Two **Haloalkanes**

1,2,3-Trichloropropane (TCP)

γ-hexachlorocyclohexane (γ-HCH, Lindane)

**Toxicity**

**Nontoxicity**
TCP

Widely used...

1, 2, 3-Trichloropropene
Propyne
Triacetin
1, 1, 2,3 - Tetrachloropropene
2 - chloropropylamine
3-Acetyl-2,4-dimethylfuran
Chlormequat chloride
Diallate
Propargyl alcohol
Tris(2-chloroethyl)phosphite
Glycerin
Toxic and Refractory Persistently contaminate Soil and Water
TCP

Serious Risk

Eco-system

Human health
Lindane

γ-HCH

Global environmental contamination issue

It has been banned, but still left over amount of environmental problem.
Lindane

Diseases

Endocrine Disruptor

Cancer

Neurotoxic properties
Question

How to deal with it?
Proposed Biodegradation of TCP

**Rhodococcus sp.**

1. **DhaA**
2. **HheC**
3. **Haloalkane**
4. **DhaA**
5. **DhaA31**

**A. radiobacter AD1**

1. **HheC**
2. **HheC/W249P**

**Haloalcohol**
Natural biodegradation of Lindane

Pseudomonas paucimobilis UT26

LinA → LinB

LinA → LinB

LinA → LinB
Polycistronic Co-expression System
2A Peptide Co-expression System

Popular co-expression system in Eukaryotic system

Translation pause

Protein release

Translation restart

2A Peptide Co-expression System

In prokaryotic system: challenging and interesting
Vectors

2A co-expression system

Polycistronic co-expression system
Results and Discussion

Transformed *E. coli*

- Toxic compounds
- Many interesting discoveries
- Low Toxicity
Colorimetric Method

Reaction with TCP

Control
DhaA+
P2A+
HheC

Lysis of transformed *E. coli*
TCP is a substrate of DhaA

Activity

Time (min)
Colorimetric Assay

Reaction with 1,3-DCP

- Control
- DhaA+
- P2A+
- HheC

Activity

Lysis of transformed *E. coli*

1,3-DCP is the substrate of HheC
GC Analysis

TCP
Degradation
Intermediate products
Degradation

![Graph showing the degradation of TCP and its intermediate products over time](image)
Degradation

TCP → 2,3-DCP

GC Analysis
GC Analysis

Degradation

2,3-DCP

Epichlorohydrin

Degradation

...
TCP Degradation

Biodegradation Pathway:

TCP → 2,3-DCP → Epichlorohydrin → Glycerol

DhaA → HheC → HheC

Confirmed

Further Work
Lindane Degradation

\[ \text{\(\gamma\)-HCH} \]

Degradation

Degradation?

Not Be Detected
Expressed LinA+F2A+LinB gene

SDS-PAGE Analysis

F2A cleaving LinA and LinB

New evidence of 2A peptide working in prokaryotic system
Soluble chimeric protein

Still active
The structure of HheC

HheC is only active as a tetramer.
Analysis of P2A Peptide

Purified using an ion-exchange chromatography

Activity Comparison

<table>
<thead>
<tr>
<th>Special Activity (U/mg)</th>
<th>Substrate 1,3-DCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HheC</td>
</tr>
<tr>
<td></td>
<td>DhaA+P2A+HheC</td>
</tr>
</tbody>
</table>
P2A Co-expression System

DhaA  P2A  HheC

An excellent linker in chimeric protein

Efficient in degradation application
Conclusion

Eight standard bioBricks have been registered

Toxic compounds have been degraded

F2A and P2A both work in prokaryotic system
Future Work

Further proving biodegradation pathway

TCP

Glycerol

Y-HCH

2,5-DDOL
Future Work

Optimizing 2A Co-expression system

To clean waste water and contaminated land.
Models

DhaA → P2A → HheC

Degradation

TCP

Intermediate product

Degradation

Prediction Model

Reaction Mathematics Model

[Graphs showing concentration over time]
First Model

- Reaction equation

\[ \begin{align*}
TCP & \xrightarrow{K_1} 2,3DCP + Cl^- \\
2,3DCP & \xrightarrow{K_2} A + Cl^-
\end{align*} \]

- Differential Equations

\[ \begin{align*}
\frac{d[TCP]}{d(t)} &= -K_1 \times x \\
\frac{d[2,3DCP]}{d(t)} &= K_1 \times x - K_2 \times y \\
\frac{d[Cl^-]}{d(t)} &= K_1 \times x + K_2 \times y
\end{align*} \]
Comparison of Theory and Experiment

Model curve

Experimental curve

Verify the accuracy of the experiment
The effects of linker length

\[ Y = Y_0 + \left( \frac{A}{w \sqrt{\frac{\pi}{2}}} \right) \times e^{-2 \left( \frac{x-x_c}{w} \right)^2} \quad (Y \leq 10) \]

The structure

α-helix > β-sheet > loop

Structure grade

α-helix  A
β-sheet  B
loop  C

The structure grade of P2A is $A(\alpha$-helix$)$

The length point of P2A is 7.9777

The optimal linker is 22 amino acids and $\alpha$-helix

We can construct new chimeric protein using the optimal linker
Human Practice

Jul 27th
• Vectors construction

• Biodegradation experiments and data analysis

• WiKi and modeling
THANKS!