Degradation of algae and microcystin – Microcystin Degradation

Aim

The biggest obstacle currently hindering the utilization of cyanobacteria is the microcystins. The 28 enzymes initially screened by our team proved to be effective for the degradation of cyanobacteria. Next we want to study how these 28 enzymes degrade the algae toxins. Which of these enzymes are capable of degrading microcystins alone. In order to explore the degradation effects of the 28 proteins screened by our team on microcystins, we designed an algae toxin degradation experiment..

Materials

microcystins, LR and RR

PBS buffer (0.02M pH = 8.2)

methanol (analytical grade)

28 proteins

Procedure

1. Dilution of microcystin samples

There are many different types of microcystins. In our group, we mainly

used two kinds of microcystins, LR and RR. The microcystin samples purchased by our group are solid powders. We need to first dissolve the microcystin into the concentration we need (50mg/mL). We used methanol (analytical grade) to solubilize microcystins. All operations are carried out in a fume hood.

(1) Pipette 1000 μL of methanol (analytical grade), add to the tube containing 50 μg of microcystin, and mix well by pipetting; (The two microcystins are treated the same way)

(2) Transfer the 1000 μL solution into a 1.5 mL centrifuge tube;

Microcystin (μg)	Methanol (μL)	Final concentration of microcystin (mg/L)
50	1000	50

Figure 1. Microcystin preparation

2. Diluting of the enzyme:

According to the experimental ideas of our group, we need to dilute the 28 enzymes to be measured to the same concentration as the microcystin (50mg/L). In order to avoid the effects of repeated freezing and thawing on enzyme activity, the remaining enzymes were stored at 4 °C for next use. The corresponding dilution ratios of the 28 enzymes used are shown in the figure below.

(1) Pipette the corresponding amount of PBS buffer (0.02M pH = 8.2) into a 1.5mL centrifuge tube;

(2) Pipette the corresponding amount of enzyme into the same 1.5mL centrifuge tube;

(3) Instantaneous centrifugation to mix the sample;

3. Construction of the reaction system

We use a 200 μL reaction system. The specific composition of the reaction system is shown in the following table. Because of the specificity of the No. 8 enzyme, all of our enzymes and No. 8 did a mixed enzyme degradation experiment (such as 1+8, 2+8). In order to ensure that the final concentration of the enzyme is the same in each centrifuge tube (50mg / L), in the mixed enzyme experiment, we use two enzymes each add 15μL (the total amount of enzyme is still 30μL, and the concentration is still It is 50mg/L). After the reaction was over, we measured the results by HPLC-MS. For the needs of HPLC, we added 1000 μL of PBS buffer to all the centrifuge tubes.

(1) Pipette 140 μL of PBS buffer (0.02 M pH=8.2) into a 1.5 mL centrifuge tube;

(2) Pipette 30 μL of enzyme (diluted in step 2) into the same centrifuge tube and mix well by pipetting;

(3) Pipette 30 μL of algal toxin solution (diluted in step 1) into the same centrifuge tube, and mix by instantaneous centrifugation;

(4) Place at 25 ° C, 40 rpm (rotary mixer) for 24h;

(5) Take the centrifuge tube after the reaction is completed, and add 1000 μL of PBS buffer to each centrifuge tube;

PBS buffer (0.02M pH=8.2) (μL)	Microcystin (μL)	Enzyme (μL)	Total capacity (μL)
140	30	30	200

Figure 3. Reaction component