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Abstract

Preparation of fungi for DNA extraction typically involves growing cultures in liquid culture in Erlenmeyer flasks, Roux bottles or even microfuge tubes (Cenis 1992 Nucl. Acids Res. 20:2380). Growing fungal cultures in liquid may require formulating new media or determining aeration requirements, and there are no rapid means of confirming the identification of the resulting mycelium. Fungi are grown routinely on agar media for identification, but agar complicates DNA extraction.

A novel method of growing fungi for DNA extraction

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Preparation of fungi for DNA extraction typically involves growing cultures in liquid culture in Erlenmeyer flasks, Roux bottles or even microfuge tubes (Cenis 1992 Nucl. Acids Res. 20:2380). Growing fungal cultures in liquid may require formulating new media or determining aeration requirements, and there are no rapid means of confirming the identification of the resulting mycelium. Fungi are grown routinely on agar media for identification, but agar complicates DNA extraction.

Solutions of 'reverse agar' (BASF pluronic polyol F-127), a block polymer of polyoxypropylene and polyoxyethylene, are solid at normal room temperatures, but liquid at 4C. When the compound is used as a replacement for agar in solid media, its unusual properties allow the separation of mycelium and medium by simply placing a mature culture in a refrigerator. The compound has been employed for isolation of heat sensitive antagonistic microorganisms (Gardner and Jones 1984, J. Gen. Microbiol. 130:731-733; Olson and Lange 1989 Opera Bot. 100:197-199), isolation of enzymes associated with basidiome formation in Coprinus (Choi and Ross 1988 Exp. Mycol. 12:80-83), and for isolating mycelium from Neurospora race tubes (Munkres 1990, Fungal Genet. Newsl. 37:26). In this note, the possibility of extracting DNA suitable for PCR amplification from mycelium grown on reverse agar media is documented.

'Reverse Malt Agar' (RMA) medium was prepared containing 30% BASF pluronic polyol F-127 substituted for agar in the Malt Extract Agar medium of Pitt (1979 The Genus Penicillium, Academic Press). The liquid was poured over the F-127 granules and the resulting suspension left in the refrigerator overnight until dissolved. The solution was then autoclaved, resulting in a thick, sludge like substance, which was again refrigerated, until a homogenous, liquid solution resulted. The liquid was then poured into petri dishes, where it solidified as it warmed to room temperature. The resulting medium is not a true solid, but rather a dense gel.

Cultures of Penicillium spinulosum Thom. (DAOM 216698), Aspergillus japonicus Saito var. japonicus (DAOM 216695), Gliocladium roseum Bainier (Doyle SB-03a, not saved) and Trichoderma harzianum Rifai (DAOM 216501) were grown on RMA for 7 days at 25C in 6 cm petri dishes. Growth rates were slightly slower than on the same medium made with 2% agar, but the resulting colonies produced microscopically typical sporulating structures. For DNA extraction, the petri dishes were placed in the refrigerator for approximately 1 hour until the medium had liquified. Subsequent handling was done on ice. Mycelium was lifted from the medium using an autoclaved pipette tip, placed on the inverted, slanted lid, and allowed to drain for 30-60 seconds. The mycelium was then cut into smaller pieces using a sterile scalpel blade, and transferred into autoclaved 1.5 ml microfuge tubes. The tubes were then spun in a cold microfuge for 5-10 minutes and the excess medium removed. The mycelium was washed twice with 750 uL cold, autoclaved distilled water followed by cold centrifugation, and then used

directly for DNA extraction. The DNA miniprep method of Edwards et al. (1991 Nucl. Acids Res. 19:1349), modified by the addition of a cold 70% ethanol wash of the final pellet, was used. The resulting DNA was treated with RNAase A for 1 hour at 37C. PCR amplification of the ITS1-ITS4 region of the ribosomal DNA was performed using the primers and conditions given by White et al. (pp. 282-287 In: PCR Protocols, Innis et al. eds Academic Press). The resulting products were digested for 2 hrs at 37C using HinfI in the buffer supplied with the enzyme.

The DNA yields obtained from mycelium grown on RMA were similar to those obtained from mycelium grown in liquid culture. Washing away excess reverse agar with cold water significantly improved yields, but DNA also was isolated from unwashed mycelium. The resulting DNA performed normally in the ITS PCR amplification and subsequent restriction digests (Figure 1).

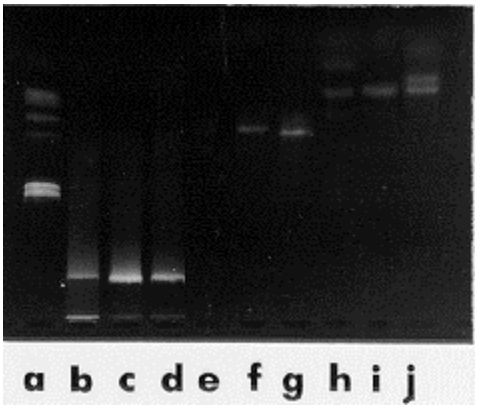

Figure 1. Miniprep DNA (b-d), ITS 1-4 amplification (e-g) and HinfI restriction digests (h-j) from fungi grown on malt extract reverse agar. b, e, h Penicillium spinulosum. c, f, i Aspergillus japonicus var. japonicus. d, g, j Trichoderma harzianum. Lane a is the marker.

Use of reverse agar for cultivation of fungi for DNA extraction may be convenient for certain studies. For fungi that do not produce characteristic structures in liquid broth, reverse agar provides a means of ensuring the correct identity of the mycelium before DNA extraction proceeds. Certain population genetics studies, for example, require the manipulation of a large number of cultures that must be cloned (eg. single-spore isolations) before genetic analysis can proceed. The use of reverse agar could eliminate one round of culture transfers, resulting in significant labour savings.

Reverse agar solutions can be stored for some time at 4C and plates poured when required. Experience has shown that poured plates do not keep indefinitely at room temperature. After 4-6 weeks, the medium no longer liquifies, presumably because of higher polymer concentrations resulting from water evaporation. Also, because the polymer itself is slightly inhibitory to fungi, it does not seem to be suitable for weak nutrient media. Our trials with the Fusarium medium SNA (Nirenberg 1981 Can. J. Bot. 59:1599-1609), for example, resulted in sparse growth that could not be harvested following liquification of the medium.

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