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Abstract

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Johnson, H. N. Identification of a third farm of ß-galactosidase by sectioning acrylamide gels.

Strickland and Shields (1967 Neurospora Newsl. 12: 15) described a method for specifically staining enzymes and matching them to proteins stained on the same acrylamide gel. This communication describer a more satisfactory method for identifying the ß-galactosidases in Neurospora. In addition to the previously described ß-galactosidases with pH optima at 7.5 and 4.2, a third form has been identified by this method which has a pH optimum at 4.5.

The standard 7.5% gels were used and after electrophoresis were frozen on dry ice or by immersion in liquid nitrogen. The gels were then split lengthwise with a razor blade and one half was stained with amido block. The other half was then sectioned into l-mm sections by a gel slicer. The slices were individually assayed using O-nitro phenyl-ß-D-galactopyranoside. The enzyme activity for each farm of the enzyme usually was localized in 2 or 3 slices. Using tracking dye (brom phenol blue) as a front marker, Rf'r for the enzymes were fairly reproducible.

Wild type Neurospora crassa (74-OR-A) was grown on 1% lactose for 5 days and the mycelio were filtered out on a Buchner funnel. The mycelio were extracted with 10 ml of 0.01 M No phosphate, pH 7.5, per g of wet weight. The mycelio were homogenized in on Omni-Mixer, sonicoted, and then stirred for 2 hrs at 4°C. After centrifugation at 20,000 × g for 20 min, the supernatant war used as crude extract. The growth medium after filtration was concentrated by dialysis against dry sucrose. Sampler for electrophoresis contained ca. 0.25 mg protein.

Crude extract gave reactions with ONPG at three distinct sites on the gel, The 7.5 enzyme hod on Rf of 0.046, the 4.2 enzyme hod on Rf of 0.250, ond a third form of the enzyme hod on Rf of 0. 150. When the growth medium was electrophoresed, activity appeared at either one or two sites, depending upon the age of the culture. Medium from a young culture shaved only the new form of the enzyme (pH 4.5) whil medium from on old culture contained both the 4.2 and 4.5 forms with a predominance of the former. Between these two extremes there were gradations in the proportions of the two forms. This work supported in port by the NIH Training Grant in Genetics (T01-GM01316) to Florida State University. - - - Genetics Laboratories, Department of Biological Science, Florida State University, Tallahassee, Florida 32306.