Antigenic Relationships Among Some *Candida* Species Studied by Crossed-Line Immunoelectrophoresis: Taxonomic Significance

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In crossed immunoelectrophoresis with the corresponding pooled rabbit antisera, *Candida tropicalis* and *Candida albicans* antigen preparations produced 65 and 63 immunoprecipitates, respectively. The cross-reactions between these antigens and antigens from 15 other yeast strains representing nine species were studied by crossed-line immunoelectrophoresis, using *C. tropicalis* and *C. albicans* antigen-antibody systems as references. We found no qualitative differences between strains of the same species or among the strains of *C. albicans*, *Candida stellatoidea*, and *Candida claussenii*. In both the *C. albicans* and *C. tropicalis* reference systems the degrees of cross-reaction between *C. albicans* or *C. tropicalis* and the nine other species revealed the following three groups: *C. albicans*, *C. tropicalis*, *C. stellatoidea*, and *C. claussenii*, which shared 85 to 100% of their antigens with *C. albicans* and *C. tropicalis*; *Candida parapsilosis*, *Candida guilliermondii*, and *Candida sake*, which shared 50 to 70% of their antigens with *C. albicans* and *C. tropicalis*; and *Candida utilis*, *Candida pseudotropicalis*, *Candida krusei*, and *Candida zeylanoides*, which shared about 30% of their antigens with *C. albicans* and *C. tropicalis*. Some antigens seemed to be species specific, whereas others seemed to be specific for a group of species within the genus *Candida* and should be useful for rapid identification of medically important yeasts.

At one time, morphological and cultural characteristics, as well as biochemical properties, were the principal criteria used in yeast taxonomy. Later, other criteria were found to be useful for species identification. Tsuchiya et al. (39-43) and Hasenclever et al. (14, 15) carried out antigenic analyses of yeast cell walls by using agglutination and absorption, and these authors identified several antigenic factors at the yeast surfaces. A new system for yeast classification was proposed based on these factors. The members of the genus *Candida* were divided into eight serological groups. Numerous discrepancies with the classification of Van Uden and Buckley (45) were noted. Spencer and Gorin (31, 32) did the first proton magnetic resonance spectroscopic studies of parietal mannans and found that the proton magnetic resonance spectra of some yeasts were consistent with serological results. The chemical structures of the immunodominant groups of some yeast mannans have been demonstrated by Ballou et al. (5), Yu et al. (51), Suzuki et al. (35), and other workers. Mannose-containing branched polysaccharides have been identified as antigenic determinants. However, the relationships between the immunochemical structures and the antigenic factors of Tsuchiya have not been defined fully (9). Yamada et al. (48-50) have stated that coenzyme Q systems are very useful for classifying yeasts. In the genus *Candida* the coenzyme Q systems vary from Q-6 to Q-10.

Another approach to yeast species identification is based on nucleic acid analyses. Determinations of the guanine-plus-cytosine (G+C) contents of deoxyribonucleic acids (DNAs) have shown the heterogeneous nature of numerous yeast genera (26-28). Such determinations allow rather coarse comparisons since strains with similar G+C contents can appear similar or not related on the basis of the results of DNA-DNA homology experiments (23).

The last method mentioned above has been promising at

the species level, but interspecific relationships cannot always be deduced from DNA complementarity since the percentages of homology between different species in one genus may be very low or very high (29). Comparisons of certain proteins, especially enzymes, have been tried and may elucidate interspecific relationships (22).

