STUDIES ON THE IMMUNO-SEROLOGY OF *RHINOSPORIDIUM SEEBERI*

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ABSTRACT

Spores of *Rhinosporidium seeberi* were used as immunogen in rabbits and the antibodies were detected in the hyperimmune serum by precipitin, agglutination and complement-fixation methods in addition to gel diffusion and immunoelectrophoresis. The extent of cross-reactivity of *R. seeberi* with other fungi comprising 3 phycomycetous 7 deuteromycetous and 2 yeast species was assessed using gel diffusion technique. Implications of these results on the taxonomy of *R. seeberi* are discussed.

INTRODUCTION

RHINOSPORIDIOSIS is a chronic infection of the mucocutaneous regions of nose, eye and pharynx, caused by *Rhinosporidium seeberi*. Various aspects of this fungal pathogen, viz., epidemiology, ecology and pathology have been worked out in detail[1]. However, this fungus is described as an unisolated and unclassified pathogen, despite its provisional inclusion under aquatic phycomycetes[2]. This is mainly based on the description of the life-cycle from histopathology of the polyps[3,4]. Among the other areas of interest regarding this fungus is immunology which is obscure and unexplored[5]. Although there are reports on cell-mediated immune responses[6] and the presence of antigenimia[7] in the patients, knowledge on the antigenic potentialities of *R. seeberi* is lacking. The present report deals with antigenicity of *R. seeberi* and its nature of cross-reactivity with other fungal antigens, for assessing its taxonomic position serologically.

MATERIALS AND METHODS

Polyps, surgically removed from the patients, were teased to release the spores from sporangia. Spores were separated from the contaminating epithelial cells by repeated sedimentation and the pure spore suspension in physiological saline was stored in a refrigerator at 5°C until further use.

Adult rabbits (1.5 kg) of either sex were immunized with spore suspension (3×10^9/ml) on alternate days through marginal ear vein. A total of five injections were given with a booster dose one week after the fifth one. Blood was collected by cardiac puncture, serum separated and stored at −20°C after inactivation.

Soluble antigen from the spores for *in vitro* tests was prepared as described elsewhere[8]. The protein content of antigen was estimated by the standard procedure[9].

The serological tests employed to estimate the antibody content in the hyperimmune serum were: Precipitin (ring) test; agglutination (direct on slide and passive); complement-fixation test; gel diffusion and immunoelectrophoresis (IE).

The fungi used for the cross-reactivity tests are: (a) Phycomycetes: *Rhizophlyctis* sp., *Pythium* sp. and *Mucor racemosus*; (b) Deuteromycetes: *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum*, *Epidermophyton floccosum*, *Aspergillus fumigatus*, *Penicillium* sp., *Phialophora jeanselmi*; (c) Yeasts: *Candida albicans*, *Cryptococcus neoformans*.

Soluble antigens from all the fungi were prepared as in *R. seeberi*; in the case of *Rhizophlyctis* sp. alone, the antigen suspension was absorbed with corn-leaves. This enabled the elimination of non-specific antigen–antibody reaction. Cross-reactivity was determined by Ouchterlony's double diffusion method. The results are recorded and compared.

RESULTS AND DISCUSSION

Table 1 summarizes the data on the serological reactivities of *R. seeberi* as assessed by the *in vitro* methods. Thus, high antibody levels have been detected in the hyperimmune serum for *R. seeberi* antigen, particularly by passive haemagglutination test (table 1). Gel diffusion and IE methods showed atleast two distinct antigenic components in the soluble antigen of *R. seeberi*. Antigen was a glycoprotein with a concentration of 3 mg/ml.

Varying degrees of cross-reactivity were detected between the *R. seeberi* antiserum and antigens of

Table 1 *Serological reactions of Rhinosporidium seeberi*

Test	Titre
Precipitin (ring) test	1:16
Direct agglutination (slide)	1.64
Passive haemagglutination	1:128
Complement-fixation	1:64

the other fungi tested (table 2). Only *Rhizophlyctis* sp. showed complete homology with the antiserum of *R. seeberi*; the reaction in the other cases was either moderate or poor. It is of interest to note that the cross-reactivity and homology between *R. seeberi* and members of deuteromycetes as well as yeasts was of the least category. Among the 3 phycomycetous members tested, moderate reactions were observed in *Pythium* sp. and *Mucor racemosus*; nevertheless, antigenic similarities between *R. seeberi* and phycomycetes were greater than the other fungi included in this work (table 2).

