A SELECTIVE MEDIUM FOR ISOLATION OF *METARHIZIUM ANISOPLIAE* FROM CATTLE DUNG

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The chief breeding sites in South India of the rhinoceros beetle, *Oryctes rhinoceros* (L.), a major pest of the coconut palm, are cattle dung heaps and farmyard organic debris. In dung, larval stages may be exposed to lethal infection by the entomogenous fungus, *Metarhizium anisopliae* (Metch.) Sorokin. Mortality occurs within 15 to 20 days of infection through the cuticle and the mummified dead grubs become completely covered with the olive green muscardine growth of the fungus. Experiments to control the grub population in breeding sites by the application of *M. anisopliae* spores, are in progress.

It was found necessary to have a selective isolation medium for assessing the population of fungal propagules in the inoculated cattle dung heaps, in untreated areas and also to determine the survival of fungal propagules in various types of breeding material used by the pest. Culture media developed elsewhere for the isolation of *M. anisopliae* from cadavers of insects (Veen & Ferron, 1966; Veen, 1968) and from soil (Pereira, Dhingra & Chaves, 1979) were found to be inadequate due to rapid overgrowth by contaminants. This report describes a selective medium for the isolation of *M. anisopliae* from cattle dung and farmyard organic debris. The basal medium consists of: chitin (Aldrich and Thomas Laboratories, U.S.A.), 0·25 g; Peptone (BDH, India), 1 g; Yeast extract (Difco), 0·5 g; K_2HPO_4, 0·55 g; KH_2PO_4, 0·93 g; $MgSO_4.7H_2O$, 1 g; rose bengal, 0·07 g; Agar (BDH, India), 18 g; distilled water, 1 l. The medium was adjusted to pH 6·2–6·5. Each litre of the basal medium was supplemented with cycloheximide, 0·2 g; chloramphenicol, 0·2 g; streptomycin, 0·1 g; chlorotetracyclin, 0·05 g and Cetyl tri-methyl ammonium bromide (CTAB), 0·35 g. The medium was autoclaved at 1·1 kg/cm^{-2} pressure for 15 min. Chitin was incorporated into the medium as a colloidal suspension prepared from partially purified chitin flakes according to the procedure of Aaronson (1970). Chloramphenicol was added to the medium prior to sterilization, whereas the other antimicrobial agents were added to the sterilized cooled medium, just prior to pouring into plates. The surface of the solidified medium was dried at room temperature for 48 h.

The selectivity of the medium was tested with composite samples drawn periodically from previously inoculated cattle dung pits and farmyard refuse heaps. Log dilutions of the sample were prepared in sterile tap water containing 0·01 % (v/v) Teepol, as dispersing agent and 0·5 cm^3 aliquots spread over the solidified medium. Plates were incubated at 28–30 °C for 8 days at 80–85 % r.h. Colonies of *M. anisopliae* could easily be recognized by the compact, cottony, snow white mycelial growth. Initiation of sporulation occurred from the centre of the mycelial growth as a light green patch which soon darkened and spread radially. Zones of chitinolysis (clearing) could be observed around the colonies of *M. anisopliae*, when viewed against light. On an average, the contaminants (fungal and bacterial) were restricted to 10 % of the total number of isolates. No appreciable increase in contamination was noted when unsterilized glassware was used. The efficiency of the medium for the reisolation of *M. anisopliae* from cattle dung, was never below 75 % in trials designed to check the percentage recovery.

The medium described by Pereira *et al.* (1979) was found satisfactory for the isolation of *M. anisopliae* propagules from soil but was inadequate when cattle dung was sampled. Consistant overgrowth with *Geotrichum candidum*, *Aspergillus* spp. and *Penicillium* spp. and a variety of gram negative bacteria occurred within 48 h. In the present medium most contaminating fungi were effectively suppressed by the use of chitin as the major carbon source. Yeast extract at 0·05 % level in the medium improved the efficiency of recovery of *M. anisopliae*, without appreciably increasing the contaminants, and also induced faster sporulation of this fungus, thereby aiding quicker identification.

Incorporation of cycloheximide and CTAB at concentrations of 200 mg and 35 mg l^{-1} respectively, along with other antimicrobial agents, greatly enhanced the selectivity of the medium by the more or less complete elimination of bacterial

colonies. The minimum inhibitory concentrations of cycloheximide and CTAB to *M. anisopliae* were determined as 600 mg and 75 mg l^{-1} respectively.

This medium is also well-suited to the study of the spread of *M. anisopliae* in organic debris. Earlier attempts (Swan, 1974) to study the spread of the fungus or to establish the presence of the fungus in a particular heap of organic matter using the bioassay test (healthy *Oryctes* grubs mixed with a sample of the organic matter), had serious drawbacks, mainly the inability to distinguish between areas where the fungus was totally absent and places where the fungal propagules were present below the threshold level for successful infection.

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SCANNING ELECTRON MICROSCOPY OF CRITICAL POINT DRIED *ACREMONIELLA VELATA*

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A previous communication (Jones, 1967) reported on the surface features of spores of the soil fungus, *Acremoniella velata* Onions & Jones (1968), examined in one of the first scanning electron microscopes. At that time very little was known about preparative techniques and the specimens were air-dried, which caused considerable shrinkage in the material. In recent years many improvements have been made in the methods used to preserve biological tissues before examining them in the scanning electron microscope. This note records vastly improved scanning electron micrographs of *A. velata* after specimens had been chemically fixed and critical point dried (Jones, 1978). After processing, the blocks of agar supporting growth of the fungus were attached to aluminium stubs, sputter coated with gold, and examined in a Cambridge Instruments S4 scanning electron microscope.

Figure 1 shows a mature conidium enveloped by a membrane (veil or epispore) with a distinct line where breakage will occur prior to it being shed. The point of attachment of conidiophore to conidium is visible in Fig. 2 which also shows the torn veil. The mode of attachment of the veil to the conidium by fibrous strands, previously seen in thin section of resin-impregnated tissue (Jones, 1968), is seen in Fig. 3, and the folded-back top of the veil is shown in the lower conidium. A mature spore without the veil, and bearing a typically short hollow stalk at the point where it was attached to the conidiophore, is illustrated in Fig. 4.

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