Deoxyribonucleic Acid Base Composition of Species in the Yeast Genus *Kluyveromyces* van der Walt emend. van der Walt

ALESSANDRO MARTINI,[1] HERMAN J. PHAFF, AND STEPHEN A. DOUGLASS

Department of Food Science and Technology, University of California, Davis, California 95616

The deoxyribonucleic acid base composition (percent guanine + cytosine [GC]) was determined for 29 strains, representing 18 species of the genus *Kluyveromyces*. It was concluded that on the basis of GC content (47.4%) and other properties *K. veronae* occupies an uncertain position in the genus *Kluyveromyces*. The GC content of the remaining 17 species ranged from 35.3 to 43.4%, and three groups of species were recognized. The GC content of the first ranged from 35.3 to 38.0%; that of the second group from 39.5 to 41.7%; that of the third group from 42.4 to 43.4%. Several species revealed a nearly identical GC content. The GC contents do not correspond in all instances with the five groups of species proposed by van der Walt.

Extensive studies have been conducted on the significance of deoxyribonucleic acid (DNA) base composition (moles percent of guanine + cytosine [%GC]) in the taxonomy and systematics of bacteria (7, 13, 15). During the past several years a number of contributions on the base composition of fungal and yeast DNA and its taxonomic implications have appeared (4, 17–21, 28). We present the results of a comprehensive study conducted on the 18 species included by van der Walt in the genus *Kluyveromyces* (33).

This genus was established (30) for a newly discovered budding, fermentative yeast species, *Kluyveromyces polysporus*, which forms large, multispored asci containing as many as seventy or more reniform to long oval spores. A second species, *K. africanus*, producing up to 16 spores per ascus, was also described (31). Characteristic of the asci of these two species was their early rupture at maturity, releasing the spores.

In 1965, following a series of controversial approaches to the taxonomy of the species forming reniform spores (2, 9, 10, 22, 34), van der Walt (32) emended the diagnosis of the genus *Kluyveromyces* on the assumption that multispored species constitute only a separate line of development among the species which normally form only four spores. He included in

Kluyveromyces all of the species formerly referred to as *Fabospora* and *Zygofabospora* Kudriavzev, *Dekkeromyces* Wickerham et Burton (nomen nudum) and *Guilliermondella* Boidin et al. Only species whose asci rupture at maturity were included; the shape of the ascospores was considered less important because the genus now includes organisms whose spore morphology may vary from crescentiform, reniform, oblong with obtuse ends, prolate-ellipsoidal, or spheroidal.

The controversy again came into focus when Santa Maria and Sanchez (25) proposed to apply the nomen nudum *Dekkeromyces* to those *Kluyveromyces* species unable to form multispored asci (*K. dobzhanskii*, *K. lactis*, *K. drosophilarum*, *K. fragilis*, *K. phaseolosporus*, and *K. wickerhamii*). They proposed *Dekkeromyces lactis* as the type of the genus.

The lack of agreement surrounding the genus suggested to us the use of the molecular approach to the taxonomy of *Kluyveromyces*, based on DNA base composition as well as on DNA-DNA homologies.

This paper reports the results of the study on DNA base composition.

MATERIALS AND METHODS

Organisms. The cultures are listed in Table 1 together with their original designations and sources. Type cultures were studied of all species accepted in the genus *Kluyveromyces* by van der Walt (33). Three additional strains of *K. lactis* were included.

TABLE 1. *Yeast strains used*

Name	Culture number and source[a]	
Kluyveromyces aestuarii (Fell) van der Walt 1961	UCD 61-29	CBS 4438[b]
K. africanus van der Walt 1956	UCD 57-16	CBS 2517[b]
K. bulgaricus (Santa Maria) van der Walt 1956	UCD 71-13	CBS 2762[b]
K. cicerisporus van der Walt, Nel et van Kerken 1966	UCD 71-14	CBS 4857[b]
K. delphensis (van der Walt et Tscheuschner) van der Walt 1956	UCD 56-2	CBS 2170[b]
K. dobzhanskii (Shehata, Mrak et Phaff) van der Walt 1955	UCD 50-45	CBS 2104[b]
K. drosophilarum (Shehata, Mrak et Phaff) van der Walt 1955	UCD 51-130	CBS 2105[b]
K. drosophilarum	UCD 71-45[c]	Meyers et al. F.S.T. 89
K. drosophilarum	UCD 71-50[c]	Meyers et al. F.S.T. 255
K. fragilis (Jörgensen) van der Walt 1909	UCD 71-58	CBS 397[b]
K. fragilis	UCD 61-293[d]	—
K. lactis (Dombrowski) van der Walt 1910	UCD 71-59	CBS 683[b]
K. lactis	UCD C-21[e]	—
K. lactis	UCD 71-73	CBS 2621
K. lactis (Dombrowski) van der Walt (synon. *Sacch. sociasii* Ramirez)	UCD 70-2	CBS 4574
K. lodderi (van der Walt et Tscheuschner) van der Walt 1957	UCD 70-3	CBS 2757[b]
K. marxianus (Hansen) van der Walt 1888	UCD 55-82	CBS 712[b]
K. phaffii (van der Walt) van der Walt 1963	UCD 70-5	CBS 4417[b]
K. phaseolosporus (Shehata, Mrak et Phaff) van der Walt 1955	UCD 50-80	CBS 2103[b]
K. polysporus van der Walt 1956	UCD 57-17	CBS 2163[b]
K. vanudenii (van der Walt et Nel) van der Walt 1963	UCD 70-4	CBS 4372[b]
K. veronae (Lodder et Kreger-van Rij) van der Walt 1952	UCD 55-41	CBS 2803[b]
K. veronae	UCD 61-518[f]	
K. veronae	UCD 68-118[g]	
K. wickerhamii (Phaff, Miller et Shifrine) van der Walt 1956	UCD 54-210	CBS 2745[b]
K. wickerhamii	UCD 61-346[h]	
K. wickerhamii	UCD 67-539[i]	
K. wickerhamii	UCD 68-821C[j]	
K. wikenii van der Walt, Nel et van Kerken 1966	UCD 71-15	CBS 5671[b]