Immunological comparison of fungal antigens for taxonomic purposes was introduced many years ago and has been used to study the genus *Candida*. Gel immunoprecipitation has been widely used as a tool for investigating fungal antigens and the corresponding antibodies. Ouchterlony analyses have revealed as many as 10 antigens (6), immunoelectrophoretic analyses (IEA) have revealed about 15 antigens (7), and quantitative immunoelectrophoresis (QIE), which is a more sensitive and resolvent technique, has allowed the study of more than 60 antigens without previous purification of components (2). QIE was used by Axelsen (2) to study yeast antigens and has been widely used to study bacterial antigens (8, 16-18). Syverson et al. (37) studied the mycelial and yeast phase antigens of *Candida albicans*, Syverson and Buckley (36) studied the soluble cell wall antigens of *C. albicans*, and Huppert et al. (19) studied *Blastomyces* and *Histoplasma* antigens. We studied the *Candida tropicalis* cell wall soluble antigens (12) and the cytoplasmic soluble antigens of *C. albicans* serotype A (10). The purpose of the present work was to study different species of the genus *Candida* by immunologically comparing proteins. We established two different antigen-antibody reference systems, one with *C. tropicalis*, which is the type species of the genus, and one with *C. albicans*, which is medically the most important yeast in humans. We compared the antigens from nine other *Candida* species in each reference system. We used a QIE method involving crossed immunoelectrophoresis (CIE) with an intermediate gel containing a second antigen (or crossed-line immunoelectrophoresis [CLIE]); this method showed reactions of complete or partial identity between individual proteins in complex antigenic mixtures.

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MATERIALS AND METHODS

Yeast strains. We studied 17 strains representing 11 species; these strains were supplied by the Centraalbureau voor Schimmelcultures (Delft, The Netherlands), the Institut Pasteur de Paris, the National Institutes of Health, and the Institut Français du Pétrole (Table 1).

Antigens. The yeasts were grown for 48 h at room temperature on a New Brunswick gyratory shaker in one of the following media: Sabouraud yeast broth (30 g of Sabouraud Institut Pasteur Production per liter, 3 g of yeast extract [Difco Laboratories] per liter) or yeast malt broth (3 g of malt extract [Oxoid Ltd.] per liter, 5 g of peptone [Difco] per liter, 10 g of glucose per liter, 3 g of yeast extract [Difco] per liter). For *C. tropicalis* and *C. albicans* the antigens obtained in the two types of broth showed no qualitative differences when they were compared by CLIE. The yeasts were harvested by centrifugation for 15 min at $15,000 \times g$ and the cellular paste obtained was washed twice with PBS buffer (pH 7.2; 7.650 g of NaCl per liter, 0.724 g of Na_2HPO_4 per liter, 0.210 g of KH_2PO_4 per liter). To a 70-ml flask (Braun Melsungen) 50 g of glass beads (diameter, 0.5 mm) was added, along with 12 to 20 g (wet weight) of yeast suspended in 25 ml of PBS buffer. The flask was shaken in a Braun homogenizer for 2 min at 2,800 rpm, using liquid CO_2 as a cooling agent. The extract was centrifuged for 1 to 2 h at $48,000 \times g$, filtered through a membrane filter (pore diameter, 0.45 μm), and freeze-dried. The colloid concentration of the extract was determined by refractometry, using human serum as a reference (2).

Antisera. Antisera were produced by immunizing four rabbits, two with a *C. tropicalis* IFP 104 extract and two with a *C. albicans* IP 886 extract, essentially as described by Harboe and Ingild (13) but with some modifications. On days 0, 14, 28, and 42, each rabbit received a mixture of 1 ml of

antigen solution and 1 ml of muramyl dipeptide (Institut Pasteur Production) intradermally at several points on its back. Every month, 45 ml of blood was taken, the animals having received an antigen-muramyl dipeptide mixture 8 days before the bleeding. Antibody titers were estimated by line immunoelectrophoresis (21). The sera from months 5 to 11 (*C. albicans*) or months 4 to 13 (*C. tropicalis*) were combined; these serum pools were the pools used in this study and were freeze-dried.

