

Screening for female-sterile mutants

L. Bhattacharya

State University of New York

J. F. Feldman

State University of New York

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Abstract

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Bhattacharya, L. and J. F. Feldman. A rapid

screening procedure for female-sterile mutants.

when one is searching for other kinds of mutants, a systematic search for such strains has been hampered by the absence of a good selection procedure. In lieu of such a selection technique, we have developed a rapid screening procedure, several steps of which may be useful for other purposes as well.

Agar plates are prepared with a medium of Westergaard and Mitchell's salts containing 0.5% sorbose and 0.1% sucrose. This sorbose/sucrose ratio induces colonial growth of wild type strains without inhibiting formation of protoperithecio. Sterile filter paper (Whatman #1) is placed over the solidified agar. Mutagenized conidio are suspended in 10 ml of overlay agar (0.8% plain agar; held at 45°C) at a concentration of about 50-100 viable conidio per 10 ml. This suspension is immediately poured over the filter paper and the agar is allowed to solidify.

Within 3-4 days colonies are visible. A day later the filter paper with the colonies on it is transferred to a second petri dish containing plain agar (1.5%) to reduce further growth of the colonies and to increase starvation. Within 2 days after transfer, the colonies contain visible protoperithecia, which are then fertilized. Within 3-4 more days, block perithecia are visible and colonies without perithecio can be easily distinguished. When unmutagenized wild type (74A) conidio are used, better than 95% of the colonies develop perithecia. To isolate the colonies without perithecia, the filter paper is lifted off the agar and the appropriate colonies are picked from the replica made in the plain agar underneath by growth of the hyphae through the filter paper.

By this technique we have isolated, so far, 11 strains after UV mutagenesis which do not form perithecia on either normal Westergaard and Mitchell's medium or on corn meal agar. All are more or less fertile and all develop protoperithecio, although some are quite slow in doing so. It also appears that an adaptation of this technique would be useful for replica plating nearly any strain of Neurospora, since colonies always grow through the filter paper and make replica in the bare agar. We have not yet done extensive study on this, however. - - - Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203.

The study of sexual differentiation in Neurospora, as with other developmental phenomena, could be greatly aided by having large numbers of mutants blocked at various developmental stages. Although unwanted female-sterile mutants often appear at times