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Serological Studies on Twelve Species of Candida

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SEROLOGICAL STUDIES ON TWELVE SPECIES OF *CANDIDA*

by

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II

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INTRODUCTION

The *Candida* are yeast-like fungi which commonly inhabit the human body, usually without causing disease. However, they do sometimes cause mild disease and occasionally even a serious one. The *Candida* were one of the earliest fungi to be associated with disease. Langenbeck in 1839 demonstrated, in thrush, the presence of a yeast-like, budding fungus which Robin in 1853, named *Oidium albicans* (1). Zoph in 1890 renamed this fungus *Monilia albicans*, while later Berkhout called the yeast *Candida albicans*, as it is known at present (1).

The concept of *Candida* is that of an organism which often lives on the human frame as a commensal or companion without causing disease, but when alterations occur in the host it spreads and causes disease (2). There are thirty species of *Candida* as reported by Lodder and Kreger-Van Rij (3), but only seven species are commonly recovered from man. *C. albicans*, *C. tropicalis*, *C. pseudotropicalis*, *C. stellotoidea*, *C. krusei*, *C. parapsilosis*, and *C. guilliermondii* have all been isolated from human sources both as pathogens and as commensals. All

seven species have been isolated from the deep lesions of humans who have died of systemic candidiasis and most have also been isolated from mucucutaneous lesions. C. stellotoidea, C. albicans, C. tropicalis and C. pseudotropicalis have been found as the etiologic agent in oral candidiasis or thrush. Vaginal candidiasis has been caused by C. albicans, C. stellotoidea, C. tropicalis, C. krusei and C. pseudotropicalis. Also, C. krusei, C. stellotoidea and C. albicans have been isolated from the urine of patients with urinary tract infections. Five species of Candida have been recovered from patients with proven mycotic endocarditis. These are C. albicans, C. parapsilosis, C. guilliermondii, C. krusei and C. tropicalis (2). Patients have died because of the general misconception that C. albicans is the only member of the species that is pathogenic. Untreated systemic candidiasis has a mortality rate approaching 100 per cent. Delay in treatment is therefore dangerous.

Drug therapy and specific debilitating illnesses are predisposing factors to candidiasis. Prolonged antibiotic, steroid, roentgen radiation, and bone marrow depressant therapy are known to enhance Candida infection;

while hypocalcemia and negative nitrogen balance may also be important factors (4). The clinicians of an earlier generation all considered thrush to be a disease of the diseased. A clinical Candida infection is often the first sign available of a deep-seated disease. The Candida are recognized as dangerous complications of open-heart surgery, leukemia, Hodgkins disease, tuberculosis, and diabetes.

Conant, as late as 1947, stated that the group of yeast-like fungi have been the most difficult to study because of the great differences of opinion concerning criteria to be used in classification (5). Emmons suggested using direct examination, direct culture, and sugar fermentations. He stated that the presence of antibodies may relate to a present or past illness or to superficial colonization of the mucosa of the gastrointestional tract. No correlation between possession of antibodies and immunity was apparent. Skin and serological tests were not specific enough or sufficiently related to a current illness to be useful in the diagnosis of candidiasis (6). Most serological studies of the Candida have been for two different purposes: to find a method of diagnosing

candidiasis by serological means; and to study the antigenic structure of the different species of Candida in order to differentiate them. In regard to the diagnosis of candidiasis, the serological approach is commonly one of the techniques used. However, a large percentage of apparently normal, healthy individuals give positive skin reactions when injected intracutaneously with vaccines or extracts of C. albicans (5). Todd, in his survey, showed that 22.5 per cent of sera collected from normal persons agglutinated C. albicans (7). Similarly, Gargani found 16 per cent of 850 normal subjects reacted positively with C. albicans with the complement fixation test (8). This is not surprising since the fungus can be isolated from the skin, mouth, and feces of normal individuals. Such wide distribution of antibodies among normal individuals, as well as patients with candidiasis make the routine serological methods almost useless in diagnosis. However, Akiba considered that skin sensitivity and serum precipitation tests were useful in the diagnosis of deep-seated human infections of Candida (9).

