Deoxyribonucleic Acid Homology in Yeasts. Genetic Relatedness within the Genus *Candida*

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SUMMARY

The DNA-RNA hybrid technique of Gillespie & Spiegelman (1965) was used to measure the genetic relatedness among ten different species within the genus *Candida* and among three of these species and their counterparts within perfect genera. Close relationships were found between *Candida albicans* (guanine + cytosine content (%GC), 35.1), *C. clausenii* (%GC, 34.9) and *C. stellatoidea* (%GC, 35.7), which showed a relative homology of 63 to 73 %, and between *C. brumptii* (%GC, 54.1) and *C. catenulata* (%GC, 54.5), relative homology 75 %. Very close relationships were seen between the three pairs of perfect and imperfect counterparts, *C. pelliculosa* (%GC, 36.8) and *Hansenula anomala* (%GC, 36.6), relative homology 81 %, *C. melinii* (%GC, 40.9) and *H. wingei* (%GC, 41.2), relative homology 97 %, and *C. pseudotropicalis* (%GC, 41.3) and *Kluyveromyces fragilis* (%GC, 41.6), relative homology 92 %. Some degree of homology (32 %) was also found between *C. pelliculosa* and *C. pseudotropicalis*. The relatedness between *C. albicans* and the seven other *Candida* species (%GC, 36.6 to 57.6) examined was low, relative homology 4 to 13 %. This also applies to *C. tropicalis* (%GC, 34.9).

INTRODUCTION

The use of nucleic acid homology techniques has proved valuable in determining genetic relatedness among micro-organisms including various bacteria (Weissman, Reich, Somerson & Cole, 1966; De Ley, Park, Tijtgat & van Ermengem, 1966) and mycoplasmas (Somerson *et al.* 1966. One of the methods for determining DNA-RNA hybrid formation is the technique described by Gillespie & Spiegelman (1965).

In this paper we present an adaptation of this nucleic acid homology method to the study of genetic relatedness among yeasts. The results are presented as percentage binding in heterologous reactions relative to that of the homologous reactions, and for some reactions also as relatedness values with confidence limits for indistinguishable DNA preparations. The concept 'relatedness value', which is defined as the product of the heterologous DNA-RNA reaction yields divided by the product of the homologous reaction yields in the reciprocal reactions between two nucleic acids, is introduced to allow the application of a two way analysis of variance (Alling, 1967). This statistical method is valid only when comparisons are being made between reactions having the same reaction rate constant. This requirement will probably demand for example an alike GC content and genome size for the compared DNAs. The method is used thus in this work only when discriminations between closely related yeast species are required. The yeasts studied were ten different species selected

from the genus *Candida*. Previous study of the DNA base composition suggested considerable genetic heterogeneity among many of the species belonging to this genus (Stenderup & Leth Bak, 1968). The expected heterogeneity in genetic relatedness among species with different DNA base compositions was confirmed by the nucleic acid homology technique. This heterogeneity was further elucidated by the fact that three of the *Candida* species examined were found to be indistinguishable from their suggested perfect forms in the genera *Hansenula* and *Kluyveromyces*. The method also allowed differentiation of species which, on the basis of biological properties and base composition, were suspected to be very closely related.

METHODS

Organisms. The name and source of each yeast species investigated together with the average per cent GC (guanine + cytosine) are listed in Table 1. All species were reclassified according to Lodder & Kreger-van Rij (1952).

Table 1. *Source, DNA base composition and % relative homology* of the yeast species investigated*

