ANTIGENIC RELATIONSHIPS OF *TORULOPSIS GLABRATA* AND SEVEN SPECIES OF THE GENUS *CANDIDA*

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Torulopsis glabrata, like a number of other yeasts and yeastlike fungi commonly associated with the human body, has been suspected of causing disease. It is an occasional inhabitant of the oral, gastrointestinal, and urinary tracts and most isolates have been obtained from human sources. Wickerham (1957) has presented the identifying characteristics of this yeast, and suggested its recognition as a potential pathogen. Although Wickerham indicated that he had identified strains of *T. glabrata* isolated from blood cultures, references to these cases were not given.

Since *T. glabrata* is a relative newcomer to the list of potential fungus pathogens, only a few studies with it have been conducted. Benham (1935) reported no pathogenicity for rabbits, but Lodder and DeVries (1938) observed some pathogenicity for rats. Lopez Fernandez (1952) concluded that mice were the most susceptible laboratory animal, and showed that in tissue sections this yeast bore a striking resemblance to *Histoplasma capsulatum*. The serological studies of Benham (1935) indicated that *Candida albicans* and *T. glabrata* possessed common antigens.

As reported here, studies of the relationships of *T. glabrata* to *C. albicans*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis*, *Candida pseudotropicalis*, *Candida stellatoidea*, and *Candida tropicalis*, indicated the presence of common antigens among these organisms. The data presented in this paper were obtained from tube agglutination reactions.

MATERIALS AND METHODS

Strains. Two strains of each species of the yeasts listed above were used in this study. The strains of *C. albicans* and *T. glabrata* were isolated in this laboratory from human sources. *C. guilliermondii* strain Y2083, *C. stellatoidea* strain Y2441, and *C. tropicalis* strain YB4434 were furnished by Dr. L. J. Wickerham. *C. krusei* strain 6258, *C. pseudotropicalis* strain 9767, and *C. parapsilosis* strain 10232 were obtained from the American Type Culture Collection. Dr. Margarita Silva supplied *C. guilliermondii* strain 3163, *C. krusei* strain 3168, *C. parapsilosis* strain 3164, *C. pseudotropicalis* strain 3169, *C. stellatoidea* strain 3165, and *C. tropicalis* strain 3166. All strains conform to the descriptions given them according to Lodder and Kreger-Van Rij (1952).

Preparation of antigens. The antigens were produced by cultivation of the various yeasts on 2 per cent glucose, 1 per cent neopeptone agar (pH 6.8) plates. Growths of the *Candida* species were harvested in 0.9 per cent NaCl solution after 48 hr incubation at 30 C. The 2 strains of *T. glabrata* required 72 hr at the same temperature for maximum growth. The yeast cell suspensions were heated at 65 C in a water bath for 6½ to 7 hr and tested for viability. If growth was observed the suspensions were reheated as before. The second exposure usually destroyed all viability. After heating, the suspensions were washed 3 times in 0.9 per cent NaCl solution and diluted to approximately 1:3 packed cell-saline ratio. These preparations were used for antisera adsorption and agglutination reactions.

The antigens used for the production of antisera were suspensions of viable yeast cells from 24 to 48-hr cultures.

Production of antisera. Thirty-two male albino rabbits, weighing 3 to 4 kg, were used in this study, and two animals were used for each strain. Rabbits injected with suspensions of all species, except *C. albicans*, received approximately 2×10^7, 5×10^7, 5×10^7, 10^8, 10^8, 5×10^8 cells on the 1st, 3rd, 5th, 8th, 10th, 12th, and 15th days, respectively, of the injection schedule. One week following the final injection of antigen, blood samples were drawn and, if the agglutinating titer of the serum was 1:480 or

more, the animals were exsanguinated. Antisera to 3 strains demonstrated a titer of 1:480, whereas, the rest were 1:960 to 1:3840.

The pathogenicity of *C. albicans* for rabbits made it necessary to inject smaller numbers of yeast cells. Accordingly, the following schedule was employed. The animals received approximately 5×10^5, 5×10^5, 2×10^6, 4×10^6, 8×10^6, 2×10^7, and 5×10^7 on the 1st, 4th, 7th, 10th, 13th, 16th, and 19th days of the injection schedule, respectively. The same criteria were applied to these sera as to the others.

