Fungal Genetics Reports

Volume 36 Article 21

An effective procedure for the preparation of Neurospora conidiophores for scanning electron microscopy.

M. L. Springer

An effective procedure for the preparation of Neurospora conidiophores for scanning electron microscopy.

Abstract

An effective procedure for the preparation of Neurospora conidiophores for scanning electron microscopy.

Springer, M. L.

An effective procedure for the preparation of Neurospora conidiophores for scanning electron microscopy.

Sample preparation for scanning electron microscopy (SEM) involves excision of a small piece of the sample, immersion in various fixatives, dehydration and critical point drying, and coating with a thin layer of gold or gold/palladium. Neurospora crassa conidiophores present special problems in that they are very fragile, and will release their conidia if they are excessively disturbed. A small tuft of conidiophores and hyphae is especially vulnerable to the violent currents caused by the addition of buffers and fixatives, and undergoes considerable buffeting when flooded repeatedly with liquid CO_2 during critical point drying. These problems are obviated by several modifications of standard protocols: (1) the cultures are grown on agar spots in 60 mm plastic petri dishes, (2) the sample viewed is cut away at the end of the procedure instead of at the beginning, and (3) the sample is dried with hexamethyldisilazane (Nation 1983 Stain Technol. 58:347-351) instead of conventional critical point drying. The following procedure is very effective for preservation of conidiophores (Springer and Yanofsky 1989 Genes Dev., 3:559-571), but has also been successfully used with hyphae, microconidia and perithecia.

The agar spots contain Vogel's minimal medium with 1.5% sucrose and 2% agar, and are made by pouring approximately 1-1.5 ml of lukewarm agar into the center of each dish without allowing it to spread to the sides. The agar should be poured when it has cooled to the point at which it can comfortably be held in the bare hand. Both temperature and agar concentration are critical; surface tension will allow spots roughly 2.5 cm in diameter and about 2 mm thick. These spot cultures have two advantages. First, the entire dish can be filled with a solution, which can be changed by drawing off the liquid with a pipette and gently pouring in the next solution near the. edge of the dish. Second, the aerial hyphae that grow away from the edges of the agar are roughly parallel to the bottom of the dish; this facilitates viewing.

Cultures are grown upside-down, to prevent drying, at 34°C. When covered with hyphae, they are turned right-side up and incubated at room temperature until maturity (usually l-2 days). To further guard against drying, about 0.5 ml of sterile water is put in the lid while the culture is upside-down. Fixative consisting of 2% gluteraldehyde and 1% paraformaldehyde in 0.1 M potassium phosphate buffer, pH 7.2, is gently poured into the dish next to the sample. After two hours at room temperature, the samples are rinsed with buffer and then post-fixed with 1% OsO_4 in the same buffer for one hour. When changing aqueous solutions, the meniscus should never come in contact with the sample; this would cause conidial scatter. Since the conidia are hydrophobic, a sheet of air will be visible over the culture while it is in the buffer. This prevents the liquid from coming in contact with the conidia, thus the aldehyde fixatives primarily fix the non-conidial regions of the culture. The conidia are fixed mainly by osmium vapor.

After being rinsed with distilled water until the solution is completely clear, the samples are dehydrated stepwise by an ethanol series consisting of 30%, 50%, 70%, 95%, and 100% ethanol, 15 minutes per step. The surface will typically wet at the 70% ethanol step. The samples are treated with fresh 100% ethanol for 30 minutes. The ethanol is completely replaced with hexamethyldisilazane (HMDS; Polysciences). After 30 minutes, the HMDS is pipetted off, and the samples are air dried in the hood for about 20 minutes or until completely dry. Blocks of samples including agar are cut from the edge with the aid of a scalpel and fine forceps. A dissection microscope is helpful at this point. Even at this stage, the conidia are capable of floating off of the conidiophores, so care should be taken not to jar the sample when cutting. The blocks are then mounted on metal stubs, sputter coated with 20 nm of gold and viewed. - - - Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.