Species	Source	% GC	% Relative homology			
			<i>C. albicans</i>	<i>C. clausenii</i>	<i>C. stellatoidea</i>	
<i>Candida albicans</i>	CBS† 2712	35.1‡	—	—	—	
<i>C. clausenii</i>	CSAV§ 29-31-1	34.9‡	63%	—	—	
<i>C. stellatoidea</i>	CBS 1905	35.7‡	73%	73%	—	
<i>C. tropicalis</i>	CBS 120/5	34.9‡	8%	7%	9%	
			<i>C. albicans</i>	<i>C. pelliculosa</i>	<i>C. melinii</i>	<i>C. pseudotropicalis</i>
<i>C. pelliculosa</i>	CBS 605	36.8‡	11%	—	—	—
<i>Hansenula anomala</i>	CBS 110	36.6	12%	81%	—	—
<i>C. melinii</i>	CBS 601	40.9‡	7%	15%	—	—
<i>Hansenula wingei</i>	CBS 2431	41.2	4%	—	97%	—
<i>C. pseudotropicalis</i>	CBS 607	41.3‡	8%	32%	11%	—
<i>Kluyveromyces fragilis</i>	CBS 397	41.6	9%	—	—	92%
			<i>C. albicans</i>	<i>C. brumptii</i>	<i>C. catenulata</i>	
<i>C. brumptii</i>	CBS 564	54.1‡	10%	—	—	
<i>C. catenulata</i>	CBS 565	54.5‡	13%	75%	—	
<i>C. zeylanoides</i>	CBS 619	57.6‡	11%	17%	22%	

* The % relative homology values are calculated from the results in Tables 2-7, as the average of the relative bindings in reciprocal heterologous reactions.

† Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

‡ Stenderup & Leth Bak (1968).

§ Czechoslovak Collection of Micro-Organisms.

|| Calculated from melting temperature (T_m) in SSC.

Medium and cultivation techniques. The yeasts were cultivated in 10-litre fermentors (Biotec, Stockholm, Sweden). The liquid medium contained (% w/v): glucose 1.0, peptone (Orthana) 1.0, yeast extract (Oxoid) 0.5 and K₂HPO₄ 0.1. The pH was adjusted to 5.6 and maintained at this level by automatic titration. The incubation temperature was 30°, and the cultures were abundantly aerated. After growth for one day,

the yeasts were harvested by centrifugation in a Sorvall continuous flow system at 4°, 10,000 g, 500 ml./min. Tests for bacterial contamination were made by direct microscopic examination and plating on blood agar immediately before centrifugation.

Preparation of DNA. The cells were washed three times in cold saline EDTA (0.15 M-NaCl + 0.1 M-ethylenediaminetetraacetate (EDTA)), at pH 8.0 and suspended in an equal volume of a buffer containing 60% sucrose, 0.5 mM-MgCl₂, 0.02 M-K₂HPO₄ at pH 7.5 and 0.02% Triton X-100. To 20 ml. of this thick suspension were added 50 g. of glass beads (0.45 mm.) and the mixture treated in a Braun homogeniser (Braun, Melsungen, Germany) for 1 min. at maximum speed. The apparatus was cooled with liquid CO₂. The broken cell suspension was diluted with the 60% sucrose buffer and centrifuged in rotor type 19 of the Spinco model L-2 preparative ultracentrifuge at 18,000 rev./min. for 2 hr. DNA was isolated from the sediment by the method of Marmur (1961) with the following modifications: Lysis with sodium dodecyl sulphate was carried out for 10 min. at 37°, after which intact cells and cell debris were sedimented and the supernatant heated at 60° for 10 min. After two deproteinizations with chloroform-isoamyl alcohol, the nucleic acid solution was treated with pronase (Calbiochem) 1 mg./ml. at 60° for 4 hr. All protein was now removed by the chloroform-isoamyl alcohol treatment and RNA digested with a mixture of pancreatic and T1 ribonuclease. To ensure that the final isopropanol precipitate was free of traces of ribonucleases, the extraction procedure was completed by treatment of the DNA with self-digested pronase 50 µg./ml. for 2 hr at 37° (Gillespie & Spiegelman, 1965) and finally two or more extractions of the DNA with water-saturated redistilled phenol. The purified DNA was dissolved in 0.01 × SSC (SSC is 0.15 M-NaCl + 0.015 M-trisodium citrate, pH 7.0) to a concentration of about 2.5 mg./ml. and stored at -20°. The average yield was about 2 mg. and the DNA was found free of RNA and protein. Determination of sedimentation velocities by the band sedimentation method (Bruner & Vinograd, 1965) yielded a molecular weight higher than 10⁷ daltons for the nuclear DNA extracted in this manner. The integrity of the DNA was judged from thermal denaturation curves and from films obtained by ultraviolet photography of DNA spun to equilibrium in a CsCl density gradient in the Spinco model-E analytical ultracentrifuge (Schildkraut, Marmur & Doty, 1962). Whole cell DNA from some of the *Candida* species investigated here contain a minor satellite DNA (Stenderup & Leth Bak, 1968). This satellite DNA may constitute up to 5% of the total DNA and is shown to be of mitochondrial origin (A. Leth Bak, O. Christiansen & A. Stenderup, to be published). Small amounts of this rapidly renaturing DNA (Borst, Kroon & Ruttenberg, 1966) could seriously affect the results of the hybridization studies. Sedimentation of the cell homogenate through the 60% sucrose buffer, containing Mg ions and Triton X-100, was in all instances able to remove all mitochondrial DNA from the DNA isolated from the sedimented nuclei.

