WEEK 9

5.26 Jianing Li

Determination of engineering bacteria by HPLC

MA-1: pLB1s-hmaS

MA-2: pLB1s-hmaS+pYB1a-aroG^{fbr}-pheA^{fbr}

MA-3: pLB1s-hmaS-tktA-ppsA+ pYB1a-aroG^{fbr}-pheA^{fbr}

LY-1: pYB1a-hmaS

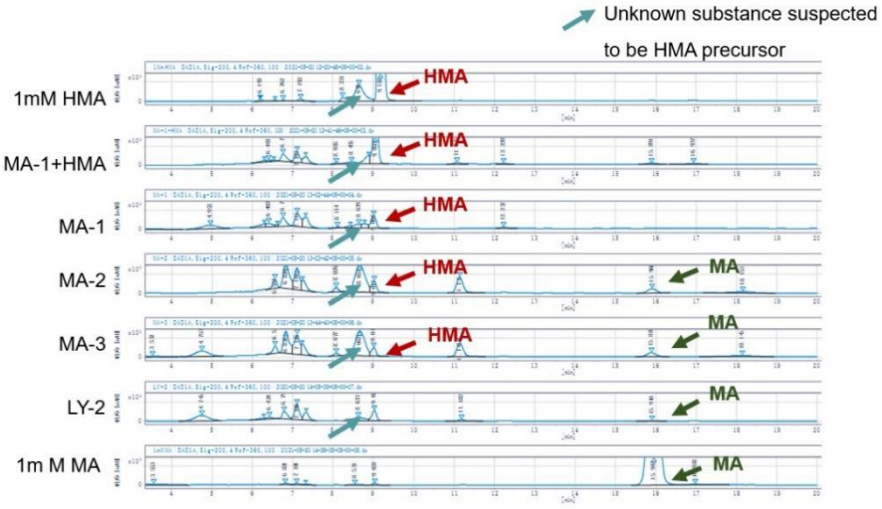

Figure 1 HPLC results of engineered bacteria

MA-1+HMA was the result of adding a small amount of HMA standard in the last HPLC sample. It was confirmed that the unknown substance was not HMA and the peak of 9.2 was HMA. The peak time is about 0.2 min earlier than last time. The yield of MA and HMA was the highest in MA-2, which was the same as last time.

5.27 Sunyue Cai

Screening

Our first screening system: Control group: 220 μL LB + 0.1 % ampicillin +1 % WT bacterial solution; 200 μL LB + 0.1 % ampicillin + 0.1 % 34 mg/mL chloramphenicol+10 % 0.6 g/L 4HB+1 % WT bacterial solution

Experimental group: 200 μL LB + 0.1 % ampicillin + 0.1 % 34 mg/mL chloramphenicol + 10 % 0.6 g/L HMA, mutant bacteria were selected.

OD600	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0249	0.0267	1.1517	0.8673	0.8153	0.8996	0.6266	0.5333	0.6019	0.5803	0.5791	0.6016
B	0.0247	0.9702	1.262	0.9126	0.9352	0.9763	0.1113	0.5303	0.6146	0.6025	0.7593	0.6124
C	0.0247	0.0659	1.2068	0.9094	0.9684	0.9906	0.5837	0.2801	0.6135	0.6082	0.5647	0.6156
D	0.9496	0.9081	0.9661	0.8613	0.9619	0.9195	0.6261	0.1135	0.5249	0.5987	0.575	0.5382
E	1.0065	0.9113	0.9866	0.9664	0.9259	0.8975	0.6184	0.6111	0.5946	0.6559	0.6597	0.615
F	1.0045	1.0084	0.9763	0.9712	0.9595	0.9799	0.6278	0.6356	0.6124	0.6277	0.6626	0.5888
G	0.9518	0.9699	0.8634	0.9559	0.892	0.8785	0.6624	0.5847	0.5575	0.7163	0.6416	0.3265
H	0.4914	0.9277	0.9712	0.8652	0.9599	0.9705	0.608	0.5705	0.6264	0.5972	0.5611	0.032

eGFP	1	2	3	4	5	6	7	8	9	10	11	12
A	9375	9596	46331	15842	15389	14782	22363	12196	13838	13890	24458	15966
B	9131	13749	46277	15368	53010	29622	10142	12628	18075	27851	14087	27095
C	9245	9707	47331	19966	34732	18872	13824	10482	22492	18338	16910	21833
D	15900	14812	24486	14777	23672	15511	16615	9655	11444	11426	12388	39319
E	35167	15411	29856	51709	29683	13396	13887	20996	23514	21451	17836	14556
F	22640	40869	23377	20732	24199	46807	22244	12267	13338	13790	20534	13412
G	17213	23171	18587	23082	13505	13715	21080	19512	12881	24634	14407	11452
H	10693	18978	31056	17300	25219	24328	13705	20315	14591	19006	16436	9822

Figure 2 Raw OD_{600} and Fluorescence values of initial screening

Since their OD values were generally higher, we speculated that the inhibition effect of 34 mg/mL chloramphenicol in liquid LB was poor.

