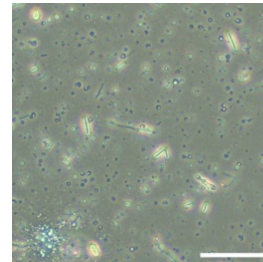


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A small scale of *Fusarium oxysporum* protoplast generation

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We are still developing and optimizing this protocol

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Abstract

The generation of fungal protoplasts is crucial for advancing fungal gene editing methods. We aim to implement the ribonucleoprotein (RNP) method for gene editing (Wang et al., 2018). Generating protoplasts is a demanding task that requires substantial resources to obtain an adequate amount for a single transformation reaction. The cost of cell wall degrading enzymes is the most significant expense in the protoplast generation process. In this study, we have developed a process for small-scale protoplast generation that not only reduces resource usage but also significantly cuts down the amount of cell wall degrading enzyme required.

Materials

Biomaterials: different species of *Fusarium oxysporum*

Growth medium: half-strength potato dextrose broth

Digestion buffer: 10 mg/mL Driselase and 15 mg/mL β -glucanase dissolved in 0.8 M NaCl solution

SuTC stabiliser buffer: 20% sucrose, 10 mM CaCl_2 , 10 mM Tris-HCl, pH 7.5

Before start

The protocol for generating protoplasts on a small scale is for *Fusarium oxysporum* and is in development. We are open to other fungal species attempting the task and providing a report on their success or any modifications to the methods.



Fungus single spore isolation

- 1 Rinse *Fusarium oxysporum* fungal spores from the two-week-old culture plate with 7 mL distilled water, and filter the spore suspension with sterilized filter paper or 4 layers of Miracloth. 5m
- 2 Count the number of spores in spore suspension with hemocytometer. 5m
- 3 The 10^6 / mL spore suspension were added into the culture medium 50 mL half-strength PDB, and grow the culture in 28 °C for 20-24 hours. 20h

Mycelium collection

- 4 Assemble sterilized collection tube and tissue filter column (Lot.: CDC25049A, FAVORGEN®Biotech Corp., Pingtung, Taiwan).
- 5 The  700 µL of germinated spores were added into the filter column.
- 6  1000 x g, 28°C 30s
Centrifuge to remove the ungerminated spores
- 7 Wash the mycelium with 700 µL of distilled water, and continue pipetting until the mycelium is suspended in the distilled water.
- 8 Centrifuge again to remove water and change new collection tubes.

Enzymes digestion

- 9 Add the digestion buffer (10 mg/mL Driselase and 15 mg/mL β-glocanase dissolved in 0.8 M NaCl solution)  700 µL
- 10 Mix  10-20 rpm  03:00:00 in room temperature 3h



Protoplast isolation

- 11 Change collection tube to 2 mL tube for collecting protoplast.
- 12  5000 x g, 4°C 10m
Centrifuge the mixture, pelleting the protoplast to the centrifuge tubes.
- 13 Remaining the pellet of protoplast, remove the digestion buffer carefully.
- 14 Add  1000 µL of SuTC stabilizer buffer (20% sucrose, 10 mM CaCl₂, 10 mM Tris-HCl, pH 7.5) for washing pellet.
- 15 centrifuge at 1000 xg for 5 min, and repeat wash process twice. 5m
- 16 dissolve pellet carefully in 500 µL SuTC buffer, and count protoplast concentration with hemocytometer.
- 17 Generally, the concentration of protoplast per column will be fall around 10⁵ to 10⁶ / mL.

Observation and following applications

- 18 The protoplasts are able to apply in CRISPR/Cas gene editing system or subject to cell staining for microscopy.

Protocol references

Wang, Q., Cobine, P. A. & Coleman, J. J. Efficient genome editing in *Fusarium oxysporum* based on CRISPR/Cas9 ribonucleoprotein complexes. *Fungal Genetics and Biology* **117**, 21–29 (2018).