Formation and detection of DNA-RNA hybrids. The procedure of Gillespie & Spiegelman (1965) was used. Radioactive complementary RNA was synthesized *in vitro* with each DNA as template. The radioactive RNA was allowed to react with single-stranded DNA immobilized on membrane filters, and the degree of hybridization determined as the radioactivity retained on these filters.

Radioactive RNA production. DNA-dependent RNA polymerase isolated from *Micrococcus lysodeikticus* was obtained from Sigma Chemical Co. Adenosine triphosphate-³H, cytosine triphosphate-³H, guanosine triphosphate-³H and uridine tri-

phosphate- ^3H were obtained from Schwartz Bio Research Inc. The corresponding unlabelled ribonucleoside triphosphates were obtained from Sigma Chemical Co. A carrier RNA for the small amounts of yeast RNA synthesized was obtained by extraction of RNA from *Escherichia coli* B by the method of Scherrer & Darnell (1962). The conditions for synthesizing radioactive RNA were those described by Nakamoto, Fox & Weiss (1964). The reaction mixture contained 30 μg . of primer DNA, 20 $\text{m}\mu\text{m} \equiv 10^{-9}$ moles of each of the labelled and unlabelled ribonucleoside triphosphates, 12.5 μmoles of tris (hydroxymethyl) aminomethane-HCl (pH 7.5), 0.20 μmoles spermidine phosphate, 0.30 μmoles of MnCl_2 and 20 units of RNA polymerase. The total volume was about 0.125 ml. The reaction mixture was incubated at 37° for 30 min., after which 0.010 ml. 0.1 M- MgCl_2 , 0.150 ml. of carrier RNA solution (containing 1.5 mg. *Escherichia coli* B RNA), and 0.050 ml. of a deoxyribonuclease solution containing 50 μg DNase I (Worthington Biochemical Corp.) were added. Incubation was continued for an additional 20 min. and the RNA isolated by the hot phenol, sodium dodecyl sulphate procedure of Scherrer & Darnell (1962). The final precipitate of radioactive RNA with the carrier RNA was dissolved in 0.5 to 1.0 ml. of $2 \times \text{SSC} + 0.05\%$ SDS (sodium dodecyl sulphate) and stored at -20° . An assay was done for each RNA synthesized. RNA solution (0.005 ml.) was mixed with 3 ml. 5% trichloroacetic acid and filtered through a membrane filter (millipore, HAWP), washed with 20 ml. 5% trichloroacetic acid and assayed for radioactivity as described below.

Gillespie and Spiegelman procedure for formation of DNA-RNA hybrids. 10 μg . of DNA in 0.5 ml. $0.01 \times \text{SSC}$ was heated at 100° for 10 min., quenched in ice and made to 5 ml. with cold $2 \times \text{SSC}$. The denaturated DNA was filtered through a membrane filter (Millipore HAWP, 20 mm.) and the filter washed with 100 ml. cold $2 \times \text{SSC}$. The filter was dried at 25° overnight, and then heated to 80° for 2 hr. Hybridizations took place in glass scintillation vials. The filters were placed flat on the bottom of these vials and overlaid with the radioactive RNA in 0.5 ml. $2 \times \text{SSC}$. Incubation was carried out for 16 hr at 73° . The filters were then washed with 73° warm $2 \times \text{SSC}$ and treated with ribonuclease as described (Gillespie & Spiegelman, 1965).