Identification and classification of microorganisms are usually carried out based on morphology, chemical nature of cells, histochemistry and GC content of DNA[10,11]. Serology has also been considered as an important adjunct to other methods so far described in literature[12]. The main criteria considered for using this method in the classification of microbes is the presence of common antigens shared by them either at the intergeneric, intrageneric or even at intraspecific levels or in the understanding of host-parasite relationships[8,9-11]. Chemical composition of such antigens may be protein, carbohydrate or nucleic acids. In the present report, spores of *R. seeberi* have been shown to be a good source of antigen for inducing antibodies in rabbits. Similarly, soluble antigens from *R. seeberi* and other fungi have exhibited fairly high degree of cross-reactivity. Strong antigenic affinities between *R. seeberi* and *Rhizophlyctis* sp. are evident by the complete homology, thereby adding an additional, though not conclusive, evidence for inclusion of *R. seeberi* under aquatic phycomycetes in the absence of the *in vitro* cultivation of the pathogen[2-4]. This study also suggests the necessity for including several other phycomycetous members, isolation of purified protein or polysaccharide antigens from these fungi and detection of antigenic relationship for evaluating the serological classification of fungi using more sensitive and sophisticated techniques.

Table 2 *Cross-reactivity of Rhinosporidium seeberi antiserum with other fungal antigens*

Fungus	Cross-reaction
Phycomycetes	
Rhizophlyctis sp.	+++
Pythium sp.	++
M. racemosus	++
Deuteromycetes	
T. mentagrophytes	+
T. rubrum	+
M. gypseum	+
E. floccosum	+
A. fumigatus	+
Penicillium sp.	+
P. jeanselmi	+
Yeasts	
C. albicans	+
C. neoformans	+

+++, Good; ++, Moderate; +, Poor.

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NEWS

MOSCOW PHYSICISTS MAKE A DISCOVERY

The State Committee for Discoveries and Inventions has registered a discovery made at the Institute of Experimental and Theoretical Physics and the Institute of High Energy Physics by corresponding member of the USSR Academy of Sciences V. Vladimirsky and by Dr I. Kapchinsky and Dr V. Teplyakov.

One major problem before the physicists is how to prevent the dispersion of particles in a linear accelerator. The method used so far makes it possible to concentrate only those particles which are moving fairly fast.

The discovery made by the Moscow physicists enables the focusing of particles moving at one-fifteenth or one-twentieth of the speed required under the old technique. That led to the development of new radiation technologies of producing materials of which nuclear reactor shells are built.

It is now possible to devise an efficient technique of regenerating uranium fuel for nuclear power plants. These accelerators can help obtain a secondary flow of electrons which will bombard heavy water "chips". That may be one way of achieving nuclear fusion. (*Soviet Features*, Science and Technology, Vol. XXVI, August 1, p. 3; Published by: Information Department, USSR Embassy in India, New Delhi 110 001.)

JEB (YOUNG SCIENTIST) PRIZE

The JEB Prize Committee headed by Dr R. C. Dalela, President, the Academy of Environmental Biology, has recommended the coveted 3rd 'JEB Prize 1987' to Dr K. S. Jagannatha Rao, Scientist, Central Food Technological Research Institute, Mysore for contributing outstanding research in the field of Toxicology, to be awarded during the 9th Annual Session of the Academy of Environmental Biology at Jai Research Foundation, Valvada.

Dr W. Rajendra, Sri Venkateswara University, Tirupati; Dr Janak Ahi, Sagar University, Sagar; Miss Jyoti Singh, Banaras Hindu University, Varanasi, and Miss Aradhana Dasgupta, M. D. University, Rohtak have been recommended for the 'Best Paper Presentation 1987 Certificate'.