WEEK 26

9.22 Jiaqi Zhang

HMA-0 constructing experiment

This week, we transformed pRB1s-HmaS-aroG-pheA, pLT1k-MP6 and pYB1a-PobR^{F5B7}-eGFP-Cmr into BL21 and BW, respectively. And then we used chloramphenicol resistance gene for adaptive evolution of the strain in order to obtain a strain with high HMA yield.

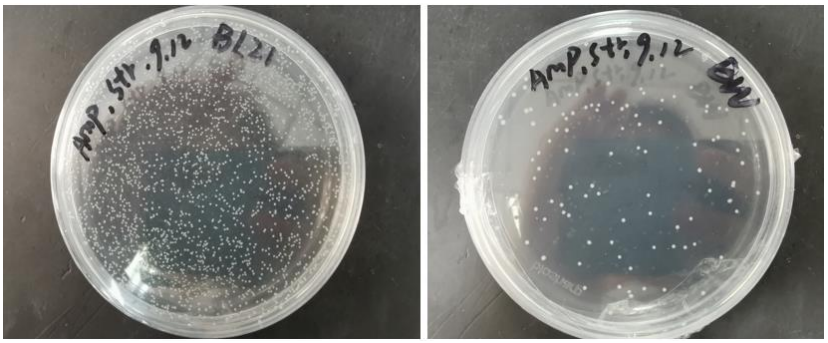

Figure 1 growth condition of the transformation plate (cultured at 37 ℃ for 12 h)

Four single colonies were selected from the BL21 transformation plate for 8 h culture, and one strain was selected as the protobacterium and named HMA-0.

Reaction system (10μL)		Reaction procedure		
Arog-Gibson-XhoI-F *pheA-Gibson-BglII-R*	0.4μL/0.4μL	98℃	5min	
microbial Plb1s-hmas-arog pheAfbr2 (positive control)	1μL	98℃ 55℃ 72℃	30s 30s 1min30s	×30
Low-fidelity enzyme	5μL	72℃	10min	
ddw	3.2μL			

Figure 2 HMA-0 PCR system (*aroG-pheA*)

Reaction system (10μL)		Reaction procedure		
pobR-mut-F/R	0.4μL/0.4μL	98℃	5min	
microbial/F5B7 plasmid、microbial (positive)	1μL	98℃ 60℃ 72℃	30s 30s 1min	×30
Low-fidelity enzyme	5μL			
ddw	3.2μL	72℃	10min	

Figure 3 HMA-0 PCR system (*pobR*)

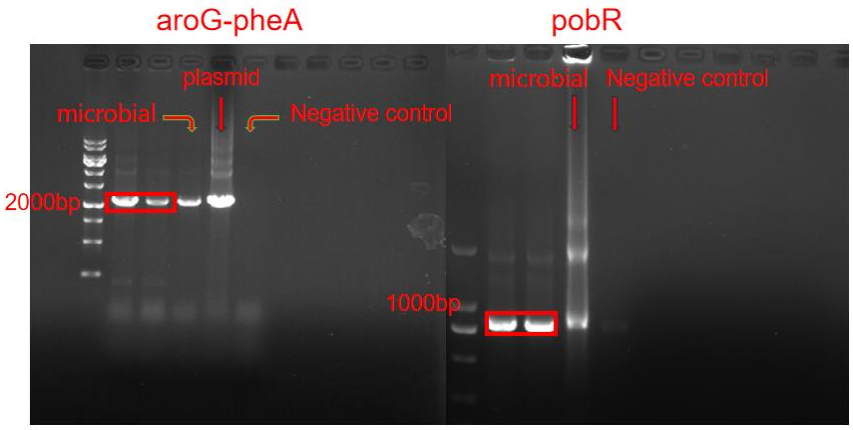

Figure 4 HMA-0 PCR electrophoresis result

AroG-pheA and *pobR* bands are correct.

9.24 Peiyao Chang

Adaptive evolution experiment under chloramphenicol pressure

The HMA-0 successfully constructed was subjected to adaptive evolution experiment under chloramphenicol pressure.

medium	M9
5xM9	8ml
20%Glu	800ul
1M $MgSO_4$	80ul
1M $Cacl_2$	8ul
20%ara	400μl
DDW	31.2ml

