

## Antigenic screening method for genetic studies

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## **Abstract**

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Garrett, M. K. An antigenic screening method of potential application in genetic studies.

It is frequently laborious to examine the segregation of isozymes which are recognized by electrophoretic criteria. The problem is aggravated when recombinants of closely linked genes are being sought, as is the case with the genes for trehalase in *N. crassa*. In this example recombinants

were being sought between the tre gene (coding for the presence of trehalase) and the mig gene (coding for the electrophoretic mobility of the enzyme, viz. fast or slow) which mapped less than one unit apart (Sussman et al. 1971 J. Bacteriol. 108: 59). An enrichment of recombinants was achieved in the usual way through the use of flanking markers, and the identification of "fast" and "slow" forms of trehalase in tre<sup>+</sup> recombinants would normally have involved electrophoresis in polyacrylamide gel. However, the use of an immunological technique made it possible to screen up to 100 ascospore isolates per day with a high degree of specificity. The technique is described here since it may have general application in *Neurospora* studies.

Numerous methods have been employed for the production of antibodies and generally require up to 10 mg of pure antigen as starting material. In the case of trehalase, 2-10 mg of pure enzyme in 0.05 M phosphate buffer (pH 5.6) was emulsified with an equal volume of Freund complete adjuvant and injected intra-muscularly into rabbits over a period of 2 weeks. Booster injections contained approximately 1 mg of antigen in incomplete adjuvant. A maximum titre was obtained in the third week and the animals were then bled by cardiac puncture or from an ear vein. The 20-30 ml of blood obtained was allowed to clot in a Petri dish and, after standing overnight at 4°C, the serum which had separated was decanted, centrifuged for 20 min at 3,000 x g to remove cells, and stored at -20°C.

The Ouchterlony double diffusion method was used to screen extracts. Degreased glass slides (2 in x 2 in) were first dipped in 0.25% Ionagar #2 (in deionized water), drained and permitted to dry. 2 ml of 1% Ionagar #2 (in 0.04 M veronal/HCl buffer, pH 7.5, + 0.003% methyl orange) was then poured to form an even layer on one side of each slide. After storage at 4°C for 12 hr, one central and four peripheral wells were cut in each slide using a template and cork-borer. The antiserum was placed in the central well and peripheral wells contained extracts of undesigned phenotypes alternating with samples of the electrophoretically identified isozymes "fast" or "slow". The extracts were prepared from acetone powdered mycelium as described previously for electrophoresis (Yu et al. 1971 Genetics 68: 473) and 10 µl samples of antisera and antigens were used throughout.

Clear-cut differences between extracts of known "fast" and "slow" strains were obtained using antiserum prepared against trehalase from the wall fraction of "fast" strains, and such differences were annulled by absorption of the antiserum with purified "fast" trehalase. A total of 574 tre<sup>+</sup> recombinants were screened by this method and only one yielded an ambiguous result on Ouchterlony plates. Electrophoresis was used to check 10% of the samples selected at random and in all cases the expected result was obtained.

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