Protocol for Protein induction in *Lactobacillus casei* ATCC 0334.
Protocol Code: InLc

Materials

- MRS medium
- Erytromicin
- Spectophotometer
- Spectophotometer cuvettes
- Flask of 100 mL
- Flasks of 2 L
- IPTG
- Fermenting jars
- Erlenmeyers de 125 mL
- Laminar Flow Hood
- Test tube
- 1.5 mL tubes
- Shaker
- Centrifuge

Procedure

Day 1.

1. Pre culture of L. casei

From a relative fresh plate of L. casei, pick a colony and growth O/N at 45 °C in 100 mL of MRS medium and 2.5 ug/mL of erythromycin. Place them in a fermenting jar to make an microaerophilic condition. Incubate in a shaker for approximately 17 hours.

Day 2.

1. Culture of L. casei

Put MRS medium in ¼ of the total volume of the flask. Add 2.5 ug/mL of erythromycin. Take 2 mL in a spectrophotometer cuvette and tag as “Blank sample”. Dilute 1:10 of preculture L. casei in the flask mention above and grow 3-4 hours at 45 °C in microaerophilic conditions in a shaker. Read the OD every 1½ hour or 2 hours.

2. Prepare IPTG

Prepare 1 M IPTG and prewarm to 45°C. Consider the culture volume taken for the readings, when calculating the volume of IPTG needed.

3. Induction

3.1. When the OD is between 0.6 and 0.8 centrifuge 2 mL for 1 min at 4000 rpm room temperature. Place the supernatant and pellet in different tubes. Label them as “supernant before induce SDS” and “pellet before induce SDS”. Freeze it at -20°C until needed.

3.2. Add 1 mM IPTG and incubate the culture overnight at 45 °C in a shaker with microaerophilic conditions.
Note: Although the protein is zinc dependent, it doesn't interfere in the recombinant production.

4. Final centrifugation
4.1. After 5 hours of incubation, centrifuge 2 mL for 1 min at 4000 rpm and 4°C, place the supernatant and pellet in different tubes. Label them as "supernatant after induce SDS" and "pellet after induce SDS". Freeze at -20°C until needed. This is for the SDS PAGE.
4.2. Centrifuge the culture in falcons of 50 ml at 12000 g and 4°C for 10 min. Place the supernatant and pellet in different tubes. Label as "supernatant after induce" and "pellet after induce".
4.3. Centrifuge the "supernatant after induce" 3-4 times more to ensure the absence of cells. Freeze at -20°C until needed.

References

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