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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

Screening for female-sterile mutants

Bhattacharya, L. and J. F. Feldman.. A rapid screening procedure for female-sterile mutants.

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 unwonted female-sterile mutants often appear at times when one is searching for other kinds of mutants, a systematic search for such strains hos 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 ore prepared with a medium of Westergaard ond Mitchell's salts containing 0.5% sorbose ond 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 ore suspended in IO ml of overlay agar (0.8% plain agar; held at 45°C) at a concentration of about 50–100 viable conidio per IO 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 doy 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 ore then fertilized. Within 3-4 more days, block perithccia ore visible and colonies without perithecio con be easily distinguished. When unmutagenized wild typ (74A) conidio ore wed, 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 mode in the plain agar underneath by growth of the hyphoe through the filter paper.

By this technique we hove isolated, so for, 11 strains after UV mutagenesis which do not form perithecia on either normal Westergourd and Mitchell's medium or on corn meal agar. All ore mole fertile and all develop protoperithecio, although some are quite slow in doing so. It also appears that on 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 bore agar. We hove not yet done extensive study on this, however. ▪ ▪ ▪ Dapart ment of Biological Sciences, State University of New York at Albany, Albany, New York 12203.