Comments on enzyme assays

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Abstract
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This enzyme methodology is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol14/iss1/33
Wild type Neurospora crassa (74-OR-A) was grown on 1% lactose for 5 days and the mycelia were filtered out on a Buchner funnel. The mycelia were extracted with 10 ml of 0.01 M Na phosphate, pH 7.5, per g of wet weight. The mycelia were homogenized in an Omni-Mixer, sonicated, and then stirred for 2 hrs at 4°C. After centrifugation at 20,000 x g for 20 min, the supernatant was used as crude extract. The growth medium after filtration was concentrated by dialysis against dry sucrose. Samples 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 had an Rf of 0.046, the 4.2 enzyme had Rf of 0.250, and a third form of the enzyme had 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. Methyl from a young culture stained only the new form of the enzyme (pH 4.5) while medium from an 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 was supported in part 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. Morgan, D. H. The assay of arginase.

Many methods of arginase assay in various organisms have been published. The following procedure has been found to work well with crude extracts of Neurospora. Frozen mycelial pods are ground in a chilled mortar with glass powder and 5-10 times their weight of Na dehydrogenase, EC. 1.3.1.1.; but with 8 mM DL-isocitrate. 

Malate synthase, EC. 4. 1.3.2.; but with 0.04 mM of Acetyl CoA. 

Phosphoenolpyruvate carboxykinase, EC. 4. 1.32.; but with 9 mM phosphoenolpyruvate. 

Citrate synthase, EC. 4. 1.3.7.; but with 0.04 mM of Acetyl CoA. 

Acetyl CoA synthetase EC. 6.2. 1.1. 

Malate dehydrogenase, EC. 1.1. 1.37. 

NADP-linked isocitrate dehydrogenase, EC. 1.1. 1.42. 

NAD-linked isocitrate dehydrogenase, EC. 1.1. 1.41.; but with 8 mM DL-isocitrate and 1 mM NAD. 

Fumarate hydratase, EC. 4.2. 1.2. 

The following assays have been used without modification of the method cited in the reference following each enzyme: 

Succinate dehydrogenase, EC. 1.3.99. 1. (King 1963 J. Biol. Chem. 238: 4032) 


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