ANTIGENIC STUDIES OF CANDIDA

I. Observation of Two Antigenic Groups in *Candida albicans*[1]

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ABSTRACT

Hasenclever, H. F. (U. S. Public Health Service, Bethesda, Md.), and William O. Mitchell. Antigenic studies of Candida. I. Observations of two antigenic groups in *Candida albicans*. J. Bacteriol. **82**:570–573. 1961.—Two distinct antigenic groups, detected by tube agglutination, have been observed among strains of *Candida albicans*. Adsorption of an antiserum with a heterologous strain did not remove the agglutinating properties for the homologous strain. Homologous adsorption of the antiserum did remove its agglutinating properties. Seventy-one isolates of *C. albicans* have been screened with this adsorbed antiserum and 38 were agglutinated at a serum dilution of 1:240 to 1:480 (group A), whereas 33 were not agglutinated at a serum dilution of 1:30 (group B). Thirty-five of the strains were studied with rabbit antisera prepared against each of six strains (three from each group). Agglutination reactions of these strains with samples of each antiserum adsorbed individually with each immunizing strain verified the results of the screening agglutination reactions. All the agglutinating properties of antisera prepared against the three group B strains were removed by adsorption with suspensions of either group A or group B strains. The identity of all isolates used in this study was confirmed by chlamydospore formation, fermentation, and carbohydrate assimilation reactions.

The presence of antigenic types or groups, a well-known phenomenon within bacterial species or viruses, to date has been observed in only one species of pathogenic yeast. Evans (1949) found, in *Cryptococcus neoformans*, three groups which were based upon differences in the capsular antigens.

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In this report we show evidence indicating the presence of two antigenic groups in *Candida albicans*. The results, upon which these conclusions are based, were obtained from tube agglutination reactions.

This study was prompted by the observation that the agglutinating properties of an antiserum to one strain of *C. albicans*, when adsorbed with the cells of a different isolate of the same species, were not removed for the homologous strain. An investigation of more isolates indicated that some were agglutinated by this adsorbed antiserum whereas others were not. The strains that were, agglutinated have been designated as group A, whereas those that were not agglutinated are group B.

MATERIALS AND METHODS

All the strains of yeasts included in this study conform to the morphological and physiological characteristics of *C. albicans*. Seventy-one strains (62 recent isolates and 9 laboratory strains) have been screened with an adsorbed antiserum to determine their antigenic group. Thirty-five of these strains have been studied extensively with unadsorbed and adsorbed antisera.

Antisera to six strains of *C. albicans* (three group A strains and three group B strains) were produced in rabbits following the intravenous injection of either viable or heat-killed suspensions of yeast cells. The schedule of immunization was the same as described previously (Hasenclever and Mitchell, 1960).

Each antiserum was adsorbed separately with each of the six strains employed in the production of antisera. Equal volumes of antiserum and 50% heat-killed yeast suspensions were mixed and placed in a water bath for 3 to 5 hr at 45 C. The mixture was centrifuged and the serum was removed. This process was repeated three more times. The first and third adsorptions were done at 45 C, whereas the second and fourth adsorp-

tions were carried out overnight at refrigerator temperatures. Following completion of the fourth adsorption, the serum was removed, diluted 1:3, and used for serological studies.

Antigens utilized for agglutination reactions were dilute suspensions of heat-killed yeast cells. Suspensions used for agglutination contained approximately 3×10^6 yeast cells per ml.

Standard agglutination procedures were used, and the lowest serum dilution was 1:30. The serum antigen mixtures were placed in a water bath at 45 C for 2 to 3 hr, refrigerated overnight, and read the following morning.

RESULTS AND DISCUSSION

Screening of the 71 strains was done with group A antiserum that had been adsorbed with a group B strain. Those results are shown in Table 1. Thirty-eight strains (group A) were agglutinated at a serum dilution of 1:240 to 1:480, whereas 33 strains (group B) were not agglutinated at 1:30.

Nineteen group A strains and 16 group B strains were studied extensively with unadsorbed and adsorbed antisera. Agglutination reactions of these strains with unadsorbed antisera may be seen in Table 2. The top line of the table indicates with each antiserum the range of agglutination titers for all 35 strains. The bottom line shows the number of strains agglutinated at each serum dilution and their antigenic category. Antisera to group A strains B311 and 857 show little difference in their agglutinating properties for either group A or group B strains, whereas antiserum 207 (group A) agglutinates, at a higher titer, most of the group A strains. Antisera to group B strains agglutinate, at a higher titer, more of the group B strains than the group A strains.

TABLE 1. *Agglutination of 71 strains by screening antiserum*

	Laboratory strains	New isolates	Total strains
Group A*..........	6	32	38
Group B†..........	3	30	33
Total............	9	62	71

* Agglutination at 1:240 or 1:480 serum dilutions.

† No agglutination at 1:30 serum dilution.

Table 3 shows the agglutination reactions of the six antisera following adsorption with group B strains. The results indicate that this adsorption of group A antisera reduces the titer for group A strains; however, the titer is the same regardless of which group B strain was used. The agglutinating properties of group A antisera for group B strains were removed by this adsorption. Adsorption of group B antisera with group B strains removed its agglutination for either group.

The results showing the effect of adsorption with group A strains of group A and group B antisera are presented in Table 4. This adsorption of the antisera removes, for either group, all their agglutinating properties.