QIE methods. CIE was performed by the method of Weeke (46), using a 1% agarose gel (Pharmacia A) in 0.025 M tris(hydroxymethyl)aminomethane-barbital buffer (pH 8.6). First-dimension electrophoresis of the *Candida* antigens (10 μl) was performed at 15°C by applying 10 V/cm for 70 min, and second-dimension electrophoresis was performed at 15°C by applying 2 V/cm for 18 h through a gel containing 10 μl of antiserum per cm^2 . In the first-dimension electrophoresis, the plates were 11 cm long and 9 cm wide, and the thickness of the gel was 0.15 cm; in the second-dimension electrophoresis, the plates were 10 cm long, 10 cm wide, and 0.1 cm thick. Line immunoelectrophoresis was performed as described by Kroll (21). The antigen was mixed with 1% buffered agarose and poured to obtain a gel that was 1 by 20.5 by 0.13 cm on a glass plate (11 by 20.5 cm). Then antibody-containing gels (7 by 2 by 0.10 cm) were poured along the antigen gel. A blind gel covered the remaining part of the plate. The antibody and antigen concentrations in the gels were 10%, and the electrophoresis conditions used were the same as described above for the second dimension of CIE. CLIE was performed as described by Kroll (20). The first dimension was done as described above for CIE, using 10 μl of *Candida* antigen. A slab (10 by 2 cm) was transferred to a coated glass plate (10 by 10 cm). A blind gel (10 by 2 by 0.13 cm) was poured along the first-dimension electrophoresis gel. After congelation a gel strip (10 by 1 cm) was cut out of the blind gel and replaced with a gel containing 10% antigen. The antibody-containing gel was formed by pouring 6 ml of agarose containing *C. albicans* or *C. tropicalis* antiserum into the remaining part of the glass plate. The antiserum concentration and the second-dimension electrophoresis conditions were as described above for CLIE. CLIE with adsorption of antibodies in situ was performed just as CLIE was, except that the amount of antigen in the gel was increased from three- to sixfold; all of the other parameters were the same as for CLIE.

The non-precipitated proteins were always removed by pressing the gel and washing it for 1 h in buffered saline and then for 15 min in distilled water. After a new pressing and drying, the plates were stained for 5 min in a solution of 0.5% (wt/vol) Coomassie brilliant blue R in ethanol-acetic acid-water (45:10:45). The excess dye was removed by repeated washing in an ethanol-glacial acetic acid-water destainer (25:10:45) (47).

RESULTS

The colloid concentrations of antigens varied from 18 to 60 g/liter. The most concentrated antigens were diluted to about 38 to 40 g/liter (Table 1). *C. albicans* and *C. tropicalis* antisera were obtained by pooling for each rabbit the serum samples that produced at least 25 precipitates in line immunoelectrophoresis gels. We detected 65 individual precipitates in the immunoplates run with *C. tropicalis* antibodies. These precipitates were numbered, beginning with 1 at the anodic end of the first-dimension electrophoresis gel (Fig. 1). In the same way, 63 precipitates were numbered on the *C. albicans* system immunoplates (Fig. 2).

TABLE 1. Strains studied, G+C contents, coenzyme Q systems, and the colloid concentration of each antigen

Strain ^a	Coenzyme Q system ^b	G+C content (mol%)	Colloid concn (g/liter)
<i>C. albicans</i> 3153A (= IP 884)	9	34.9 ^c	20
<i>C. albicans</i> NIH A207	9	34.9 ^c	24
<i>C. albicans</i> CBS 562 ^T	9	34.9 ^c	18
<i>C. albicans</i> 3156B (= IP 886)	9	34.9 ^c	30
<i>C. albicans</i> NIH B526	9	34.9 ^c	32
<i>C. clausenii</i> CBS 1949 ^T	9	36.8, 35.4 ^{c,d}	40
<i>C. guilliermondii</i> CBS 566 ^T	9	46.3 ^d	36
<i>C. krusei</i> CBS 573 ^T	7	39.6 ^e	26
<i>C. parapsilosis</i> CBS 604 ^T	9	40.8 ^e	38
<i>C. pseudotropicalis</i> CBS 607 ^T	6	41.3 ^e	38
<i>C. sake</i> CBS 617	9	38.8 ^f	18
<i>C. stellatoidea</i> CBS 1905 ^T	9	35.7 ^e	40
<i>C. stellatoidea</i> CBS 2990	9	35.7 ^e	28
<i>C. tropicalis</i> CBS 94 ^T	9	36.1 ^d	38
<i>C. tropicalis</i> IFP 104	9	36.1 ^d	36
<i>C. utilis</i> CBS 621 ^T	7	45.8 ^e	38
<i>C. zeylanoides</i> CBS 619 ^T	9	57.6 ^e	26

^a CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; IP, Institut Pasteur, Paris, France; NIH, National Institutes of Health, Bethesda, Md.; IFP, Institut Français du Pétrole, Lyon, France.