Lay-out of experiments, counting of radioactivity and statistical analysis of results. Equal amounts of radioactive RNA were added to three or four DNA filters for each DNA to be compared. Nonspecific binding of radioactivity was determined by adding the same amount of RNA to four blank filters in each experiment. The nonspecific binding was generally lower than 0.05% of input radioactivity. The filters were assayed for radioactivity in a liquid scintillation counter (Packard Tri-Carb scintillation spectrometer, model 3320). They were counted in plastic vials and a toluene scintillation solution containing 0.03% POPOP and 0.4% PPO (Packard). The efficiency of counting was 19% with a background of 22 c.p.m. The statistical counting error was less than $\pm 1\%$. All individual counts were corrected for nonspecific binding by subtraction of average blank. The statistical methods used are described in detail by Alling (1967) and exemplified in the paper of Weissman *et al.* (1966).

RESULTS

Relationships among Candida albicans, C. clausenii, C. stellatoidea and C. tropicalis. Table 2A gives the mean counts for reactions between DNA filters containing single-stranded DNA from *C. albicans*, *C. clausenii*, *C. stellatoidea* and *C. tropicalis*,

respectively, and radioactive complementary RNA synthesized with DNA from each of these organisms as template. The mutual DNA-RNA reaction yields between *C. albicans*, *C. claussenii* and *C. stellatoidea* were high and of the same order of magnitude. By contrast, surprisingly low reaction yields were found between nucleic acids from these species and nucleic acids from *C. tropicalis*. This result was confirmed by experiments with two different DNA preparations from the same strains of *C. albicans*

Table 2. *Relationships among Candida albicans, C. claussenii, C. stellatoidea and C. tropicalis*

Source of template for radioactive RNA	Source of DNA tested			
	<i>C. albicans</i>	<i>C. claussenii</i>	<i>C. stellatoidea</i>	<i>C. tropicalis</i>
<i>C. albicans</i>	1,726 (100)	1,188 (69)	1,391 (81)	147 (9)
<i>C. claussenii</i>	1,479 (56)	2,647 (100)	2,089 (79)	267 (10)
<i>C. stellatoidea</i>	985 (65)	993 (66)	1,515 (100)	188 (12)
<i>C. tropicalis</i>	135 (6)	82 (4)	134 (6)	2,264 (100)

B. Relatedness values for *C. albicans*, *C. claussenii* and *C. stellatoidea*†

	<i>C. albicans</i>	<i>C. claussenii</i>		
<i>C. claussenii</i>	0.38	—	—	—
<i>C. stellatoidea</i>	0.51	0.51	—	—

* Different amounts of RNA were employed for each separate experiment (190,000 to 265,000 c.p.m.), making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

† See Methods for method of calculation. The 99% confidence interval for indistinguishable DNA preparations was 0.54 to 1.87.

and *C. tropicalis*. The experiment was also repeated with another strain of *C. tropicalis* (CBS 180/88). The DNA from this strain and the strain of *C. tropicalis* in Table 1 were found to be indistinguishable. Whether the small counts in the *C. tropicalis* heterologous reactions were significant was tested in experiments in which filters containing DNA isolated from *Escherichia coli* B were reacted with radioactive RNA synthesized with DNA from different yeast species as templates. The amounts of radioactivity retained in these experiments did not differ significantly from the non-specific bindings to blank filters. The relative homology (average per cent binding in heterologous reactions) between *C. albicans*, *C. claussenii* and *C. stellatoidea* is 63 to 73% (Table 1), indicating that these species must be closely related. The three species are, however, distinguishable at the 99% confidence limit as seen from the relatedness statistics (Table 2B) (relatedness values from 0.38 to 0.51, lower 99% confidence limit 0.54); *C. tropicalis* is only remotely related to any of the species *C. albicans*, *C. claussenii* and *C. stellatoidea* (7 to 9% relative binding (Table 1)).

