group V: No change 20, Down 60

Group VI: Down 20, Down 60



Results and Discussion

- **Group I : Up 20 min; Up 60 min**
  - **Genes encoding 30 and 50s ribosomal proteins, translation initiation and elongation factors.**
  - **Membrane transport proteins: *sec*Y, *sec*E and *sec*G.**
  - **Virulence genes: *hit*A (ferric iron binding periplasmic protein); *hit*B (Iron III transport system permease).**
  - **Type IV pilus assembly proteins: *pil* C, D, G, I, M, N, O and P.**
- **Upregulation of virulence genes => protective response to OPP treatment.**

Results and Discussion

- **Group II : Up 20 min; No change 60 min**
  - ***nor*B- nitric oxide reductase subunit B: 4-fold upregulation.**
  - **Nitric oxide reductase enzyme :expressed under anaerobic conditions in *P. stutzeri*.**
  - **Possible shift to anaerobic respiration after 20 min : Nitrate used as final electron acceptor – Denitrification.**
  - **Rhamnosyl transferase chain A (*rhl*A): 2.4 fold upregulation**
  - ***rhl*A: critical for the exhibition of swarming motility by *P. aeruginosa* –Environmental adaptation.**

Results and Discussion

- **Group III : Down 20 min; No change 60 min**
  - ***hcn*A, *hcn*B, *hcn*C: Approximately 2-fold downregulation.**
  - ***hcn*ABC encodes a cyanide synthase, which forms hydrogen cyanide from glycine.**
  - ***P. aeruginosa* does not produce cyanide under anaerobic conditions: nitrate being used as the terminal electron acceptor.**
  - **Supports theory: Possible transient switch to anaerobic respiration after 20 minutes of OPP treatment.**

Results and Discussion

- **Group IV : No change 20 min; Up 60 min**
  - **Amino acid biosynthesis genes: *arg*G, *arg*H, *gln*A, *lys*A, *lys*C, *pro*A, *glt*P, *his*B, *his*E, *aro*K, *ser*C, *gly*A3.**
  - **Contrasting results: In *S. aureus* exposed to OPP, amino acid biosynthesis genes are downregulated.**
  - **Specifically lysine and diaminopimelic acid (DAP) biosynthesis were markedly downregulated : Inhibition of peptidoglycan layer formation =>Possible mode of action of OPP on *S. aureus*.**
  - **This suggests that the effect of 0.82mM OPP in *P. aeruginosa* and *S. aureus* differ.**
  - **Implications for Disinfectant choices for eliminating different bacteria in hospitals.**

Results and Discussion

- **Group V : No change 20 min; Down 60 min**
  - ***nap*A, B, D and F genes: Components of the *nap* operon that encodes a periplasmic nitrate reductase.**
  - **The periplasmic nitrate reductase supports anaerobic growth in the presence of nitrate : Denitrification.**
  - **Implication: Anaerobic respiration is not favored after 60 minutes of OPP exposure**
  - **Contrast to after 20 minutes when anaerobic growth is favored.**

Results and Discussion

- **Group VI : Down 20 min; Down 60 min**
  - **Most downregulated gene: ribosome modulation factor (*rmf*) Fold change: -6.25 after 20 minutes and -25.9 after 60 minutes.**
  - **RMF: promotes efficient protein synthesis**
  - **The *rpoS* gene: Encodes RpoS, an alternative sigma factor of RNA ploymerase.**
  - **RpoS: Master regulator of gene expression in exponentially growing *E. coli* cells exposed to osmotic stress.**
  - **In *E. coli*: Mutations in *rmf* and *rpoS* => Decreases in cell viability.**
  - **Downregulation of *rmf* and *rpoS* may be related to the mechanism by which OPP causes growth inhibition in *P. aeruginosa*.**

Conclusions

- When exposed to 0.82mM OPP, *P. aeruginosa* may switch to anaerobic respiration after 20 minutes and resume aerobic respiration after 60 minutes.
- Downregulation of *rmf* and *rpo*S may be related to the mechanism by which OPP causes growth inhibition in *P. aeruginosa*.
- Response to OPP exposure includes the upregulation of translation leading to the production of membrane transport and virulence proteins.
- Effect of OPP on *P. aeruginosa* and *S. aureus* differ.
- This gene expression profile can be used for a better understanding of:
  - The target cellular pathways of OPP in *P. aeruginosa*.
  - How *P. aeruginosa* develops resistance to OPP.

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