^b Data from reference 49.

^c Data from reference 28.

^d Data from reference 24.

^e Data from reference 33.

^f Data from reference 23.

FIG. 1. CIE reference pattern of *C. tropicalis*. (A) Coomassie brilliant blue-stained glass plate. The antigen was 10 μ l of *C. tropicalis* IFP 104 reference extract in the cathodic well, and the antibody was 10 μ l of rabbit antiserum against an extract of *C. tropicalis* (*C. tropicalis* reference serum) per cm^2 . (B) Drawing of the 65 precipitates that regularly occurred in repeated CIE runs.

FIG. 2. CIE reference pattern of *C. albicans*. (A) Coomassie brilliant blue-stained glass plate. The antigen was 10 μ l of *C. albicans* IP 886 reference extract in the cathodic well, and the antibody was 10 μ l of rabbit antiserum against an extract of *C. albicans* (*C. albicans* reference serum) per cm^2 . (B) Drawing of the 63 precipitates that regularly occurred in repeated CIE runs.

The degrees of cross-reaction between antigenic mixtures from *C. tropicalis* and 10 other antigens were studied by using CLIE and *C. tropicalis* serum (Fig. 3). In the same

way, CLIE was also used in the *C. albicans* system with 13 antigens (Fig. 4). The cross-reactive antigens (which showed reactions of total or partial identity) were numbered in each

FIG. 3. Antigenic comparison between extracts from *C. tropicalis* and *C. parapsilosis* by using CLIE and *C. tropicalis* antiserum. The antigen was 10 μ l of *C. tropicalis* IFP 104 extract in the cathodic well. The intermediate gel was a blind gel (A) or contained 50 μ l (B), 100 μ l (C), or 150 μ l (D), of *C. parapsilosis* extract. The antibody was 10 μ l of *C. tropicalis* reference serum per cm^2 for each plate. Three types of reactions are shown. In the reactions showing identity, the antigen applied to the intermediate gel caused a horizontal precipitation line which fused with the precipitation peak originating from the antigen applied to the well (e.g., peak 56); increasing the antigenic concentration in the intermediate gel resulted in anodic migration of the corresponding precipitates (compare gels B, C, and D). In the reactions showing partial identity, the antigen applied to the intermediate gel caused a horizontal precipitation line which fused only partially with the precipitation peak or caused an increase in area of the same peak (e.g., peak 27). In the reactions showing lack of identity, some peaks were unaffected by the application of an antigenic mixture in the intermediate gel (e.g., peak 6). No change occurred in 22 of the 65 antigens of the *C. tropicalis* reference pattern; *C. tropicalis* IFP 104 and *C. parapsilosis* CBS 604^T shared 66% of the proteins studied, and these two strains are therefore related.

FIG. 4. Antigenic comparison between extracts from *C. albicans* and *C. krusei* by using CLIE and *C. albicans* antiserum. The antigen was 10 µl of *C. albicans* IP 886 extract in the cathodic well. The intermediate gel was a blind gel (A) or contained 200 µl of *C. krusei* extract (B). The antibody was 10 µl of *C. albicans* reference serum per cm² for each plate. The following three types of reactions are shown (see legend to Fig. 3): identity (e.g., peak 37), partial identity (e.g., peak 9), and lack of identity (e.g., peak 1). No change occurred in 43 of the 63 antigens of the *C. albicans* reference pattern; *C. albicans* IP 886 and *C. krusei* CBS 573^T shared only 31.7% of the proteins studied, and thus these two strains are not related.

case. All of the antigens from *C. tropicalis* IFP 104 (reference system) cross-reacted with the antigens from *C. tropicalis* CBS 94^T (T = type strain). All of the antigens from *C. albicans* serotype B strain 3156B (reference system) cross-reacted with the antigens from different *C. albicans* strains (serotypes A and B and strain CBS 562^T), *Candida stellatoidea* strains and a *Candida claussenii* strain. With the other species, the percentages of cross-reactive antigens varied from 81.5 to 26.1% in the *C. tropicalis* system and from 84.1 to 28.5% in the *C. albicans* system (Tables 2 and 3).

