A METHOD FOR INDUCING SPORULATION OF ALTERNARIA SOLANI IN CULTURE[1]

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Alternaria solani (Ell. & Mart.) Jones & Grout has the reputation of being a difficult laboratory test organism because of its reluctance to produce, under ordinary cultural conditions, sufficient quantities of uniform spores for bioassay or plant inoculation purposes. Various workers have attempted, with more or less success, to improve its sporulation by various methods including mutilation of the mycelium (1,2,3,4), addition of vitamins to the medium (3), and exposure to sunlight (3,4), ultraviolet light (3), or fluorescent light (2).

A relatively simple method developed at this Research Institute has given good results consistently over a period of 10 years. The fungus is first grown for 2 weeks on V8 juice agar in Petri plates. The aerial mycelium is then removed by scraping the surface of the medium with the end of a glass slide, The plates, with lids removed, are then washed for 24 hours in running water, If necessary they may be wrapped individually in cheese cloth to keep the medium in place. The purpose of washing is to remove some unidentified antisporulating factor which may or may not be associated with the pigment which also leaches out.

After washing, the open plates are stacked on a tray in an inverted, slanted position so that each plate is partially closed by the bottom of the plate against which it leans. This arrangement appears to provide optimum humidity conditions for sporulation, The plates are kept in the open under the normal temperature and light conditions of the laboratory. Within 2 days a velvety layer of spores can be seen covering the agar surface. These are washed off by means of a jet of water from a wash bottle connected with an air line. By this means a minimum of immature spores or debris is removed. The plates are then restacked as before, Several successive crops of spores can be harvested at 1 to 2-day intervals before the medium becomes too dry.

The spores in suspension are collected on filter paper by suction through a sintered glass or Buchner funnel, air dried, and stored in closed Petri plates in a refrigerator, Under these conditions they have been found to retain their viability and pathogenicity for a period of 1 year or longer. Although aseptic conditions are not maintained throughout the process, contamination has not been found to interfere with bioassays or inoculations.

Occasionally the sporulating capacity of the stock cultures, maintained on P. D. A. slants stored in a refrigerator, was found to decline. This was not surprising in view of the frequency of mutations in this organism.

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Whenever this happened we were able to recover a good heavily sporulating stock selection from a large number of single spore isolates, These were obtained simply by pouring a series of dilution plates and transferring from individual colonies that were well separated from their neighbours, We have also applied the dilution plate procedure to increase sporulation in stocks of Helminthosporium sativum and Glomerella cingulata and to reduce the production of microconidia by Monilinia fructicola,

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