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Aspergillus nidulans protoplast isolation for transfections

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Protocol status: Working
We have used this protocol in our group and it was working very well.

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Abstract

Modified *Aspergillus nidulans* protoplast isolation using Novozyme VinoTaste Pro as an enzyme source. Protocol from previously established method adapted to produce viable protoplasts for transfections from an inexpensive and commercially available cellulase/chitinase source.

Image Attribution

Andrew W. Liu

Materials

MATERIALS

- VinoTaste Pro
- 1.1M Potassium Hydroxide
- 2M Potassium Chloride
- Citric Acid (Anhydrous)
- Aspergillus Media (MM & CM)
- 1.2M Sucrose Solution (filter sterile)

Grow Overnight Culutre

15m

1 **Late afternoon-evening the day before**, grow scraped condispores (two arms from MM complete agar plate, ~ 1 × 10^8 total spores) in 30ml liquid CM, supplemented with pyridoxine and riboflavin, overnight at 25°C 18-20 hrs or 30°C 11-12 hrs on orbital shaker at 150rpm. Growth can be arrested at 4°C for an hour or two prior to protoplasting if needed.

Hyphae formation after 12 hours shaking and incubation at 30°C.

Prepare Protoplast Solution

50m

2 **An hour before protoplast isolation**, prepare 25ml fresh 2x PP Solution in 50ml conical tube as follows: 10m

- 13.7ml 2M KCL
- 480mg Citric Acid (Anhydrous)
- 6.4ml 1.1 M KOH
- 3.2g VinoTaste Pro (Novozymes)
- ~3.0ml dd$H_2$0 (final volume 25ml)

3 Shake vigorously in 50ml conical tube. Filter sterilize through 125 or 250ml single use 0.22µm low-binding SFCA filter unit (or rinse CA filter with liquid CM prior to remove surfactant). Allow 30-40min to filter using house vacuum. During this time, harvest hyphae. 40m

