

Saptasense Protocol

Isolation of Dextran

Background

The maple syrup solution following incubation will be filled with many contaminants in addition to the desired dextran. The standard exopolysaccharide (EPS) isolation procedure involves precipitation with cold ethanol to isolate the crude EPS product from the cellular debris. Precipitation steps are followed by centrifugation, resuspension in warm DI water, and refrigeration. Centrifugation and precipitation is repeated again on the following day after which the isolated supernatant undergoes dialysis and 36 hour refrigeration.

It is important to note that a trichloroacetic acid precipitation step is frequently used in EPS isolation protocols in order to remove any protein impurities from the sugar chain. For the purposes of this project, this step has been omitted.

PROTOCOL: Dextran Isolation

Materials

Cold 95% ethanol (C₂H₅OH)
Distilled water

Instruments

Centrifuge
Dialysis bag/tube (3.5 kDa
MWCO)
Heat block
2mL and 50mL centrifuge
tubes
Lyophilizer

Methods

- Remove the remaining cultured samples from the culture tubes and place them in labeled 2mL microcentrifuge tubes. Heat tubes using heat block at 100°C for 30 minutes to heat kill any remaining active enzymes.
- Centrifuge at 12,000 x g at 4°C for 15 minutes. Transfer supernatant to a new labeled 50 mL centrifuge tube. Add an equal volume of cold ethanol. Refrigerate overnight at 4°C. Adding cold ethanol will precipitate the dextran out of solution.
- Centrifuge sample at 12,000 x g at 4°C for 15 minutes. Remove supernatant. Redissolve product in 25 mL of distilled water with gentle heating (below 50°C).
- Add 25 mL of cold ethanol. Centrifuge at 25,000 x g at 4°C for 25 minutes. Remove supernatant.
- Redissolve pellet in 15 mL of distilled water with gentle heating.
- Seal the bottom of a small dialysis tube (10 cm in length) with a clip. Place the solution in a dialysis tube (3.5 kDa MWCO). Seal the top of the tube with a second stopper and place it in a beaker of distilled water. Cover and keep at 4°C for 24 hours. The distilled water must be changed

twice throughout this dialysis period. The remaining retentate represents the crude dextran product with small mw impurities removed.

- Recover dialyzed dextran through lyophilization for use in hydrogel preparation.

Protocol adapted from Ahmed et al., doi: 10.1016/j.foodhyd.2012.06.009