Relationships among Candida albicans, C. melinii, C. pelliculosa and C. pseudotropicalis. Mean counts for the DNA-RNA reactions for nucleic acids from *C. albicans*, *C. melinii*, *C. pelliculosa* and *C. pseudotropicalis* and the relative homology between these species are seen in Table 3 and Table 1, respectively. In the reactions between *C. pelliculosa* and *C. pseudotropicalis* an average of 32% relative binding was found in the heterologous reactions. The relative homology for the other combinations in this series was low (7 to 15%).

Relationships among Candida albicans, C. brumptii, C. catenulata and C. zeylanoides. Single-stranded DNA on filters and radioactive RNA synthesized with DNA from each of *C. albicans*, *C. brumptii*, *C. catenulata* and *C. zeylanoides* as template were reacted under conditions for DNA-RNA hybrid formation. The reaction yields for these reactions (Table 4A) and the calculated relative homology (Table 1) show that *C. brumptii* and *C. catenulata* are closely related (75%). These two species were distinguishable (relatedness value 0.56 lower 99% confidence limit 0.57) (Table 4B). *C. zeylanoides* showed a somewhat higher relationship to *C. brumptii* and *C. catenulata* (17% and 22%), than did any of the three species to *C. albicans* (10 to 13%).

Table 3. *Relationships among Candida albicans, C. melinii, C. pelliculosa and C. pseudotropicalis*

Mean counts of triplicate assays of radioactivity retained on filters after reaction between DNA and radioactive RNA synthesized with each DNA as template*

Source of template for radioactive RNA	Source of DNA tested			
	<i>C. albicans</i>	<i>C. melinii</i>	<i>C. pelliculosa</i>	<i>C. pseudo- tropicalis</i>
<i>C. albicans</i>	2,710 (100)	211 (8)	318 (12)	268 (10)
<i>C. melinii</i>	254 (5)	4,918 (100)	577 (12)	532 (11)
<i>C. pelliculosa</i>	96 (9)	185 (18)	1,042 (100)	358 (34)
<i>C. pseudotropicalis</i>	158 (5)	310 (10)	957 (30)	3,180 (100)

* Different amounts of RNA were employed for each separate experiment (250,000 to 400,000 c.p.m.) making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

Table 4. *Relationships among Candida albicans, C. brumptii, C. catenulata and C. zeylanoides*

A. Mean counts of triplicate assays of radioactivity retained on filters after reaction between DNA and radioactive RNA synthesized with each DNA as template*

Source of template for radioactive RNA	Source of DNA tested			
	<i>C. albicans</i>	<i>C. brumptii</i>	<i>C. catenulata</i>	<i>C. zeylanoides</i>
<i>C. albicans</i>	973 (100)	41 (4)	48 (5)	103 (11)
<i>C. brumptii</i>	172 (15)	1,112 (100)	767 (69)	302 (27)
<i>C. catenulata</i>	280 (20)	1,144 (81)	1,419 (100)	498 (35)
<i>C. zeylanoides</i>	240 (10)	158 (7)	135 (6)	2,291 (100)

B. Relatedness value for *C. brumptii* and *C. catenulata*.†

	<i>C. catenulata</i>			
<i>C. brumptii</i>	—	—	0.56	—

* Different amounts of RNA were employed for each separate experiment (175,000 to 225,000 c.p.m.), making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

† The 99% confidence interval for indistinguishable DNA preparations was 0.57 to 1.76.

Relationships among Candida albicans, C. pelliculosa and Hansenula anomala. Reaction yields (Table 5A) and relative homology (81%) (Table 1) show a close relationship between *C. pelliculosa* and *H. anomala*. The two species could not be distinguished by the accuracy obtained in this experiment (relatedness value 0.66, lower 99%

confidence limit 0.53) (Table 5B). The relative homology between *C. pelliculosa* and *C. albicans* (14%) is close to that found earlier (11%), and close to that found for *H. anomala* and *C. albicans* (12%) (Table 1).

