Gratzner, H. G. A convenient method for α-amylase and invertase electrophoresis on cellulose acetate strips.

Cellulose acetate serves as an attractive electrophoretic medium for mpid separation of enzyme proteins. However, difficulty is encountered when one wishes to visualize an enzyme on the strip. For this reason, we have developed a simple technique for use with α-amylase from Neurospora culture media.

The culture medium (Vogel's minimal, or phosphate buffer) from induction experiments is placed into a dialysis sack and concentrated against solid sucrose in the cold room overnight (Horowitz and Fling 1962 Neurospora Newsl. 2: 19). Twelve lambda samples of this concentrate are placed on pre-soaked, blotted sepraphore 111 (Gelman) strips at a position near the cathode and electrophorized for 1 hour at 300 v (or approximately 75 volts/strip). The electrolyte is 0.3 M borate buffer, pH 8.2.

At termination of the run, the strips are blotted and placed upon 0.5% starch ▪ 2% agar blocks and incubated for about IO minutes. The strips are then peeled from the ogor blocks and stained in iodine vapor for several minutes. This procedure giver a smooth, blue background with white bands corresponding to amylase activity. The block. can also be stained as a duplicate.

For the simultaneous localization of invertase, the strip, or a longitudinal half of the amylase strip, is sliced into 3 mm lateral strips, which are then sequentially eluted in small tubes with distilled water. An equal volume of buffered, 10^{-2} M sucrose is added to the tuber, and the tubes are incubated at 37°C for 1 hour. Reducing sugar is assayed in each tube by means of o-nitrosalicylate reagent (Bernfeld, in Methods in Enzymology, Vol. I).

By this method, it is possible to localize the two enzyme bands in a relatively short time. Although obviously the resolution of acrylamide gel electrophoresis is not obtained, the cellulose acetate procedure is useful, particularly for comparison of amylases from various strains of Neurospora. With many experiments, it is possible to electrophorize the unconcentrated growth medium with excellent results. ▪ ▪ ▪ Department of Zoology, University of South Florida, Tampa, Florida. 33620