WEEK 8

5.19 Shuning Guo

Determination of engineering bacteria by HPLC

The main process:

LY-A: pLB1s-hmaS

LY-B: pLB1s-hmaS+pYB1a-aroG^{fbr}-pheA^{fbr}

LY-C: pLB1s-hmaS-tktA-ppsA+ pYB1a-aroG^{fbr}-pheA^{fbr}

1. The 1 μL plasmids were transferred to 50 μL BW competent cell.
2. They were transferred to liquid LB for 12 h on a 37 ℃ shaking table.
3. Zym-5052 medium was used for induction.
4. OD=6, and M9 medium was used for resuspended.
5. HPLC detection

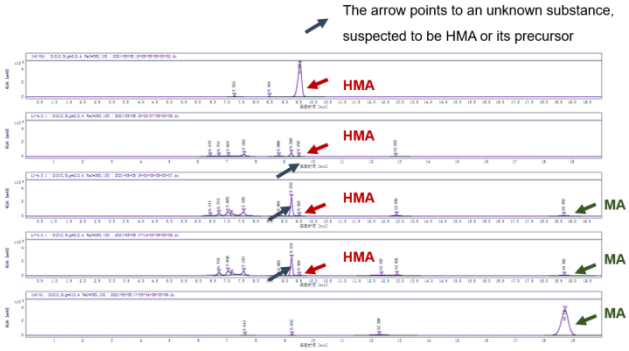

Figure 1 The peak diagram of HPLC results

We infer from the peak area that the HMA yield of LY-B was about 0.098 mM, or 16.4 mg/L and the MA yield of LY-B was about 0.22 mM, or 37.0 mg/L. (All the above were the yields of glucose added 4 g/L in 200 μL M9 medium)

5.21 Linshan Cao

Protein expression of engineering bacteria

The expression of *pheA* and *tktA* in MA-2 and MA-3 strains was unknown and the expression of *hmaS* in the three strains was not clear, so we think that protein detection was needed again.

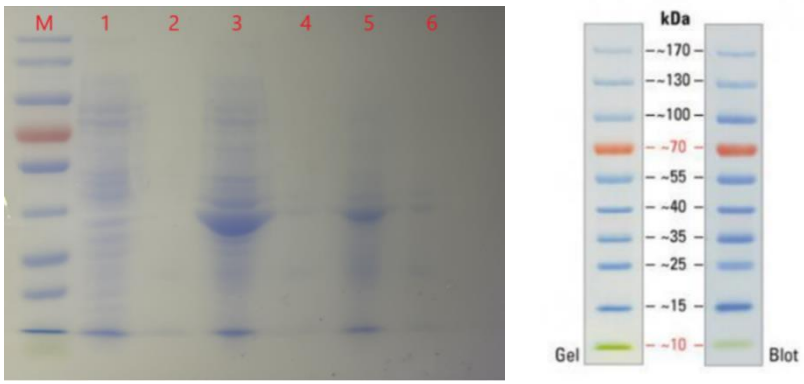

Figure 2 The SDS-PAGE graphical results of MA-1, MA-2 and MA-3 1 channel was

the supernatant of MA-1; 2 channel was the precipitation of MA-1; 3 channel was the supernatant of MA-2; 4 channel was the precipitation of MA-2; 6 channel was the precipitation of MA-3. 5 channel was the supernatant of MA-3; hmaS: 38.9 kDa aroG: 40.3 kDa pheA: 43.1 kDa tktA: 64.8 kDa ppsA: 87.4 kDa

5.22 Zhihao Wang

Response condition groping

1. Test the toxicity of 4HB and HMA.

In order to verify whether 4HB and HMA with ethanol could affect the growth of bacteria, we measured the growth curve overnight with a microplate tester and compared it with the system without inducer. We could see that 4HB and HMA has no toxicity to our bacteria.

