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SHORT PAPER

Selection for conditional lethals: A general negative selective system for *Schizophyllum commune*

BY MIRIAM RICH ANDERSON AND CAROL S. DEPPE

Department of Genetics and Cell Biology, 250 Bio Science Center, University of Minnesota, Saint Paul, Minnesota 55108, *U.S.A.*

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SUMMARY

This paper describes a method for selecting for conditional lethal mutations in the filamentous fungus *Schizophyllum commune*. The method is, to our knowledge, the only successful application of filtration enrichment to Basidiomycetes. In addition, it is far more powerful than filtration enrichment methods reported previously for any fungus; it permits recovery of up to 80 % auxotrophs starting with material untreated with any mutagen.

1. INTRODUCTION

Until recently, there has been no general, negative selective system available for the filamentous fungus *Schizophyllum commune*, in spite of numerous attempts to develop such a technique. This paper describes a filtration enrichment negative selective system developed in this lab. Another involving actidione has been developed by Shneyour (personal communication). The technique described here is a modification of the filtration enrichment selection used with *Ophiostoma* by Fries (1947), and with *Neurospora* by Woodward, De Zeeuw & Srb (1954). It is, to our knowledge, the only successful application of filtration enrichment to Basidiomycetes. In addition, it is far more powerful than filtration enrichment methods reported previously for any fungus; it permits recovery of up to 80 % auxotrophs starting with material untreated with any mutagen.

The procedure involves two main steps: First, spores are incubated in shake-flask cultures under conditions where the wild-type can grow and the desired mutants cannot. Those spores that germinate are removed in a filter and discarded while the ungerminated ones slip through and are saved. Secondly, the ungerminated spores are plated under conditions where the mutants can grow. Those that now germinate are isolated and retested under both conditions to verify that they are mutants.

The conditions for this procedure were worked out exclusively in the context of selecting for auxotrophs. However, the technique should also be applicable to selections for other kinds of conditional lethals.

2. MATERIALS AND METHODS

Standard culture methods are described by Raper & Hoffman (1974) and media and supplement concentrations are described by Ostlund *et al.* The spores used in the selection were from a dikaryon produced by crossing wild-type strains M4 *A41 B41* and M5 *A43 B43*, which are descendants of and largely isogenic to the Raper strain 699. No mutagenic treatment was used so the mutants obtained are spontaneous.

Filters consisted of a layer (0·2–2 mm thick) of glass wool supported by a 'basket' of

gauze pushed 4–5 cm into the neck of a 1-litre Erlenmeyer flask. The density of growth in the culture to be filtered determines the thickness of the layer of glass wool that will provide the most effective filtration. Each filtration should require between 15 and 35 sec; less time is required when the filter is too thin to be effective or the culture is not dense enough to require filtration, but if more time is required the risk of contamination is too high.

3. BASIC PROCEDURE

(i) *Removal of wild-type spores*

A 1-litre flask containing 500 ml of minimal medium and plugged with cotton is inoculated with the spores of one dikaryon. (This results in up to 10^{10} spores per flask.) Then it is placed at 30 °C on a gyratory shaker set at about 180 rev/min. After about 14 h, some spores will have germinated and some germlings will be large enough to be caught in glass wool. Before the culture becomes too dense with germlings it must be poured through a filter into another 1-litre flask. The filter is then discarded and the cotton plug is switched to the new flask. Subsequent filtrations are required at roughly 20, 30 and 60 h, depending on the density of the inoculum and the thickness of the filters used. There are two signs that a culture is ready for filtration: when it first becomes noticeably thick like a soup instead of watery like medium, and when a ring of germlings appears on the sides of the flask where they are washed up during shaking. Several hours after the appearance of these signs the culture may be too dense to filter.

