2024.3.1-2024.3.17

Construction of PBPs Defective Strains

1. **Two pairs of forward and reverse primers 0959-gr-F/0959-gr-R and 1089-gr-F/1089-gr-R are annealed at 95 ℃ for 5 minutes to obtain the 0959 and 1089 gene fragments.**

2. **In the T4 ligase system, the 0959 and 1089 fragments are ligated to the enzymatically cut vector pL2R using T4 enzyme at 22℃ for 4-5 hours, resulting in the edited plasmids pL2R-0959 and pL2R-1089.**

3. **The ZMO0959 and ZMO1098 genes located at the positions of ZMNPΔ0038 are deleted using the edited plasmids pL2R-0959 and pL2R-1089, respectively, to obtain PBPs defective strains ZMNPΔ0959 and ZMNPΔ1098.**

4. Electroporation

The plasmid pEZ-PtZT1 is electroporated into ZMNPΔ0959 and ZMNPΔ1098, and the electroporated bacteria are sealed with parafilm and incubated at 30℃ in a shaker for 4-6 hours. Then, they are spread onto RS solid culture plates and marked respectively with 100 µL and 200 µL, for a total of 4 plates. The plates are incubated at 30℃ upside down in a constant temperature incubator.

5. Monoclonal Colony PCR

The electroporation plate is verified by monoclonal colony PCR. The water-soluble bacteria of the correct strain are inoculated into a 50 mL centrifuge tube containing 8 mL of RS Liquid culture meduim , and placed in a 30℃ shaker for overnight culture. Then, in a super-clean bench, 1 mL of the bacterial liquid is aspirated and mixed with 1 mL of 60% glycerol in a cryopreservation tube and stored in a -80℃ refrigerator to obtain the ZMNPΔ0959-PtZT1 and ZMNPΔ1089-PtZT1 strains.

6. Fermentation

(1) Firstly, 1 mL of the bacterial liquid is taken to a 50 mL centrifuge tube containing an appropriate amount of RMG5 (with corresponding antibiotics) medium as the fermentation seed liquid and statically cultured in a 30℃ incubator until the middle and late logarithmic phase. It is inoculated into the RMG5 (with corresponding antibiotics) medium with 80% bottling volume in a 50 mL Erlenmeyer flask, with the initial OD_{600nm} controlled at 0.1, and fermented at 100 rpm and 30℃.

(2) At fixed intervals, 1 mL of the sample is taken out in a super-clean bench for collection. The OD_{600nm} of the bacterial liquid is detected using a UV-1800 UV spectrophotometer and recorded. The remaining samples are frozen at -80℃ for subsequent detection.

(3) The data graphs are plotted using Graphpad 9.0 (Insightful Science, CA, USA) software.

7. Analysis by High Performance Liquid Chromatography (HPLC)

The samples are centrifuged at 12,000 rpm for 4 minutes, and the supernatant is collected and filtered through a 0.22 µm filter. 400 µL samples are taken into an HPLC injection vial for the detection of the contents of glucose, ethanol, and 3-HB in the HPLC.