

Nasrallah, J.B. and A.M. Srb. Microelectrophoresis

of extracts from single perithecia.

Electrophoresis of proteins from a single perithecium can 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 outside 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 50 μ Hamilton syringe, the capillary tubes are filled with separating gel solution to a depth of .5 cm, and proportionately twice as much stacking gel is used. Pieces of cellulose acetate film (Sepraphore III, Gelman), cut to fit into the capillary tubes, are washed with 0.1 M phosphate buffer, pH 7.0. A single perithecialium is quashed onto a cellulose acetate piece. If desired, microscopic analysis of ascus and ascospore morphology can be performed before the cellulose acetate piece is placed on the surface of the stacking gel. A small volume of stacking gel solution is then layered on top of the cellulose acetate piece to prevent diffusion of the proteins back into the electrophoresis buffer. The upper part of the capillary tube 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 bath to delay band diffusion. The gels are removed from the capillary tubes using a syringe filled with ethylene glycol and provided with a fine needle (No. 27 or 30). The gels are 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 National Institute of General Medical Sciences, USPHS.) - - Section of Botany, Genetic and Development, Cornell University, Ithaca, New York 14853.