

Isolation of sterility mutants

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Abstract

Isolation of sterility mutants

Vigfusson, N. and J. Weier. Improved
method for the isolation of sterility mutants.

Recent work in this laboratory has involved, in part, the isolation of sterility mutants in Neurospora crassa. The standard method for isolating these mutants is very laborious: conidia, after mutagenesis, are plated on sorbose medium, individual colonies are isolated after 48-72 hours and subsequently tested for sterility by crossing them with the appropriate mating type. Crosses are made by inoculating dry conidia on to a plate on which the other mating type is already growing.

A more rapid method of isolation involving replica plating has now been introduced. Conidia after mutagenesis are plated on sorbose medium, but at a lower concentration (approximately 20 colonies per plate). After 96 hours these plates are replicated with a velvet pad to a plate containing a lawn of growth of the other (but aconidial) mating type. Master plates are now held in the freezer to avoid any further growth. After 14-18 days at 25°C, crossing plates can be inspected for reduction of fertility of the replicated colonies. Such colonies are then picked from the master plate for further testing. Colonies showing no reaction whatsoever on the crossing plate are ignored, on the assumption that they were insufficiently replicated by the velvet. (Our observations indicate that mutants showing an extreme degree of sterility still will react with the opposite mating type; however, in such cases the sex reaction does not proceed beyond the formation of very small protoperithecia.)

The above method reduces drastically the number of isolates that must be handled individually as the following figures indicate: 1,103 colonies on 80 plates have been replicated to date. From these, 23 colonies have been isolated as "suspects", four of which have turned out to be mutations to reduced fertility. By comparison, 6,357 colonies have been isolated by the conventional method, yielding 27 mutants. The method has been tested with good results using sterile, semi-sterile, and fertile strains. - * - Department of Genetics, University of Alberta, Edmonton 7, Alberta, Canada.