Aspergillus niger protoplast protocol

Introduction

This protocol is adapted from a protocol used by DTU Bioengineering.

Materials

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  - Fungal plates (either streaked or 3-point)
  - Drigalski spatula
  - 500 ml shake flasks
  - Counting chamber
  - Solutions (APB, ATP, PCT, milli-Q, TM)
  - 30 °C incubator with shaking
  - sterile tea spoon
  - mira cloth in funnel (sterile)
  - Glucanex
  - magnet stirrer
  - magnets
  - 50 ml sterile falcon tubes
  - 0.45 µm filters
  - 50 ml syringe
  - centrifuge for falcon tubes
- **Media**
- Aspergillus transformation buffer (ATB)
- Aspergillus protoplastation buffer (APB)
- YPD media

Procedure

Initiation

1. Streak spore suspension of host strain on YPD plates supplemented with uridine (for this particular fungus) and let grow for a week (there should be black spores!)
2. All solutions should be sterile.

Day 1 (inoculation)

3. Add 95 ml of YPD media supplemented with uridine to a shake flask and transfer 5 ml of the YPD media to a plate with A. niger. Collect conidia and spores from plate by carefully scraping off the conidia using a drigalski (the spores are hydrophobic and would therefore rather just fly around than actually get into suspension so be careful not to make a mess here). This should give a concentration of around 10^8 spores/100 ml. It is a good idea to make more than one shake flask at a time.

4. Incubate shake flasks at 30 °C, 150 RMP for 48 h.

Day 3 (mycelial harvest)

5. Place the sterile funnel with a mira cloth in to a sterile blue cap bottle and transfer the contents of the shake flasks to the mira cloth (content should be brown and thick).

6. Wash the mycelia using *Aspergillus* protoplastation buffer (APB) to remove residual glucose from the mycelia (this can inhibit protoplastation). You need to use quite a bit of APB. squeeze out remaining liquid using a sterile spoon.

   Then, transfer the mycelium to falcon tubes (≈2 g per tube => 1 shake flask ≈ 2 falcon tubes).

Protoplastation

7. Add glucanex to APB to get a final concentration of 40 mg glucanex per ml APB and dissolve glucanex via gentle magnetic stirring and no heat.

8. Sterile filter 20 ml of APB+glucanex to each falcon tube using a 0.45 µm filter and a 50 ml syringe (there is a bit of resistance in the filter but that's ok)

9. Shake/incubate enzyme-mycelium mix at 30 °C, 150 RPM for 2-3 h.

10. From now on, whenever you pipette anything with the cells in it, cut of the edge of the pipette tip and CAREFULLY(!) pipette the cells. If you don't do this, they break as they don't have a cell wall to keep them stable.

11. Evaluate the number and quality of protoplasts in a microscope and discard a batch that is too diluted (i.e. < 10^5 protoplasts/ml). Approved protoplast solutions are then diluted by pouring APB up to 40 ml. and the tubess are balanced. Dilute *Aspergillus* transformation buffer (ATB) to 1/2x with sterile milli-Q H_2O and carefully place 5 ml of this on top of the APB, creating an overlay. Centrifuge samples on rotor settings rotor code Sla-600TC; time: 13 min; Speed: 3000g; Temperatire: 16 °C, Acc: 2, Brake: 2 (NB! due to slow acc and brake, this takes forever!)

12. In the interphase between the two liquids, a halo of white slurry consisting of concentrated protoplasts should be observed. If there is cell wall debris mixed in with the protoplasts, that's ok. They can still be used. Withdraw the protoplasts with a pipette and wash them in a new falcon tube. Add ATB up to 40 ml and pellet the protoplasts at 3000g for 13 min (acc. 2, brake 2). Discard supernatant by decanting.

13. Count protoplasts in microscope by diluting a small sample 1:100.

14. Resuspend protoplasts in 4 ml ATB to obtain concentrated solution.