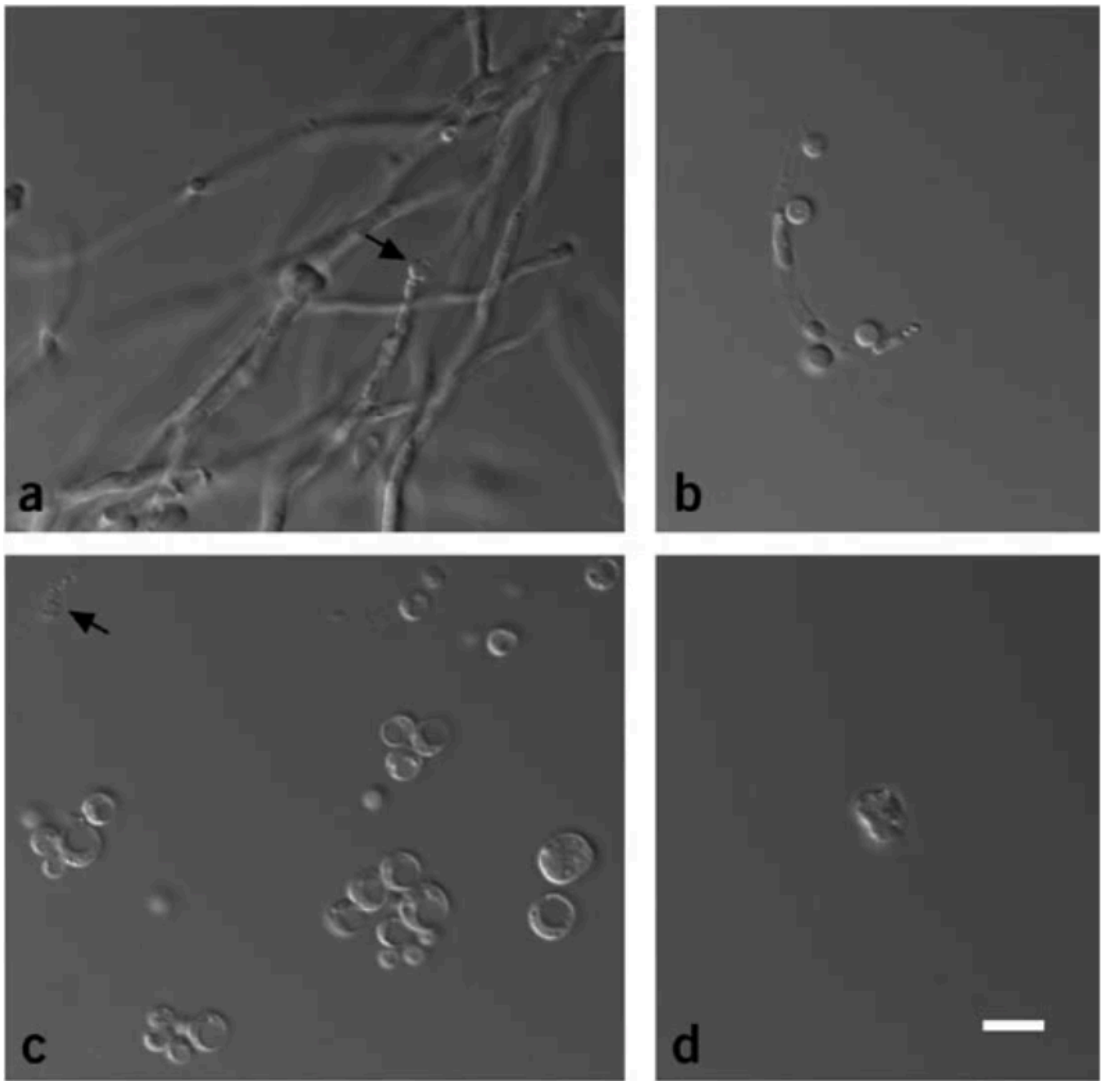
Harvest Hyphae & Cell Wall Digestion 2h 15m

4 Collect hypha over a fine mesh or sterile filter paper (or single use polyester tea-bag sprayed with 70% Ethanol). 5m

5 Using a heat sterilized spatula, wash with 10ml CM, gather hyphae and place into 3ml of CM in a 15ml conical tube; when completed, note volume. 5m

6 Add an equal volume of room temp 2x PP solution. 2m

7 Digest cell wall in shaking incubator set at 30°C 80rpm for approximately 2 hours (28°C; 2-3 hours), checking the progress under a microscope. 2h



Hyphal mat, pre-digestion (a); protoplasts and undigested hypha (b); collected protoplasts after running digested prep over sucrose cushion, vacuole formation is typical at this step, arrow: hyphal remnant (c); fused protoplasts after PEG treatment (d). Bar:10µm. From Szewczyk *et al.* 2006 Nat. Protoc.1(6):3111- 3120.

Isolate Protoplasts

45m

8 When satisfactorily digested, filter undigested material and hyphal clusters with 70µm MACS Smart Strainer (catalog number 130-110-916) into 15ml tube, rinse mesh and

5m

remaining residue with 2ml CM.

9 Slowly underlay 1-2ml filter sterile 1.2M sucrose solution using a sterile 9-inch Pasteur pipette. 2m

10 Spin at 1800 x g for 10 min at 4°C. 12m

11 Collect free protoplasts into 1.5ml Eppendorf tube at the interface with sterile Pasteur pipette. Place on Ice in 2.0ml Eppendorf tube. 5m

12 Add cold STC (1.2M sorbitol, 10mM Tris pH7.5, 10mM $CaCl_2$) to 1.5ml and mix by very gentle inversion 3 times, spin at 1800 x g for 10min at 4°C. 12m

13 Carefully remove supernatant and resuspend cell pellet in cold STC 300-500µl STC for 3-5 transfections. 2m