The study of the antigenic structure and the

differentiation of the various species of Candida was attempted as early as 1931 by Stone and Garrod (10). They used both complement fixation and precipitin methods without much success. The agglutination experiments of Almon and Stovall, in 1934, led them to believe that C. albicans and C. tropicalis were identical but differed from C. parapsilosis (11). This was generally confirmed by the studies done by Rawson and Norris in 1947 (12). Jonsen, Thjotta, and Rasch, in 1953, showed that C. albicans and C. stellatoidea are closely related and distinct from C. pseudotropicalis (13). There is some belief that C. pseudotropicalis is the imperfect state of Saccharomyces fragilis. C. claussenii and C. albicans are identical biochemically. In a series of agglutination experiments in 1956, van Uden, Matos-Faia, and Assis-Lopes were unable to show any serological difference between them. One of the most extensive studies was done by Tsuchiya and his colleagues, in their detailed systemic study of the antigenic structure of the Candida group using agglutination methods. They used a variety of absorbed and monospecific antisera in slide agglutination tests, and showed

that it was possible to identify some, but not all, members of this group (14). Hasenclever and Mitchell, in 1961, observed the existence of two distinct sero-types of C. albicans, designated A and B (15). This was confirmed by Stallybrass in 1964 (16).

In 1964, Taschdjian and his co-workers, found that precipitating antibodies were of value in diagnosis. They examined sera from healthy rabbits, sera from healthy people, and sera from patients with systemic candidiasis. They found precipitating antibodies only in the sera of patients with systemic candidiasis (17). Stallybrass, in 1964, examined a very large number of sera and came to the conclusion that only patients with Candida septicemia developed precipitins (16). In 1966, Murray and Buckley, examined 160 sera obtained from a blood bank and found no precipitans to any Candida. Then they did precipitin tests for candidiasis on 40 patients; two of these had proven systemic lesions. Only these last two showed positive results (2).

The studies on the serology of the Candida emphasize the fact that their antigenic structure is complicated. The results of the agglutination, precipitin

reactions and complement fixation tests are confusing, contradictory and, so far, too impractical for the clinical laboratory. This study is an attempt to clarify the situation by using the newer techniques of the Ouchterlogy agar gel-diffusion precipitin method, (18) the hemolysin method, as well as the usual agglutination procedure. Since the precipitin method appears of possible value in diagnosis, this will be the main emphasis in this work.

MATERIALS AND METHODS

The following species of Candida were used:

1. C. albicans
2. C. krusei
3. C. parapsilosis
4. C. tropicalis
5. C. guilliermondii
6. C. stellotoidea
7. C. pseudotropicalis
8. C. parakrusei
9. C. utilis
10. C. intermedis
11. C. mycotherma
12. C. humicola

Antisera were prepared by injecting rabbits three times a week with formalin killed suspensions of the yeasts in saline. The organisms were grown on Sabouraud-Dextrose slants for 72 hours at room temperature. They were harvested, washed with distilled water, and formalin killed. They were again washed three times with distilled water

and a 1 per cent suspension was made with saline. One cc. of 1:1000 merthiolate was added to insure sterility. The rabbits were bled weekly until a sufficiently reactive titer was reached. At this point the animals were exanguinated and the separated sera stored at -20C.

