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Agar gel electrophoresis of amylases

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Abstract

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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.).

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