HMA-Responsive Sensor-Driven adaptive laboratory evolution (ALE)

9.22 Jiaqi Zhang

HMA-0 constructing experiment

This week, we double transformed pRB1s-hmas-aroG^{fbr2}-pheA^{fbr2} and pYB1a-pobR$^{mut(P5-B7)}$-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.

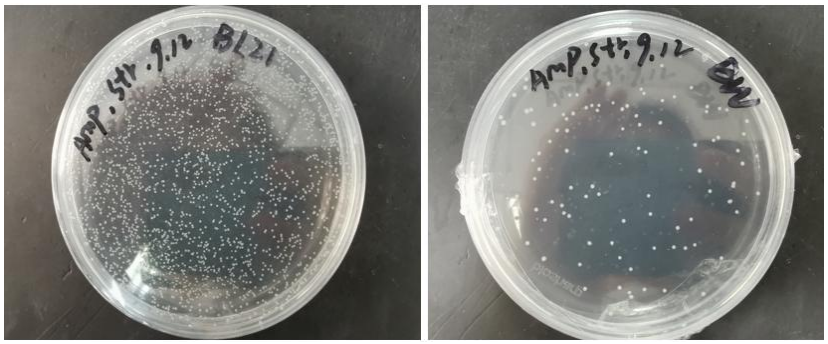

Figure 1 growth condition of the transformation plate (cultured at 37℃ for 12 h)