Whole cell agglutination was done by using a 1 per cent and a 0.5 per cent suspension. One drop of yeast suspension and one drop of serum were mixed well on a slide. They were read for agglutination after 1/2 hour and 1 hour at room temperature. The degree of agglutination was recorded as follows:

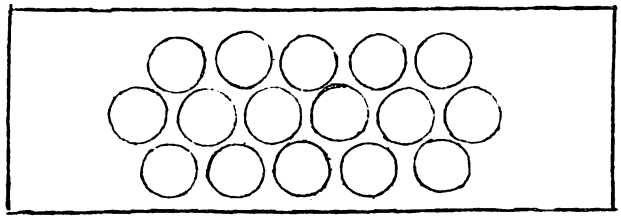
++++	100 per cent agglutination
+++	75 per cent agglutination
++	50 per cent agglutination
+	25 per cent agglutination
±	Trace of agglutination
-	No agglutination

Since there was so much cross-reaction among these yeasts, only +++ or ++++ reactions were considered in the final comparison of the agglutination and precipitin reactions.

An attempt was made to use the hemoagglutination method of Neter (19). However, no hemoagglutination was

observed with either whole or sonic disintegrated yeast cell suspensions. These suspensions were tested with chicken, sheep, baboon, Rhesus monkey, Guinea pig, and Group "0" human blood cells at both 37C and at room temperature. Hemolytic reactions were tested and observed with each of the above combinations. The most complete hemolysis was observed with the disintegrated cell suspensions and a 0.5 per cent suspension of human Group "0" blood cells at 37C. These reactions were read for hemolysis at 1 hour and again at 2 hours. The broken cell suspensions were obtained by subjecting a 1 per cent yeast suspension to at least 50 minutes of sonic vibration by a Sorvall Sonicator.

The modified Ouchterlony agar gel precipitin technique was used to test for precipitins. Usually the Ouchterlony technique consists of wells for antisera and antigens cut into the agar. In this modified method, sterile filter paper discs were placed on the agar according to the following template. The position of the discs and distance between them is very important for optimum production of the precipitin bands.



The antigen is placed in the middle row and the antibody is in the outer rows. Double agar slides were prepared of Noble Agar. The sterile paper discs were impregnated with the desired serums and antigens. The serums and antigens were placed in position on the slide and kept at room temperature for 24 hours in a wet Petri dish, to prevent the agar from drying out. The discs were washed off and the slides read for precipitin bands. The information on Tables 3 and 4 were recorded as follows:

\+ Precipitin band present.

\- No precipitin bands formed.

Each serum was tested for cross reactions to each of the Candida species, using agglutination and precipitin methods. The sera were then absorbed with each yeast found to react. Whole yeast suspensions did not absorb high titer serum satisfactorily, so absorptions were

carried out by mixing one volume of antisera with 1/2 volume of disintegrated cell suspension for 2 hours at 37C. This mixture was placed in the refrigerator overnight, then centrifuged at 2,000 RPM for 15 minutes. The recovered antiserum was used to retest for the three types of reactions.

RESULTS

The agglutination procedure showed a high degree of cross-reactivity between the twelve yeasts. Even after absorption each serum would cross-react, although in a reduced amount. (See Tables 1 and 2) Further absorption attempts resulted in a complete loss of agglutinating ability. However, it appears to be due solely to the unavoidable dilution of the antibodies by the absorbing suspension.

Each serum was tested for hemolysins both before and after absorption. Hemolysins were demonstrated in each serum in both cases. The absorption procedure apparently didn't remove any hemolysins, and they may be group specific rather than species specific reactions.

Strongly defined precipitin bands were obtained for each of the yeasts against its specific antiserum. A lot of cross-reaction was also noted (see Table 3).

1. *Candida albicans* rabbit antiserum reacted also with *C. parapsilosis*, *C. tropicalis*, *C. parakrusei*, and *C. intermedis* antigen.

2. *C. krusei* antiserum reacted with *C. humicola*.

3. C. parapsilosis antiserum reacted with C. albicans, C. tropicalis, C. parakrusei, and C. intermedis.

4. C. tropicalis antiserum reacted with C. albicans and C. intermedis.

5. C. guilliermondii antiserum reacted with C. utilis.

6. C. stellotoidea antiserum reacted with C. albicans, C. krusei, C. parapsilosis, C. tropicalis, C. pseudotropicalis, C. parakrusei, and C. intermedis.

