Detection and degradation of Microcystin LR

By Jilin_China
Directory

Water Safe Jilin-China

Our team
Project
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Our Team
Team

We come from Jilin University, Which is located in the north of China.
Our team

Mr. Wu
Ms. Jiang
Mr. Gao
Advisor:

Students:

Instructors:
Our Team
Project
Although we got dozens of ideas about the project and had heated discussions, we focused on one toxin in water pollution eventually.
Algae blooming in Lake Erie

- In this summer, Lake Erie has a worsening problem with toxic bacteria commonly known as blue-green algae.
Microcystin LR threatens water safety

- Contrast concentration of Microcystin LR:
  - Safe limit: 1.045 µg/L
    —— The World Health Organization
  - Lake Erie: reaching 2.5 µg/L
    —— Toledo-Lucas County Health Department

- Terrible influence:
  Microcystin LR was found in Toledo’s treated water, which caused 30,000 southern residents living without fresh water for two days.
Here, comes the QUESTION:

Why we still work on it?

More easy
More intelligent
More efficient
So, what we do?

1. We find the promoter which can detect the existence of Microcystin LR in drinking water.

2. We construct a gene reporter which can express GFP to let us know.

3. We synthesized MlrA gene which can degrade Microcystin-LR in high efficient.
A. How to find it?

The Mlr gene cluster of *Sphingomonas sp.*

*Environ Toxicol.* 2001;16(6):523-34
We synthesized a RFP-promoter-GFP gene cluster to detect the relationship between MLR promoter and microcystin LR.
The fragment between mlrC and mlrA contains a promoter, which can express GFP in *E. coli* and *Lactococcus lactis* Induced by Microcystin LR.
B. How to report it?

We put GFP gene into our system, which is situated upstream of Mlr A gene and downstream of Mlr promoter.

When the Microcystin LR reaches the safe limit, we can know it by green fluorescence.
C. How to degrade it?

- **Mlr C**: Hydrolyse the tetrapeptide degradation product.
- **Mlr A**: Cut the Microcystin LR cycle structure to a line.
- **Mlr D**: Facilitate the transport of Microcystin LR or its degradation product.
- **Mlr B**: Catalyse the degradation of linear Microcystin LR to a tetrapeptide degradation product.
Degraded by MlrA
Degraded by MlrA Toxin ↓ 160 times!

Environ Toxicol. 2001;16(6):523-34
The mlrA gene was synthesized according to the sequence of *Sphingomonas sp. ACM-3962* containing 336 codons. 246 codons were optimized for increasing expression of mlrA in *Lactococcus lactis*. 

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SYNTHESIZED mlrA GENE

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1. Fusion expression cluster of mlr promoter-GFP-mlrA

2. Using *E. coli*-L. *lactis* shuttle expression vector pMG36e

Expressed in *E.coli* and *L.lactis*.

3. *L.lactis* could avoid secondary pollution in the natural environment.
The fusion protein GFP-mlrA was expressed in *E.coli* BL21 and it could be even more efficiently expressed in *Lactococcus lactis*.
Detection ability

The detection limit is 0.01 µg/L.
Degradation ability

**E.coli**

Degradation: about 12 hrs at 10 µg/L; about 8 hrs at 1 µg/L

**L.lactis**

Degradation: about 16 hrs at 10 µg/L; about 8 hrs at 1 µg/L
Practical application in future

Water Sample → Freeze-drying *Lactococcus lactis* → UV lamp bulb → Fluorescence Spectrophotometer

1-2 hrs
3 Human Practice
Human Practice

2014

Survey: Apr 15th

Observation: May 1st

Cooperation: Jun 6th

Communication: Aug 23rd

Propagate: Sep 15th

Nov 2nd
A. Survey

We did a survey in the neighborhood community.

**OBJECT:** 2000 nearby lake’s residents

**FORM:** Questionnaire Survey

**Conclusions:**

1. Worried about drinking water and dozens of complaints on the polluted water

2. Residents really want detection kits to test drinking water in their home.
A simple method to detect drinking water?

Are you drinking water safe?
- Safe: 71%
- Not Safe: 18%
- I did not know: 11%

Do you need simple decetive method at home?
- Yes, I need: 14%
- No, I did not: 33%
- I did not care: 53%
B. Field visits

In order to find the current situation about the algae bloom, we visited several lakes to find it out.

◆ The South Lake in Changchun City

◆ The SongHua Lake in Jilin City
The South Lake in Changchun
The SongHua Lake in Jilin
C. Propagation

- To propagate and share iGEM, this great competition to more students, we prepare a special lecture to high school students from Affiliated Middle School of Jilin University.
D. Communication

• NanJing University
  ----For communication and support

• Huazhong Agricultural University, Wuhan University and some other IGEM teams
  ---- For exchanging ideas

• Lanzhou University
  ---- Assistance in theoretical analysis
Human Practice
4 Acknowledgement
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Welcome other team members!
Q&A

Congratulation for IGEM’s 10th anniversary!