Fungal Genetics Reports

Volume 6 Article 19

Crossing techniques for large numbers of isolates

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Abstract

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Pittenger, T. H. Crossing techniques for large numbers of isolates.

The procedure described below is not a new crossing technique, but is a method that we have found useful when both mating-type and growth requirements have to be determined on a large number of progeny at the same time. Growth tests are routinely made in 10 x 75 mm culture tubes containing 1 or 1.5 ml of liquid supplemented media. The tubes containing the supplements on which a particular isolate grows contain an abundance of conidia if the inoculum is allowed to grow for several days. These conidia can readily be used as the male parent in making crosses if these two different tests are made at the same time. At the time the mutants are tested in liquid media, we inoculate separately a corresponding number of 15 x 120 mm culture tubes with strains of mating type A and a which are selected as good protoperithecium formers. By the time these cultures have formed protoperithecia, the progeny isolates being tested for their growth requirements have formed conidia. These liquid-grown cultures are then shaken vigorously to suspend the conidia and mycelia. Since these tubes are still all plugged with cotton, all of the conidia from a large number of cultures can be put into suspension without ever having to remove the cotton plugs. This is not only an aid to minimizing contamination, but it also makes it unnecessary to prepare separate cultures of all the isolates as a source of conidia for crossing.

To aid further in reducing contamination and as an aid in crossing, conidia from the tubes containing the protoperithecial parents are removed with an aspirator device which traps the conidia in a water-lysol mixture in a 1000 ml filtering-type Erlenmeyer flask. The use of aconidial strains as the female parent would make this step unnecessary, but if large masses of conidia are present in the tube containing the female parent it is difficult to add only a small suspension of conidia and to get it well spread over the agar surface containing the protoperithecia.

Approximately 0.5 ml of the conidial suspension is removed from the small culture tubes with a sterile pipette and added to the tubes containing the protoperithecial parents. A separate pipette should be used to add conidia to each of the A and a cultures. We have found 5 3/4-inch disposable Pasteur-type pipettes (Scientific Products) sterilized in 6-inch test tubes with aluminum closures very useful for this procedure.

The use of cultures of the mutant spray (Smith 1962 NN#1:14) as the female parent could work equally well since the primary advantage of the above method is simply to take advantage of conidia for crossing that are already present in cultures that have been used in determining the growth requirements of the isolates being tested. A modification for more general use would simply be to inoculate cultures in liquid medium when they are to be used as the male parent in a cross and these conidia can then be readily put into suspension by shaking or using a vortex-type mixer prior to being used as a fertilizing parent. - - - Department of Agronomy, Kansas State University, Manhattan, Kansas.