7. C. pseudotropicalis antiserum reacted with C. krusei and C. parakrusei.

8. C. parakrusei antiserum reacted with C. krusei.

9. C. utilis antiserum reacted with C. guilliermondii, C. mycotherma, and C. humicola.

10. C. intermedis antiserum reacted with C. albicans, C. parapsilosis, and C. tropicalis.

11. C. mycotherma antiserum reacted with C. krusei and C. humicola.

12. C. humicola antiserum reacted with C. parapsilosis, C. tropicalis, C. guilliermondii, C. stellotoidea, and C. mycotherma.

An attempt to absorb each antiserum with the other eleven disintegrated cell suspensions was unsuccessful. Apparently the large amount of suspension needed, diluted the antibodies in the serum and no precipitin bands were noted. Each serum was then absorbed with disintegrated cell suspensions of the cross-reacting yeasts. The following results were noted when agar-gel precipitin tests were repeated with the absorbed serum (see Table 4).

1. C. albicans reacted with its own antigen and weakly with C. tropicalis.

2. C. krusei reacted only with its own antigen.

3. C. parapsilosis reacted with its own antigen and weakly with C. albicans.

4. C. tropicalis reacted with its own antigen.

5. C. quilliermondii reacted with its own antigen.

6. No precipitin bands were formed with C. stellotoidea. Such a large volume of suspension was required for the seven reactive yeasts that the antibody was probably too dilute.

7. C. pseudotropicalis reacted with its own antigen.

8. C. parakrusei reacted with its own antigen.

9. C. utilis reacted with its own antigen and weakly with C. guilliermondii.

10. C. intermedis reacted only with its own antigen.

11. C. mycotherma reacted with its own antigen.

12. C. humicola didn't show any precipitin bands and this was probably due to dilution of the antibodies, also.

TABLE 1

RESULTS OF AGGLUTINATION REACTIONS

Antiserum	Cell Suspension											
	alb.	krusei	para-psil.	trop.	guill.	stell.	pseu-trop.	para-krusei	util.	inter.	myco.	humi.
C. albicans	++++	++++	++++	+++	+	+++	+++	+++	++++	++++	++	++
C. krusei	++++	++++	++++	±	±	+++	++	+	+++	+++	+	++++
C. parapsilosis	++++	++++	++++	++++	±	+++	+++	++++	+++	+++	++	++++
C. tropicalis	++++	++	++	++++	±	+++	+++	+	+++	++++	±	+
C. guilliermondii	+++	+++	—	+++	++++	+	+++	+	++++	+++	++	+
C. stellotoidea	++++	+++	+++	+++	+	+++	+++	+++	++	+++	+	+
C. pseudotropicalis	++	+++	++	+	++	+++	++++	+++	++	++	+	++
C. parakrusei	++	++++	++	+	+	++	++	++++	+++	++	+++	++
C. utilis	++	+++	±	++	++++	+	+	+	+++	+	+++	+++
C. intermedis	+++	++	+++	+++	++	+	+++	++	+++	++++	+	±
C. mycotherma	++	+++	++	++	+++	+	++	+	++	++	++++	+++
C. humicola	++	++	+++	+++	+++	+++	+++	+	++	++	+++	++++