Table 5. Relationships among *Candida albicans*, *C. pelliculosa* and *Hansenula anomala*

Source of template for radioactive RNA	Source of DNA tested		
	<i>C. albicans</i>	<i>C. pelliculosa</i>	<i>H. anomala</i>
<i>C. albicans</i>	2,797 (100)	394 (14)	491 (18)
<i>C. pelliculosa</i>	375 (13)	2,994 (100)	2,650 (89)
<i>H. anomala</i>	162 (5)	2,196 (73)	3,014 (100)

B. Relatedness value for *C. pelliculosa* and *H. anomala*†

<i>C. pelliculosa</i>	—	—	<i>H. anomala</i> 0.66
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* Different amounts of RNA were employed for each separate experiment (190,000 to 265,000 c.p.m.), making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

† The 99% confidence interval for indistinguishable DNA preparations was 0.53 to 1.88.

Table 6. Relationships among *Candida albicans*, *C. pseudotropicalis* and *Kluyveromyces fragilis*

Source of template for radioactive RNA	Source of DNA tested		
	<i>C. albicans</i>	<i>C. pseudotropicalis</i>	<i>Kl. fragilis</i>
<i>C. albicans</i>	2,424 (100)	322 (13)	320 (13)
<i>C. pseudotropicalis</i>	190 (7)	2,537 (100)	2,554 (101)
<i>Kl. fragilis</i>	368 (5)	5,794 (82)	7,101 (100)

B. Relatedness value for *C. pseudotropicalis* and *Kl. fragilis*†

<i>C. pseudotropicalis</i>	—	—	<i>Kl. fragilis</i> 0.81
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* Different amounts of RNA were employed for each separate experiment (285,000 to 304,000 c.p.m.), making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

† The 99% confidence interval for indistinguishable DNA preparations was 0.70 to 1.43.

Relationships among *Candida albicans*, *C. pseudotropicalis* and *Kluyveromyces fragilis*. The results in Table 6A and Table 1 indicate a close relationship between *C. pseudotropicalis* and *Kl. fragilis*, relative homology (92%). The relatedness value for these two species is 0.81 and the lower 99% confidence limit for indistinguishable DNAs 0.70, showing that the two species are not distinguishable. The relative bindings in the reactions between *Kl. fragilis* and *C. albicans*, average (9%), are close to those for *C. pseudotropicalis* and *C. albicans* (10%), and are a reproduction of the value for these two species listed in Table 1 (8%).

Relationships among *Candida albicans*, *C. melinii* and *Hansenula wingei*. Also *C.*

melinii and *H. wingei* (relative homology 97%) are seen to be very closely related (Table 7A and Table 1). The relatedness value between these two species is 0.92, lower 99% confidence limit 0.62, showing that the species are not to be distinguished, in this experiment (Table 7B). The relative homology between *C. albicans* and *C. melinii* (5%) and between *C. albicans* and *H. wingei* (4%) is very close to the value for *C. albicans* and *C. melinii* (7%) found in another experiment (Table 1).

Table 7. Relationships among *Candida albicans*, *C. melinii* and *Hansenula wingei*

A. Mean counts of quadruplicate assays of radioactivity retained on filters after reaction between DNA and radioactive RNA synthesized with each DNA as template*

Source of template for radioactive RNA	Source of DNA tested		
	<i>C. albicans</i>	<i>C. melinii</i>	<i>H. wingei</i>
<i>C. albicans</i>	3,063 (100)	9 (0)	76 (2)
<i>C. melinii</i>	687 (9)	7,985 (100)	7,504 (94)
<i>H. wingei</i>	57 (6)	931 (100)	929 (100)

B. Relatedness value for *C. melinii* and *H. wingei*†

<i>C. melinii</i>	—	—	<i>H. wingei</i> 0.92
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† Different amounts of RNA were employed for each separate experiment (200,000 to 300,000 c.p.m.), making comparisons possible only within rows. Values in parentheses are per cent bindings in heterologous reactions relative to the bindings in homologous reactions taken as 100%.

* The 99% confidence interval for indistinguishable DNA preparations was 0.62 to 1.61.

DISCUSSION

The genus *Candida* has previously been considered to be a heterogenous group composed of members with different morphological, biochemical and ecological characteristics. This heterogeneity is reflected in a great variation in DNA base composition among several of the different members of the group. (Stenderup & Leth Bak, 1968). The nucleic acid homology technique confirms this suggested heterogeneity in genetic relatedness for several of the *Candida* species investigated in this study, close relationships being observed only between *C. albicans*, *C. claussenii*, and *C. stellatoidea* and between *C. brumptii* and *C. catenulata*.

