A method for obtaining high specific activity radioactive extracts from perithecia

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Abstract
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mersion of perithecia results in uptake via the ostiole.

The following is a description of our current procedure. Perithecia are collected from crosses made by spreading a conidial suspension of both parental strains on Westergaard’s medium in petri plates. After the onset of ascus formation, but before the beginning of ascospore formation, perithecia are collected with the aid of fine forceps and transferred unto 4% agar. After removing any EXCESS Westergaard’s agar medium adhering, a flattened inoculating needle is used to transfer the perithecia to 10 x 75 mm test tubes filled with 7.5 ml submersion medium. Usually 15 perithecia are transferred to each of two tubes. The perithecia in one tube are left submerged and the perithecia contents are checked at appropriate intervals to determine whether further development, as evidenced by the formation of ascospores, occurs during submersion. The perithecia in the second tube are submerged for a specific length of time (hrs, works well), then poured into a filter-line funnel, washed with about 30 ml distilled water, and transferred to a slant of 2% purified agar. Perithecial contents are checked at intervals (usually 1, 2, and 3 days after transfer) to determine whether ascospore formation proceeds normally.

The following parameters have been tested for their effect on perithecial development. a) Preliminary handling of perithecia -- when perithecia that have been "cleaned" by quickly removing excess agar and by washing in distilled water both before and after submersion compared to perithecia not cleaned, no differences in ascospore production were seen. Thus, if care is taken to avoid dessication, the perithecia can survive without ill effect. b) Perithecial stage -- in general, the older the perithecia, the better they will survive submersion. When 3-4 day old material is submerged and subsequently transferred to agar, a few mature and form spores, but the majority deteriorate before forming asc. Most 4-5 day old perithecia survive submersion and form ripe spores, but the frequency of ascospore abortion is often higher than in unsubmerged perithecia. Perithecia that already contain asc, but no spores, usually survive submersion well. c) Submersion medium -- in most experiments involving several submersion media, distilled water was used as the "control"; and thus as a standard of comparison for evaluating the other media tested. Following is a list of submersion media tested. In each case, perithecial contents were examined qualitatively to determine the presence or absence of detrimental effects, such as increased ascospore abortion, variation in size or shape, or increased frequency of 5-spored asc. -- * Best, subsequent perithecial development: 5 units aminid/10.5% NaCl, Squibb mineral oil; Good: 0.05% NaCl, 50 units aminid/10.05% NaCl; Average: distilled water, liquid Westergaard’s medium containing 0%. 2%, or 4% sucrose, liquid Westergaard’s medium diluted 1:1 with distilled water, 5mM caffeine, 10 mM caffeine; Poor: liquid Westergaard’s medium containing 8% sucrose, 8% sucrose solution, 0.5M colchicine; Toxic: 0.1 M colchicine, 0.1M phosphate buffer (pH 6.7), 0.3M acetic acid buffer (pH 5.2). Requirements for completion of perithecial development during continuous submersion are stricter than those for survival after temporary submersion. -- Good, comparable to unsubmerged culture: 5 units aminid/10.5% NaCl, Squibb mineral oil, 0.05% NaCl; Average: distilled water, 50 units aminid/10.05% NaCl; Poor, only a few spores form or those spores or ac are abnormal; liquid Westergaard’s medium containing no sucrose, 8% sucrose solution, 5mM caffeine; little or no further development; liquid Westergaard’s medium containing 2%, 4%, or 8% sucrose, liquid Westergaard’s medium (2% sucrose) diluted 1:1 with distilled water, 10 mM caffeine.

Several interesting facts emerge. Although Westergaard’s medium is a widely used crossing medium, continuous submersion of developing prithecia within Westergaard’s medium inhibits further development, even when 6-8 day-old prithecia, which already contain young spores, are submerged. Sucrose in the submersion medium also seems detrimental to further development. Also, once perithecia start forming asc, they seem to be self-contained, requiring no obvious source of nutrients (i.e., they will develop in distilled water) and little or no external oxygen (good development in mineral oil). Perithecia that have been submerged in distilled water for up to 7 days -- with no further development during submersion -- will, upon transfer to agar, resume development and form normal spores. There do, however, seem to be a correlation between the length of submersion before transfer and the amount of ascospore abortion. A six hour submersion seems to provide adequate uptake of the compound of interest and does not usually result in increased ascospore abortion.

There is also a relationship between the age of the peritheium and the length of submersion tolerated. For example, perithecia which contain only sterile hyphal and crosers will tolerate a six hour submersion in liquid Westergaard’s medium quite well; but, if submerged for 24 hours, approximately 80% of the peritheium degenerate. Older perithecia, which already contain asc, will survive quite well after a 24-hour submersion in the same solution.

No differences in development were detected between perithecia transferred to purified agar and those transferred to agar containing Westergaard’s medium. The advantage of purified agar is that subsequent hyphal growth and de novo perithecial formation are kept to a minimum.

