Use of a serologically distinct strain of *Thermoactinomyces vulgaris* in the diagnosis of farmer's lung disease

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SYNOPSIS During investigations into farmer's lung disease it was noticed that *Micropolyspora faeni* (MF) was isolated and identified with ease, but difficulty was encountered in the identification of *Thermoactinomyces vulgaris* (TV), although an organism resembling TV was frequently isolated. Extracts prepared from the isolates resembling TV (called *Thermoactinomyces vulgaris* variant, TVV) when tested against the standard TV antiserum by double diffusion, did not produce any precipitin lines. When TVV extracts were tested against the serum of a patient from whom TVV had been isolated from the sputum, +++ precipitin lines were observed. With the TVV extracts the authors have demonstrated the existence of a serologically distinct strain of TV, and also that it would be a useful addition to the routine testing of antibodies to farmer's lung disease.

Farmer's lung disease (FLD) is a form of pulmonary extrinsic alveolitis due to the inhalation of antigenic material from mouldy hay. Clinically, FLD is identified as a well-defined group of symptoms and signs including dyspnoea, cough, malaise, chills, fever, and loss of weight, an acute attack following some hours after contact with mouldy hay. Radiology of the lungs may show a widely distributed miliary infiltration or ground-glass appearance (Pepys and Jenkins, 1965). Surveys have shown that precipitins to the thermophilic actinomycete *Micropolyspora faeni* (MF) are most frequently encountered in people exposed to mouldy hay (Pepys and Jenkins, 1965). It was also reported that in 50% of these cases precipitins to *Thermoactinomyces vulgaris* (TV) were also present. The authors failed to confirm this finding. With the routinely used TV extract only 20% (3 out of 16) of the sera which contained antibodies to MF also had antibodies to TV. With the *Thermoactinomyces vulgaris* variant (TVV) extract used as well, however, the percentage was increased to 33 (5 out of 16). Pepys and Jenkins (1965) suggested the possibility that other antigens (apart from MF and TV) were involved in the pathogenesis of farmer's lung, and Wenzel et al (1974) have described antigenically different types of TV which may be implicated in the disease. It was decided to assess the usefulness of the TVV extract to reinforce the diagnosis of FLD in addition to the routinely used extracts of TV and MF.

Material

MF and TV extracts and positive antisera to these two organisms were supplied by the Mycology Reference Laboratory and used at the recommended dilutions. The original TVV positive serum was obtained by screening sera from cases of FLD that had been found to have antibodies to MF. This serum (from Mr. A in table II) did not contain antibody to TV.

**DOUBLE DIFFUSION TESTS**

Double diffusion tests were performed in 50 mm diameter plastic petri dishes in borate buffered agar with sodium azide as a preservative. A seven hole perspex template with one central and six peripheral 6 mm holes, together with seven brass dies, was used to position wells in the agar.

**SERA**

Two hundred and forty sera received at the laboratory accompanied by a request for antibody to FLD in the 12 months beginning April 1974 were included in the investigation. These sera were examined for antibodies to MF, TV, and TVV.

**SPUTA**

One thousand three hundred sputa received in the laboratory from April 1974 to April 1975 were examined for MF, TV, and TVV organisms. Sputa from known cases of tuberculosis and postoperative cases were not included.
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TVV extract

TVV was isolated and an extract was prepared as described in the methods section of this paper. The organism was kindly identified by Dr. M. R. Hollingdale of the Mycology Reference Laboratory as belonging to the type III strains of the aerobic actinomycetes (Becker et al., 1965).

Methods

Isolation of TVV from sputum and preparation of extract

A large loopful of sputum was shaken vigorously with 2 ml of sterile saline in a bijou bottle containing small glass beads. A loopful of the mixture was inoculated onto two nutrient agar plates (Oxoid No. 2). The plates were placed in a plastic bag to prevent drying and incubated at 55°C for four days. Growth of TVV was rapid; in 24 hours, 2 mm white colonies appeared with a wrinkled centre and powdery periphery. They were indistinguishable from those of a control culture of TV. The culture was scraped off the medium to make a dense suspension in nutrient broth. The suspension was evenly pipetted over the surface of another nutrient agar plate, allowed to dry, and incubated in a plastic bag at 55°C for one week. The agar with, by now, confluent growth was cut into 6 mm³ blocks and placed in a sterile ‘universal’ container (UVC). Nutrient broth was added to the neck of the UVC, thoroughly mixed with a blender and again incubated at 55°C for one week. Finally the container was repeatedly frozen (−20°C) and thawed over a period of one week; the resultant liquid was easily pipetted.

