Fungal Genetics Reports

Volume 14 Article 25

Agar gel electrophoresis of amylases

D. N. Fass

Agar gel electrophoresis of amylases

Abstract

Agar gel electrophoresis of amylases

Fass, D. N. Agar gel electrophoresis of Neurospora amylases.

The procedure of Kikkawa (1963 Ann. Rep. Scient. Works, Fac. Sci. Osaka Univ. 11:41) previously used for the production of zymograms of Drosophila amylase hos been modified for use with Neurospora crassa. A gloss plate ca. 13 X 10 cm is frosted on one ride by grinding with household cleanser (Comet). The electrophoretic medium consists of 2% Difco Purified Agar in 0.01 M citric acid buffered to pH 5.0 with NaOH. This some buffer is used in the electrode reservoirs. A layer of tempered agar 1 mm thick is pipetted onto the frosted side of the prewarmed plate and is evenly with the ride of a pipette. Whatman #2 filter paper strips 1 x 0. 15 cm soaked with enzyme ore applied too line 3 cm from a long edge of the plate. The enzyme is absorbed for 10 min, after which the strips are removed. The plate is connected to the reservoirs by double thicknesses of Whatman #1 filter paper. A potential gradient of 40V/cm is applied and maintained for 2 hours. The amylases will migrate to the cathode.

The plate is then immersed in a 4 mg/ml soluble starch solution for 15 min, followed by a brief water rinse. Digestion of the starch is allowed to occur for 20 min in a 37°C incubator, after which the agar is stained in a solution of 0.3% KI - 0.03% 12. Two types of bonds will Lx visible; clear (against dork blue) and faint pink. The former ore the Y-amylases (gluc-amylases) and the latter are the a-amylaser.

Enzyme may be obtained by growing most strains for 8 days in Vogel's salts plus 2% sucrose with necessary supplements in stationary culture. The medium is decanted and the pads are washed for 1 hr in Vogel's salts. This also is decanted and Vogel's salts plus 1% maltose is added to the pad. After 24 hrs of shaking at 25°C, easily detectable quantities of enzyme will be present in the medium. This medium should be concentrated 20-50 fold by dialysis against air or sucrose before electrophoresis. Strain inos 89601a (FGSC#498), grown for 4 days with shaking in Vogel's salts + 1% sucrose and inositol, will produce sufficient enzyme in the medium for electrophoresis without concentration. Strain inos 89601A (FGSC#497) does not produce elevated levels of amylase (H. G. Gratzner, person.1 commun.).

This work supported in part by the NSF and NIH Training Grant in Genetics (T01-GM01316) to Florida State University. Genetics Laboratories, Deportment of Biological Science, Florida State University, Tallahassee, Florida 32306.