A summary of agglutination reactions with adsorbed antisera is shown in Table 5. The figures indicated by the asterisk represent the observed upper and lower extremes. The majority of these reactions were titers of 1:120 to 1:240.

The antigenic differences described in this report have been observed utilizing tube agglutination as the serological procedure. Studies with other techniques for demonstrating antigen-antibody reactions, such as complement fixation, precipitation, and agar gel diffusion have been

TABLE 2. *Agglutination reactions of 35 strains* with unadsorbed antisera*

	Strain (antigenic group) at indicated titers† of antisera:																
	B311(A)		207(A)			857(A)			792(B)			3171(B)			526(B)		
	480	960	240	480	960	480	960	1920	120	240	480	120	240	480	120	240	480
No. strains of group A..	17	2	1	1	17		16	3	1	16	2	9	10		7	12	
No. strains of group B..	16		14	2		3	13			3	13	2	7	7	1	4	11

* Group A strains, 19; group B strains, 16.

† Reciprocals of serum dilutions.

TABLE 3. *Agglutination reactions of 35 strains* with antisera adsorbed† with group B strains*

	Strain (antigenic group) and titers of antisera					
	B311 (A)	207(A)	857(A)	792 (B)	3171 (B)	526 (B)
A strains	60–120	120–240	240–480	<30	<30	<30
B strains	<30	<30	<30	<30	<30	<30

* Group A strains, 19; group B strains, 16.

† Each antiserum individually adsorbed with each B strain.

TABLE 4. *Agglutination reactions of 35 strains* with antisera adsorbed† with group A strains*

	Strain (antigenic group) and titers of antisera					
	B311 (A)	207 (A)	857 (A)	792 (B)	3171 (B)	526 (B)
A strains	<30	<30	<30	<30	<30	<30
B strains	<30	<30	<30	<30	<30	<30

* Group A strains, 19; group B strains, 16.

† Each antiserum individually adsorbed with each A strain.

made, but have not been consistent in showing the differences observed with agglutination reactions. Culture filtrates, French press extracts, extracts of candida cells ground with alumina, and acid hydrolyzates have been investigated for antigenic activity, but without reproducibility.

There has been some question as to the validity of agglutination reactions with yeast cells. Martin (1942) concluded that agglutination with *Candida* species was unreliable, and preferred to use precipitation or complement fixation. Other investigators, Tsuchiya et al. (1954), Jonsen, Thjotta, and Rasch (1953), and Rosenthal and Furnari (1958), have obtained consistent and reproducible results with agglutination. The latter has been our experience with this, and other studies (Hasenclever and Mitchell, 1960).

A serum dilution of 1:30 as the lowest dilution was used to obviate possible nonspecific reactions. Heating serum at 56 C for 30 min also helped eliminate these factors. Heated, pooled normal rabbit serum diluted 1:5 did not agglutinate any of 20 strains tested.

TABLE 5. *Summary of agglutination reactions with adsorbed antisera*

Antiserum	Adsorbed with	Titers with	
		Group A cells	Group B cells
Group A	Group B cells	60–480*	<30
Group A	Group A cells	<30	<30
Group B	Group B cells	<30	<30
Group B	Group A cells	<30	<30

* These figures represent the extreme range of positive titers as shown in Table 3. The majority of the reactions were from 120 to 240.

Seventy-one strains of *C. albicans* have been categorized as to group. Of these isolates all have been found to fall within group A or B. Antisera to six strains, three group A and three group B, unadsorbed and reciprocally adsorbed, were utilized. Thirty-five strains, 19 group A and 16 group B, were employed for agglutination reactions with the unadsorbed and cross-adsorbed antisera. There were quantitative differences existing among the antisera, both before and after adsorption, but the reactions were consistent and reproducible.

It is apparent that, under the experimental conditions described, the group A strains possess an antigen or antigens that are not present in the group B strains, but still contain all of those associated with group B isolates. It is the presence of this antigen or antigens in the group A strains and the absence of the antigen(s) in group B strains that make these two groups distinguishable. Strains of *C. albicans* can be easily separated into their respective groups using tube agglutination reactions and adsorbed antisera.

LITERATURE CITED

EVANS, E. E. 1949. An immunologic comparison of twelve strains of *Cryptococcus neoformans* (*Torula histolytica*). Proc. Soc. Exptl. Biol. Med. **71**:644–646.

HASENCLEVER, H. F., AND W. O. MITCHELL. 1960. Antigenic relationships of *Torulopsis glabrata* and seven species of the genus *Candida*. J. Bacteriol. **79**:677–681.

JONSEN, J., T. THJOTTA, AND S. RASCH. 1953. Quantitative agglutination studies in fungi. II. Serological relationship between *C. albicans* and *C. stellatoidea*. Acta Pathol. Microbiol. Scand. **33**:86–91.

MARTIN, D. S. 1942. Studies on the immunological relationships among species of the genus *Candida* (*Monilia*). Am. J. Trop. Med. **22**:295–303.

ROSENTHAL, S., AND D. FURNARI. 1958. Slide agglutination as a presumptive test in the laboratory diagnosis of *Candida albicans*. J. Invest. Dermatol. **31**:251–253.

TSUCHIYA, T., S. IWAHARA, F. MIYASAKI, AND Y. FUKAZAWA. 1954. Studies on the classification of the genus *Candida*. I. Antigenic analysis of seven species of the genus *Candida*. Japan. J. Exptl. Med. **24**:95–103.