How to obtain microconidia.

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Background

Microconidia are predominantly uninucleate (Horowitz and Macleod 1960, Baylis and DeBusk 1967) This makes them useful for determining nuclear ratios in heterokaryons, extracting the components of heterokaryons, and recovering new transformants or mutations in pure culture. Microconidia were used in the earliest mutant hunts with Neurospora and the first quantitative studies of the effects of radiation (Lindegren and Lindegren 1941a, b, Hollaender *et al.* 1945, Sansome *et al.* 1945). In these studies, microconidia were obtained using strains of the *fluffy* mutant, which produces no macroconidia. Barratt and Garnjobst (1949) increased the yield of microconidia greatly by combining *fluffy* with *peach*. (peach as a single mutant produces both macroconidia and abundant microconidia.) Microconidia from *pe fl* were used by Horowitz and Macleod (1960) for chemical determination of the DNA content of the Neurospora genome. Another double mutant, *fluffy; dingy*, resembles *pe fl* and may be preferred because it is fertile in homozygous crosses (Perkins and Bjorkman 1979). Other genotypes and procedures used to obtain microconidia have been described and reviewed by Maheshawari (1991, 1999).

Microconidia have the disadvantage of showing low germination and rapid loss of the ability to form colonies (Barratt 1964). This may reflect the homology of microconidia to spermatia, which in related genera such as Podospora function solely as male gametes and are incapable of vegetative growth (Table 1 in Maheshwari 1999). Neurospora strains that show increased microconidial plating efficiency have been selected by Munkres (1977) and by Pandit (1993). Germination exceeding 80% has been obtained when microconidia are spread on a dialysis membrane overlying sorbose medium (Kalpana *et al*. 1998). Germination and plating efficiency can be increased by amino acid supplementation (Pitchaimani *et al.* 2000).

Introduction of a prokaryotic thymidine kinase gene into Neurospora has made it possible to select monokaryotic macroconidia, thus providing an alternative to using microconidia for resolving heterokaryons: Wild type Neurospora is unable to make thymidine kinase. When a thymidinc kinase positive helper strain is used as one component of a heterkaryon, the thymidine kinase negative component can be extracted in pure condition (Metzenberg and Sachs 2002). (See *How to separate the components of a heterokaryon*.)

Procedures

Microconidia obtained using wild type strains. (Pandit and Maheshwari 1993):. Cultures are initiated by placing a trace quantity of macroconidia at the pierced center of a circular piece of cellophane which covers the surface of 2% water-agar in a Petri dish. (Cellophane is a transparent cellulose sheet made from viscose.). The circles are cut from cellophane sheets boiled for 5 min in 1% KOH to remove soluble impurities, washed by swirling in distilled water, spread between wet Whatman filter paper, and autoclaved before laying over the surface of solidified agar. The Petri dishes are inverted and kept for 7 to 12 days at 22º-25º C in a humid atmosphere.

The cellophane is peeled off after approximately 10 days of mycelial growth. Within 3 to 5 hours, microconidiophores develope from the thin mycelium which has grown in the agar under the cellophane. The young microconidiophores are generally associated with a liquid droplet.

The microconidia are harvested 24 hours after removing the cellophane by adding 1 or 2 ml sterile water, tilting the Petri dish and using a pipette to remove the microconidial suspension. A second and third crop of pure microconidia can be obtained after harvesting the previous crop.

The production of microconidiophores is increased substantially if water-agar is replaced by Westergaard and Mitchell synthetic crossing medium (SC) supplemented with iodoacetate (Rossier *et al.* 1973, as modified by Ebbole and Sachs 1990.).

Ebbole and Sachs (1990) describe the purification of microconidia from macroconidiating strains grown on 150 mm slants (0.1× SC, 0.5% sucrose, 2% agar, 1 mM iodoacetate) and freed from macroconidia and mycelial fragments by passing a suspension through Millipore Durapore Millex 5 μm filters (Millipore Catalog No. SLSV025LS). Slants for growth are prepared by adding 60 μl of 0.1M sodium iodoacetate (Sigma No. 12512, freshly prepared and filter-sterilized in water) to 6 ml sterile, molten medium in 16 x 150 mm culture tubes adjusted to 60°C after autoclaving. Viability varies from 1 to 20%.

George Bistis (1990 personal communication to D. Perkins) has developed a procedure for inducing germinating macroconidia to produce microconidia. This is based on his studies with oidia in Ascobolus (Bistis 1957). (1) Pour a shallow plate of 2% agar in water (~6 ml per plate). (2) Spot or streak macroconidia from a dilute suspension. Preincubate 6 hours at 25°C. (3) Cut out a small block holding 6 to 12 conidia. (4) Place the agar block face-up on a pregrown receptor plate of water-agar with a sparse *fluffy* or *eas* lawn of the same mating type as the macroconidia. (5) Observe microconidiation 1 or 2 days later. The receptor plate is prepared by inoculating a water-agar plate with *eas* or *fl* and incubating it for 6 to 8 days at 25°C before introducing the agar block.

Microconidia obtained using the* mcm *mutant (Maheshwari 1991): Strains that contain the *mcm* (*microcycle microconidiation*) mutation are wild type in other respects, including morphology and production of macroconidia. Macroconidia from strains containing the *mcm* allele are suspended in sterile water. The suspension may be stored at 4°C until needed. Ehrlenmeyer flasks one-fifth filled with liquid Vogel's medium N containing 1.5% glucose as carbon source are inoculated with macroconidia to give a concentration of 5×10^6-10^7 macroconidia per ml. Initially, germ tubes grow out from the macroconidia, Then after ~12 hours, polarized growth is arrested and the filaments begin budding off microconidia, exclusively. This occurs at 18° or 22°C but not at higher temperatures. Over 90% of microconidia are uninucleate, the remainder binucleate. Using microconidia produced in liquid culture by an *mcm* strain (FGSC 7455) that was obtained by backcrossing to Oak Ridge wild type for nine generations, Pandit (1993) obtained 60% colony formation. Microconidia produced aerially by the same strain showed only 36% plating efficiency.

Microconidia obtained using* peach fluffy *or* fluffy; dingy *double mutants (Barratt and Garnjobst 1949, Perkins and Bjorkman 1979): These double mutants produce abundant microconidia and no macroconidia. Cultures appear grey rather than orange. The double-mutant strains have been found useful in situations where the mutant morphology is not a serious disadvantage. The yield of microconidia is better on complete medium than on minimal (Baylis and DeBusk 1965). Freshly grown cultures should be used. *pe fl* strains FGSC 3072 A and 3073 a were selected for high plating efficiency (Munkres 1977). *pe fl* strains FGSC 4169 A and 4170 a are progeny of 6 or 7 backcrosses to OR (Kafer 1982).

Microconidia obtained using the* fluffy *single mutant (Sansome *et al.* 1945, following Lindegren and Lindegren 1941): Seven- to 10-day-old *fluffy* cultures are wetted down with sterile water and microconidia are harvested 2 days later. The microconidia are washed off the surface with sterile saline solution and filtered through absorbant cotton to remove mycelial fragments..

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