Submersion may be a useful procedure for a variety of studies. It can be used as a means for effecting perithecial uptake of nutrients, inhibitors, etc., and would be especially suited for compounds that are too unstable to be added directly to a crossing medium, or, conversely, for compounds that inhibit crossing per se. Results from continuous submersion may provide insight into the nutrients, etc. necessary for in vitro development of isolated asc. (This research was supported by a Predoctoral Training Grant, T1 GM-01035, from the National Institute of General Medical Sciences, U.S.P.H.S., and by Grant GM-12953 to A.M. Squibb from the National Institute of General Medical Sciences, U.S.P.H.S.) -- - - Section of Botany, Genetics and Development, Cornell University, Ithaca, NY 14853.

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One way of dissecting a biological process is by isolating mutants which are blocked at different points in that process. These mutants are then used to determine what alterations have occurred in the molecular components of that process.

Many mutants have been isolated which block perithecial development as either the male or female component of a cross [review in Johnson, T. E. (1978) Genetics 88: 27-47]. One very general 1 ppmch to understanding the molecular alterations in these mutants is to determine what changes have occurred in the proteins which are synthesized by those mutants. This report presents a reliable way to label perithecial proteins with radioactive amino acids.

The desired maternal component is inoculated on crossing medium supplemented with 2% sucrose and appropriate growth requirements. The desired maternal component is inoculated on crossing medium supplemented with 2% sucrose and appropriate growth requirements. The desired maternal component is inoculated on crossing medium supplemented with 2% sucrose and appropriate growth requirements. The desired maternal component is inoculated on crossing medium supplemented with 2% sucrose and appropriate growth requirements. The desired maternal component is inoculated on crossing medium supplemented with 2% sucrose and appropriate growth requirements.
Figure I. Cultures were harvested and incubated, for six hours, total activity taken up i.e., overage of three repeats ± Standard Deviation; total radioactivity incorporated into TCA precipitable counts (o-o-o).

then ground (Nasrallah and Srb (1973) Proc. N. Acad. Sci. USA 70: 1891-1893), centrifuged at 5,000 g for 5 minutes and the supernatant frozen at -60°C. Before use, the sample is thawed and centrifuged again at 5,000 g for 5 minutes.

The amount of label incorporated into TCA precipitable material plateau at about four hours. Labelled proteins can be obtained at any point in development by using this technique; there is, however, a drop off both in uptake and in incorporation at later points in the development (Figure 1). Using 35S-methionine (2.2μCi, 700μCi/ml), I have obtained extracts containing as much as 1000 cpm/μg protein. This technique provides a very efficient uptake of label with minimum handling after labeling.

The desired maternal component strain is inoculated on day 0 into petri plates containing standard Westergaard's crossing medium (Am. J. Bot. 1974 34: 573-577) supplemented with 2% sucrose and 4% agar.

On the fifth day or whenever protoperithecia appear, 2 ml of a suspension of conidia of 108/ml or greater is pipetted over the plate and spread gently with a small piece of moist cotton. This preparation is relatively pure; a small amount of conidia is removed with a dry cotton swab.

Perithecia can be harvested at any time, by gently scraping the surface with a broad, blunt spatula that has a burr turned under. This preparation is relatively pure, but additional purification can be obtained by chopping the material in an Omnifor mixer for twenty seconds at 2,000 rpm. The perithecia are then allowed to settle out in a graduated cylinder and collected and concentrated on a filter apparatus. * * * Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

This procedure describes a convenient method for the isolation of crude nuclear pellets from N. crassa. The method, an adaptation of one developed by Hauzela et al. (1977) J. Biol. Chem. 252:704-713), utilizes the Braun Homogenizer to disrupt cells. The main advantages of the technique are that the cells need not be frozen, large amounts of material can be handled, the homogenization is fast and easily controlled, fewer steps are required to release the nuclei, and yields are comparable (75%) to those obtained using the French pressure cell, and lower concentrations of Fixall will stabilize the nuclei. The crude nuclear pellets are used to prepare DNA and pure nuclei.

Germinated conidia (14 h) are harvested by filtration and rinsed. The Braun homogenizer disrupts cells via high speed shaking (4000 rpm) with glass beads. Typically, 90 g wet weight of cells are used in each isolation. The 90 g are distributed among four 75 ml glass homogenizer bottles. Each bottle contains 50 g acid-washed glass beads (45-50 mm), 10-15 g cells and 11 ml of isolation buffer (Hauzela et al., 1977). The isolation buffer, however, contains only 5% Fixall 400. The cells are kept cold during the homogenization by a jar of ice with siphoned CO2. The cells are homogenized in 30 sec pulses followed by 30 sec rests. Table 1 shows that optimum yields without

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<th>Efficiency of Cell Disruption with the Braun Homogenizer</th>
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Homogenizations were performed in 30 sec pulses followed by 30 sec rests.