Mating type tests on plates with wild type testers

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Abstract
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Replication of the conidiating colonial strains \( cr \) (crisp, B123) and \( rg \) strains (ragged, crisp 853, B123) has been described by Maling (1960) J. Gen. Microbiol., 23:257. \( cr \) and \( rg \) crosses are fertile, but colonies are too large to make it worthwhile to replicate directly from ascospore plasmids. \( cr \) colonies are smaller and replicate well with velveteen or filter paper, but \( rg \) crosses are sterile, so that applications are limited to conidial platings. Maling employed \( cr \) effectively to study recombination by using a 32-prong replicator and specially prepared master plates. \( rg \) or derivative microconidating strains (\( rg \); see fl.) have been used for selecting auxotrophic (Maling) and radiationsensitive mutants (Chang and Tuvesson 1967 Genetics 56:801; Schroeder 1970 Mol. Gen. Genet., 107:291).

I have found another combination of genes that seems to possess the favorable features of both \( cr \) and \( rg \). When \( sn \) (snowflake, C136; Mitchell 1959 Genetics 44:847) is combined with \( cr \) (B123), the double mutant is homozygous fertile, and \( sn \) ascospores or conidia form compact conidiating colonies similar to \( rg \), that plate and replicate efficiently. (\( sn \), like \( rg \), is located close to the centromere of linkage group I, not for from \( cr \).

Although \( sn \) hasn't yet been tried out in actual mutant hunts or recombination experiments, its potential usefulness prompts this preliminary account.

CROSSES are conveniently made in 15 cm tubes on slants of minimal synthetic cross media using mixed suspensions of \( sn \) and \( cr \) A and \( sn \) and a conidio. Perithecia are abundant, mature, and ascospores are oozed but not shot from the perithecia. Ascospores from well-aged crosses were suspended, surface-spread and heat-shocked on pm-poured plates. The colonies resemble those of \( rg \) figured by Maling, and conidiate and pigment well. Conidio do not become airborne. Well over 100 colonies per plate should be resolvable when replicated as described by Maling (filter paper) or Schroeder (velveteen).

\( m_{cr} \) stocks of both mating types have been deposited with the Fungal Genetics Stock Center (FGSC#2001 and 2002).  

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Mating type tests can be made on agar plates by a method similar to that described by Smith (1962 Neurospora NewsL. 1:14), except that wild-type strains are used as tester female parents instead of the mutant spray. The medium consists of Westergaard and Mitchell's inhibits spreading and conidiation without inhibiting the production of mycelia. In addition, the plates are kept in the dark to further reduce conidiation. Protoperithecia are formed within 5 days after inoculation. A grid can then be made on the petri dish and the plate can be fertilized with the strains to be tested in the usual manner.  

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One of the major difficulties encountered in attempting to obtain \( 32p \) labeled DNA from Neurospora conidia is the fact that conidio cannot usually be produced in a liquid medium. The use of a liquid medium is desirable because when conidia are produced on a solid agar medium containing \( 32p \), the uptake of radioisotope from the medium by the conidio is not efficient enough to give a high specific activity unless very large amounts of \( 32p \) are used. A method for growing Neurospora in a liquid medium under conditions that would favor the production of conidio rather than mycelia is what is needed. We have found that a modification of the procedure described by Baker (1969 Neurospora NewsL. 15:13) gives satisfactory results. Conidia were produced on Fries minimal medium (Ryan, Beadle and Tatum 1943 Amer. J. Botany 30:784), containing 1/20th the normal concentration of phosphorus and absorbed in rolls of cotton. Absorbent cotton sheets were rolled into cylinders of such a size that they formed tightly wedged rings when placed in one-liter wide-mouthed conical flasks (KIMAX No. 26650). Before being placed in the flasks, the cotton cylinders were washed thoroughly with distilled water and squeezed hard to get rid of excess water. After being autoclaved, each flask received the following additions: 50 ml of sterile 8% sucrose solution and 50 ml of sterile 2x Fries minimal medium, containing 1/20th the normal 0.1g% KH2PO4 and to which 2 x 106 conidia/ml have been added. Approximately 100 ml of water from the washing is retained by the cotton cylinder in each flask. Thus the final concentrations of sucrose, phosphate and conidia were 2g%, 0.05g% and 1 x 106, respectively.

The flasks were incubated at 30°C in the dark for two days and then at ambient temperature in the light for 4-6 days, depending upon the extent of conidiation. Conidia were harvested from the medium by the usual method (Baker ibid.). Specially ground sea sand was used to break open the conidio. Highly purified conidial DNA was isolated using hydroxyapatite chromatography as described previously (Chattopadhyay and Dutta 1969 Neurospora NewsL. 15:11 and Dutta 1969 Neurospora NewsL. 14:9). It was possible to obtain 50 to 100\( \mu g \) DNA having approximately 10,000 cpm per \( \mu g \) DNA, by using 2 to 3 mc \( 32p \) (NEN, Boston, Mass) per flask.

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