DISCUSSION

In this study we confirmed the superiority of CLIE over other precipitation methods in analyses of microbial extracts since more than 60 antigens were demonstrated in both the *C. tropicalis* and *C. albicans* systems.

We found no qualitative antigenic differences between strains belonging to the same species since five strains of *C. albicans* and two strains of *C. tropicalis* shared common components. The same methods have previously demon-

TABLE 2. CLIE results in the *C. tropicalis* system

Species	Non-cross-reactive antigens		Cross-reactive antigens		% Of cross-reactivity
	Precipitate no.	Total no.	Precipitate no.	Total no.	
<i>C. tropicalis</i>	None	0	All	65	100
<i>C. albicans</i>	3, 4, 6, 9, 10, 18, 23, 41, 45, 46, 48, 55	12	All other antigens	53	81.5
<i>C. stellatoidea</i>	3, 4, 6, 9, 10, 18, 23, 41, 45, 46, 48, 55	12	All other antigens	53	81.5
<i>C. parapsilosis</i>	1, 2, 3, 4, 5, 6, 9, 10, 18, 23, 24, 28, 36, 37, 41, 45, 46, 48, 52, 55, 62, 64	22	All other antigens	43	66.1
<i>C. guilliermondii</i>	1, 2, 3, 4, 6, 7, 9, 10, 18, 21, 22, 23, 24, 26, 28, 29, 30, 31, 32, 33, 35, 36, 37, 41, 45, 46, 48, 52, 55, 56, 62, 64, 65	33	All other antigens	32	49.2
<i>C. sake</i>	1, 2, 3, 4, 6, 9, 10, 18, 22, 23, 24, 25, 28, 29, 31, 32, 33, 35, 40, 41, 42, 43, 45, 46, 48, 52, 54, 55, 59, 61, 62, 63, 64	33	All other antigens	32	49.2
<i>C. pseudotropicalis</i>	All other antigens	47	8, 11, 13, 14, 19, 26, 30, 39, 42, 44, 49, 51, 53, 54, 57, 58, 60, 61	18	27.6
<i>C. krusei</i>	All other antigens	48	8, 11, 13, 14, 19, 36, 39, 42, 43, 44, 47, 50, 51, 54, 57, 58, 59	17	26.1
<i>C. zeylanoides</i>	All other antigens	45	7, 8, 11, 13, 14, 19, 21, 25, 26, 34, 37, 42, 43, 44, 53, 54, 58, 59, 63	20	30.7
<i>C. utilis</i>	All other antigens	46	8, 11, 13, 14, 15, 19, 30, 34, 36, 37, 38, 42, 44, 47, 49, 51, 53, 59, 63	19	29.2

strated no differences between various *C. albicans* strains (2) or different *Pityrosporum ovale* strains (11). Previously (10), we found qualitative antigenic differences between *C. albicans* strains 3153A and 3156B, which belong to serotypes A and B, respectively. These differences were not corroborated by CIE with an intermediate gel containing the corresponding antisera prepared in rabbits (data not shown). Using CLIE (intermediate gel containing antigen) and more concentrated new antigenic preparations, we found that all of the antigens of the two serotypes cross-reacted, and it is likely that in the first experiments the antigen concentration in the intermediate gel was not sufficient to allow complete adsorption of antibodies. Since the serotype specificity resides in surface parietal mannans (34), our antigenic cellu-

lar extracts may have lacked these mannan antigens or may have contained insufficient quantities of them. All of the antigens from *C. albicans* 3156B cross-reacted with the antigens from the closely related organisms *C. stellatoidea* and *C. clausenii*. These results agree with those of Bak and Stenderup (4) and Saez and Andrieu (30).