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*）

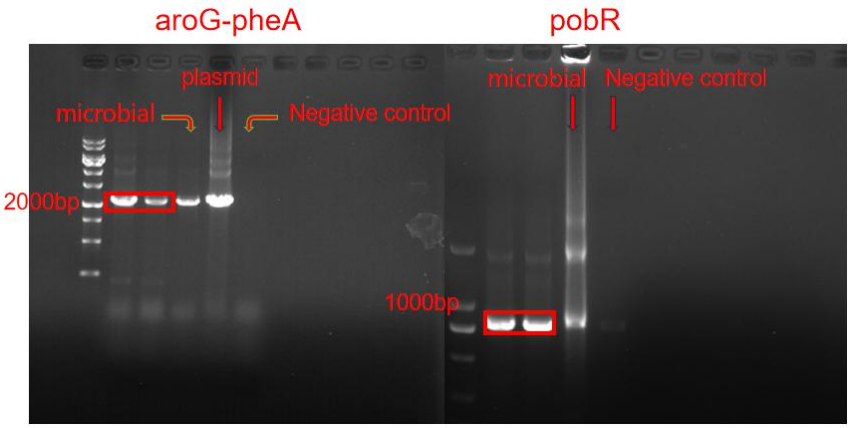

Figure 4 HMA-0 PCR electrophoresis results

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

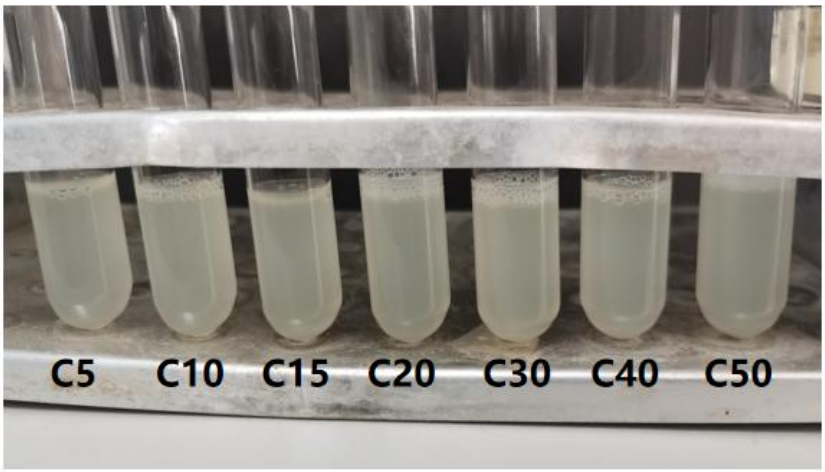

Figure 6 Gradient growth condition

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

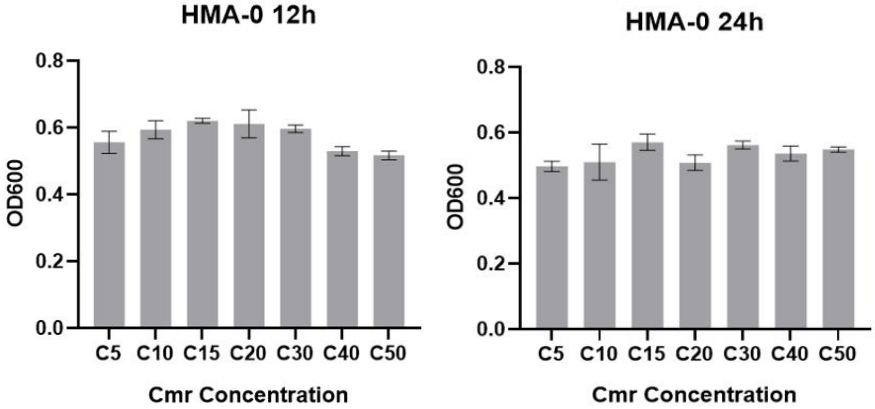

Figure 7 OD_{600} of HMA-0 (left); OD_{600} of HMA-0 (right)

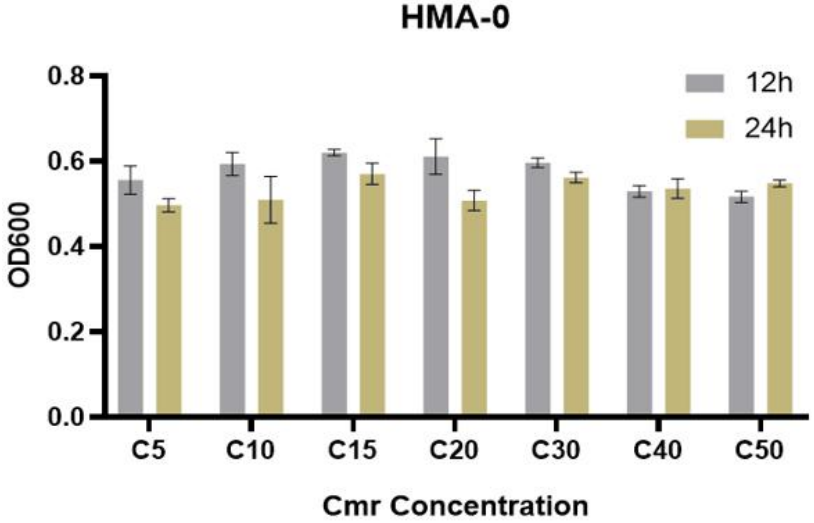

Figure 8 OD_{600} comparison condition of HMA-0 after 12 h VS 24 h

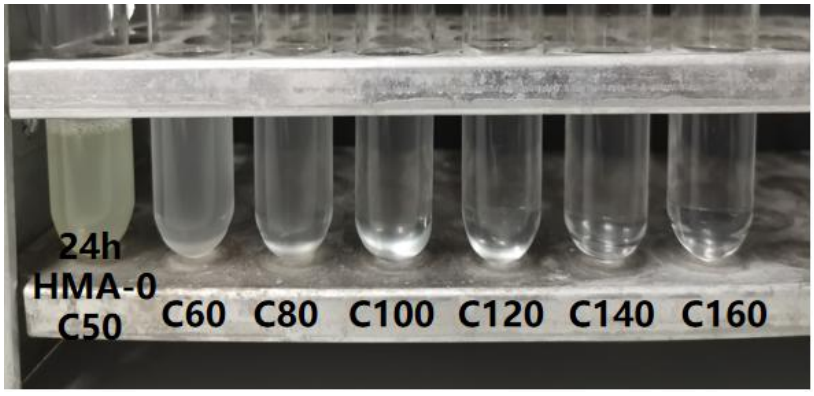

Figure 9 Gradient growth condition (12h)

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

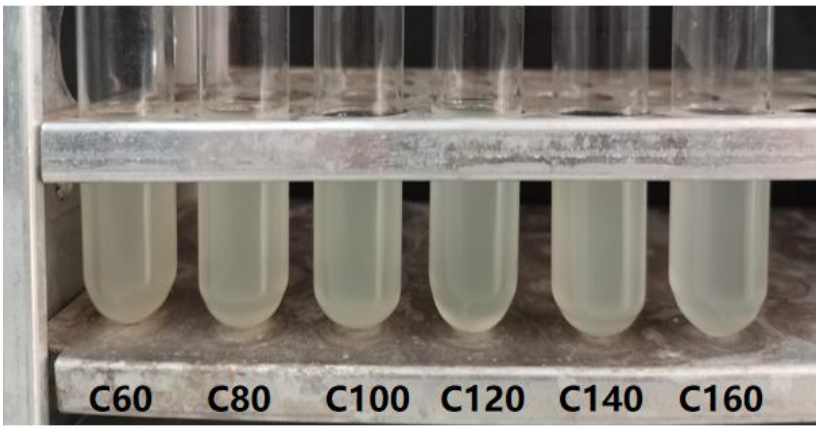

Figure 10 Gradient growth condition（24 h）

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

Chloramphenicol (60 μL/mL) was selected for subsequent experiments and was named HMA-C60-1.