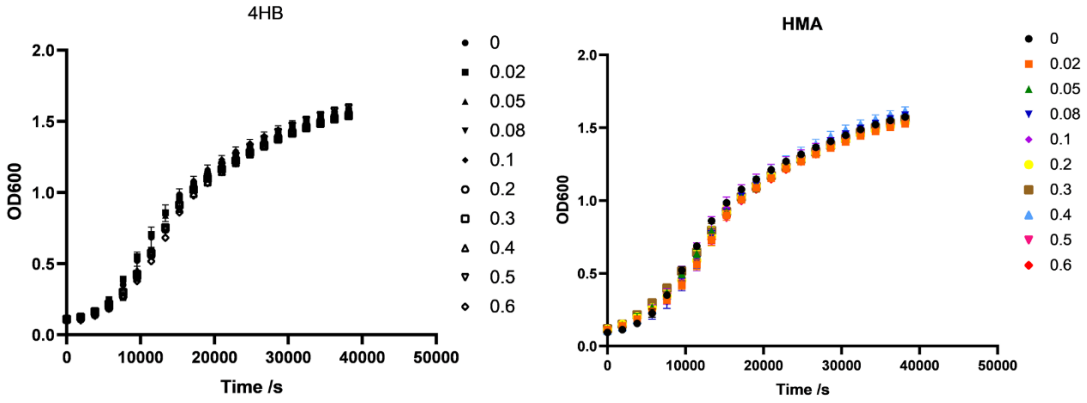

Figure 3 The growth curve of E. coli was added with a 10 % gradient of 4HB (left) and HMA (right)

2. Find the appropriate concentration of 4HB

In order to find the best 4HB concentration for the induction of control bacteria and verify that HMA has no induction effect on control bacteria, we did the gradient experiment of 4HB and HMA, and measured the growth curve overnight with enzyme tag and observed the fluorescence value.

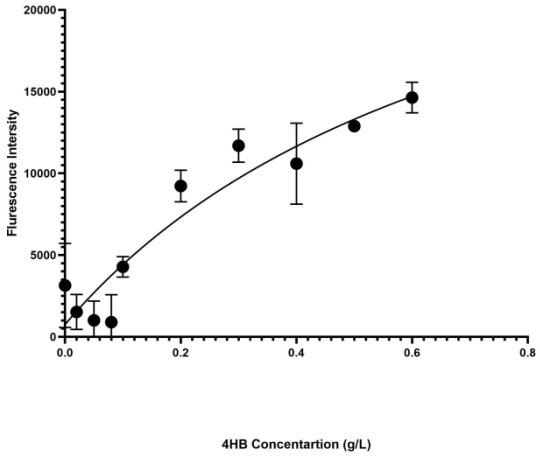

Figure 4 The relationship between fluorescence intensity and 4HB concentration on control bacteria

3. HMA validation of control bacteria

It was verified that HMA had no induction effect on control bacteria.

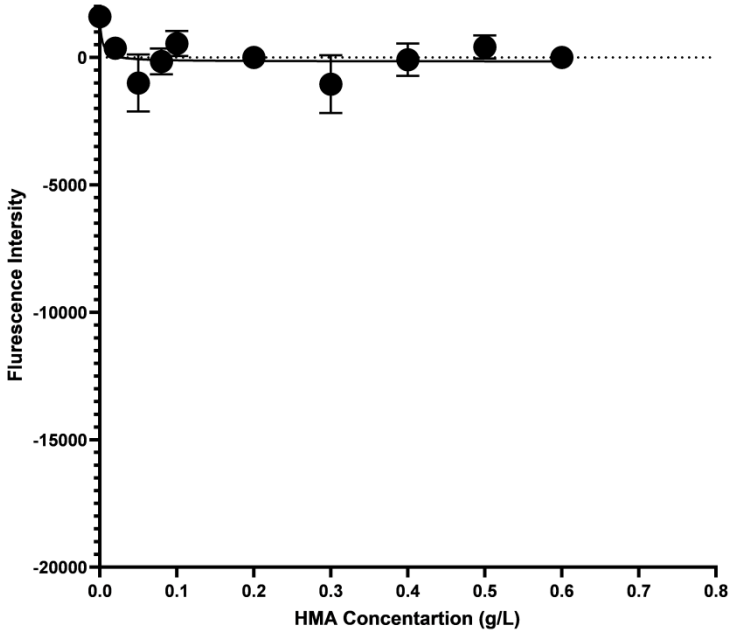

Figure 5 The relationship between fluorescence intensity and HMA concentration on control bacteria

5.23 Yifan Bian

chloramphenicol gradient experiment

In order to find out the range of chloramphenicol that can relieve the effect of chloramphenicol inhibiting the growth of bacteria, we conducted a chloramphenicol gradient experiment, adding 0.1 g/L of 4HB to induce the control bacteria, and select the bacteria that can grow.