Antigenic comparisons between the *C. albicans* or *C. tropicalis* antigen-antibody system and different species showed three groups of species (Fig. 5). The four species of group I were *Candida zeylanoides*, *Candida utilis*, *Candida pseudotropicalis*, and *Candida krusei*, which shared only a few components with *C. tropicalis* and *C. albicans*. Group 2, which included *Candida parapsilosis*, *Candida guilliermondii*, and *Candida sake*, constituted an intermediate group

TABLE 3. CLIE results in the *C. albicans* system

Species	Non-cross-reactive antigens		Cross-reactive antigens		% Of cross-reactivity
	Precipitate no.	Total no.	Precipitate no.	Total no.	
<i>C. albicans</i> (all strains)	None	0	All	63	100
<i>C. stellatoidea</i> (all strains)	None	0	All	63	100
<i>C. clausenii</i>	None	0	All	63	100
<i>C. tropicalis</i>	1, 2, 3, 10, 15, 17, 21, 27, 44, 62	10	All other antigens	53	84.1
<i>C. parapsilosis</i>	1, 2, 3, 6, 7, 10, 12, 13, 15, 16, 17, 21, 22, 27, 28, 38, 44, 56, 61, 62	20	All other antigens	43	68.2
<i>C. sake</i>	1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 15, 17, 20, 21, 25, 26, 27, 29, 30, 34, 35, 38, 42, 43, 44, 53, 54, 55, 61, 62	31	All other antigens	32	50.7
<i>C. guilliermondii</i>	1, 2, 3, 4, 6, 7, 8, 10, 12, 13, 15, 16, 17, 18, 20, 21, 25, 26, 27, 29, 30, 34, 35, 36, 37, 38, 42, 44, 51, 53, 54, 55, 61, 62	34	All other antigens	29	46
<i>C. pseudotropicalis</i>	All other antigens	45	9, 13, 14, 22, 24, 28, 31, 32, 37, 38, 41, 42, 43, 45, 49, 55, 57, 59	18	28.5
<i>C. krusei</i>	All other antigens	43	9, 13, 14, 22, 24, 28, 29, 31, 32, 37, 38, 41, 42, 43, 45, 49, 55, 56, 59, 63	20	31.7

FIG. 5. Comparison between the G+C contents (gc%) and the similarity coefficients (sc) of yeasts in the *C. tropicalis* system. The similarity coefficients were calculated from the numbers of proteins shared by *C. tropicalis* and 10 other yeast species and are expressed as percentages. tr, *C. tropicalis*; al, *C. albicans*; st, *C. stellatoidea*; cl, *C. clausenii*; pa, *C. parapsilosis*; sa, *C. sake*; gu, *C. guilliermondii*; kr, *C. krusei*; ps, *C. pseudotropicalis*; ut, *C. utilis*; ze, *C. zeylanoides*.

since *C. tropicalis* and *C. albicans* shared about 50 to 70% of their antigens with these species. Group 3 included *C. tropicalis*, *C. albicans*, and related species (*C. clausenii* and *C. stellatoidea*), and close relationships were found in each system (Tables 2 and 3). In both systems, some individual antigens cross-reacted totally or partially with the corresponding antigens from all species; these antigens may be common to many yeast species. Other individual antigens were common to only a group of species, and some antigens did not cross-react with any antigen from the species tested and seemed to be species specific (Table 4).

