MUTATIONAL STUDIES ON PHYTOPHTHORA INFESTANS

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SUMMARY
Attempts were made to induce and isolate auxotrophic mutants of Phytophthora infestans, using ultra-violet light and ethyl methane sulphonate as mutagens. Low doses of ultra-violet light had little effect on the germination of the spore but markedly reduced the number of spores forming colonies and the growth rate of these colonies. Ethyl methane sulphonate did not affect the growth rate of the surviving colonies. No auxotrophic mutants were isolated but differences were noted in the ability of some survivors to initiate growth on the minimal medium, although once growth was established the colonies grew quite readily.

1. INTRODUCTION
Adaptation of Phytophthora infestans (MONT.) DE BARY to true resistance of the cultivated potato is well established (Troxopeus, 1956) and there is some evidence that adaptation to field resistance may occur also (Niederhauser, 1964). Similar changes have been reported in laboratory experiments. Galleghy and Eichenmuller (1959) reported that they could readily change isolates to include the race 4 characteristic in addition to those previously recognised, while Graham et al., (1961) reported the addition of the characteristics of several races to isolates after short periods of training on the appropriate R-gene varieties. Wilde (1961) reported the changes from race 1 to races 1, 2 and 1,4 following treatment with ultra-violet light and also put forward some evidence for the occurrence of heterokaryosis. In contrast, McKee (1963) was unable to detect any change from race 4 to race 1,4 among 500,000 zoospores surviving ultra-violet irradiation and several other workers have failed to detect changes in race characteristics in laboratory experiments (Black, 1952; Muller et al., 1955). The interpretation of these results awaits an analysis of the genetic basis of variation and pathogenicity in this organism. This situation might be partly resolved by a genetic analysis carried out in culture, using auxotrophic and drug resistant mutants as nuclear markers, as has been done in studies on other fungal pathogens. This study has been hampered by the lack of a medium on which widely spaced zoospores will germinate and continue growth to form compact colonies within a reasonably short period of time. A suitable medium, the pectin-glucose medium, has now been devel-

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oped (Clarke, 1966) and this paper is the report of an attempt to induce and isolate auxotrophic mutants of P. infestans.

2. METHODS

2.1. The isolate

A culture of race 4 was kindly provided by Mr. J. Bean of the Botany School, Cambridge. A single zoospore isolate was obtained and maintained, and zoospore suspensions were prepared, following the procedures described previously (Clarke, 1966). Estimations of the number of zoospores present was made using a haemocytometer, and standard microbiological techniques were used for diluting the spore suspensions. Quantities of 0.2 ml of the appropriate dilutions, giving not more than 150 colonies per plate, were spread on the pectin-glucose medium using a glass spreader. Approximately 98.5% of zoospores were found to be uninucleate when stained and examined in acridine orange, the remainder being binucleate.

2.2. Media

The concentrations are given in g/- or ml/litre distilled water.

Minimal medium: KH₂PO₄, 0.5 g; MgSO₄.7H₂O, 0.25 g; asparagine, 1 g; glucose, 25 g; thiamin, 0.001 g; Davis agar (David Gelatine Ltd.), 20 g.

Complete medium: Minimal medium + “Difco” yeast extract, 5 g; “Difco” casamino acids, 2.5 g; nucleic acid hydrolysate (prepared by the method of Pontecorvo et al., 1953), 3 ml; vitamin mixture (Pontecorvo et al., 1953), 1 ml.

Pectin glucose medium: Minimal medium + “Difco” yeast extract, 5 g; “Difco” casamino acids, 2.5 g; apple pectin, 250 grade (British Drug Houses), 10 g. The pH of all media was adjusted to 5.5, using N HCl or N NaOH as required, before autoclaving at 10 lb. for 10 min.

2.3. Mutagens

Ultra-violet light: A 9 cm petri dish containing 10 ml of a zoospore suspension was exposed, with constant agitation, under a Hanovia XI low-pressure mercury lamp at a distance of 22 cm. An exposure time of 40 sec killed 95–99% of the zoospores. Approximately 2,000,000 spores were irradiated and 548 of the survivors were isolated and assayed for changes in their growth requirements.

Ethyl methane sulphonate: Approximately 3,000 zoospores were treated with 0.12 M solution for 3 min, after which the reaction was stopped by diluting 1:10 with sterile water. This treatment killed about 70% of the spores. 155 of the survivors were isolated and assayed for changes in their growth requirements.

Other techniques are described in the text.