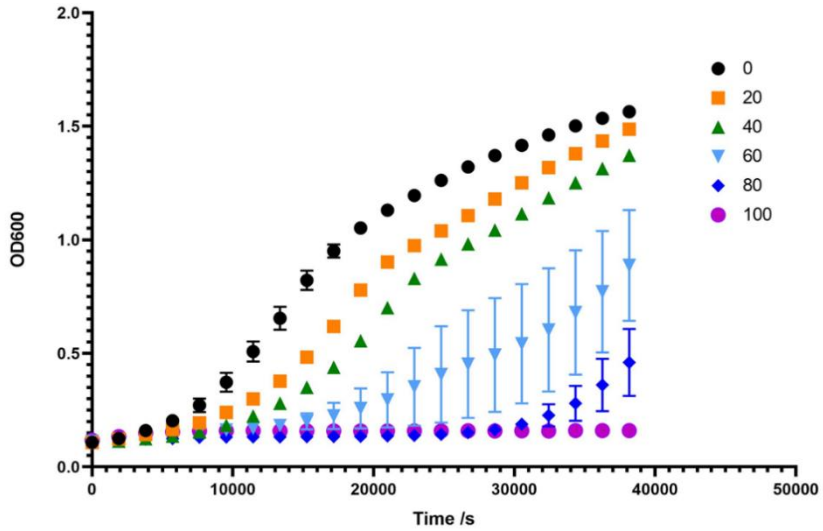

Figure 6 The growth curve of control bacteria in different chloramphenicol concentrations

5.25 Juan Luo

Exploration of first screening on solid medium

In the first screening condition of solid medium, we prepared chloramphenicol in the concentration of 10, 20, 34, 40 and 50 mg/mL. We added 20 mL LB solid medium, 2 mL 0.1 g/L 4HB solution, 20 μL chloramphenicol of various concentrations and 50 mg/mL ampicillin into 50 mL centrifuge tube and inverted plate. Each concentration plate was coated with 200 μL diluted 10^8-fold BW bacterial solution incubated for 10 hours and the colony growth of the plate was observed after inverted culture for 12 hours. It was observed that the colony grew well at 34 mg/mL chloramphenicol and isolated single colony appeared.

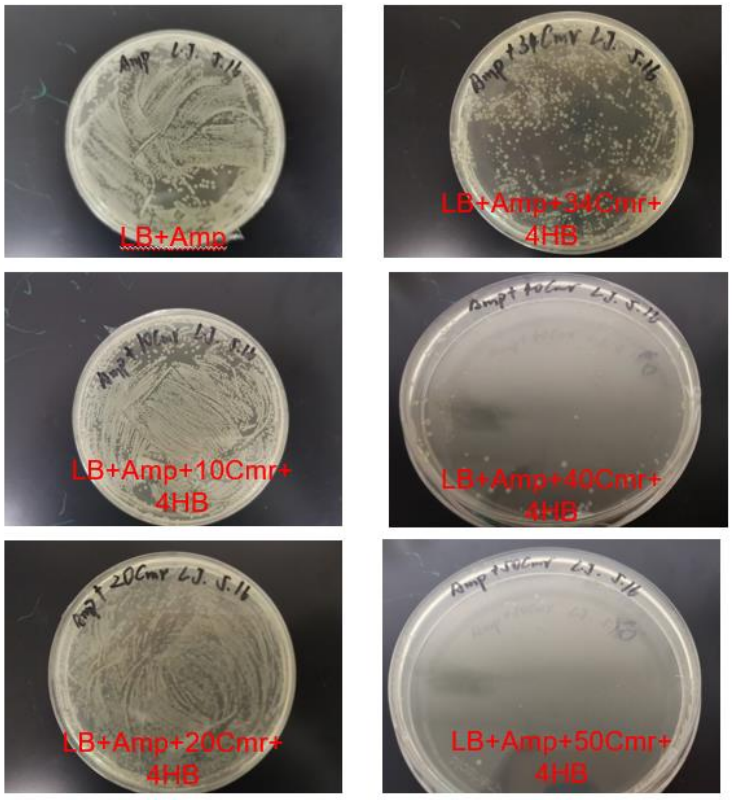

Figure 7 Growth of control bacteria in different concentrations of chloramphenicol