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Storage of ascocarps and ascospore progeny of *Cochliobolus heterostrophus*.

Cochliobolus heterostrophus is a filamentous ascomycete that causes southern leaf blight of maize. Media and techniques for crossing this pathogen, isolating ascospores and storing cultures were summarized by Yoder, (1988 Adv. Plant Pathol. 6:93-112). The sexual cycle is induced on naturally senescent maize leaves pressed to the surface of a mineral salts medium. At 23°C, ascocarps appear within 5-7 days. Maturation of asci within the ascocarps is asynchronous. Most asci mature in about 21 days. After about the 25th day, many ascospores become fragile and difficult to isolate intact. Ascospores are isolated by dissecting open the ascocarps and spreading the asci on the surface of an agar block. Asci may either be teased open with a sterile needle or induced to rupture by treatment with a solution of ß-glucuronidase. Asci containing complete tetrads (7 or 8 ascospores) will often split open spontaneously. Ascospores may then be teased apart. The release of the ascospores onto the agar surface induces germination in about 1 hour. Colonies reach a size sufficient for storage and phenotype testing in about 5 days. Progeny are commonly stored by suspending mycelia and conidia in vials of 15 to 25% glycerol at -65°C. This note describes additions to these procedures that our laboratory has found useful.

1. Storage of ascocarps

Ascospores of *Cochliobolus* can be easily harvested for only about 4 days. If this window of optimum maturity is missed, or if it becomes clear after completion of phenotype tests that additional progeny are needed, it is usually necessary to restart the cross. We have attempted to prolong this window by refrigeration of the ascocarps, but without success. We have found, however, that air drying ascocarps seems to stop the maturation process and induces a reversible state of dormancy. When the ascocarps are remoistened, vigorous ascospores can again be harvested. We have taken advantage of this for the temporary storage of crosses. The method we have used is as follows:

When the majority of asci have formed viable spores and few or none are overripe (21-25 days at 23°C) leaf tissue bearing the ascocarps is stripped off the surface of the agar medium, placed in a sterile vial and allowed to air dry. The vial is capped and stored at 23°C (preferably with desiccant). When additional progeny are desired, a small section of leaf bearing ascocarps is placed on the surface of an agar block for several minutes until the leaf softens and the ascocarps regain their previous swollen appearance. Ascocarps can then be picked off the leaf and asci and ascospores isolated normally. We have noticed no obvious changes in ascus or ascospore phenotype or ascospore viability in crosses stored by this method for up to 4 months. The turgidity of asci and viability of ascospores begins to decline after about 6 months. This extension of the time ascospores can be harvested is generally in great excess of practical needs. We have not tested the effect of refrigeration or freezing on viability during dry storage.

2. Storage of ascospore progeny

A second problem that we have encountered is maintaining all progeny while the necessary phenotype tests and test crosses are performed. If-possible, we try to store progeny until all research on the project is complete. Previous storage methods that we have tried either didn't ensure viability for more than a few months or required too much valuable freezer space. We now routinely store progeny in 96-well plates at -65°C. Our method is an adaptation of the glycerol storage method recommended by Yoder (1988).

Progeny cultures are stored as soon as they are big enough to transfer to avoid any undesirable changes due to sectoring or senescence. Small mycelial fragments are inoculated into the wells of the 96-well plates. Each well contains 125 ul of complete medium (CM, as described by Yoder, 1988) prepared either with or without agar. The plates are sealed with parafilm to reduce drying and incubated under cool white fluorescent lights at 23°C. After 3 days, or when growth is heavy, 100 ul of 15 or 25% glycerol is added to each well containing solid medium or 50% glycerol to each well containing liquid medium. The wells are then stirred with a sterile toothpick or pipette tip to suspend the mycelium in the glycerol. The dishes are sealed with a low-temperature-resistant tape and frozen at -65°C. To retrieve the cultures, the plates are placed on a bed of crushed dry ice to prevent thawing and a small crystal of ice is scraped off the surface. Virtually all of approximately 1000 progeny stored by this technique were viable when tested after 3 years. We do not yet know the limit of viability.

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