2024.3.18-2024.3.31

The introduction of exogenous ethanol utilization pathways

1. **Polymerase chain reaction** The Pe^{EUP} fragment is obtained via PCR.

2. T5 Enzyme Ligation

The Pe^{EUP} fragment is ligated with the vector pEZ39p at a molar ratio of fragment to vector of ≥ 3:1, with a total mass not exceeding 120 ng. The required amounts of the fragment and the vector are calculated and added to a 4 μL system, complemented with water. Subsequently, 0.5 μL of buffer and diluted T5 enzyme are added each. Through the T5 enzyme ligation reaction conditions, the ligation is carried out to form the plasmid pEZ39p-Pe^{EUP}:

$$Pe^{EUP} + pEZ39p \text{ —— } pEZ39p\text{-}Pe^{EUP}$$

The mixture is spread on an LS medium plate in a super-clean bench and incubated overnight in a 37℃ constant temperature incubator.

3. Monoclonal Colony PCR

The T5 enzyme ligation plate is verified by performinutesg monoclonal colony PCR. The water-soluble bacteria of the correct strain are inoculated into a 50 mL centrifuge tube containing 8 mL of LS Liquid culture meduim and incubated overnight in a 37℃ shaker.

4. Electroporation

The plasmid is extracted from the DH5a pEZ39p-Pe^{EUP} strain and electrotransformed into the NPTΔ1-PtZT1 competent cells. 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 incubated overnight in a 30℃ shaker. Subsequently, in a super-clean bench, 1 mL of the bacterial liquid is aspirated and mixed with 1 mL of 60% glycerol in a cryotube and stored in a -80℃ freezer to obtain the strain ZMNPTΔ1-PtZT1^{EUP}.

5. Fermentation

(1) Firstly, 100 μL of glycerol bacteria are inoculated into a cryotube containing 1 mL of RMG5 (containing 100 μg/mL spectinomycin) medium and statically activated in a 30℃ incubator until turbidity is achieved.

(2) The activated bacterial liquid in the cryotube is poured into 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 OD600nm controlled at 0.1, and fermented at 100 rpm and 30℃.

(3) At fixed intervals, 1 mL of the sample is taken out in a super-clean bench for collection. The OD600nm of the bacterial liquid is detected using a UV-1800 UV spectrophotometer and recorded. The remaining samples are frozen at -80℃ for subsequent detection.

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

6. 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 (High Performance Liquid Chromatography).