Bacterial Cellulose Beads

This protocol describes the growth of bacterial cellulose (BC) beads with *Komagataeibacter sucrofermentans* DSM 15973, and their cleaning before use.

Materials

- *K. sucrofermentans* DSM 15973
- YPD medium
- Demi water
- 0.1 M NaOH

Equipment

- 50 mL falcon tubes
- Shaking incubator (30 °C, 500 rpm)
- Heating plate or water bath (80 °C)
- Serological pipettes and pipette boy
- Tweezers
- Autoclave
- Nitrile gloves

Protocol

Growing the beads:

1. Inoculate 10 mL of YPD medium with *K. sucrofermentans* DSM 15973 in a 50 mL falcon tube.
2. Incubate at 30 °C and 500 rpm for 74 h.

Cleaning the beads:

3. Remove the culture medium with a serological pipette.
4. Wash three times with demi water. The water now contains cells and must be autoclaved before it is discarded.
5. Replace the water with 0.1 M NaOH and heat to 80 °C, swirling the flask every 5 min.
6. After 30 min, replace the NaOH with fresh 0.1 M NaOH.
7. Keep washing at 80 °C for at least 60 min in total. The beads are clean once they turn white or transparent.
8. Take the beads out of the NaOH and rinse them with demi water to neutralise the pH.

CBD Binding Assay

This protocol tests the binding of a fluorescent cellulose-binding domain (CBD) fusion protein to bacterial cellulose beads.

Materials

- Functionalising strain expressing the fluorescent CBD fusion
- Growth medium with the appropriate antibiotic
- Cleaned BC beads (see the previous protocol)
- Protease inhibitor
- B-PER Bacterial Protein Extraction Reagent (Thermo Scientific)
- PBS or tap water

Equipment

- Shaking incubator
- Spectrophotometer (OD_{600})
- 1.5 mL Eppendorf tubes
- Tweezers
- Fluorescence microscope or fluorescence imager
- Pipettes and pipette tips

Protocol

1. Grow the functionalising strain overnight.
2. Dilute the culture to an OD_{600} of 1.
3. Transfer 1 mL of the diluted culture into a new 1.5 mL tube for each planned BC bead.
4. Add protease inhibitor to each tube.
5. Lyse the cells with a suitable protocol; here, B-PER Bacterial Protein Extraction Reagent.
6. Incubate one BC bead in each protein suspension for 30 min.
7. Wash the beads by replacing the suspension with PBS or tap water, twice.
8. Determine the fluorescence with a fluorescence microscope or a fluorescence imager.