Concentration of chromatographic effluent for gel electrophoresis

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Abstract
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Taylor, C. W. A more efficient procedure for scoring mating type and aberrations.

A technique for scoring aberrations among isolates by crossing them with standard fluffy strains (fl or P605) and fPA was described by Perkins et al. (1962 Can. J. Genet. Cytol. 4: 187).

Strains to be tested were spotted on plates containing fertile fluffy testers, and aberrations scored by the frequency of white spores on the lid of the plate. It has now been found to be technically advantageous for large numbers of sex tests and scoring aberrations to make the crosses in 3-inch tubes rather than on plate.

The advantages of tubes over plates are these. The tests can be done by relatively unskilled help without the possibility of scatter in the formation of perithecia or in ascospore shooting. There is no problem of spores moving in the condensate that forms on the lid of a plate. Any error is thus eliminated in determining which spores came from a particular isolate. Positive tests can be used for progeny testing, if necessary.

Tubes inoculated using a suspension of fluffy mycelia prepared as follows: the two fluffy strains are grown 4 days at 34°C in 300 ml flasks containing 50 ml of a liquid glycerol complete medium (a modification of Medium 2 described by Tatum et al., 1950 Am. J. Botany 37:38) and a 15 cm filter paper cone (for greater aerial growing surface). Several flasks can be prepared and grown up at one time and then refrigerated until needed (good for several weeks). A dense mycelial suspension is made by adding about 50 ml sterile water to each flask and vigorously shaking, first by hand and then with a vortex mixer. Three-inch tubes of SC agar (Synthetic Cross Medium, Westergaard and Mitchell 1947 Am. J. Botany 34:573) are inoculated with a drop of the mycelial suspension, using a wide-bore Pasteur pipette with a squeeze bulb. SC is color-coded with Schilling food color before dispensing to identify the two fluffy mating types and minimize mix-ups.

Tubes inoculated with the fluffy testers are incubated at 25°C until proterithidia are formed. They are then ready for fertilization, or they may be stored at 5°C and used up to one week later. Sex tests are scorable after four days at 25°C and aberrations (fertility and frequency of aborted spores) can be determined within 12-14 days after crossing by examining ascospores shot to the wall of the tube. Tests are routinely made against testers of both mating types to reveal false negatives, infertility, or bisexuality.


This is a technique we have developed for use in the purification of dehydrogenases from Neurospora but it may have other applications. In the purification of enzyme extracts the fractions of effluent collected from the chromatographic column are much too dilute for direct application to supporting material for gel electrophoresis. A simple method of concentrating the effluent was needed to allow detection of bands of protein or isoenzymes after electrophoresis. This method is based on the technique first described by Horowitz and Fling (1962 N. N. 2:19) who placed dilute solutions of tyrosinase in dialysis bags to dialyze against solid sucrose. We reversed the positions of the sucrose and the sample. Sucrose granules are placed in a dialysis bag of large diameter. The top surface of the bag is flattened so that a row of filter paper pieces can be placed in close contact with the surface. The bag is chilled and cold fractions of effluent are pipetted onto the surface until a layer of liquid is formed on top of each piece. Water and electrolytes are absorbed into the sucrose below and in on hour or so a second application, if necessary, can be made or the filter paper piece can now be inserted into starch or acrylamide gel for electrophoresis. This method allows a large number of samples to be concentrated simultaneously and requires very little expenditure of equipment or material. The pipetted amounts of effluent also give a control of the amount of protein or enzymes to be subjected to electrophoresis.


During an investigation of arginine synthesis in N. crassa, it became necessary for us to assay for arginosuccinate synthetase. A method based on the disappearance of citrulline from reaction mixtures has been described (Newmeyer 1962 J. Gen. Microbiol. 28: 215), but little information was presented concerning conditions for optical activity. The present report describes a modified, more sensitive procedure developed in this laboratory.

Culture conditions and the method of preparing acetone powders have appeared elsewhere (Fairley and Wampler 1964 Arch. Biochem. Biophys. 106: 153). The acetone powder is added to 0.01 M phosphate buffer pH 7.4 (1 g/9 ml), homogenized in a Servall Omnimixer and centrifuged for 15 min at 23,000 x g. The residue is resuspended in a small volume of buffer and recentrifuged. The combined supernatant liquids containing 10-15 mg protein/ml are added to a column of Sephadex G-25 (a 2.2 x 25 cm column is adequate for a 15-m1 sample) which has been equilibrated with 0.01 M phosphate buffer, pH 7.4. The type of buffer is not critical and any pH between 7.0 and 8.2 is satisfactory. Protein is eluted with the same buffer and tubes containing more than 2 mg protein per ml as measured in the Biuret reaction, are saved.

Preparations which are not parsed through Sephadex (or dialyzed for several hours) do not exhibit activity which is linear with enzyme concentration.

The Sephadex-treated enzyme is assayed in a 2-ml reaction mixture which contains: 38 µmoles potassium aspartate, pH 8.0; 22 µmoles MgSO 4.1.5 µmoles L-citrulline; 2.0 µmoles ATP; 20 µmoles 3-phosphoglyceric acid and 100 µmoles Tris-chloride buffer, pH 8.0. In this crude stage at least 0.5 mg of protein must be used for appreciable reaction to occur. The mixture is incubated at 37°C for 10 minutes and the reaction is stopped with 1 ml of 1N perchloric acid. Three ml of water is added (final volume 6 ml) and the mixture is centrifuged. A one-ml sample of the supernatant is assayed for citrulline by the method of Gerhord and Pardee (1962 J. Biol. Chem. 237: 891). The reaction can be run with 0.5 µmoles citrulline and arrayed without diluting (final volume 3 ml) but these conditions result in slightly lower apparent activity.

The reaction is linear with respect to protein concentration within the range used and with respect to time up to 10 minutes (Fig. 1). One of the reagents, ATP, is both a substrate and an inhibitor for the reaction as shown in Fig. 1. Fig. 2 shows the