How to make very small inocula

Dorothy Newmeyer and David D. Perkins

Background

Tiny inocula are desired in several situations, such as scoring nutritional requirements or determining sensitivity or resistance to killing by UV or toxic chemicals (e.g., Schroeder 1970, Stadler and Smith 1968, Newmeyer *et al.* 1978). Supplements required by some auxotrophs are very low, and the carry-over of nutrients with large inocula may obscure test results (e.g.,). Macroconidia provide a convenient inoculum for testing.

Procedure

Conidiating strains

Suspended conidia. Dilute suspensions are suitable when a loop or pipette is used to spot inocula onto sorbose agar test media in plates, or aliquots are to be delivered to tubes and uniform inocula are desired. See Schroeder (1988) for detailed instructions for making spot tests.

Dry conidia. Where numerous cultures are to be tested for ability to grow on the test medium, it is unnecessary to make a suspension of macroconidia from each of the cultures to be tested. Either of two procedures is quick and effective:

- A tube culture is held horizonally and tapped to obtain a film of powdery macroconidia visible on the clear glass surface opposite the culture. For inoculating slants, a fine-gauge inoculating needle is touched to the film and used to pick up a very small quantity of conidia, which are inoculated at a single point on the agar surface. For tests on plates, a. small loop of sterile water is touched to the film and used for spotting on the sorbose agar test medium.

- An alternative procedure applies when tests are being made in culture tubes. A dry stiff inoculating needle is used to pick up a small mass of conidia directly from the culture to be tested. The needle is then held over an open tube of test medium and tapped gently. If the tube used for testing is held vertically in a strong beam of a light coming from the side, the resulting quasi-darkfield illumination enables individual conidia to be seen drifting down like snowflakes or dust particles onto the surface of the medium. Inoculation is thus known to have been accomplished, even though the needle never touched the medium and the inoculating material on the surface is so sparse that it is invisible to the naked eye. Carrying over vast numbers of loose, unneeded conidia can be avoided if the needle is tapped vigorously before being removed from the tube of origin when the conidia are picked up.

Nonconidiating strains

Small mycelial fragments can be separated using a sharp-pointed transfer needle. With platinum-iridium, a fine, narrow point is obtained by hammering the needle to form a thin spatulate end, then using sharp scissors to make two cuts at an acute angle. If sufficiently sharp, the needle can be used to tease out a very small, barely visible fragment from an aconidiate colony, even when the colony is hard and dense. See *How to make tapered platinum-iridium needles and use them for isolating ascospores***.**

References

Newmeyer, D.,. and D. R. Galeazzi. 1978. A meiotic UV sensitive mutant that causes deletion of duplications in Neurospora. Genetics 89: 245-269.

Newmeyer, D., A. L. Schroeder, and D. R. Galeazzi. 1978. An apparent connection between histidine, recombination, and repair in Neurospora. Genetics 89: 271-279.

Schroeder, A. L. 1970. Ultraviolet-sensitive mutants of Neurospora. 1. Genetic basis and effect on recombination. Molec. Gen. Genet. 107: 291-304.

Schroeder, A. L. 1988. Use of Neurospora to study DNA repair. In *DNA Repair. A Laboratory Manual of Research Procedures, Vol. 3*. E. C. Friedberg and P. C. Hanawalt, eds. Pp. 77-98. Dekker, New York.

Stadler, D. R., and D. A. Smith. 1968. A new mutation in Neurospora for sensitivity to ultraviolet. Can. J. Genet. Cytol. 10: 916-919.