Efficient Method for Selection of Auxotrophic Mutants of Neurospora

Abstract. A technique for the selection of induced and spontaneous auxotrophic mutants of *Neurospora* in relatively high frequency is described. The method takes advantage of the reduced rate of death of germinating conidia of an inositol-requiring strain when a secondary mutation is imposed.

One of the shortcomings of *Neurospora crassa* for genetic and biochemical investigation has been the difficulty of obtaining large numbers of auxotrophic mutants of an induced or spontaneous origin. Most of the methods employed (*1*) have been cumbersome, tedious, and inefficient and have had only limited usefulness in the selection of spontaneous mutants.

Conidia of the inositol-requiring strain 37401 were observed to die rapidly when allowed to germinate in a medium devoid of inositol (*2, 3*). The loss of viability of the conidia is sharply reduced by inhibiting extensive germination with amino acid analogs in the incubation medium (*3*) or by imposing an amino acid requirement on the inositol-requiring strain by synthesizing double mutants through the appropriate crosses. The double mutant *inos-tryp-3* (37401-S1952), which requires tryptophan in addition to inositol for growth, has a conidial inactivation rate 70-fold less than that of the *inos* strain.

The inhibition of "inositol-less death" by the superimposition of an additional growth-factor requirement suggested that there would be a selective advantage for induced auxotrophic mutants in an *inos* genetic background under conditions of inositol deprivation. The enrichment for new auxotrophic mutants under selective circumstances would be, in principle, analogous to the successful mutant enrichment procedure employing a mutant of *Escherichia coli* blocked prior to the synthesis of the essential cell wall component diaminopimelic acid (*4*).

The inositol-requiring strain 89601a was used in the irradiation experiments described below in order to minimize the number of reversions to prototrophy (*5*). Conidia were harvested from 4-day liquid cultures grown at 30°C in a synthetic medium (*6*) with 2 percent sucrose and 5 μg of inositol per milliliter. The conidia were filtered through sterile cotton to remove mycelial bits and washed three times by alternate centrifugation and resuspension in sterile distilled water. The spores, adjusted to a concentration of 10^7 per milliliter, were irradiated (*7*) with ultraviolet light until a survival of 2 to 20 percent was obtained. To minimize the loss of induced mutations because of the possible involvement of a delay in phenotypic expression, the conidia, at a concentration of 10^6 per milliliter, were incubated in 1-percent sucrose synthetic medium with 0.25 μg of inositol per milliliter for 6 hr at 25°C. The suspension was vigorously shaken to prevent heterocaryosis. The suspension was then filtered through glass wool to remove germinated conidia and washed three times by alternate centrifugation and resuspension in sterile distilled water. After the suspension had been adjusted to a concentration of 10^7 to 10^8 conidia per milliliter, 1-ml aliquots were plated in 12 ml of synthetic minimal sorbose medium (*8*). The plates were incubated for 2 to 4 days at 34°C and supplemented with "4X" complete sorbose medium (*8*). After incubation for 3 days at 34°C, the colonies were counted and transferred to glycerol-complete slants and subsequently tested for their phenotype.

Table 1. Frequency of mutants obtained from irradiated and nonirradiated conidia of an inositol-requiring strain of *Neurospora* after subjection to "inositol-less death."

Survival frequency after irradiation	Viable conidia per plate (No.)	Incubation (day)	Frequency of survivors after incubation	Cultures tested (No.)	Mutants (No.)	Mutants: Frequency among cultures tested
			Experiment 1			
*	5.6×10^6	4	0.75×10^{-6}	39	6	0.15
0.21	1×10^6	4	2.70×10^{-5}	188	152	0.81
			Experiment 2			
*	7.8×10^6	4	0.90×10^{-6}	6	1	0.17
0.21	1.6×10^6	4	3.10×10^{-5}	30	27	0.90
			Experiment 3			
*	4.8×10^7	2	1.10×10^{-6}	200	3	0.015
0.027	1.3×10^6	2	7.70×10^{-6}	52	24	0.46
			Experiment 4			
*	1.2×10^7	4	9.10×10^{-7}	32	1	0.03
0.33	4×10^6	4	5.50×10^{-6}	71	51	0.72
0.20	2.6×10^6	4	1.90×10^{-6}	41	31	0.76

* Nonirradiated sample.

The results of a number of experiments are summarized in Table 1. In every case the frequency of mutants obtained was equal to or greater than that reported for any of the procedures previously employed. Since each colony represents a single macroconidium plated, each induced mutant obtained probably represents an individual mutational event. The results seem encouraging, particularly since many mutants are not recovered as a result of the effective multinucleate character of many of the macroconidia. It should be noted that there has evidently been an extensive enrichment of spontaneous mutants.

Although the method as described seems efficient, minor modifications may further improve the yield of mutants. The necessity of preincubation for phenotypic expression has not been unequivocally demonstrated, but when this step was omitted the yield was occasionally comparatively low. The time of incubation on minimal medium is apparently significant and might also be modified. The spectrum of mutant types recovered includes a high frequency of amino acid auxotrophs, some purine- and pyrimidine-requiring mutants, 3 to 4 percent morphological mutants, and an occasional vitamin-requiring mutant. A high frequency of leucine-requiring mutants has been observed in all experiments (*9*).

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References and Notes

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3. B. S. Strauss, *J. Gen. Microbiol.* 18, 658 (1958).
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6. H. J. Vogel and D. M. Bonner, *Microbial Genetics Bull. No. 13* (1956), p. 43.
7. A Westinghouse 3-w "germicidal" lamp at a distance of 30 cm was the ultraviolet light source.
8. The sorbose medium suggested to us by P. St. Lawrence contained 1.5 percent sorbose, 0.2 percent glycerol, 0.2 percent glucose, and 2 percent agar in Vogel's N synthetic minimal medium. The "4X" complete medium contained 5 mg of inositol, 10 g of Difco yeast extract, 4 g of Sheffield N Z Case, and 40 ml of a synthetic vitamin mixtu→[E. L. Tatum, R. W. Barratt, N. Fries, D. Bonner, *Am. J. Botany* 37, 38 (1950)] per liter of sorbose medium.
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