TABLE 2

RESULTS OF AGGLUTINATION REACTIONS AFTER ABSORPTION

Antiserum	Cell Suspension											
	alb.	krusei	para-psil.	trop.	guill.	stell.	pseu-trop.	para-krusei	util.	inter.	myco.	humi.
C. albicans	++++	+++	+++	+++	±	+	++	++	+++	++	+	+
C. krusei	+++	++++	+++	-	-	+	+	±	++	++	-	++
C. parapsilosis	++	+++	++++	+++	-	++	++	+++	++	++	±	++
C. tropicalis	++	±	+	++++	-	+	++	-	+	+++	-	-
C. guilliermondii	+	++	-	+	++++	-	++	-	++	++	+	-
C. stellotoidea	+++	++	++	+	-	+++	+	++	±	++	±	±
C. pseudotropicalis	+	++	±	-	+	++	++++	++	+	±	-	+
C. parakrusei	+	+++	+	-	±	+	+	++++	++	+	++	+
C. utilis	±	++	-	+	++	-	±	+	+++	-	++	+
C. intermedis	++	+	++	+	+	-	++	+	++	++++	-	-
C. mycotherma	+	++	+	±	++	-	+	+	+	+	++++	++
C. humicola	+	+	++	+	++	++	++	-	+	±	++	++++

TABLE 3

RESULTS OF PRECIPITIN REACTIONS

Antiserum	Disintegrated cell suspensions											
	alb.	krusei	para-psil.	trop.	guill.	stell.	pseu-trop.	para-krusei	util.	inter.	myco.	humi.
C. albicans	+	-	+	+	-	-	-	+	-	+	-	-
C. krusei	-	+	-	-	-	-	-	-	-	-	-	+
C. parapsi-losis	+	-	+	+	-	-	-	+	-	+	-	-
C. tropi-calis	+	-	-	+	-	-	-	-	-	+	-	-
C. guillier-mondii	-	-	-	-	+	-	-	-	+	-	-	-
C. stello-toidea	+	+	+	+	-	+	+	+	-	+	-	-
C. pseudo-tropicalis	-	+	-	-	-	-	+	+	-	-	-	-
C. parakrusei	-	+	-	-	-	-	-	+	-	-	-	-
C. utilis	-	-	-	-	+	-	-	-	+	-	+	+
C. intermedis	+	-	+	+	-	-	-	-	-	+	-	-
C. mycotherma	-	+	-	-	-	-	-	-	-	-	+	+
C. humicola	-	-	+	+	+	+	-	-	-	-	+	+

TABLE 4

RESULTS OF PRECIPITIN REACTIONS AFTER ABSORPTION

Antiserum	Disintegrated cell suspensions											
	alb.	krusei	para-psil.	trop.	guill.	stell.	pseu-trop.	para-krusei	util.	inter.	myco.	humi.
C. albicans	+	-	-	+	-	-	-	-	-	-	-	-
C. krusei	-	+	-	-	-	-	-	-	-	-	-	-
C. parapsi-losis	+	-	+	-	-	-	-	-	-	-	-	-
C. tropi-calis	-	-	-	+	-	-	-	-	-	-	-	-
C. guillier-mondii	-	-	-	-	+	-	-	-	-	-	-	-
C. stello-toidea	-	-	-	-	-	-	-	-	-	-	-	-
C. pseudo-tropicalis	-	-	-	-	-	-	+	-	-	-	-	-
C. parakrusei	-	-	-	-	-	-	-	+	-	-	-	-
C. utilis	-	-	-	-	+	-	-	-	+	-	-	-
C. intermedis	-	-	-	-	-	-	-	-	-	+	-	-
C. mycotherma	-	-	-	-	-	-	-	-	-	-	+	-
C. humicola	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

This study suggests a promising aspect of diagnosing candidiasis. Further investigation, using cultures isolated from patients, would be necessary. A Candida albicans antisera for the agglutination method is available commercially. The further development of the precipitin procedure might produce a valuable diagnostic tool, since it appears to be more species specific.

Several antigenic relationships among these twelve Candida might be worth further study.

1. C. albicans, C. intermedis, and C. tropicalis appear to have similar precipitin reactions. C. albicans and C. tropicalis, in particular, seem to be closely related. There was no cross-reaction between C. albicans and C. stellotoidea, although they are considered to be closely related.

2. C. guilliermondii and C. utilis appear to share common precipitin antigens.

3. C. stellotoidea and C. humicola are the most antigenically active among these twelve yeasts. Further

investigation might produce antigenic relationships not presently considered.