Figure 5 M9 liquid medium formulation

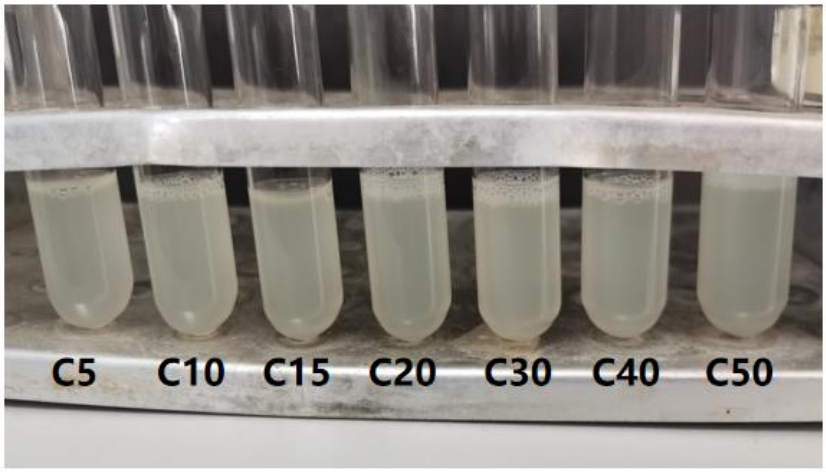

Figure 6 Gradient growth condition

The chloramphenicol concentrations in transfer culture were 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml, 50 μg/ml. After 12 h shake culture at 37 ℃ (inoculation amount 2%), C50 was selected for subsequent compression experiment, named HMA-C50-1.

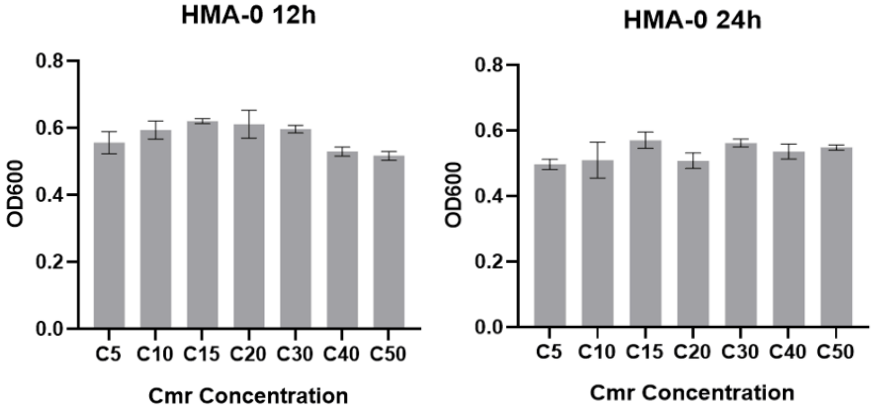

Figure 7 OD_{600} of HMA-0 (left); OD_{600} of HMA-0 (right)

