Metals of interest

Copper: maximum drinking water level standard 1.3 mg/L

aquatic chronic exposure limit 30.3 µg/L

Human tolerance relatively high
Used for microbial pest control
Some lower organisms acute toxicity (phytoplankton, zooplankton, amphibians)

Cadmium: maximum drinking water standard 0.005 mg/L

aquatic chronic exposure limit 2.7µg/L

Higher animal toxicity: accumulates in kidneys and liver, produces bone and blood problems
Carcinogenic (Group B1)
Minor use as pesticide
Cu is a trace element: required for certain enzymatic and carrier proteins

Cd no known cellular use—toxic- may disrupt Ca and Zn metabolism

Both cause oxidative stress
Both act on –SH proteins

Cell concentrations regulated by chelation, sorption, uptake, and efflux mechanisms
Biosensors

**Final goals:**

1) An array of promoter fusions that respond differentially and specifically with light output upon exposure to toxic metals

2) A gene chip array to detect transcript abundance from cells responding to toxic metals. Pattern of gene activation would specify the metal.
Test organisms: *P. putida* strains

Cells of both pseudomonads are killed by concentrations of Cu starting between 1 and 10 mg/L
The pseudomonad cells show little change in culturability with Cd even at doses of 100 mg/L.

Both wild type isolates behave similarly showing resistance to Cd
Generation of Luciferase Biosensor

Metal stimulus

promoter  ORF

Transcript

Protein product

Normal cell

lux A/B

Transcript

Light

Biosensor
$P. \textit{putida}$ Corvallis mutants-Cu [in .01M CaCl2]

![Graph showing the Lux activation by different concentrations of Cu²⁺.](image)
Cadmium detection

FeSOD mutant is most sensitive

Ca inhibits the Cd response
Response of KT2440 mutants to Cu

<table>
<thead>
<tr>
<th>Mutant strain</th>
<th>10 mg/L</th>
<th>1 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>18</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>45</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>47</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>41</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>43</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>46</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>31</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>66</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>70</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>69</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>30</td>
<td>0.1</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Response of KT2440 mutants to Cd

[Bar chart showing the relative change in lux (fold) for different mutants and Cd concentrations (100 mg/L, 10 mg/L, 1 mg/L).]
Control Treatment

(10 mg/l Cu++)

A

B

pI 4

pI 7

97.0

66.0

45.0

30.0

20.1

14.4

97.0

66.0

45.0

30.0

20.1

14.4
Spot B: increased three fold

Corresponded to lipoamide dehydrogenase in TCA cycle
Cd responses

Mr (kDa)

97.0
66.0
45.0
30.0
20.1
14.4

pI 4 4-15% pI 7

Peptides of increased Intensity.

KT2440 0.01M Ca(NO₃)₂ 100 ppm Cd
Conclusions

1) The two approaches for biosensor development are feasible

2) *luxAB::* Insertional mutants that detect Cu and Cd differentially have been identified
   Gene loci await determination

3) Peptides that increase upon Cu exposure are detected and one has been correlated with a specific function