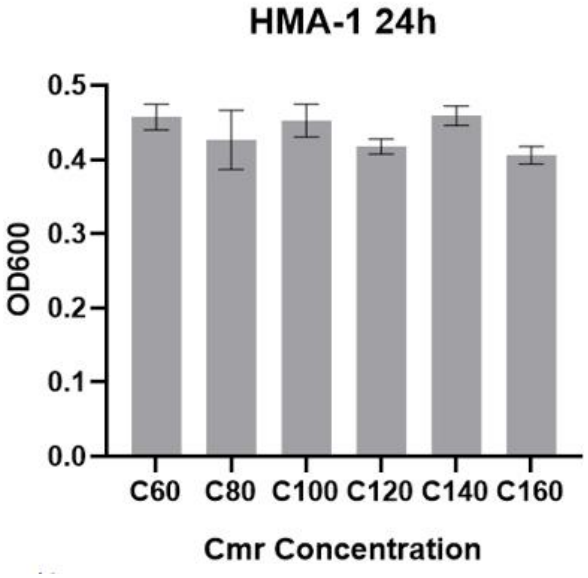

Figure 11 OD_{600} of HMA-C50-1（12 h）

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

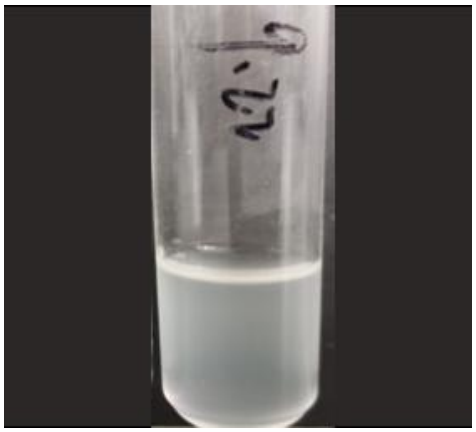

Figure 12 HMA-C60-1 growth condition under pressure (19 h)

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).

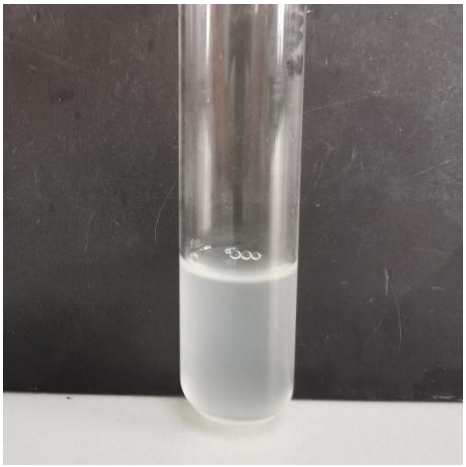

Figure 13 HMA-C60-2 growth condition under pressure（20 h）

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).

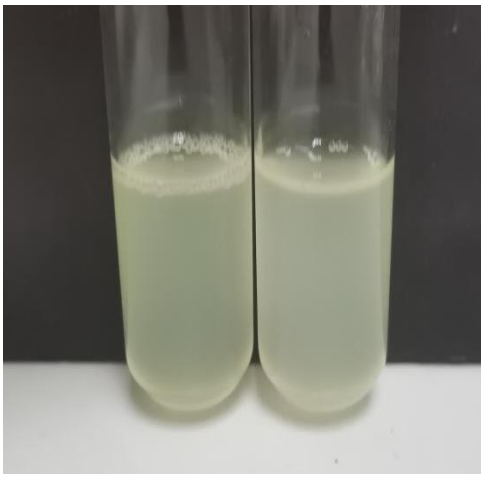

Figure 14 HMA-C65-1 growth condition under pressure (24 h)

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).

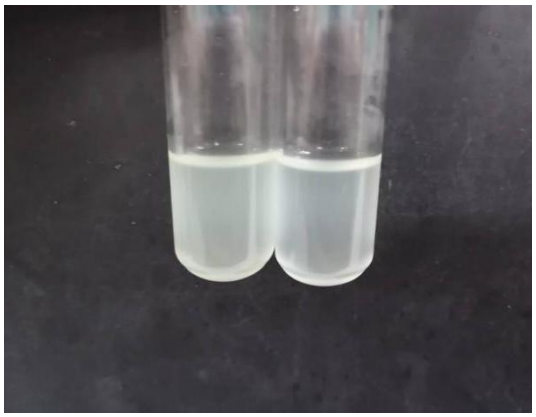

Figure 15 HMA-C65-2 growth condition under pressure

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.

