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Microelectrophoresis of extracts from single perithecia

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Abstract
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Electrophoresis of proteins from a single perithecium con be Performed in capillary tubes, according too modification of Grossbach's procedure (1965, Biochim. Biophys. Acta 107: 180-182). Glass capillary tubing with 5-6 mm outride diameter and 1/4-3/4 mm inside diameter (Thomas Co.) ore cut into 4 cm lengths. The Column Coot (Canalco) coated tuber are sealed at one end with parafilm. The electrophoretic system and procedure employed ore essentially as described for the standard electrophoretic analysis of perithecial extracts (Nasrallah and Srb (1973), Proc. Not. Acad. Sci. USA 70:

1891-1893) with the following modifications: using a 50μ Hamilton syringe, the capillary tuber are filled with separating gel solution to a depth of , .5 cm, and proportionately twice as much stacking gel is used. Piecer of cellulose acetate film (Sepraphore III, Gelman), cut to fit into the capillary tuber, are washed with 0.1 M phosphate buffer, pH 7.0. A single perithecium is quashed onto a cellulose acetate piece. If desired, microscopic analysis of ascus and ascospore morphology con be performed before the cullulose acetate piece is placed on the surface of the stocking gel. A small volume of stacking gel solution is then layered on top of the cellulose acetate piece to prevent diffusion of the proteins bock into the electrophoresis buffer. The upper port of the capillary tuber is filled with electrophoresis buffer containing Bromphenol Blue as tracking dye. Electrophoresis is run in a standard Canalco disc electrophoresis apparatus, at 1/2 ma per gel. After the tracking dye has migrated 1 cm down the separating gel, the current is stopped and the capillary tubes are immediately submerged in an ice both to delay band diffusion. The gels are removed from the capillary tuber using a syringe filled with ethylene glycol and provided with a fine needle (No. 27 or 30). The gels ore fixed in 10% (w/v) trichloroacetic acid and stained with a 1:10 dilution of a 1% (w/v) aqueous solution of Coomassie Brilliant Blue in 10% (w/v) trichloroacetic acid.

This technique has been successfully applied to: 1) genetic analysis of electrophoretic variants of perithecial proteins, and 2) to their distribution in single perithecia produced by mycelia heterokaryotic for the determinants of the protein variants and for ascus and ascospore shape. (Supported by Grant GM-12953 from the Notional Institute of General Medical Sciences, USPHS.) - - Section of Botany, Genetic and Development, Cornell University, Ithaca, New York 14853.