(ii) *Isolation of the mutants*

Four to five days after inoculation, the culture is filtered a final time through two thick filters. By this time virtually any spore that can germinate in minimal medium at 30° has been removed and the number of viable spores has decreased by a factor of about 10^6. The spores are concentrated by centrifugation and plated on complete medium at a density of about $2–8 \times 10^5$ spores per 15×100 mm petri plate, as counted on a hemacytometer. Most of these are inviable. The plates are incubated at 30 °C and checked daily up to the fifth day for the appearance of colonies. An auxonography test is done on each colony as soon as it is large enough to yield two tiny (1 mm^3) pieces. Up to some 80 % of the colonies tested may be reasonably tight auxotrophs; the rest include very leaky auxotrophs and various morphological and slow-growing mutants as well as wild-type colonies. Many of the morphological and slow-growing mutants may be recognized and avoided during the isolation procedure by waiting until the colonies are larger, but this limits the number of filtered spores which can be spread on each plate to fewer than 10^5.

4. RESULTS

Factors which influence the outcome of a filtration selection include the filtration medium, the plating medium, and the filtration period (the total length of time the spores are in the filtration medium). These can be altered to provide useful variants of the basic selection (described below). Different sets of spores (from different dikaryons) that are handled the same way yield approximately the same varieties and frequencies of mutants, so this factor is assumed to be insignificant. The effects of these parameters are illustrated in Table 1. (More detailed tables are available on request.)

One variant allows the elimination of overabundant types of auxotrophs. Adenine and pyridoxine auxotrophs have appeared as much as 52 % and 59 % respectively of the mutants obtained in a selection. Addition of adenine and pyridoxine to the filtration medium allows the removal of such mutants along with the wild-type and thus increases the final frequency of the other kinds of auxotrophs. Other unneeded auxotrophs can be avoided in like manner.

Table 1. *Conditions and results of various selections*

Experiment no.	Flask no.	Filtration* medium	Plating medium*	Filtration period (h)	Auxotrophs: wild type	Auxotrophs obtained*
1	1	Min	CYM	92	75:19	44 *hop*, 21 *ad*, 5 *not*, 2 *met*, 1 *pab*, 2 unk.
	1	Min	CYM	132	39:15	19 *hop*, 15 *ad*, 3 *not*, 2 *met*
2	1	Min	CYM	118	46:31	14 *hop*, 24 *ad*, 2 *met*, 6 *pab*
	2	Min	CYM	118	19:7	11 *hop*, 8 *ad*
3		Min + ad + pyr	CYM	—		11 *lys*, 13 *leu*, 2 *arg*
4		Min + ad + pyr	CYM	—		2 *lys*, 4 *leu*, 3 *not*
5		Min + ad + pyr	Min + his	—	45:53	45 *his*
6		Min + ad + pyr	Min + leu + ile + val + thr + nic + arg	102	17:28	13 *arg*, 3 *not*, 1 *leu*
		Min + ad + pyr	Min + phe + tyr + trp + pab + nic + arg	102	19:31	18 *arg*, 1 *not*
7	3	Min + ad + pyr	Min + leu + ile + val + thr + nic + arg	92	1:107	1 *leu*
	4	Min + ad + pyr	Min + leu + ile + val + thr + nic + arg	92		9 *leu*
	3	Min + ad + pyr	Min + phe + tyr + trp + pab + nic + arg	92	23:107	23 *not*
	4	Min + ad + pyr	Min + phe + tyr + trp + pab + nic + arg	92	19:71	19 *not*, 1unk.
	3	Min + ad + pyr	Min + bio + arg + cho + ura + pro	92	11:24	11 *arg*
	4	Min + ad + pyr	Min + bio + arg + cho + ura + pro	92	10:25	10 *arg*
	3	Min + ad + pyr	CYM	92	31:149	32 *not*, 1 *leu*
	4	Min + ad + pyr	CYM	92	25:133	25 *not*
8	1	Min + ad + pyr	CYM	80	1:62	1 *not*
	2	Min + ad + pyr	CYM	82	3:35	1 *not*, 2 unk.
	3	Min + ad + pyr	CYM	82	2:17	2 *not*
	4	Min + ad + pyr	CYM	—	1:28	1 *not*
	5	Min + ad + pyr	CYM	—	6:68	6 *not*
	6	Min + ad + pyr	CYM	—	2:39	2 *not*
	7	Min + ad + pyr	CYM	—	6:33	5 *not*, 1 unk.
	1	Min + ad + pyr	Min + leu + ile + val + thr	80	5:82	5 *leu*
	2	Min + ad + pyr		82	5:64	5 *leu*
	3	Min + ad + pyr		82	2:81	2 *leu*
	4	Min + ad + pyr		—	3:47	3 *leu*
	6	Min + ad + pyr		—	1:106	1 *leu*
	7	Min + ad + pyr		—	2:43	2 *leu*
	1–7	Min + ad + pyr	Min + phe + tyr + trp + pab		0:473	