9.29 Peiyao Chang

Adaptive evolution experiment under chloramphenicol pressure
HMA-C75-1 (1% inoculation) was transformed into 80 μg/mL chloramphenicol M9 liquid medium. After 12 h of shaking culture at 37℃, the bacterial condition was as follows.

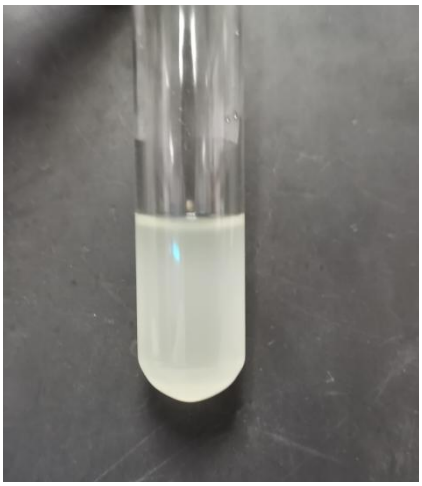

Figure 16 HMA-C75-1 growth condition under pressure
We preserved the HMA-C75-1 which was cultured in 80 μg/mL chloramphenicol M9 liquid medium for 12 h and named it HMA-C80-1.

10.2 Zhongyue Li

Adaptive evolution experiment under chloramphenicol pressure
HMA-0, HMA-50, HMA-60-1, HMA-60-2, HMA-65-1, HMA-65-2, HMA-70-1, HMA-75-1 and HMA-80-1 were resuscitated in M9 medium (inoculation amount 2%, no chloramphenicol was added). 12 h later, the growth curve was measured in the 96-well plates (adding different concentrations of chloramphenicol).

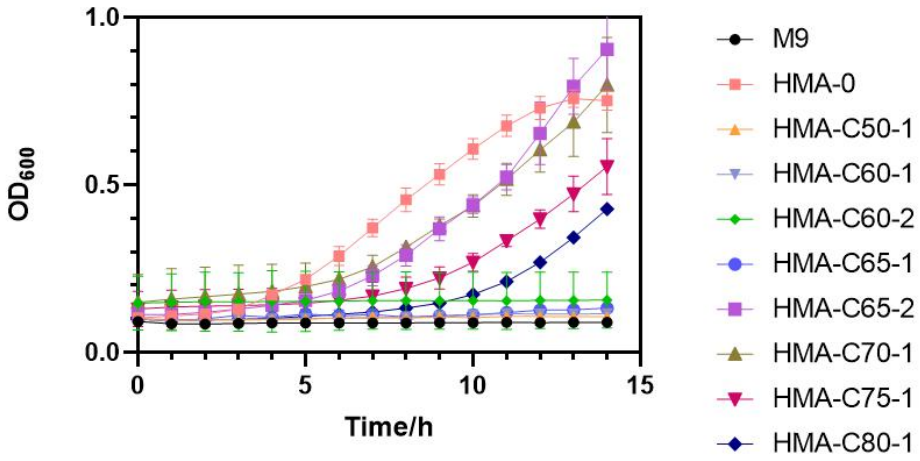

Figure 17 Growth curve

HMA-0, HMA-50, HMA-60-1, HMA-60-2, HMA-65-1, HMA-65-2, HMA-70-1, HMA-75-1 and HMA-80-1 were resuscitated in M9 medium (inoculation amount 2%, no chloramphenicol was added).

10.4 Xin Xin

Adaptive evolution experiment under chloramphenicol pressure

The above strains were transferred to M9 medium (inoculation amount 1%, without chloramphenicol), 1 mL of bacterial solution was taken after 12 h culture, and the supernatant was taken after centrifugation of 10000 g/L for 3 min. Samples were prepared and detected by HPLC.

