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A more efficient procedure for scoring mating type and aberrations

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Abstract

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A technique for scoring aberrations among isolates by crossing them with standard fluffy strains (fl (P605) a and flPA) was described by Perkins et al. (1962 Can. J. Gene+. Cytol. 4:187). Strains to be tested were spotted on plater containing fertile fluffy testers, and aberrations scored by the frequency of white spores shot to the lid of the plate. It has now been found to be technically advantageous for large numbers of sex tests and far scoring aberrations to make the crosses in 3-inch tuber rather than on plater.

The advantages of tubes over plater are these. The tests can be done by relatively unskilled help without the possibility of scatter either in the formation of perithecia or in ascospore shooting. There is no problem of spores moving in the condensate that forms on the lid of a plate. Any error is thus eliminated in determining which spores came from a particular isolate. Positive tests can be used for progeny testing, if necessary.

Tubes are inoculated using a suspension of fluffy mycelia prepared as fallows: the two fluffy strains are grown 4 days at 34°C in 300 ml flasks containing 50 ml of a liquid glycerol complete medium (a variation of Medium 2 described by Tatum et al., 1950 Am. J. Botany 37:38) and a 15 cm filter paper cone (for greater aerial growing surface). Several flasks can be prepared and grown up at one time and then refrigerated until needed (good for several weeks). A dense mycelial suspension is mode by adding about 50 ml sterile water to each flask and vigorously shaking, first by hand and then with a vortex mixer. Three-inch tubes of SC agar (Synthetic Cross Medium, Westergaard and Mitchell 1947 Am, J. Botany 34:573) ore inoculated with a drop of the mycelial suspension, using a wide-bore Pasteur pipette with a squeeze bulb. SC is color-coded with Schilling food color before dispensing, to identify the two fluffy mating types and minimize mix-ups.

Tubes inoculated with the fluffy testers are incubated at 25°C until protoperithecia are formed. They are then ready for fertilization, or they may be stored at 5°C and used up to one week later. Sex tests ore scorable after four days at 25°C and aberrations (fertility and frequency of aborted spores) can be determined within 12-14 days after crossing by examining ascospores shot to the wall of the tube. Tests are routinely made against testers of both mating types to reveal false negatives, infertility, or bisexuality. - - - Department of Biological Sciences, Stanford University, Stanford, California.