Bioaugmentation experiments with *Pseudomonas stutzeri* KC: lessons learned and new insights

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Pseudomonas stutzeri KC


Denitrifying bacterium (NO₃⁻ → NO₂⁻ → N₂O → N₂)

Secretes a biomolecule that provides pathway control: degrades carbon tetrachloride without chloroform production.

Motile rod chemotactic toward nitrate

Secreted biomolecule identified by Lee et al. (1999)

Pyridine-2,6-(bis)thiocarboxylate (PDTC)

Chang-Ho Lee, Thomas A. Lewis, Andrzej Paszczynski, Ronald L. Crawford

Identification of an Extracellular Catalyst of Carbon Tetrachloride Dehalogenation from Pseudomonas stutzeri Strain KC as Pyridine-2,6-bis(thiocarboxylate)

Biochemical and Biophysical Research Communications

Volume 261, Issue 3, 11 August 1999, Pages 562-566
Genes for PDTC production were independently identified by Lewis et al. (2000) and Sepulveda-Torres et al. (1999).

The KC genome was recently sequenced; now under analysis.

Lewis et al. reported that a KC mutant (CTN1) spontaneously lost the ability to degrade CT and produce PDTC.

The lost ~148 kbp fragment contained the pdt genes.
Current understanding of mechanism (Criddle et al., 2013)

1. Slightly alkaline conditions (pH ~8)
2. Decreased availability of Fe
3. Activation of fur
4. Transcription of pdt locus
5. Translation of mRNA to produce protein products
6. Synthesis of PDTC
7. Export of PDTC
8. Cu binding to PDTC
9. PDTC-Cu activation (reduction)
10. CO₂ + nonvolatile products

*Actively respiring cells containing electron transport chains*
Schoolcraft, Michigan

Nitrate and carbon tetrachloride-contaminated aquifer
At pH 8, KC was favored over native Schoolcraft microflora under denitrifying conditions.

KC only wins in the first step of denitrification.

<table>
<thead>
<tr>
<th></th>
<th>KC</th>
<th>Schoolcraft Flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate to nitrite</td>
<td>3.12± 0.69</td>
<td>0.81± 0.21</td>
</tr>
<tr>
<td>Nitrite to nitrogen</td>
<td>0.23± 0.09</td>
<td>0.67± 0.08</td>
</tr>
</tbody>
</table>

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

KC wins  Indigenous microflora win


- Closely-spaced wells (1 m apart) installed
- Weekly base addition
- Inoculation
- Weekly addition of base plus acetate.

Hydraulic Characterization and Design of a Full-Scale Biocurtain

by David W. Hyndman, M.J. Dybas, L. Forney, R. Heine, T. Mayotte, M.S. Phanikumar, G. Tatar, J. Tiedje, T. Voide, R. Wallace, D. Wiggert, X. Zhao, and C.S. Criddle

GROUND WATER 38, no. 3: 462–474

Development, Operation, and Long-Term Performance of a Full-Scale Biocurtain Utilizing Bioaugmentation

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JAMES TIEDE,† KATRINA LINNING,†
DAVID WIGGERT,† THOMAS VOICE,†
XIANDA ZHAO,† LESLIE DYBAS,† AND CRAIG S. CRIDDLE†

Weekly alkalinity addition prepared the site for bioaugmentation with strain KC.
Inoculation (Day 117)

- Colony on agar plate → Shake flask
- Pressure monitoring at well heads during injection
- Starter culture: 50 gallons grown on nutrient broth
  - Steam-sterilized tanks aerated with filter-sterilized air
  - Growth in filter-sterilized groundwater, supplemented with acetate, phosphate
- Inoculation (Day 117)
Downstream degradation of carbon tetrachloride

Appearance of the biocurtain

Strain KC was initially only detected in a 0.3 m strip close to the injection well gallery.