The great genetic relatedness between *Candida albicans*, *C. claussenii* and *C. stellatoidea* is in agreement with the conventional classification. Although distinguishable, similarity in morphology, antigenic composition and biochemical reactions is seen in these species. The GC content of their DNA is also very similar (Table 1).

Although morphologically different from *Candida albicans*, *C. claussenii* and *C. stellatoidea*, the serology, biochemical reactions and DNA base composition of *C. tropicalis* might have suggested a closer relationship to these species. The close relationship between *C. brumptii* and *C. catenulata* might have been expected on the basis of their characteristic thermal denaturation curves (Stenderup & Leth Bak, 1968).

The genetic similarity was in all other cases surprisingly low. This low genetic relatedness might in some cases be explained by the fact that a number of *Candida* species are considered imperfect counterparts of species within various perfect genera. Thus *C. pelliculosa*, *C. pseudotropicalis* and *C. melinii* are considered the imperfect

stages of *Hansenula anomala*, *Kluyveromyces fragilis* and *H. wingei* respectively (Wickerham & Burton, 1954; Diddens & Lodder, 1938; L. J. Wickerham, personal communication). The results of the DNA-RNA hybrid experiments indicating that the DNA from these pairs are indistinguishable, give strong support for the transfer of these *Candida* species to the respective perfect genera.

It appears from Table 1 that all the very closely related species also have nearly identical GC content of their DNA, the only exceptions are *Candida pelliculosa*-*C. pseudotropicalis*, which show some 30% relative bindings despite of a difference in GC content of about 4.0%.

The low reactions (5 to 10%) between species which actually may belong to different genera might indicate some degree of genetic relatedness among all yeasts. If this be so, it might indicate a common phylogeny of these organisms. Part of these common base sequences might be explained by the findings that ribosomal RNA cistrons seem to have been highly conserved among fungi (Bicknell & Douglas, 1969) and that the genes coding for the ribosomal RNA occupy about 2.5% of the whole genome in yeast (Schweizer, MacKecknie & Halvorson, 1969).

The relative homology ideally constitute a quantitative measure of the fractional similarity between genomes. However, there are several reservations to be made. The degree of homology measured is not independent of the incubation temperature (Johnson & Ordal, 1968; Kingsbury, Fanning, Johnson & Brenner, 1969). A hybridization temperature of 73° (which is $T_m - 25^\circ$ in $2 \times$ SSC for *Candida zeylanoides*) have been chosen for all hybridizations in this study. This temperature may be unnecessarily high for the reactions with the DNAs of the lower GC content. However we believe that higher hybridization temperatures (although the bindings may be somewhat lower) will only heighten the accuracy and discrimination in these experiments.

The possibility may also exist that the kind of RNA polymerase (from *Micrococcus lysodeikticus*) used in these experiments would not synthesize RNA complementary to both DNA strands in equal amounts or would not copy all regions on the yeast primer DNA with equal frequency. Such asymmetric and selective transcription has already been shown for the *in vitro* transcription of intact native λ phage DNA (Cohen & Hurwitz, 1967) and T4 phage DNA (Geiduschek, Snyder, Colvill & Sarnat, 1966) by *Escherichia coli* RNA polymerase. No such selective copying has been reported for the *in vitro* transcription of bacterial DNA. Dubnau, Smith, Morell & Marmur (1965) have in DNA-RNA hybridization studies found the same degree of homology between *Bacillus subtilis* and several other members of the genus *Bacillus*, using either 'pulse labelled' RNA or RNA synthesized *in vitro*.

Generally, the results reported here prove the applicability and significance of the nucleic acid homology technique as a tool in a rational taxonomy of yeasts. The results were found reproducible, and the accuracy of the determinations is comparable to that of others using similar methods for the detection of nucleic acid homology among bacteria and mycoplasmas. (Somerson *et al.* 1966; Weissman *et al.* 1966).

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