ANTIGENICITY AND CROSS-REACTIVITY IN *EPIDERMOPHYTON FLOCCOSUM*

IMMUNITY in dermatophytoses has been a debated subject[1], although circulating antibodies to dermatophytes in humans and experimental animals have been demonstrated earlier[2]. Water soluble and neutral polysaccharides from species of *Trichophyton* and *Microsporum* have been shown to be antigenic in rabbits[3]. Information on the antigenic potentialities of *Epidermophyton floccosum* on the other hand is meagre and poorly understood. The present study reports the antigenic structure of several clinical isolates of *E. floccosum* and the cross-reactivity among themselves and between dermatophytes.

The antigen for immunizing rabbits was prepared from a 15-day old shake culture of the fungus grown at 30° C in Sabouraud's glucose broth. Vacuum dried mycelial powder suspended in saline (100 mg/ml) was autocalved at 15 lb for 20 minutes. The suspension was then homogenized and mixed with an equal volume of complete Freund's adjuvant. Soluble antigens for *in vitro* tests were prepared by grinding mycelial powder with glass powder followed by extraction with borate-saline buffer (pH 7·2). The antigen extract contained 6 mg protein/ml.

Each of four rabbits (weighing 1·5 to 2·0 kg) in a group was injected with 0·2 ml of antigen-adjuvant mixture (0·6 mg protein) subcutaneously on days 1 and 8. The dose was doubled and trebled on 15th and 22nd days, respectively. One booster dose of soluble antigen (1·2 mg protein in 0·2 ml) was injected through the ear vein on the 29th day. During the entire immunization each rabbit received antigen equal to 5·4 mg protein. The blood was collected through the ear vein, one week after the last injection and the serum was separated.

The antibodies in the serum were detected by ring (qualitative precipitin) test, haemagglutination and complement-fixation tests, and by gel diffusion and immunoelectrophoretic methods as described by Campbell *et al.*[4]. The banding pattern of antigen-antibody interaction in gel diffusion plates and recording the results are according to the method of Kalyanasundaram *et al.*[5].

The serum from the rabbits immunized with *E. floccosum* showed the presence of antibodies. The titre values by ring test, haemagglutination and complement-fixation tests were 1:80, 1:256 and 1:32, respectively. The titre values remained unchanged after second and third booster doses of antigen. Gel diffusion and Immunoelectrophoretic tests revealed the presence of three distinct antigenic components (numbered 1 to 3) in *E. floccosum*. The soluble antigen used in *in vitro* testing for the presence of antibodies, contained glycoprotein comprising of 6% protein and 35–40% polysaccharides.

The results revealed that all dermatophytes tested with the exception of *Trichophyton violaceum* HM 354, have cross-reacted with antiserum raised against *E. floccosum* HM 308*a* (Table I). The study further

TABLE I

Antigenic dissimilarities among isolates of Epidermophyton floccosum *and between* E. floccosum *and other dermatophytes*

Antigen isolated from		Antiserum against	Precipitin bands observed
Species	Isolates		
E. floccosum	HM 308 *a*, HM 116 *a*, 269	*E. floccosum* HM 308 *a*	1, 2, 3
do.		do.	1, 2, 3
do.	HM 14, 219	do.	1, 3
do.	HM 145, 167, 358	do.	2, 3
do.	HM 78, 264, 289, 290 *a*, 314	do.	3
Trichophyton mentagrophytes	HM 115	do.	3
T. rubrum	HM 123	do.	3
T. violaceum	HM 354	do.	0
*Microsporum gypseum**		do.	3
M. canis	HM 382	do.	3

* A strain obtained from London School of Hygiene and Tropical Medicine, London.

points out the commonness of one antigenic component (band 3) for all the fungi. Two isolates of *E. floccosum* (HM 116*a* and HM 269) are in complete homology with HM 308*a*, serologically, whereas the homology in five other cases has been only partial being represented by two of the three antigenic components only. In five other isolates of *E. floccosum* the cross-reactivity is indicated by only one antigen (band 3) which is also present in species of *Trichophyton* and *Microsporum*. Thus the cross-reactivity varied greatly among isolates of *E. floccosum*. *T. mentagrophytes*, *T. rubrum*, *M. gypseum* and *M. canis* possessed only one antigen common with *E. floccosum*. These isolates were cultured from clinical lesions of types tinea cruris, t. circinata, t. corporis and onychomycoses. But no correlation was observed between the antigenic composition of dermatophytes and clinical lesions they produced. The antigenic differences among isolates of a single species must be borne in mind while preparing test vaccines.

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BERLESIELLA (FAM. PLEOSPORACEAE) A NEW MYCOPARASITE FROM INDIA

DURING the mycological collection tour of Goa in 1971, an unusual fungus was observed, in the form of whitish lesions overgrowing the stromata (discothecia) of another fungus, *viz.*, *Rhytodhysteron* (= *Tryblidiella*) which on microscopic examination was identified as a species of *Berlesiella* Sacc. (Fam. Pleosporaceae), an ascomycete which was found to be hitherto undescribed from India[2]. The taxonomy of this fungus is in dispute. Chenantais[1], in his classic paper, discussed this aspect of speciation in this genus at length and accepted only two valid species, *i.e.*, *B. setosa* Wint. and *B. nigerrima* (Blox.) Sacc.

