Conidiating colonial strains suitable for replication

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Abstract
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Replication of the conidiating colonial strains (crisp, B123) and cr (ragged, crisp 853, B123) has been described by Maling (1960) J. Gen. Microbiol. 23:257. cr x cr crosses are fertile, but colonies are too large to make it worthwhile to replicate directly from ascospore platins. cr colonies are smaller and replicate well with velveteen or filter paper, but cr x cr crosses are sterile, so that applications are limited to conidial platings. Maling employed cr effectively to study recombination by using a 32-pronged replicator and specially prepared master plates. rg cr or derivative microconidiating strains (rg cr; pe fl) have been used for selecting auxotrophic (Maling) and radiation-sensitive mutants (Chang and Tuveson 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 cr. When sn (snowflake, C136; Mitchell 1959 Genetics 44:847) is combined with cr (B123), the double mutant is homozygous fertile, and sn cr ascospores or conidia form compact conidiating colonies similar to rg cr, that plate and replicate efficiently. (sn, like rg, is located close to the centromere but not from cr). Although sn cr 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 cr A and sn cr C, a conidio. Peritheciora are abundant, mature and spreading and conidiate rather slowly, and ascospores are oozed but not shot from the ostioles. Ascospores from well-aged crosses were suspended, surface-spread and heat-shocked on pm-poured plates. The colonies resemble those of rg cr 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 rg cr 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. -- - Department of Biological Sciences, Stanford University, Stanford, California 94305.

Feldman, J. F. and M. N. Hoyle. Mating type tests on plates with wild-type strains as testers. Salts with 0.5% sorbose and 0.1% sucrose. This sorbose/sucrose ratio inhibits the production of mycelia. In addition, the plates are kept in the dark to further reduce conidiation. Protopertiochla 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. - - - Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203.

Mandodza, M. T. and S. K. Dutta. An efficient method for 32p labeling of conidial DNA. One of the major difficulties encountered in attempting to obtain 32p labeled DNA from Neurospora conidia is the fact that conidia 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 conidia 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 News1.15:13) 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 &sorbed 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%, 0.05 g% 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 News1.15:11 and Dutta 1969 Neurospora News1.14:9). It was possible to obtain 50 to 100 µg DNA having approximately 10,000 cpm per µg DNA, by using 2 to 3 mc32p (NEN, Boston, Mass) per flask.

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