C.J.R. Klittich and J.F. Leslie

Multiwell plates for complementation tests of Fusarium.

Vegetative compatibility group (VCG) is a phenotype used to subdivide and categorize populations of *Fusarium* species (Correll et al. 1987 Phytopathology 77:1640-1646). Strains are vegetatively compatible if alleles at all loci controlling vegetative compatibility are identical. At least 10 loci control this trait in *F. moniliforme* (Puhalla and Spieth 1985 Exp. Mycol. 9:39-47). Identification of VCG requires a complementation test for heterokaryosis between auxotrophic mutants with known VCGs and field isolates with unknown VCGs. Mutants unable to reduce nitrate (*nit* mutants) are easily generated from *F. moniliforme* and *F. oxysporum* using medium containing chlorate, a toxic analog of nitrate (Klittich and Leslie 1988 Genetics 118: in press). The clearest complementation reactions are obtained when one *nit* mutant is deficient in a gene for production of a molybdenum cofactor (*nitM*) and the other *nit* mutant is deficient in either the nitrate reductase structural locus (*nit1*) or the pathway-specific regulatory locus (*nit3*). Population studies may require pairings of scores of VCG standards with hundreds of field isolates, making VCG testing an arduous task. We have reduced the effort and space required for these pairings by using disposable, plastic multiwell plates. Although we developed this technique to screen populations of *F. moniliforme,* it should be useful with other fungi as well.

Sterile, 24-well tissue culture plates are filled with melted minimal agar medium at 1.0 ml per well using a repeating syringe. After the medium has solidified, the plates can be used immediately or stored for several weeks in plastic bags at 5°C. To initiate complementation tests, each well of a plate is inoculated with a drop of spore suspension from an auxotrophic mutant (usually nitM) of a VCG standard strain. A sterile Pasteur pipette is used for inoculations. A drop of spore suspension from a complementary auxotrophic mutant (usually nit1 or nit3) of a field isolate is then added to the inoculated wells. Thus, 24 different field isolates can be paired with a VCG standard on one multiwell plate. As a positive, one well per VCG standard is inoculated with a compatible, complementing auxotrophic strain. The plates are incubated seven days at 25°C in a plastic bag to prevent drying. Pairings giving wild type growth are repeated on minimal agar plates (Correll et al. 1987) to confirm the complementation reaction. Complementation reactions are more definitive when the auxotrophic mutants are separated on a plate and meet to from a line of heterokaryotic growth. By screening field isolates in multiwell plates and retesting positive reactions, we save time in labeling, inoculating, and scoring pairings, and dramatically reduce the space required to conduct hundreds of pairings simultaneously.

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