Fungal Genetics Reports

Volume 3 Article 6

Procedure for filtration-concentration experiments

Mary Case

Recommended Citation
Case, M. (1963) "Procedure for filtration-concentration experiments," *Fungal Genetics Reports*: Vol. 3, Article 6. https://doi.org/10.4148/1941-4765.2150

Procedure for filtration-concentration experiments

Abstract

Procedure for filtration-concentration experiments

Case, Mary. Procedure for filtration-concentration experiments.

The concentration of mutants by filtration from treated conidia was first utilized by Fries (Nature 159:199, 1947) for *Ophiostoma multiannulatum* and later adapted for Neurospora (Catcheside, J. Gen. Microbiol. 11: 34, 1954; Woodward, DeZeeuw, and Srb, Proc. Nat. Acad. Sciences, 40: 192, 1954). The principle involves the placing of treated conidia into a minimal medium and the filtering off of the growing mycelia as the wild type conidia germinate. Details have been described for these experiments by the above authors for Neurospora. Through the years, however, certain refinements of the experimental procedure have been made at Yale. (Various ideas for these experiments were contributed by Dr. F. J. de Serres while at Yale University and by Dr. Val Woodward).

Wild type strain: The wild type strain 74A is grown for 8 days on Fries minimal agar (1.5% sucrose). Conidia are harvested in distilled water, filtered through cotton, centrifuged, and washed before treatment with mutagens such as X-rays, ultraviolet, or chemicals. This filtering through cotton reduces the number of large conidia (presumed multinucleate) as well as the mycelial fragments. The conidial suspension is counted with a hemacytometer.

Filtration flasks: 500 ml. narrow mouth Erlenmeyer flasks are used for these experiments with not more than 300 ml. of Fries minimal medium/flask (1.5% sucrose). The concentration of conidia added to 300 ml. of medium should be no more than 30×10^6 viable conidia. Higher concentrations of conidia appear to reduce the efficiency of the recovery of mutants and to delay the germination of wild type conidia. In general, regardless of the mutagen used, a better recovery of auxotrophs appears to be obtained in the range of 40% to 60% survival.

Filtration process: The filtration flasks are incubated at 25°C on a low-speed reciprocating shaker. Since the control conidia begin to germinate within 8 to 10 hours after treatment, it is desirable to begin the incubation of the flasks at night, so that the first filtration occurs the next morning. For the next 20 hours the filtration flasks are filtered every 3 to 4 hours. After this period the flasks can be filtered every 8

to 10 hours for the duration of the experiment. Under no circumstances, however, are the conidia permitted to germinate sufficiently to form a mycelium which can fragment.

Filtration is accomplished by pouring the contents of the incubated flasks through 4 layers of cheese cloth (grade 60) into an empty sterile 500 ml. flask. The cheese cloth, held in place by a rubber band, forms a bag hanging into the mouth of the empty flask. The filter is covered with aluminum foil or paper during autoclaving and storage. To aid the germination of chemical-or ultraviolet-treated conidia, one-third of the original volume of fresh Fries minimal medium (e.g., 100 ml.) is added to the incubation flask after the first 24 hours. Both chemical and ultraviolet treatments appear to cause delayed erratic germination of conidia. An initial washing of the treated conidia prior to placement in the filtration flasks does not seem to eliminate this effect. The addition of the new medium, however, appears to help overcome this delayed germination phenomenon.

Plating: The suspension is plated on a Fries-sorbose minimal medium with added supplementation for the type of mutant desired. Under the experimental conditions described the first sample from the filtration flasks can be plated after 40 hours. At this time the sample should be clear with no obvious germination. Mutant recovery should be adequate if in a treated sample of 0.5 ml. plated at 40 hours there are no more than 50 colonies present. At 40 hours the number of colonies from a control sample should be no more than 5 to 10/0.5 ml. It is advisable to begin plating as early as feasible if the survival of the mutant type which is desired is not optimal under starvation conditions. ---Biology Department, Yale University, New Haven, Connecticut, U.S.A.