Levan Production and Isolation

Introduction

This protocol details steps for levan production and isolation from *Bacillus subtilis* cultures grown with a sucrose-containing "productive medium" (previously optimized for maximal levan production by Abou-Taleb et al., 2014).

Materials

- For 300 mL of "productive medium"
    - 1.35 g ammonium sulphate
    - 18 g sucrose
    - 0.18 g MgSO4
    - 6.48 g K2HPO4
- *Bacillus subtilis* culture (grown in LB medium)
- Ice-cold absolute ethanol

Levan Culture

1. Prepare 300 mL sterile "productive medium" containing (g/L) in LB: ammonium sulphate, 4.5; sucrose, 60; MgSO4.7H2O, 0.6; K2HPO4, 21.6. (optional: adjust pH to 7.8 before sterilization)

    Notes:

    - *Sulphates are used for maximizing levansucrase production*
    - *The optimal production of the levansucrase is at 30°C. The effect of different nitrogen sources showed that baker's yeast with 2% concentration gave the highest levansucrase activity. Addition of 0.15 g/L MgSO(4) was the most favorable for levansucrase production.*

2. Add *B. subtilis* (grown in LB nutrient broth medium) to "productive medium" at 5% v/v ratio in 250 mL Elenmeyer flask at 37°C on rotary shaker for 96h

Levan Isolation

3. Take samples of 10 mL to determine cell dry weight & levan concentration.
4. Centrifuge samples at 10,000 rpm for 10 min at 4°C.
    - Pellets will be sources of dry cell weight— wash twice with distilled water and dry at 80° to a constant weight.
    - Supernatant will be used to precipitate levan polymer by adding 1.5 volumes of ice-cold absolute ethanol to supernatant and left for an hour. Wash precipitated pellets twice with distilled water, then collect pellets by centrifugation at 10,000 rpm/10 min. Precipitate will be polymer dry weight after oven-drying at 110°C for 24 hours.

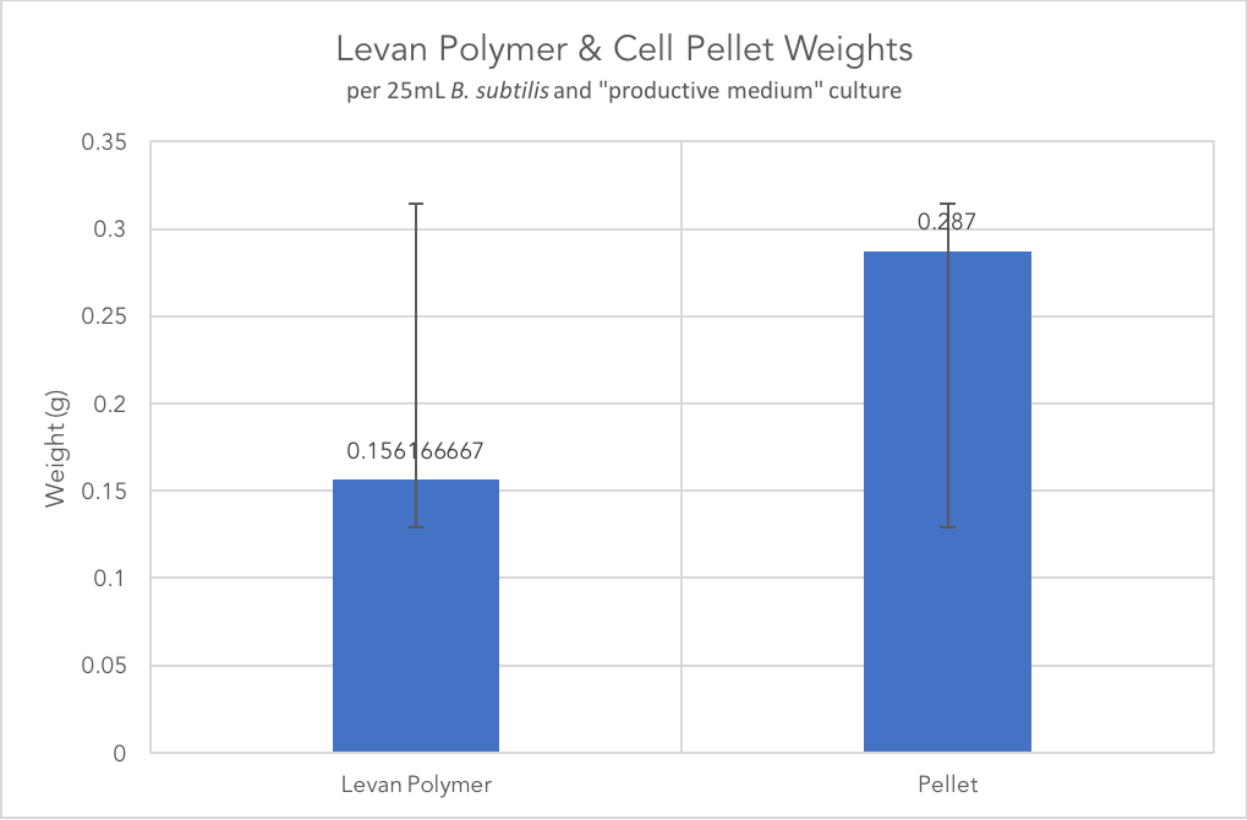
Revised and Consolidated Levan Protocol

8/15/17:

1. Prepared 150 mL of “productive medium”
2. Grew 7.5 mL of s.c. *B. subtilis* in 150 mL of "productive medium" for 96h

8/18/17:

1. Split culture into six 45 mL falcon tubes (25 mL each).
2. Centrifuge for 15 min at max speed, 4°C.
3. Decant supernatant into new Elenmeyer flask. The cell pellets were kept and oven dried at 110°C for 24 hours to determine cell dry weight. Supernatant was used to precipitate levan polymer by adding 1.5 volumes of ice-cold absolute ethanol to supernatant and left for an hour.
4. For each 25 mL *B. subtilis* culture in “productive medium” → added 37.5 v/v absolute ethanol (236.25 mL) : 62.5 mL total volume
5. Collect polymer pellets by centrifugation for 30 min at max speed, 4°C
6. Weigh precipitated polymer pellet
    a. Resulting cell pellet (step 3) & polymer weights will be per 25 mL culture of *B. subtilis* and “productive medium”



References:

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