Fungal Genetics Reports

Volume 19 Article 11

Antigenic screening method for genetic studies

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Recommended Citation
Garrett, M. K. (1972) "Antigenic screening method for genetic studies," *Fungal Genetics Reports*: Vol. 19, Article 11. https://doi.org/10.4148/1941-4765.1869

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Abstract

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

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 mopped 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+ 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 studier.

Numerous methods have been employed for the production of antibodies and generally require up to IO 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 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 gloss 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) war 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 wing a template and cork-borer. The antiserum was placed in the central well and peripheral wells contained extracts of undesignated phenotypes alternating with sampler of the electrophoretically identified isozymes "fart" or "slow". The extracts were prepared from acetone powdered mycelium as described previously for electrophoresis (Yu et al. 1971 Genetics 68: 473) and IO 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+ recombinants were screened by this method and only one yielded on 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.

This work was carried out whilst the author was a Research Associate in the Deportment of Botany at the University of Michigan, and was supported by NSF grant GB 6811X to A. S. Sussman, to whom I am indebted for support and facilities. The cooperation of S. Liu and T. Jepson in the preparation of antisera is gratefully acknowledged. - - - Deportment of Botany, The Queen's University, Belfast, Northern Ireland.