Cyanobacteria Converter & MCs Detection with Yeast

2009 Tianjin University  IGEM Team
Motivation

Electricity Generation

- US
- India
- China
- Japan
- US-share
- India-share
- China-share
- Japan-share

Terawatt-hours vs. Share of the total world
World primary energy demand will grow 45% between 2009 and 2030; 50% of this increase from China and India; China account for 40% of world coal demand and is expected to increase to 50% by 2030.

By IEA World Energy
Carbon Dioxide Emissions

Carbon Dioxide Emissions

Carbon Dioxide Emissions in 2008

- Total (million tonnes)
- Per capita (tonnes)

US: 20.92 million tonnes, 6.371 per capita
China: 6.897 million tonnes, 5.21 per capita
India: 1.23 million tonnes, 1.419 per capita
Japan: 10.89 million tonnes, 1.391 per capita
China’s Electricity Generation

Carbon Dioxide Emissions

Algal Bloom in Main Water Resource in China
Wu Xi: Cyanobacteria assault the city
As the outbreak of the algal bloom in Tai Hu lake in 2001, the city’s tap water is heavily polluted, threatening the safety of drinking water system.
Background

- Water quality deterioration
- Microcystin
- Increase incidence of liver cancer
- Water intoxication
- Animal death
## MCs Detection with Yeast

### Toxicity of Microcystin

<table>
<thead>
<tr>
<th>Exposure Mode</th>
<th>Time</th>
<th>Place</th>
<th>People poisoned</th>
</tr>
</thead>
<tbody>
<tr>
<td>drink water</td>
<td>1975</td>
<td>United States</td>
<td>5000 people suffer from acute gastroenteritis</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>Australia</td>
<td>149 people suffer from hepatitis</td>
</tr>
<tr>
<td>1972-1995</td>
<td>Jiangsu and Guangxi, China</td>
<td>A high incidence of primary liver cancer</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>Brazil</td>
<td>2000 people suffer from gastroenteritis, and 88 people died</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>Australia</td>
<td>Many people suffer from barcoo fever</td>
<td></td>
</tr>
<tr>
<td>touch directly</td>
<td>1989</td>
<td>Britain</td>
<td>16 people suffer from throat ulcers, headaches and vomiting</td>
</tr>
<tr>
<td>1995</td>
<td>Australia</td>
<td>Many people suffering from gastroenteritis, fever, and eyes and ears problems</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Britain</td>
<td>11 people suffer from fever and skin rash</td>
<td></td>
</tr>
<tr>
<td>hemodialysis</td>
<td>1974</td>
<td>United States</td>
<td>23 people suffer from muscle pain, vomiting, chills, skin rash</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>Brazil</td>
<td>116 people suffer from blurred vision, nausea, vomiting, and liver damage; 63 died</td>
</tr>
</tbody>
</table>
Our Project

Bait: BD-PP1  AD: (Activation domain)
Prey: AD-GSH+MC  BD: (Binding domain)
MCs Detection with Yeast

Mechanism of Detecting Process

Detoxicity mechanism (GSH glutathione)

Toxicity mechanism (PP1 protein phosphatase 1)
MCs Detection with Yeast

Our Design

[Diagram showing the interactions between PGADT, PGBKTK7, GSH, and PP1 in yeast.]
MCs Detection with Yeast

Inside the nucleus of Yeast

Promoter Gal4

RNA Polymerase II

β-Gal

Outside the cell

Reporter LacZ

MCs

GSH

AD

BD

PP1
MCs Detection with Yeast

PP1 and GSH Auto-inducing Detection

Positive control (PGBKKT7/PP1+PGADT7/GSH)

SD/–Trp/–Leu/–His/–Ade (A)

SD/–Trp/–Leu (B)

Inside the nucleus of Yeast
Authentication of the interaction between PP1 and GSH+MCLR

1-4
Medium with MC-LR+Yeast
After 6h → Liquid N₂ → X-gal

5
Medium without MC-LR+Yeast
After 6h → Liquid N₂ → X-gal

6
Yeast of p53-BD+ T-antigen--AD
After 6h → Liquid N₂ → X-gal

1 2 3
10 μg/L, 0.1 μg/L, 0.01μg/L
without MCs, Positive control

4 5 6
## Existing microcystin detection methods: advantages and disadvantages

<table>
<thead>
<tr>
<th>Method</th>
<th>Qualitative</th>
<th>Quantifiable</th>
<th>Easy to handle</th>
<th>Lower detection level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse method</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HPLC</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ELISA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Liver cell</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PPIA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tianjin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Further probe of Cyanobacteria

- Contains photosynthetic pigments & Consume CO₂
  - Chlorophyll-a and Phycobilins
- Contain gas vesicles  Grow faster
  - optimize growth based on sunlight and nutrients
- Easy to manipulate
- low cost
The way we do it

Acetyl CoA

E2

Pyruvic dehydrogenase

E1

pyruvate

Thpp

Acetyl CoA synzyme

S-Acetyl dihydrolipoamide-E

Dihydrolipoamide dehydrogenase

Dihydrolipoamide-E

Lipoamide-E

PDC: pyruvate decarboxylase
ADH: alcohol dehydrogenase

Cyanobacteria

Convertor

glyoxylate

acetaldelhyde

Ethanol
Construction of plasmid T7 RNA Polymerase
Selection of transgenic Anabaena

Growth condition of the wide type and transgenic Anabaena sp. PCC 7120 with the KANA concentration 30ug/ml, 30ug/ml respectively
## Experiment results

<table>
<thead>
<tr>
<th>Table 1</th>
<th>OD600</th>
<th>[Ethanol] in g/l</th>
<th>Average ethanol production mg.L-1/OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top10</td>
<td>2.956</td>
<td>0.0165</td>
<td>5.581</td>
</tr>
<tr>
<td>PSBAI-pdc-adh -Top10</td>
<td>2.096</td>
<td>0.0501</td>
<td>23.9026</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>OD750</th>
<th>[Ethanol] in g/l</th>
<th>Average ethanol production mg.L-1/OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT7120</td>
<td>1.547</td>
<td>0.491</td>
<td>0.3174</td>
</tr>
<tr>
<td>PA-489-7120</td>
<td>1.421</td>
<td>0.6919</td>
<td>0.4869</td>
</tr>
</tbody>
</table>
Conclusion

- The contribution of the prevention of algal bloom
  - A simple approach of the detection of MCs
  - Set up a lower detection level

- The contribution of environmental problems and energy crisis
  - Take advantages of greenhouse gas
  - Generate bio-energy
Acknowledgement

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