Fungal Genetics Reports

Volume 26 Article 21

Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium

O. C. Yoder
Cornell University

Recommended Citation

Yoder, O. C. (1979) "Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium," *Fungal Genetics Reports*: Vol. 26, Article 21. https://doi.org/10.4148/1941-4765.1709

Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium

Abstract

Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium

Yoder, O. C.

Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium.

The high-sorbose, filtration concentration method of mutant enrichment reported by Applegate et al. (Neurospora Newsl. 25: 17, 1978) was modified and evaluated for its efficiency of mutant selection. Conidia were suspended in water, filtered through four layers of cheesecloth (20 mesh/inch), adjusted to 2x 10^6-2x 10^7/ml, and 10 ml of the suspension was placed in a 10 cm diameter glass petri dish for a one min exposure to W-light (48 ergs/sec/mm^2) that resulted in 70 to 90%

kill. The suspension was transferred to a 500 ml flask containing 250 ml Vogel's minimal salts plus 6% sorbose, 0.5% glucose, and 0.5% fructose. The flask was incubated in a waterbath shaker at 100 reciprocations/min; restriction of growth was greater at 37° C than at 25° C. At 12 hr intervals (at either temperature), the suspension was passed through a single layer of Nitex #53 nylon monofilament screen with 35 μM openings, (Baylis et al. Neurospora Newsl. 7: 7, 1965, Turtox 73-511-4); four layers of cheesecloth also filtered effectively but were less convenient to handle. When the filtration was complete, nongerminated conidio were collected by one of two methods. 1) If the filtration medium contained no inhibitor, the suspension was mixed with an equal volume of warm (60° C) complete agar medium, or appropriately supplemented minimal, and poured into petri dishes (15 to 20 ml/dish). 2) To wash conidia, a dense suspension of "carrier conidia" (killed by 12 hr incubation at 60° C) was added, and the conidio were pelleted by centrifugation and resuspended in 15 to 20 ml water (additional washing by centrifugation was sometimes necessary). Carrier conidio aided in locating the pellet and reduced the likelihood that living conidia would stick to each other during centrifugation or would be lost in the supernatant. (Carrier conidio hove previously been used in this way D.E.A. Catch&de.) Aliquots (1 ml) of resuspended conidio were placed in Petri dishes and mixed with warm agar medium.

After a 60hr filtration period at 37° C, there were 500 to 600 survivors, of which about 30% were auxotrophs; after 84 hr there were less than 100 survivors ond up to 92 % ouxotrophs. Averages from several experiments show that among all survivors 11% were temperature sensitive, 57% were auxotrophic, ond about 30% produced white ascospores indicative of chromosome aberrations, in tests carried out by D.D. Perkins. Specific selection for guanine ouxotrophs or for caffeine sensitivity yielded 2% in each care.

Three isolates with Oak Ridge background, al-2 (15300; FGSC #3448), eas (UCLA 191) and eas;mts (UCLA 191; MN'), were compared for utility in mutant hunts. All three were equally easy to handle and were efficient for selection of auxotrophs; overages for two experiments with 84 hr filtration-enrichment periods were: al-2 89%. eas 73%, and eas;mts 92% (for each isolate a few of the auxotrophs were leaky and a few were morphs).

The method is thus extremely efficient, in addition to being convenient. A modification of the method hos been useful with another ascomycete, Cochliobolus heterostrophus. By replacing 1.5% glucose in the filtration medium with 2% sorbose and 0.2% glucose and adjusting procedures slightly, the rate of auxotroph selection was increased from about 0.1% to 1 to 2%.

- - - Department of Plant Pathology, Cornell University, Ithaca, NY 14853.