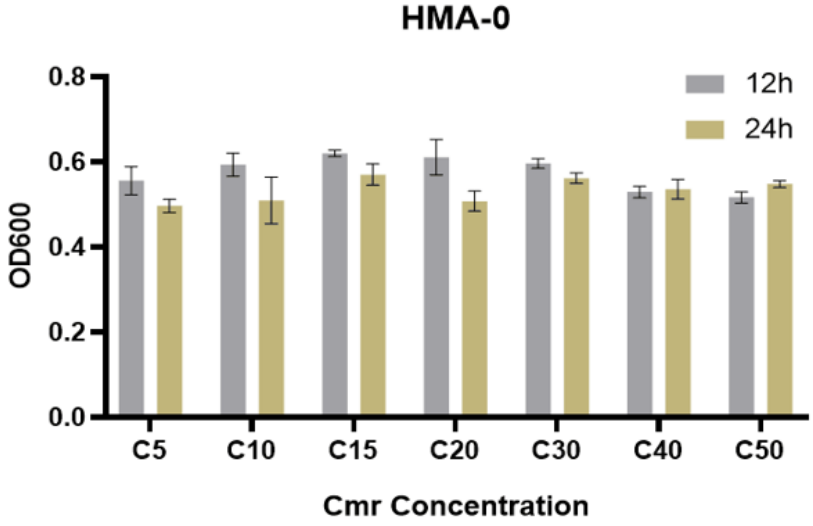

Figure 8 OD_{600} comparison condition of HMA-0 after 12 h VS 24 h

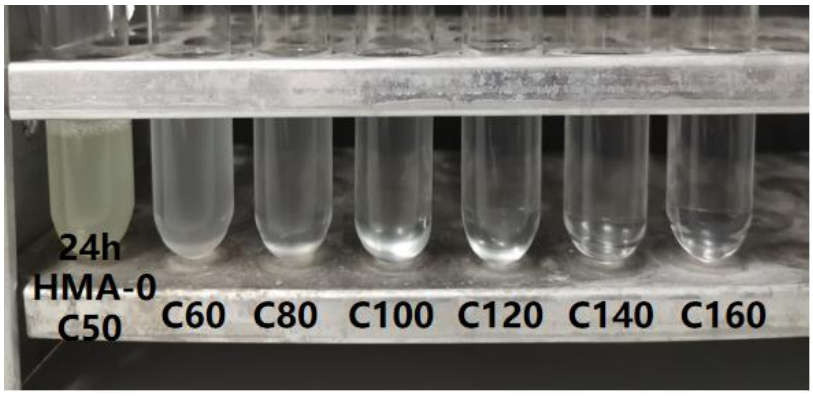

Figure 9 Gradient growth condition (12 h)

The chloramphenicol concentrations in transfer culture of HMA-C50-1 were 60 μg/ml, 80 μg/ml, 100 μg/ml, 120 μg/ml, 140 μg/ml, 160 μg/ml. Figure 9 is the shake culture growth condition after 12 h (inoculation amount 1%).

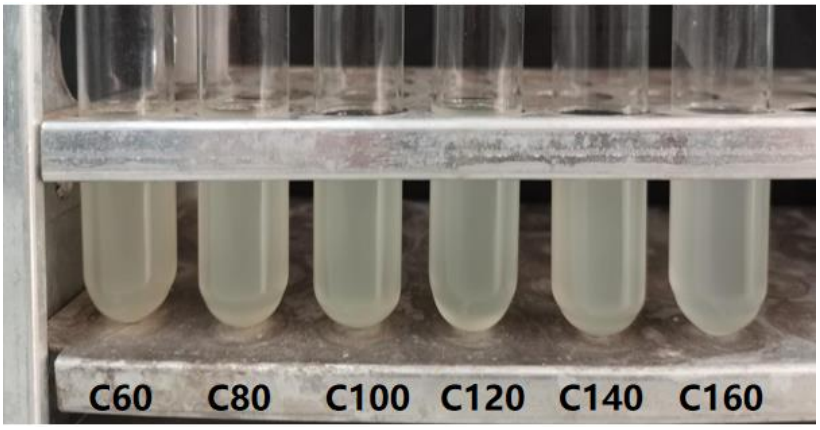

Figure 10 Gradient growth condition (24 h)

The chloramphenicol concentrations in transfer culture of HMA-C50-1 were 60 μg/ml, 80 μg/ml, 100 μg/ml, 120 μg/ml, 140 μg/ml, 160 μg/ml. Figure 9 is the shake culture growth condition after 12 h (inoculation amount 1%).

C60 was selected for subsequent experiments and was named HMA-C60-1.

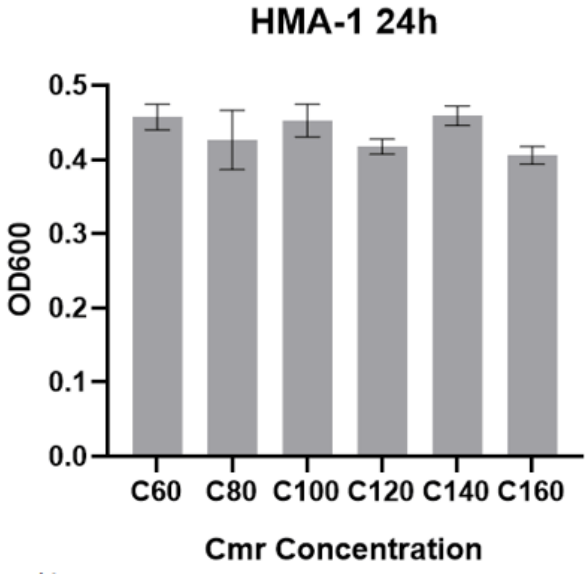

Figure 11 OD_{600} of HMA-C50-1 (12 h)

HMA-C60-1 (1% inoculation) was transformed into 60 μg/ml chloramphenicol M9 liquid medium and shaken at 37 °C (Figure 12).