In the following comparison of reactions, each antiserum is listed, with the species which gave cross-reactions in the next column. As is readily apparent, the agglutination column contains more cross-reactions than the other two. The agglutination after absorption was not listed as there were still too many reactive members.

COMPARISON OF REACTIONS

ANTISERUM	AGGLUTINATION	PRECIPITIN	PRECIPITIN AFTER ABSORPTION
C. albicans	C. albicans C. krusei C. parapsilosis C. stellotoidea C. pseudotropicalis C. parakrusei C. utilis C. intermedis	C. albicans C. parapsilosis C. tropicalis C. parakrusei C. intermedis	C. albicans C. tropicalis
C. krusei	C. albicans C. krusei C. parapsilosis C. stellotoidea C. utilis C. intermedis C. humicola	C. krusei C. humicola	C. krusei
C. parapsilosis	C. albicans C. krusei C. parapsilosis C. tropicalis C. stellotoidea C. pseudotropicalis C. parakrusei C. utilis C. intermedis C. humicola	C. albicans C. tropicalis C. parakrusei C. intermedis C. parapsilosis	C. albicans C. parapsilosis

ANTISERUM	AGGLUTINATION	PRECIPITIN	PRECIPITIN AFTER ABSORPTION
C. tropicalis	C. albicans C. tropicalis C. stellotoidea C. pseudotropicalis C. utilis C. intermedis	C. albicans C. tropicalis C. intermedis	C. tropicalis
C. guilliermondii	C. albicans C. krusei C. tropicalis C. guilliermondii C. pseudotropicalis C. utilis C. intermedis	C. guilliermondii C. utilis	C. guilliermondii
C. stellotoidea	C. albicans C. krusei C. parapsilosis C. tropicalis C. stellotoidea C. pseudotropicalis C. parakrusei C. intermedis	C. albicans C. krusei C. parapsilosis C. tropicalis C. stellotoidea C. pseudotropicalis C. parakrusei C. intermedis	No reaction, too dilute
C. pseudo-tropicalis	C. krusei C. stellotoidea C. pseudotropicalis C. parakrusei	C. krusei C. pseudotropicalis C. parakrusei	C. pseudotropicalis
C. parakrusei	C. krusei C. parakrusei C. utilis C. mycotherma	C. krusei C. parakrusei	C. parakrusei

ANTISERUM	AGGLUTINATION	PRECIPITIN	PRECIPITIN AFTER ABSORPTION
C. utilis	C. krusei C. guilliermondii C. utilis C. humicola C. mycotherma	C. guilliermondii C. utilis C. mycotherma C. humicola	C. guilliermondii C. utilis
C. intermedis	C. albicans C. parapsilosis C. tropicalis C. pseudotropicalis C. utilis C. intermedis	C. albicans C. parapsilosis C. tropicalis C. intermedis	C. intermedis
C. mycotherma	C. krusei C. guilliermondii C. mycotherma C. humicola	C. krusei C. mycotherma C. humicola	C. mycotherma
C. humicola	C. parapsilosis C. tropicalis C. guilliermondii C. stellotoidea C. pseudotropicalis C. mycotherma C. humicola	C. parapsilosis C. tropicalis C. guilliermondii C. stellotoidea C. mycotherma C. humicola	No reaction, too dilute

SUMMARY

The three main types of immunological reactions are present in all twelve species of Candida. The hemolytic type appears to be species specific, since it was found in all the reactions tested. The agglutination reaction produces agglutination varying from $\pm$, or trace to ++++ or 100 per cent agglutination. This is due to cross-reactions among the Candida and cannot be completely removed by absorption. The precipitin reactions seem to give the most specific results, and most of the cross-reaction bands are absorption removable. The hemoagglutination reaction could not be demonstrated with any of these twelve species of Candida.

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