

Microcystin Terminator 微囊藻毒素杀手

Team Nanjing_NFLS

Nanjing Foreign Language School × Southeast University



Introduction

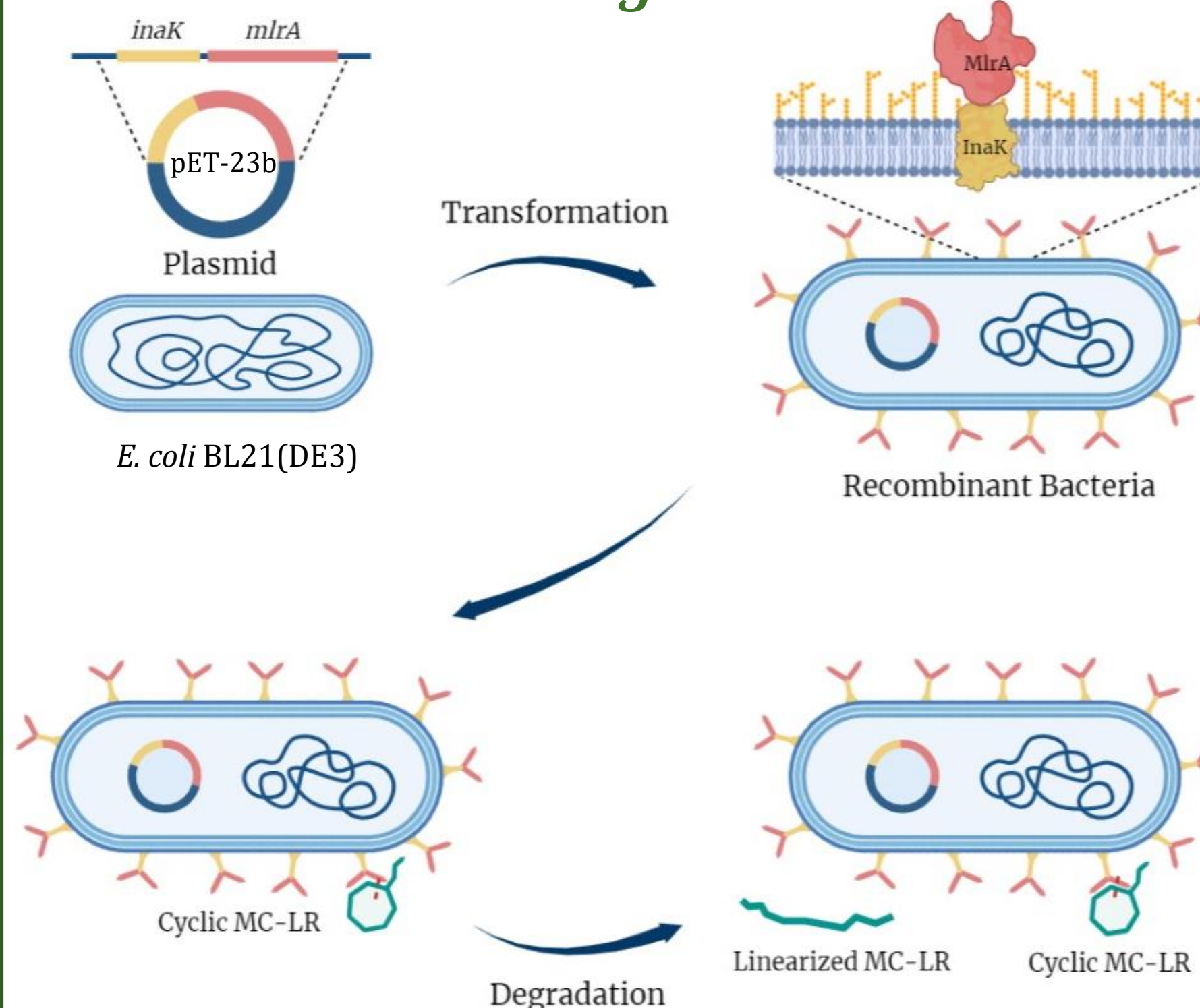
- Harmful Algal Blooms (HABs) have become a major issue for water body contamination. Cyanotoxins are secondary metabolites of cyanobacteria, among which Microcystin-LR is the most toxic. MC-LR has been listed as a Class 2B carcinogen and may cause liver failure; they pose a severe threat to ecological stability and public health.
- As residents near Qinhuai River, a victim of HABs, we aim to utilize bioremediation to eliminate MC-LR by using a specific degradation enzyme: MlrA. It linearizes the cyclic toxin by breaking a peptide bond. The product, linearized MC-LR, is 20 times less toxic. In order to optimize the gene expression, we link *mlrA* to *inaK* (*ice nucleation protein*), producing an cell-surface display system of the enzyme.

Methods

- Construction of cell-surface display system**
 - Fusion of *inaK*-N-*mlrA* by overlap PCR
 - pET23b used as plasmid backbone
 - pET23*mlrA* → *E. coli mlrA*
 - pET23b → *E. coli* Control Group
- Verification of MlrA expression**
- Verification of engineered *E. coli* activity**
 - Experiment groups
 - E. coli inaK*-N-*mlrA*
 - E. coli inaK*
 - Sphingopyxis sp.m6* (MC-LR natural degrader)
 - System components

<i>E. coli</i> or <i>Sphingopyxis sp.m6</i>	0.1mL
Synthesized MC-LR	1 μg/mL
Mineral Salt Mixture	0.1mL
Total	1mL
 - Cultivation at 30°C and rotary shaker 150rpm
 - Sample retrieval at each hour, for 8 hours
 - Centrifuge 12000rpm for 15min at 4°C
 - MC-LR assay by H.P. Liquid Chromatography

Design



Follow-Up

Influence of *E. coli* on *microcystis aeruginosa* (MC-LR producer)

- Survival rate of the cyanobacteria
 - Direct counting
- Metabolic activity, especially photosynthesis
 - Retrieval of relevant RNA
 - Reverse transcription to complementary DNA
 - Quantitative Real-Time PCR
- Future production levels of MC-LR
 - High Performance Liquid Chromatography analysis
 - Comparison with previous data

Human Practice

- ✓ **Freshwater Bioremediation Alliance**
Initiator: Nanjing_NFLS, XJTU
Teams: GXU UM_Macau CHINA-FAFU Nanjing-China
Goals:
 - Education of biosafety via videos/workshops
 - Online co-interview with environment experts
 - Potential mentorship on modeling, hardware, etc.
- ✓ **World Environment & Ocean Day**
Initiator: CHINA-FAFU
Teams: Nanjing_NFLS NPU-CHINA BUCT-CHINA
Speakers: FAFU, Minjiang Univ., Tsinghua Univ.
Sessions:
 - Introduction & discussion between teams
 - Novel research intro by keynote speakers
 - Proposal of water protection methods

Team

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References

- Liu, M., Feng, P., Kakade, A., Yang, L., Chen, G., Yan, X., Ni, H., Liu, P., Kulshreshtha, S., Abomohra, A. and Li, X., 2020. Reducing residual antibiotic levels in animal feces using intestinal *Escherichia coli* with surface-displayed erythromycin esterase. *Journal of Hazardous Materials*, 388, p.122032.
- Wang, R., Li, J., Jiang, Y., Lu, Z., Li, R. and Li, J., 2017. Heterologous expression of *mlrA* gene originated from *Novosphingobium sp. THN1* to degrade microcystin-RR and identify the first step involved in degradation pathway. *Chemosphere*, 184, pp.159-167.