Cross-reactions among *C. albicans*, *C. tropicalis*, and other *Candida* species have been studied by several workers. In a study by Axelsen (3) in which he used CLIE, the degrees of cross-reaction between *C. albicans* and different species agreed with our results, except the results for *C. guilliermondii*. Other strains of *C. guilliermondii* are being studied to elucidate this discrepancy. In two other studies, in which IEA was used (7, 25), the degrees of cross-reaction with *C. albicans* and *C. tropicalis* sera were much lower. These differences could have several origins. IEA is much less sensitive than QIE; in addition, IEA compared 13 (7) or 16 (25) fungal antigens, whereas more than 60 antigens were compared by QIE. Thus, the basis for protein comparison is different in the two techniques; in IEA, similar proteins from

different antigenic mixtures are identified only by their positions. CLIE allows direct observation of identity reactions just as the Ouchterlony technique does, but it also offers more information than the Ouchterlony method. Thus, it appears clear that among all gel precipitation techniques, QIE is the most useful technique for antigenic comparisons of complex antigenic mixtures. Other criteria that also have been found to be useful for species identification include agglutination and adsorption, proton magnetic resonance spectroscopic methods, and the structure of the coenzyme Q system. Although these methods also pointed out heterogeneity among numerous yeast genera, mainly in the genus *Candida*, they allowed only scant comparisons among the 11 species tested; 7 of these species had the same coenzyme Q type, and 5 had similar proton magnetic resonance spectra (31, 49). *C. albicans*, *C. tropicalis*, and *C. sake* could not be distinguished by agglutination-adsorption experiments (41).

The strains which we investigated had G+C contents of 34.9 to 57.5 mol%. Some species with almost identical G+C contents such as *C. tropicalis* and *C. stellatoidea* or *C. parapsilosis* and *C. pseudotropicalis* (24, 33), appeared to be clearly different when CLIE and the *C. tropicalis* or *C. albicans* system were used. However, it must be emphasized that species which are very different from the species that are used for antiserum preparation cannot be compared among themselves; four species (*C. krusei*, *C. pseudotropicalis*, *C. utilis*, and *C. zeylanoides*) with very different G+C contents, different coenzyme Q systems, and perfect states belonging to various genera had identical similarity coefficients (Fig. 5). Corresponding antisera should be prepared for the study of these species. Such antisera are under development in our laboratory; we are also performing immunological studies with antigens obtained from several reference strains for each species.

DNA-DNA reassociation is a useful technique that allows strain comparisons at the species level, as has been done for *C. tropicalis* (24). QIE results have suggested close affinity between species that show no relatedness in DNA-DNA homology studies (for example, *C. tropicalis* and *C. stellatoidea*) (1, 4). Van der Walt and Johannsen (44) and Price et al. (29) showed that interbreeding *Kluyveromyces* species had low levels of sequence similarity. Thus, DNA reannealing experiments can detect only close relationships among the species of one genus. Another approach, immunological comparison of enzymes, was proposed by Lachance and Phaff (22) (i.e., β -glucanases in the genus *Kluyveromyces*). QIE allows simultaneous study of more than 50 proteins without previous purification. Many of these proteins are probably enzymes. Tran Van Ky et al. (38) found 14 enzymatic activities in 17 precipitates obtained with *C. albicans* antigen by using immunoelectrophoresis. Since our antigens were prepared in a similar way, most of the antigens compared in our study could be enzymes. QIE seems to be capable of elucidating relationships among the species in a genus. These techniques could allow selection of specific antigens for each species or group of species, which could be used for rapid identification of medically important yeasts.

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TABLE 4. Antigens in the *C. tropicalis* system^a

Antigens common to:	Antigen no.
<i>C. tropicalis</i> , <i>C. albicans</i> , <i>C. stellatoidea</i> , and <i>C. clausenii</i>	1, 2, 24, 28, 52, 62, 64
<i>C. tropicalis</i> , <i>C. albicans</i> , <i>C. stellatoidea</i> , <i>C. clausenii</i> , and <i>C. parapsilosis</i>	22, 29, 35
<i>C. tropicalis</i> , <i>C. albicans</i> , <i>C. stellatoidea</i> , <i>C. clausenii</i> , <i>C. parapsilosis</i> , and <i>C. guilliermondii</i>	12, 20, 27
All 11 species	8, 11, 13, 14, 19

^a In addition, the following antigens were non-cross-reactive: 3, 4, 6, 9, 10, 18, 23, 41, 45, 46, 48, and 55.

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