The yeast cell numbers in the suspensions were determined by direct count in a Levy hemocytometer.

Adsorption of sera. The sera collected from two rabbits injected with a single strain of yeast were pooled and heated at 56 C for 30 min. The antisera to all strains were treated in this manner. Each antiserum was adsorbed with each strain of yeast used in this study, that is, homologous and heterologous adsorptions. The heated 33 per cent yeast cell suspensions were utilized for this process. For the adsorption procedure of each antiserum, 2 ml of serum were placed in each of 17 test tubes, and 2 ml of yeast suspension were added to a specified tube. Since 16 strains were included in the study, each of the 16 tubes of sera was adsorbed with a specified yeast, and the 17th tube was included as the unadsorbed sample. The resulting mixtures, and unadsorbed control, were incubated at 45 C in a water bath for 6 to 7 hr, and then placed in a 4 C refrigerator overnight. The following morning the cell-serum mixtures were centrifuged, the serum removed, and each sample was mixed with 2 ml more of the same yeast suspension. This procedure was repeated 3 times. After the third adsorption, the serum was removed, diluted 1:3, and stored at −10 C. Failure of the homologously adsorbed antiserum to agglutinate the specific yeast indicated the efficiency of this procedure.

Serological procedures. For the determination of the agglutinating titers of the antisera, twofold dilutions of the sera were employed. One-half ml of the diluted serum was added to ½ ml of the antigenic suspension. The final serum dilution in the first tube of each determination was 1:30. The use of this initial dilution was necessary to avoid nonspecific reactions. Heating of the sera at 56 C also helped reduce these reactions.

The antigenic suspensions for the agglutination tests were prepared by appropriate dilution of the heated cell suspensions. The numbers of yeast cells in these suspensions were determined by direct count and were approximately 3×10^6 cells per ml. Tubes containing the antigen-serum dilution mixtures were placed in a 45 C water bath for 2 to 3 hr, removed to 4 C, and refrigerated for about 20 hr. The titer was read as the highest serum dilution causing a definite aggregation of the yeast cells. Sera drawn from some of the rabbits before injection of the yeast suspensions were used as negative controls. Saline-antigen controls were also used.

RESULTS AND DISCUSSION

The results of the homologous and heterologous agglutination with unadsorbed antisera are presented in table 1. These data illustrate the antigenic heterogeneity that existed among these species of yeasts. They also indicated that *T. glabrata* possessed antigens common to all the *Candida* species studied.

Table 2 presents the agglutination reactions of *T. glabrata* with adsorbed antisera to the *Candida* species. The data are presented in a simplified manner to expedite evaluation, and to illustrate with reasonable accuracy the magnitude of the titer. Each block represents the results of 8 agglutination reactions since antisera to 2 strains were produced, each antiserum was adsorbed with both strains, and 2 different isolates of *T. glabrata* were used. It is apparent that there was some variation between the strains, and there were some equivocal reactions. Since the initial dilution in all tests was 1:30, it is likely that subtle antigenic differences were not detected, but nevertheless a consistent pattern can be noted. It appears that a closer relationship exists with *C. tropicalis*, *C. guilliermondii*, and *C. albicans*, but these species will not adsorb the reactivity of *C. parapsilosis*, *C. krusei*, or *C. pseudotropicalis* antisera for *T. glabrata*. *C. tropicalis*, however, appears to have the broadest antigenic pattern of all the *Candida* species studied. This is in agreement with the results of Tsuchiya *et al.* (1955), Trimble (1957), Gordon (1958), and Rosenthal and Furnari (1958).

The agglutination reactions of the *Candida* species and *T. glabrata* with adsorbed *T. glabrata* antisera are presented in table 3. Although the

TABLE 1

Homologous and heterologous agglutination reactions with unadsorbed antisera of Candida species and Torulopsis glabrata

