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Production and regeneration of G. lucidum protoplasts

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Abstract

Production and regeneration of G. lucidum protoplasts, which is important to G.lucidum monokaryotization

Introduction

G.lucidum have two different types hypha in mycelium--the haploid one and the diploid one. Using a protoplast regeneration method can generate an isolate from a single cell which maybe only contain a suit of chromsomes. Firstly, we digest the mycelium into a single protoplast. Then, dilute the protoplast suspension into an appropriate concentration. Third, regenerate these protoplasts on MYG medium.

Reagents

Lywallzyme

Mannitol

PDB broth

MYG agar medium

Equipment

Shaker

Incubator

Procedure

1. Inoculate mycelia into 100 ml PDB media (200 g potato, 20 g dextrose, 1 L water) in 250 ml flasks.
2. Cultivate the mycelia were cultivated in the dark at 28°C under continuous shaking at 50 rpm.
3. Harvest the culturesby filtering through sterile gauze.
4. Place approximately 5 g of mycelia in a 50 ml tube containing 20 ml of filter-sterilized 1.5% lywallzyme (Guangdong Institute of Microbiology, China) in 0.6 M mannitol.
5. Incubate the mixtureat 30°C for approximately 2 h with occasional agitation.
6. Remove hyphae fragments after 2 h of digestion. MYG agar medium (2% malt extract,

0.2% yeast extract, 2% dextrose, 1.5% agar) was used for G. lucidum protoplast regeneration.

7. Dilute the protoplast suspension was diluted to 1 x 10^4 cells/ml, and 50 µl of the diluted protoplast suspension was added to a MYG medium plate and inoculated at 25°C in the dark.