* 'Min' is minimal medium and 'CYM' is complete yeast medium. Symbols for auxotrophs are given in the text. All flasks of any one experiment were handled uniformly.

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Another variant allows selection for specific kinds of auxotrophs. If the filtered, concentrated spores are plated on minimal medium plus specific supplements, instead of on complete yeast medium, the frequency of the specific mutant among total isolates is greatly increased. For example, arginine auxotrophs, which characteristically appear in low frequency on complete yeast medium, will appear as virtually all of the mutants obtained on arginine medium. Occasionally a leaky adenine auxotroph has appeared on medium that contains no adenine just as wild-type colonies appear. Some of the auxotrophs obtained on supplemented minimal media will scarcely grow on complete yeast medium, and it seems unlikely that they could have been obtained there. These include *his*, *leu* and *aro* mutants.

5. DISCUSSION

To date, auxotrophs of at least 38 different complementation groups have been obtained by the technique described in this paper. There are also several unidentified auxotrophs, and many auxotrophs which have been identified but not classed by complementation group. The groups identified to date are: *ad-2*, *ad-4*, *ad-5*, *ad-7*, *ad-8*, *ad-9*, *ad-12*, *arg-1*, *arg-2*, *arg-6*, *arg-7*, *arg-8*, *aro-1*, *asp-1*, *cho-1**, *gua-1*, *his-1*, *his-2*, *his-3*, *hop-1*, *hop-2*, *leu-1*, *lys-1*, *lys-2*, *lys-3*, *met-1*, *met-2*, *met-3*, *nic-1**, *nic-2**, *nic-3**, *nic-4**, *nic-6**, *not-1*, *pab-1*, *pan-1**, *pan-2**, and *trp-1* (Ostlund *et al.*, in preparation). The symbols stand for *ad*enine, *arg*inine, *aro*matic (phenylalanine + tryptophan + tyrosine + p-aminobenzoic acid), *asp*aragine, *cho*line, *gua*nine, *his*tidine, *h*istidine or pyridoxine (*hop*), *leu*cine, *lys*ine, *met*hionine, *nic*otinamide, *n*icotinamide or tryptophan (*not*), *p-a*mino*b*enzoic acid (*pab*), *pan*tothenate, and *tryp*tophan.

Growing wild-type germlings leak a variety of products into the medium. These products can accumulate and crossfeed the corresponding auxotrophs allowing them to grow and be removed in the filter. The possibility of losing auxotrophs this way increases with the use of larger inocula or less frequent filtration. Some auxotrophs appear to be much less susceptible to crossfeeding than others, for example, *leu-1* and *not-1*. Those that are most susceptible probably cannot be obtained by filtration enrichment. Thus crossfeeding effects the variety and frequency of mutants obtained in a selection.

This technique has permitted isolation of very high frequencies of spontaneous auxotrophs. Others who have used filtration enrichment generally report considerably lower yields, despite their use of mutagens to increase the initial frequency of mutations. *Schizophyllum* basidiospores have two haploid nuclei per cell, are extremely uniform in size, and germinate relatively synchronously. We speculate that the efficiency of our technique depends at least in part upon this uniformity of starting material.

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* Not spontaneous.