Antisera to:	Antigen															
	C. tropicalis strain:		*C. parapsilosis* strain:		*C. stellatoidea* strain:		*C. guilliermondii* strain:		*C. albicans* strain:		*C. krusei* strain:		*C. pseudotropicalis*		*T. glabrata* strain:	
	3166	YB4434	3164	10232	3165	Y2441	3163	Y2083	B311	857	3168	6258	3169	9767	B331A	3753
C. tropicalis																
Strain 3166	*480*	*480*	120	120	60	60	240	240	240	480	60	30	60	60	240	480
Strain YB4434	*960*	*1920*	120	240	240	240	960	1920	960	960	60	120	120	120	960	960
C. parapsilosis																
Strain 3164	240	480	*480*	*960*	480	480	120	240	240	240	160	60	120	120	120	120
Strain 10232	240	240	*480*	*480*	120	120	240	480	240	240	30	30	120	60	240	480
C. stellatoidea																
Strain 3165	240	240	240	480	*960*	*480*	120	240	120	240	240	240	480	240	240	480
Strain Y2441	480	240	960	960	*960*	*960*	120	240	240	240	120	240	240	480	120	240
C. guilliermondii																
Strain 3163	480	480	30	30	60	120	*960*	*960*	240	240	30	30	30	30	480	480
Strain Y2083	480	960	60	30	60	120	*1920*	*3840*	960	960	60	30	60	30	960	1920
C. albicans																
Strain 3311	960	960	240	480	240	240	1920	1920	*1920*	*1920*	240	240	120	240	1920	1920
Strain 857	480	960	240	240	480	240	960	480	*480*	*960*	60	60	60	60	480	960
C. krusei																
Strain 3168	240	240	240	120	240	480	30	30	120	120	*960*	*1920*	240	240	480	240
Strain 6258	120	120	240	480	480	240	30	30	60	30	*480*	*960*	480	480	240	240
C. pseudotropicalis																
Strain 3169	240	120	120	60	240	480	120	60	120	240	960	960	*1920*	*960*	240	240
Strain 9767	60	30	120	240	240	240	60	60	120	60	240	240	*480*	*480*	120	240
T. glabrata																
Strain B331A	480	960	120	240	120	120	960	960	960	480	120	120	240	240	*1920*	*1920*
Strain 3753	960	960	120	240	240	60	960	1920	480	960	60	60	240	120	*1920*	*1920*

The values in the table represent the reciprocal of the serum dilutions.
The reactions for homologous species are indicated by *italic* figures.

TABLE 2

Agglutination reactions of Torulopsis glabrata with adsorbed antisera to Candida species

Antisera to:	Antisera Adsorbed with:							
	C. tropicalis	*C. parapsilosis*	*C. stellatoidea*	*C. guilliermondii*	*C. albicans*	*C. krusei*	*C. pseudotropicalis*	*T. glabrata*
C. tropicalis	−	2+ to 3+	2+ to 3+	+ to 2+	− to +	2+ to 3+	2+ to 3+	−
C. parapsilosis	+	−	2+	+	+	2+ to 3+	2+ to 3+	−
C. stellatoidea	−	−	−	−	− to +	+ to 2+	2+ to 3+	−
C. guilliermondii	−	2+ to 3+	2+ to 3+	−	+ to 2+	2+ to 3+	2+ to 3+	−
C. albicans	−	2+ to 3+	2+	+	−	2+ to 3+	3+	−
C. krusei	+ to 2+	+ to 2+	+ to 2+	+ to 2+	+ to 2+	−	2+	−
C. pseudotropicalis	+	+	+	+	+ to 2+	+ to 2+	−	−

− = No agglutination at 1:30 serum dilution; + = agglutination at 1:30 serum dilution; 2+ = agglutination at 1:60 or 1:120 serum dilution; and 3+ = agglutination at 1:240 or 1:480 serum dilution.

heterologous adsorptions reduced the titers of the homologous reactions, *T. glabrata* had antigens that were not detected in the species of *Candida* used in this study. There were some discrepancies between the results of table 2 and table 3. *T. glabrata* was agglutinated by *C. parapsilosis*, *C. krusei*, and *C. pseudotropicalis* antisera after adsorption with *C. tropicalis*, *C.*

TABLE 3

Agglutination reactions of Candida species and Torulopsis glabrata with adsorbed T. glabrata antiserum