5.28 Juan Luo

Liquid initial screening conditions

Since the inhibition effect of 34 mg/mL chloramphenicol in liquid LB was poor, the suitable concentration of chloramphenicol in liquid LB was explored by gradient chloramphenicol concentration.

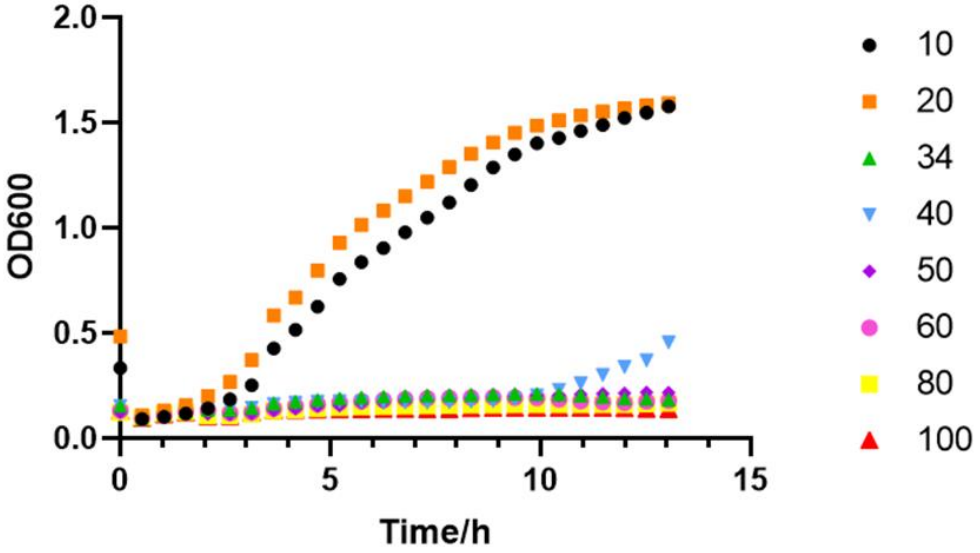

Figure 3 Growth curves of control bacteria in different concentration without induction

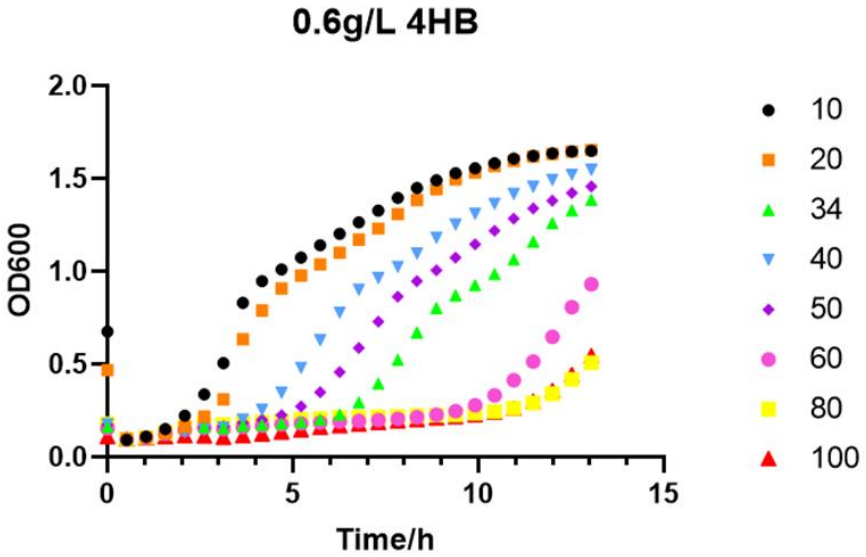

Figure 4 Growth curves of control bacteria in different concentration with induction

5.29 Chao Chen

Re-screening verification

We did a re-screening verification, but none of the strains met our requirements.