The writers' collection was, therefore, studied in detail, which on comparison with the two known species of *Berlesiella*, was found to differ significantly in morphology and dimensions of various fruiting structures (*vide* Table I) and as such described here as new to science.

Berlesiella castl.-rockii sp. nov. (Fig. 1, A–E)

Pseudothecia pulvinata, hemispherica vel oblonga, carbonacea, discreta, solitaria, ostiolata, magnit. 68–104 × 108–144 μm; asci octosporae clavatae, bitunicati, magnit. 44–52 × 6–10 μm; ascospora fusoidia vel oblonga muriformia, olivacea, magnit. 12–16 × 4–8 μm.

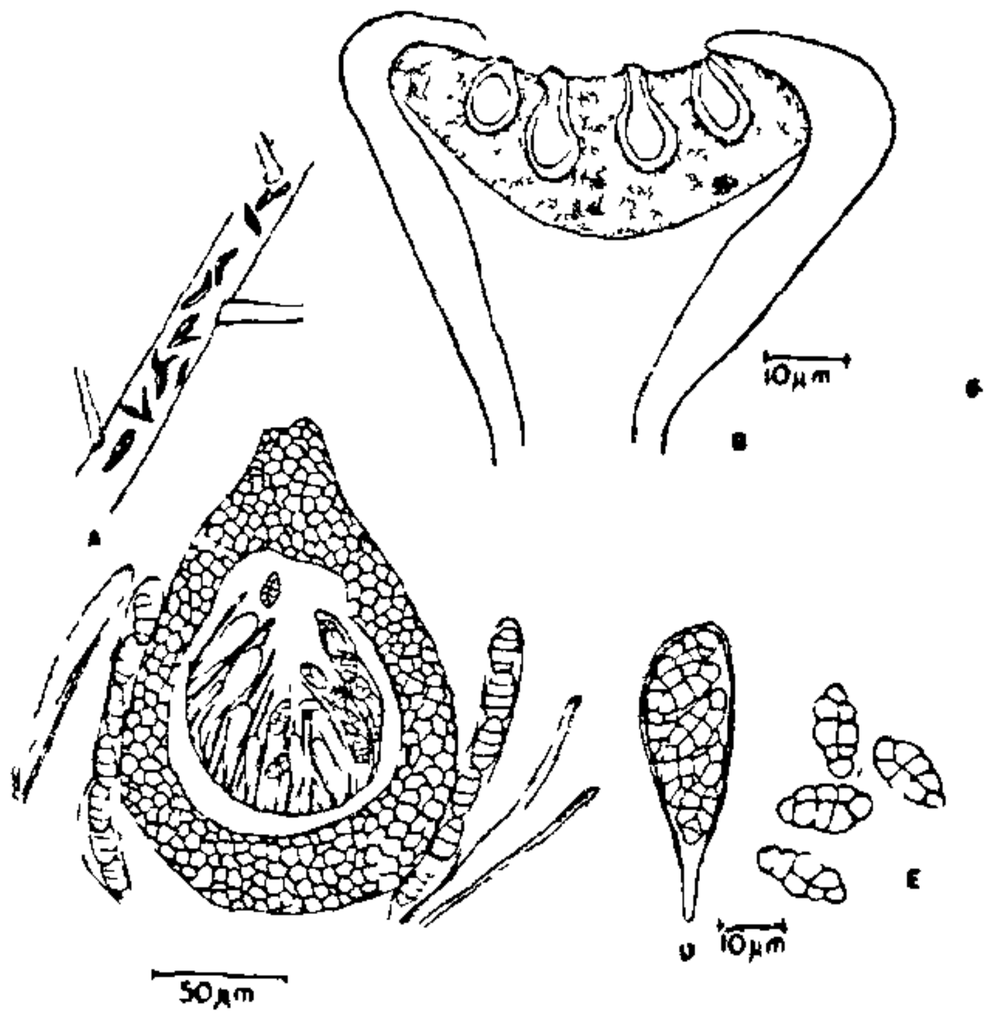

FIG. 1. (A–E): *Berlesiella castle-rockii* sp. nov. A, Habit. B, V.S. of *Rhytidhysteron* showing relation of mycoparasite; C. Pseudothecium of *Berlesiella* in section; D, Ascus; E, Ascospores.

Pseudothecia pulvinate, hemispherical to oblong, carbonaceous, discrete; solitary, more or less beak-like structures with an ostiole, measure 60–104 × 108–144 μm; asci octosporous, clavate, bitunicate, measure 44–52 × 6–10 μm. ascospores fusoid to oblong, muriform, greenish, measure 12–16 × 4–8 μm. *Matrix*: Mycoparasite on the discothecia of *Rhytidhisteron rufula* Spreng, growing on stems of *Eleagnus conferta* Roxb. (Fam. Eleagnaceae) Legit. V. G. Rao at Castle Rock, 1971, No. AMH 2074 (Holotype).

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TABLE I

Comparison between species of Berlesiella

Species	Stroma	Asci	Ascospores	References
B. nigerrima (Blox.) Sacc. (type spp.)	Upto 1 mm across	80 × 12 μm	12–17 × 5–6 μm	Chenantais[1]
B. setosa Wint.	..	120 × 15 μm	24–26 × 8–10 μm	Chenantais[1],
B. castle-rockii (under study)	108–144 × 68–104 mμ	44 – 52 × 6–10 μm	12–16 × 4–8 μm	Authors