

A selective plating technique for determining the recombination frequency between crisp and linked mutant loci

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Abstract

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This technique allows a quicker and less laborious analysis of a large number of progeny than the isolation of single random spores. Its accuracy depends on regular Mendelian segregations and a high percentage of viable ascospores which germinate simultaneously after the heat

shock. These factors were checked by the full analysis of 100 single random spore isolates from the same batch as that plated.

Analysis of the cross cr (F945), +, +, x +, aur (34508), nic-1 (3416) will serve as an example. Random ascospores were plated (about 100 per plate) on a medium containing per litre:- 4 gms sorbose, 7.5 gms sucrose, 50 mgms bacto-peptone (British Drug Houses), 20 gms agar (Oxoid Ionagor No. 2) and Fries salt solution with trace elements and biotin (Beadle and Tatum, 1945, Amer. J. Bot., 32, 678-685). The sorbose to sucrose ratio and the bacto-peptone favoured development of cr progeny but inhibited growth of nic-1 progeny. After 2 days at 25°C conidiating crisp colonies were distinguishable from non-conidiating, non-crisp colonies. The latter which grew faster were subcultured onto complete medium in 3" x 3/8" tubes and incubated for 2 days at 30°C then exposed to daylight for a few hours in order to distinguish pink (+, +, + recombinant) from aurescent (+, aur, + recombinant) cultures. The plates were incubated at 25°C in the light for a further 24 hours permitting the scoring of pink crisp (cr, +, + parental) and white crisp (cr, aur, + double recombinant) colonies.

Therefore the parents detected gave the frequency of half parents and the recombinants detected gave the frequency of half recombinants since they were one of each reciprocal type resulting from a cross-over in region I or II or a double cross-over. If the single random spore isolates did not give significant differences from the expected 1:1 ratios of mutant to non-mutant progeny for each marker then the frequencies of half parents and half recombinants detected by the plating method were compared by the 2 x 2 x² test with the same data from the single spore isolates. Provided there were no significant differences in any of these tests then recombination frequencies were determined from the frequencies of half parental and half recombinants detected by the plating method.

This method was used successfully to determine the recombination frequencies in 6 crosses of the type cr, +, +, x +, aur, nic-1; 5 crosses of the type cr, +, +, x +, al (G2), nic-1; 5 crosses of the type cr, +, +, x +, me-6 (36809), aur and 3 crosses of the type cr, +, x +, aur.

This technique allows the use of any biochemical mutant which can be inhibited or whose growth is greatly restricted by appropriate alteration of the plating medium. With cr and a single biochemical or suitable morphological mutant half parental and half recombinant progeny can be scored directly from the plates.

A high percentage of viable ascospores which germinated simultaneously was obtained by crossing one mutant which had been successively backcrossed at least six times to Lindegren 1A wild type with another mutant which had been backcrossed similarly to Lindegren 1A, Abbott 4A or Abbott 12a wild type. In contrast, crosses between these same mutants with Abbott ancestry, whether Abbott 4A or Abbott 12a or both, often yielded few viable ascospores which germinated sporadically over several days.

It may be desirable, to overcome poor viability and germination, to breed strains as above so that the selective plating analysis may be used. It was found that, unlike Lindegren 1A, abortion and low fertility factors carried by Abbott 4A and 12a hindered backcrossing. Also, backcrossing to any one of the wild types may introduce factors which alter significantly the frequencies of recombination between the same linked markers.

This work was carried out at the Department of Botany, Bristol University and is reported in detail in the author's M.Sc. Thesis, 1962, University of Bristol. ---Microbiological Research Establishment, Porton, Salisbury, Wiltshire, England.