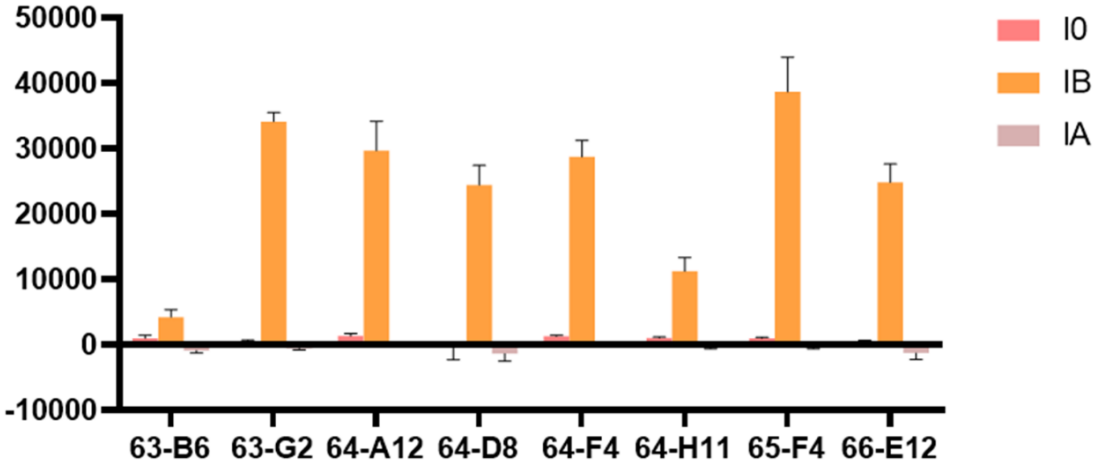

Figure 5 The I0, IB and IA value of each mutant

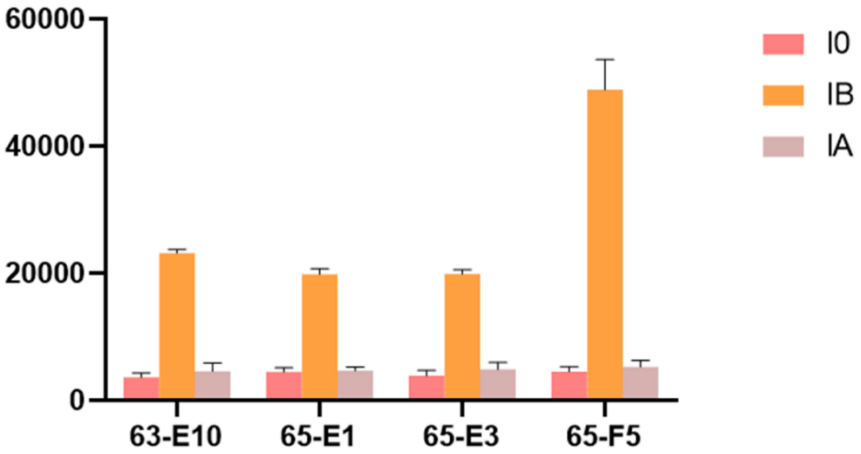

Figure 6 The I0, IB and IA value of each mutant

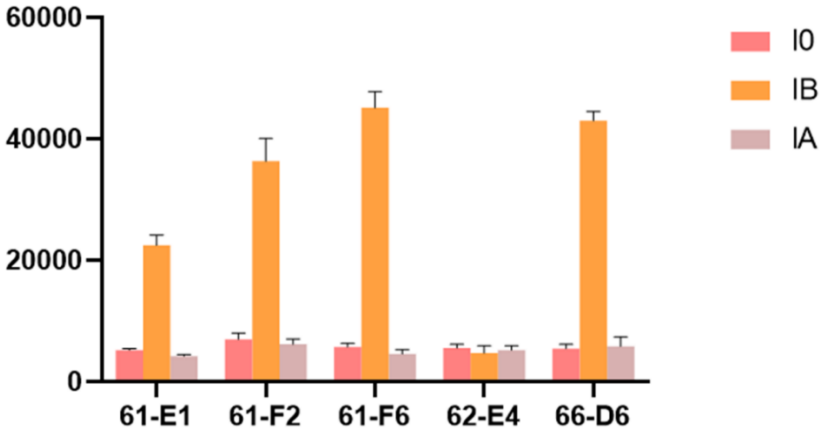

Figure 7 The I0, IB and IA value of each mutant

5.30 Xiangxin Li

First screening of solid medium conditions

In order to determine the growth of BW bacteria in solid medium under normal conditions, the control bacteria were cultured in liquid LB in 37 ℃ shaking table for 10 h to OD=4 to ensure the consistent growth of the control bacteria each time. ampicillin resistant plate was coated with 200 μL bacterial solution with different dilution ratios of 10^8、10^7、10^6、10^5、10^4

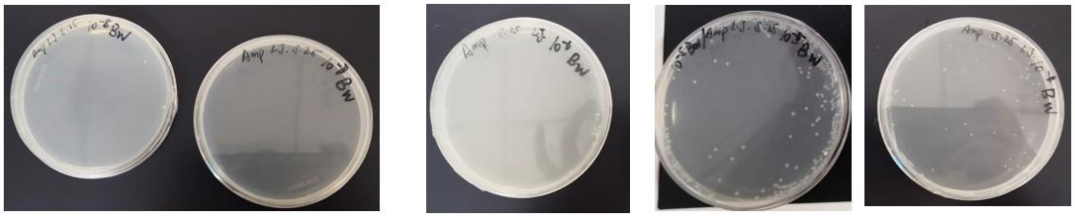

Figure 8 Growth of control bacteria in different dilution ratios

According to the results of the previous step, the plate with a dilution ratio of 10^4 was selected. The number of mono clones was about 120 under the dilution ratio.