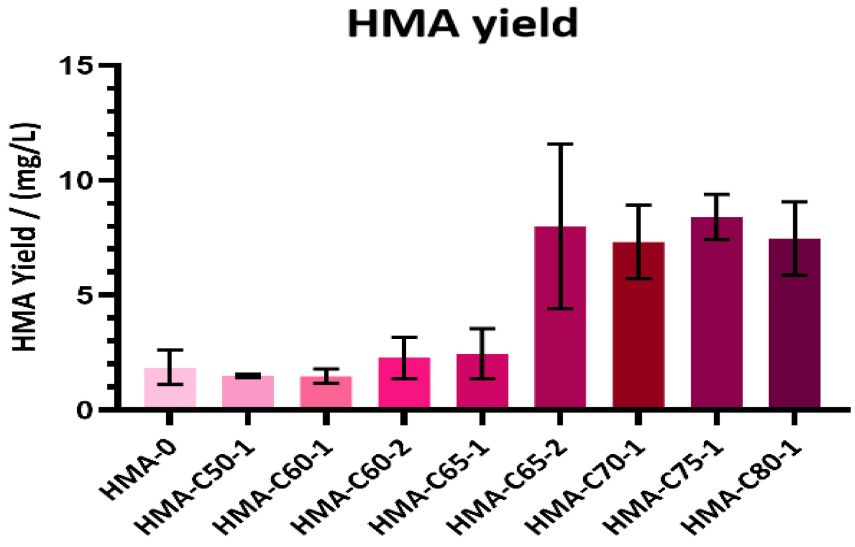

Figure 18 HPLC detection result

According to HPLC results, compared with HMA-0 strain, the yield of HMA in HMA-C65-2 and later strain were significantly increased by more than three times.

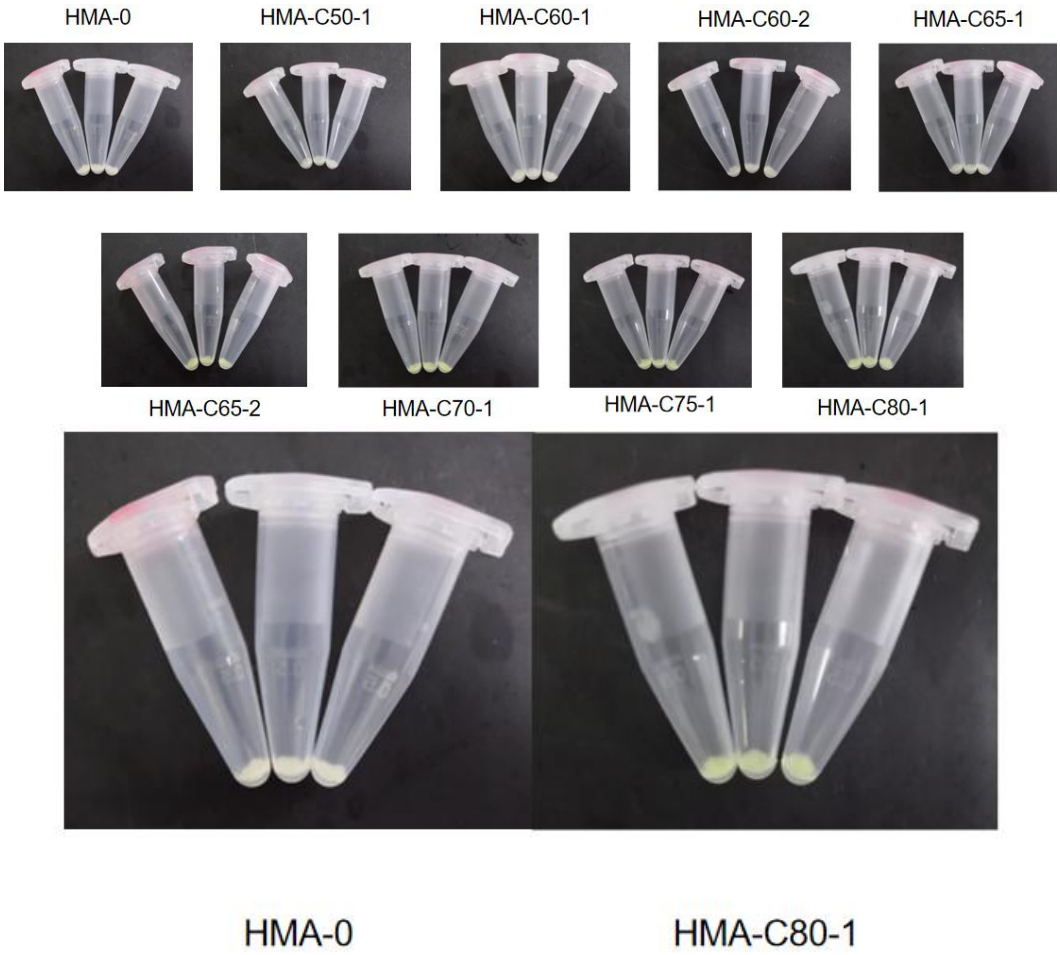

Figure 19 Bacteria color comparison

The comparison of HMA-0 and HMA-C80-1 bacteria showed that the latter was obviously green, which further indicated that the increase of product yield resulted in the enhancement of *eGFP* expression.