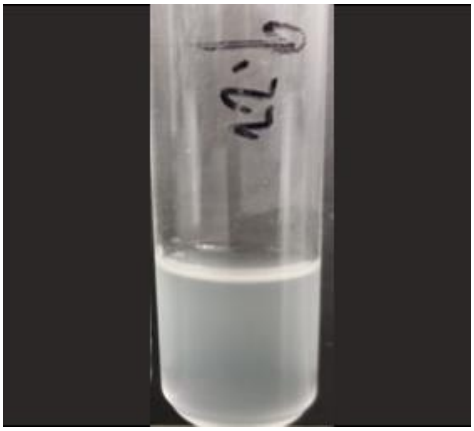

Figure 12 HMA-C60-1 growth condition under pressure (19 h)

9.26 Xin Xin

Adaptive evolution experiment under chloramphenicol pressure

We preserved the HMA-C60-1 which was cultured in 60 μg/ml chloramphenicol M9 liquid medium for 24 h and named it HMA-C60-2. 65 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C60-2 (1% inoculation) and shock culture was conducted at 37 °C (Figure 13).

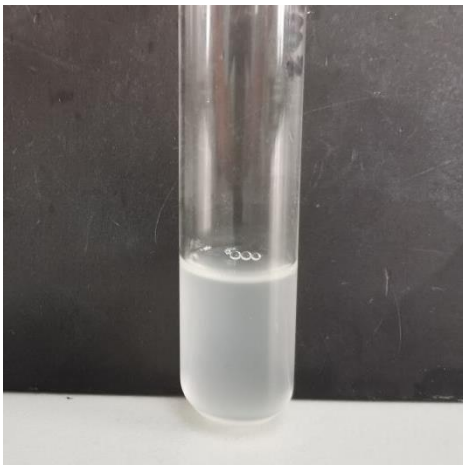

Figure 13 HMA-C60-2 growth condition under pressure (20h)

We preserved the HMA-C60-2 which was cultured in 65 μg/ml chloramphenicol M9 liquid medium for 20 h and named it HMA-C65-1. 65 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C65-1 (1% inoculation) and shock culture was conducted at 37 °C (Figure 14).

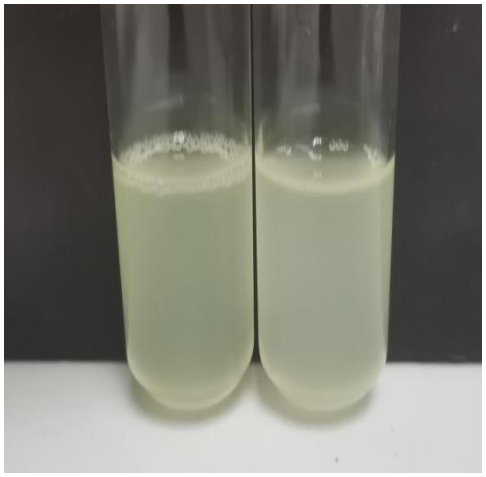

Figure 14 HMA-C65-1 growth condition under pressure (24 h)

9.27 Zhongyue Li

Adaptive evolution experiment under chloramphenicol pressure

We preserved the HMA-C65-1 which was cultured in 65 μg/ml chloramphenicol M9 liquid medium for 24 h and named it HMA-C65-2.

65 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C65-2 (1% inoculation) and shock culture was conducted at 37 °C (65 μg/ml chloramphenicol, cultured for 11 h on the left).
70 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C65-2 (1% inoculation) and shock culture was conducted at 37 °C (70 μg/ml chloramphenicol, cultured for 10 h on the right) (Figure 15).

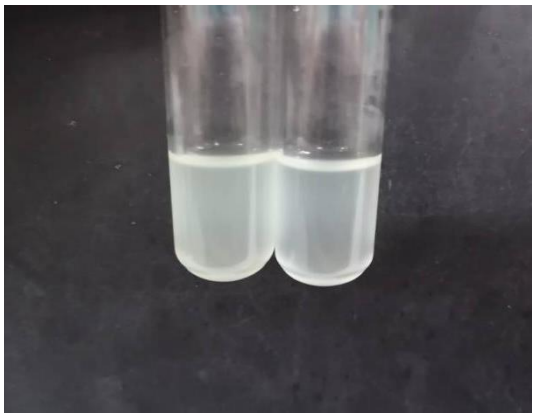

Figure 15 HMA-C65-2 growth condition under pressure

9.28 Yulong Zhang

Adaptive evolution experiment under chloramphenicol pressure

We preserved the HMA-C65-2 which was cultured in 70 μg/ml chloramphenicol M9 liquid medium for 20 h and named it HMA-C70-1.
75 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C70-1 (1% inoculation) and shock culture was conducted at 37 °C.
We preserved the HMA-C70-1 which was cultured in 75 μg/ml chloramphenicol M9 liquid medium for 20 h and named it HMA-C75-1.
80 μg/ml chloramphenicol M9 liquid medium was prepared to transfer HMA-C75-1 (1% inoculation) and shock culture was conducted at 37 °C.