Mating type tests using a plating technique

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Abstract

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The following method of using plates for determining the mating types of isolates has been employed successfully for some time. It enables

large numbers of cultures to be tested rapidly with a great reduction in the quantity of glassware, time and medium required.

Thick suspensions of conidia of each mating type of the morphological mutant spray (B132) are made in sterile water. A 9 cm. Petri dish containing Westergaard's crossing medium (Westergaard, M. and Mitchell, H.K., 1947, Amer. J. Bot. 34, 573) is spread evenly with 0.2 ml. of conidial suspension of spray mating type a and a similar plate spread with spray A. Inoculated plates are incubated for five to six days at 25°C until a mat of mycelium is formed and protoperithecia are produced. Should the mycelium tend to spread over the sides of the plate, it may be checked by wiping with sterile cotton wool dipped in alcohol.

Incubated plates are marked on the back with wax pencil or ink into a convenient grid (of 24 or 25 parts) with suitable orientation marks. Conidia from the cultures to be tested are inoculated with a loop in the centre of corresponding portions of plates of each mating type. In this way two plates are sufficient to test 24 or 25 cultures. The plates are then sealed with transparent adhesive tape and incubated at 25°C until perithecia are formed. Sometimes plates may be scored after only 48 hours incubation but more usually three to six days are needed.