TVV extracts were titrated against the original TVV serum diluted 1:2; a titre of 1 in 8 of the extract was found to give +++ precipitins with this serum. The TVV extract was used at a dilution of 1 in 8 to test sera for antibody to TVV, to identify TVV isolates, and in cross absorption tests. TVV isolates were stored on nutrient agar slopes at room temperature after overnight incubation at 55°C. TVV extracts were stored frozen at −20°C.

Immunodiffusion

The method of immunodiffusion was basically that of Ouchterlony (Pepys et al., 1963). Borate buffered agar, 4-5 ml, was pipetted into a 50 mm diameter petri dish. The perspex template was placed at once over the open dish and the seven sterilized brass dies were put into the holes in the template while the agar was still molten. The plate was then placed at 4°C for 30 min, after which the dies and template were carefully removed. Any excess agar was cleared from the holes and the bottom of the holes was resealed by running in molten agar and removing it immediately. The double diffusion plates could be stored in a plastic sealed container at 4°C for up to two weeks. After inoculation the plates were placed in a sealed container next to a small beaker half filled with water, incubated at 30°C, and examined daily for four days for precipitin lines.

Examination of Hay and Dust

Samples of musty hay and dust were taken from the farms of Mrs. C and Mr. A. These two patients were clinically diagnosed as cases of FLD, subsequently found to have antibody to TVV, and also to have TVV isolated from their sputum. Sterile UVCs were half-filled with hay and dust and ‘topped up’ with sterile nutrient broth. After allowing for absorption, more nutrient broth was added to the level of the bottle neck. These samples were then incubated at 55°C for one week, subcultured onto nutrient agar, and incubated again at 55°C. The plates were examined the following day. All white colonies were subcultured onto nutrient agar plates and, if uniform, were extracted as above.

Results

It was demonstrated (table I) by cross-absorption tests that TVV was antigenically distinct from TV. An extract of TV did not absorb out antibody to TVV, nor did an extract of TVV diminish antibody to TV. No cross-reactions were demonstrated between TVV and MF.

<table>
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<tr>
<th>Antiserum</th>
<th>Extract</th>
<th>Precipitin lines</th>
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<td>TVV absorbed 1 hr with MF</td>
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Table I Tests to demonstrate antigenic differences between T. vulgaris (TV), T. vulgaris variant (TVV) and M. faeni (MF)

Examination of sera

Four patients with clinical FLD were found to have antibody to TVV; TVV was also isolated from the sputum of all four. Precipitins to TVV were strong (+ + +); in two cases antibody to TV could not be demonstrated. All four patients had antibody to MF but it was noted that in two cases the precipitins to TVV were stronger than to MF (see table II). Antibody to TV was present in the sera of 23 patients but these were all weak (+), and only three of these 23 patients had clinical FLD (table III).
variant of *Thermoactinomyces vulgaris* was in four
patients found to be of relatively greater diagnostic
value than the TV extract commonly used. Strongly
positive (+++) precipitin lines were regularly
observed. The significance of the isolation of MF,
TV, and TVV from the sputum of patients with
FLD is hard to assess. The presence of the thermo-
actinomycetes may activate the production of
antibody to the organisms without causing clinical
disease. It is postulated that though these organisms
are frequently inhaled, it is only when antibody is
produced, sufficient to precipitate in gel, that the
cl临ical disease of FLD becomes evident. A quantita-
tive relationship between the amount of precipitating
antibody and the degree of hypersensitivity pneu-
momitis, as suggested by Moore and Fink (1975) for
pigeon breeders’ disease, may occur, but ‘other
factors are possibly involved’. If the level of precipi-
tating antibody is of major importance, the variant of
*T. vulgaris* identified by the authors may be
relevant to the development of the disease process
since in our small series the presence of antibodies
was associated with FLD to a greater extent than
were antibodies to TV. Our failure to demonstrate
a particularly good association of antibody to TV
and patients with FLD may be, perhaps, a reflection
of geographical variation in the microflora of
mouldy hay (Wenzel et al, 1974).

The authors wish to thank the many clinicians who
kindly provided data and extra specimens from their
patients. The director, Dr. D. W. R. Mackenzie, and
staff of the Mycology Reference Laboratory are
also thanked for their helpful assistance and the
provision of standard organisms and antiserum.

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