### Strain KC on sediments – Day 152

<table>
<thead>
<tr>
<th>Distance from gallery</th>
<th>Depth (m)</th>
<th>Strain KC (cfu/gram)</th>
<th>Aquifer flora (cfu/gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 meter</td>
<td>13.7</td>
<td>1.35 x 10^6</td>
<td>1.6 x 10^6</td>
</tr>
<tr>
<td></td>
<td>19.8</td>
<td>2.5 x 10^5</td>
<td>2.1 x 10^6</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>1.1 x 10^6</td>
<td>3.9 x 10^6</td>
</tr>
<tr>
<td>1 meter</td>
<td>13.7</td>
<td>0</td>
<td>2.5 x 10^4</td>
</tr>
<tr>
<td></td>
<td>19.8</td>
<td>0</td>
<td>1.8 x 10^5</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>0</td>
<td>8.5 x 10^4</td>
</tr>
<tr>
<td>2 meters</td>
<td>13.7</td>
<td>0</td>
<td>9.0 x 10^4</td>
</tr>
<tr>
<td></td>
<td>19.8</td>
<td>0</td>
<td>1.0 x 10^5</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>0</td>
<td>1.4 x 10^5</td>
</tr>
</tbody>
</table>
but as pH increased downstream of the injection well gallery, KC was detected downstream.

Strain KC on sediments – Days 336-342

<table>
<thead>
<tr>
<th>soil boring location</th>
<th>center of grid, 1.5 m downgradient D8</th>
<th>center of grid, 3.0 m downgradient D8</th>
<th>northeast section, 1.5 m downgradient D11</th>
<th>southwest section, 1.5 m downgradient D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth (m)</td>
<td>PKC(^a) (cfu/g)</td>
<td>native flora (cfu/g)</td>
<td>PKC (cfu/g)</td>
<td>native flora (cfu/g)</td>
</tr>
<tr>
<td>10.7</td>
<td>2.1 \times 10^5</td>
<td>5.8 \times 10^5</td>
<td>9.2 \times 10^4</td>
<td>1.2 \times 10^6</td>
</tr>
<tr>
<td>13.8</td>
<td>nd</td>
<td>9.5 \times 10^4</td>
<td>2.5 \times 10^5</td>
<td>6.5 \times 10^5</td>
</tr>
<tr>
<td>16.8</td>
<td>3.0 \times 10^4</td>
<td>2.1 \times 10^5</td>
<td>4.1 \times 10^4</td>
<td>3.3 \times 10^5</td>
</tr>
<tr>
<td>19.8</td>
<td>1.6 \times 10^5</td>
<td>3.8 \times 10^5</td>
<td>1.6 \times 10^5</td>
<td>3.7 \times 10^5</td>
</tr>
<tr>
<td>22.9</td>
<td>1.7 \times 10^5</td>
<td>5.2 \times 10^5</td>
<td>1.8 \times 10^4</td>
<td>1.4 \times 10^5</td>
</tr>
<tr>
<td>25.9</td>
<td>1.3 \times 10^5</td>
<td>4.1 \times 10^4</td>
<td>nd</td>
<td>7.1 \times 10^4</td>
</tr>
</tbody>
</table>

\(^a\) Microbes extracted and PKC identified as described in ref 20.
We now know that KC possesses an integrative and Conjugative Element (ICE): Mobile chromosomal elements that have the ability to be transferred between cells by conjugation like a plasmid.
Integrative and Conjugative Element (ICE):

- attL
- Integration & Excision
- Mobility
- Addiction
- Toxin/Antitoxin
- Accessory
- pdt genes

Module

Direct Repeat

Target Gene:
tRNA-pro

ICE found in *P. stutzeri* strains.

Accessory modules identified:

- KC – pdt genes
- SLG510A3-8 - Cu resistance
- 19SMN4
Lessons and questions

• Application of strong selection pressures (pH adjustment to 8) constrained the organism, locking it into a specific location.

• KC is an example of natural genetic engineering involving horizontal gene transfer of an ICE. Such elements could potentially escape constraints that limit spread of strain KC.