Antigen:	Antiserum Adsorbed with:							
	C. tropicalis	*C. parapsilosis*	*C. stellatoidea*	*C. guilliermondii*	*C. albicans*	*C. krusei*	*C. pseudotropicalis*	*T. glabrata*
C. tropicalis	−	3+	3+	+ to 2+	+ to 2+	2+ to 3+	2+ to 3+	−
C. parapsilosis	−	−	−	−	− to +	2+	2+	−
C. stellatoidea	− to +	−	−	−	−	− to +	− to +	−
C. guilliermondii	−	3+	3+	−	+ to 2+	3+	3+	−
C. albicans	−	3+	2+	− to +	−	3+	3+	−
C. krusei	−	−	− to +	−	−	−	−	−
C. pseudotropicalis	− to +	−	− to +	2+	− to +	−	−	−
T. glabrata	2+ to 3+	3+	3+	2+ to 3+	3+	3+	3+	−

− = No agglutination at 1:30 serum dilution; + = agglutination at 1:30 serum dilution; 2+ = agglutination at 1:60 at 1:120 serum dilution; and 3+ = agglutination at 1:240 or 1:480 serum dilution.

TABLE 4

Agglutination reactions of Candida species with Candida antisera adsorbed with Torulopsis glabrata

Antisera to:	Antigen							
	C. tropicalis	*C. parapsilosis*	*C. stellatoidea*	*C. guilliermondii*	*C. albicans*	*C. krusei*	*C. pseudotropicalis*	*T. glabrata*
C. tropicalis	2+ to 3+	+ to 2+	− to +	+ to 2+	+ to 2+	− to +	− to +	−
C. parapsilosis	+ to 2+	3+	2+ to 3+	− to +	− to +	− to +	− to +	−
C. stellatoidea	+ to 2+	3+	3+	+ to 2+	+ to 2+	2+	+ to 2+	−
C. guilliermondii	2+ to 3+	− to +	− to +	2+ to 3+	2+ to 3+	−	−	−
C. albicans	2+	2+	+ to 2+	+ to 2+	2+	− to +	− to +	−
C. krusei	−	− to +	− to +	−	−	2+	− to +	−
C. pseudotropicalis	−	− to +	+ to 2+	−	−	+ to 2+	2+ to 3+	−

− = No reaction at 1:30 serum dilution; + = agglutination at 1:30 serum dilution; 2+ = agglutination at 1:60 − 1:120 serum dilution: and 3+ = agglutination at 1:240 or 1:480 serum dilution.

guilliermondii, or *C. albicans*, but the reverse was not true. It was possible that *T. glabrata* had surface antigens common to deep antigens of *C. parapsilosis*, *C. krusei*, and *C. pseudotropicalis*, and was agglutinated by their antisera adsorbed with the other species. On the other hand if some of the antigens of *C. parapsilosis*, *C. krusei*, and *C. pseudotropicalis* common with *T. glabrata* were located more deeply within the cell, these species probably would not be agglutinated by the adsorbed antisera since agglutination is a surface phenomenon.

The agglutination of *Candida* species with Candida antisera adsorbed with *T. glabrata* are shown in table 4. Adsorption of the sera with *T. glabrata* reduced the titers of all the homologous reactions and eliminated some of the heterologous reactions. These data support the evidence presented in tables 1, 2, and 3 that illustrate the broad antigenic relationship of *T. glabrata* to the species of *Candida* utilized in this study.

For these studies viable yeast cells have been utilized as antigens in the production of the antisera, whereas nonviable yeast cell suspensions have been used for adsorption and agglutination studies. The rabbits gave a better antibody response to the viable cells, but little or no difference could be detected between the agglutination reactions of viable and of nonviable suspensions.

Two typical strains of each species were used in this study. The two strains of *T. glabrata* used bear a close antigenic relationship to the 7 species (14 strains) of *Candida* used. The study does not preclude the possibility of more antigenic variation within these species than is shown here.

The identification of these organisms by reliable serological means, simplifies and expedites laboratory procedures, but carefully adsorbed antisera are necessary.

SUMMARY

A comparison of the antigenic relationship of *Torulopsis glabrata* with *Candida tropicalis*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida guilliermondii*, *Candida albicans*, *Candida krusei*, and *Candida pseudotropicalis* has been made. Two strains of each species were studied. The results indicated that *T. glabrata* has antigens common to all the species of *Candida* included in this investigation, in addition to those that were specific for this organism. Other species of *Torulopsis* were not studied.

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