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Abstract

In order to examine the nuclear types that may be carried in strains of Neurospora collected from nature, we desired a method of selectively inducing microconidiation in mycelia whereby uninucleate microconidia could be obtained for isolating homokaryotic derivatives. We have devised a simple and highly effective method of obtaining pure microconidia from wild-collected strains as well as from *N. crassa* 74-OR23-1VA.

A simple method of obtaining pure microconidia in *Neurospora crassa*

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In order to examine the nuclear types that may be carried in strains of Neurospora collected from nature, we desired a method of selectively inducing microconidiation in mycelia whereby uninucleate microconidia could be obtained for isolating homokaryotic derivatives. We have devised a simple and highly effective method of obtaining pure microconidia from wild-collected strains as well as from *N. crassa* 74-OR23-1VA.

The cultures were initiated by placing a trace quantity of macroconidia at the center of a cellophane circle which covered the surface of 2% water agar in a Petri dish. The cellophane (a transparent cellulose sheet made from viscose) was pierced at the point of inoculation. The circles were cut from cellophane sheets purchased from market, 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 were inverted and kept for 7-12 days at 22-25 C in a room which received intermittent illumination. The stacked Petri dishes along with a beaker containing water were covered by a bell jar to provide a humid atmosphere.

A thin mycelial growth developed over cellophane and produced patches of aerial microconidiophores in 7-12 days (Figure 1). However, some macroconidiophores were also produced. If the cellophane was peeled off after approximately 10 days of mycelial growth on cellophane, then microconidiophores developed selectively within 3-5 h from the thin mycelium which had grown in the agar under the cellophane. The young micro- conidiophores generally were associated with a liquid droplet.

The microconidia were harvested 24 h after removing the cellophane by adding 1-2 ml sterile water, tilting the Petri dish, and removing the microconidial suspension with a pipette. A second and third crop of pure microconidia could be obtained after harvest of the previous crop.

The production of microconidiophores (Figure 1) both on cellophane and subsequently on agar was increased substantially if water agar was replaced by Westergaard and Mitchell synthetic crossing (SC) medium supplemented with iodoacetate (Rossier, Oulevey and Turian 1973 Arch. Mikrobiol. 91:345-353) as modified by Ebbole and Sachs (1990 Fungal Genet. Newsl. 37:17-18). This medium contained 0.1 x SC, 0.5% sucrose, 1 mM iodoacetate (IAA) and 2% agar. Since the development of macroconidiophores precedes that of micro-conidiophores (Springer and Yanofsky 1989 Genes Dev. 3:559-571), the initiation of mycelial growth on cellophane followed by its removal was expedient for the production of virtually pure microconidiophores. All strains tested produced microconidiophores by this method. Microconidia were counted by a haemocytometer and 150-200 were spread on sorbose plating medium (Davis and de Serres 1970 Meth. Enzymol. 17A:79-143). The number of colonies formed was used to estimate their viability. The yield and viability of microconidia obtained in four strains by growth on cellophane/SC+IAA is given in Table 1.

Table 1. Yield and germination of Neurospora microconidia

Strain	Crop	Yield/ Petri dish [x 10(6)]	Viability (%)
N. crassa 74-OR23-1VA*	1	13.2	35
	2	14.2	42
N. crassa RM 124-2A*	1	16.6	36
	2	21.9	37
N. crassa Vickraman A** (FGSC 6688)	1	1.0	38
N. intermedia Maddur 1991-101A**	1	7.7	17

* Average values from two experiments, each replicated 2 or 3 times
**Single experiment consisting of 3 replicates

Macroconidia (4-7 um) can be distinguished from microconidia (2.5-3.5 m) by their larger diameter. Based on microscopic examination, we did not find any significant contamination by macroconidia in the population of microconidia obtained by this method. It is likely that this method will generally be useful for obtaining microconidia from strains of Neurospora. The use of cellophane, SC medium containing iodoacetate and the maintenance of a humid atmosphere were important in obtaining microconidiation.

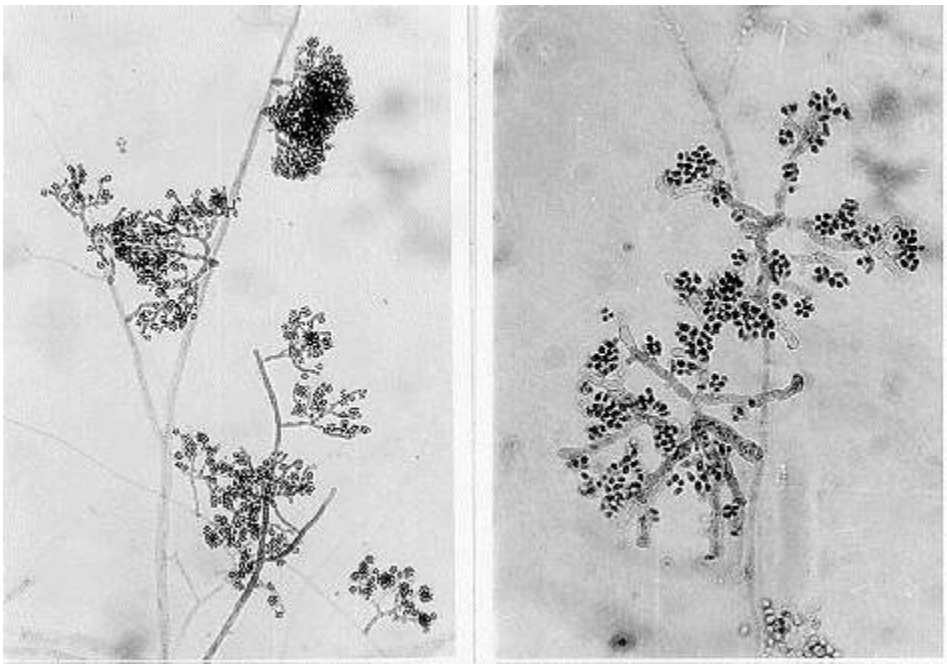

Figure 1. *N. crassa* 74-OR23-1VA. Left: clusters of microconidiophores on cellophane placed on SC + IAA medium after 10 days growth. Right: Enlarged view of branched microconidiophores and microconidia. Bright-field micrographs of samples stained with acid fuchsin. Bar length is 50 um.

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