[a] Abbreviations: UCD, Collection of the Department of Food Science and Technology, University of California, Davis; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.

[b] Type culture.

[c] Isolated from spartina grass in the coastal marshlands of Louisiana.

[d] Isolated from *Drosophila melanogaster* in California.

[e] Isolated from unpasteurized sour milk, California.

[f] Isolated from crop contents *Drosophila melanogaster*, Lake Berryessa, California.

[g] Isolated from a prune conveyer belt, Yuba City, California.

[h] Isolated from *Salix* slime exudate, Sacramento River, California.

[i] Isolated from *Acer* slime exudate, Morioka, Japan.

[j] Isolated from *Betula* slime exudate, British Columbia, Canada.

One of these was originally described as *Saccharomyces sociasii* (23) but later considered synonymous with *K. lactis* (33). The study also included an additional, characteristic strain of *K. fragilis*, two of *K. veronae* and three of *K. wickerhamii*.

Because *K. drosophilarum* had been previously isolated only from crop contents of *Drosophila* in California, two strains were included that were recently isolated from spartina grass (*Spartina alterniflora*) growing in the coastal marshlands of Louisiana

where it comprised about 20% of the yeast flora (S. P. Meyers, P. Miles, and D. G. Ahearn, Bacteriol. Proc. G75, 1971).

Growth conditions. The yeasts were grown in YAG (0.5% yeast autolysate [Pfizer-Albimi] and 5.0% glucose; 500 ml of medium in 1-liter Erlenmeyer flasks) on a rotary shaker at room temperature. Late logarithmic-phase cells were harvested by centrifugation and washed three times with a saline solution containing 0.1 M ethylenediaminetetra-

acetate (EDTA).

Extraction of DNA. (i) Lysis was carried out by suspending 40 to 45 g of wet cells in 100 ml of saline-EDTA (0.15 M sodium chloride, 0.1 M EDTA, pH 8.0) containing 2% (w/v) of sodium dodecyl sulfate (SDS) and 1% of mercaptoethanol. The thick suspension was placed in a 3-liter Fernbach flask. A large cotton plug soaked with 50 ml of chloroform was inserted in the neck, and aluminum foil was placed over the plug. The flask was incubated at 37 C for 4 to 20 hr with periodic checks for thickening of the cell suspension. (ii) Isolation and purification of DNA from the viscous cell suspension were performed by a modification of Marmur's procedure (16). In every step DNA was precipitated with 95% ethanol (except for one isopropanol precipitation before dialysis) and separated by spooling around a glass rod. (iii) *K. aestuarii, K. africanus, K. delphensis,* and *K. veronae* did not yield DNA by this method. For these strains extraction was performed by preparing an acetone powder of the freshly harvested yeast, followed by rotary shaking of the dry powder with 4-mm glass beads for approximately 24 hr. The broken cells were then treated with Pronase to destroy the activity of deoxyribonucleases. The DNA was then purified by the normal routine. Full details of this modified method will be published elsewhere.

Good yields of spoolable DNA were obtained by these methods. Selective extraction of parts of the nuclear genome does not appear to be a problem in view of the very good agreement between GC percentages of different strains of the same species and literature values available for some of the species (see Table 2). DNA extracted by the modified Marmur method (16) contained very low levels (from barely detectable to 5%) of mitochondrial DNA (m-DNA), based on scanning distributions of DNA molecules subjected to isopycnic centrifugation in cesium chloride. DNA extracted from acetone powder contained a higher level of m-DNA (up to approximately 12% in the case of *K. delphensis*). The presence of m-DNA does not appear to interfere with the determination of %GC by the melting procedure (14) as seen in Table 2 by the excellent agreement of %GC for *K. delphensis* obtained by the Marmur method (16), the Knittel (8) method (which is not affected by m-DNA), and by buoyant density determination (26). The rather low %GC of the m-DNA in this genus (approximately 25%, based on unpublished observations in our laboratory) is undoubtedly an important reason for its noninterference in measuring %GC of the nuclear DNA.

Determination of the melting point (T_m). The thermal denaturation temperature was determined by the method of Marmur and Doty (14) with a Gilford model 2400 automatic recording spectrophotometer, the samples containing ca. 20 µg of DNA/ml. A standard DNA preparation (*Candida lusitaniae* UCD no. 68-36, T_m = 88.5, representing the mean value of more than 200 determinations) was included in every determination as a control.

In addition to the usual graphical procedure for the determination of the melting point, another technique (8) was used, which is based on the plot-ting of absorbancy values against temperature on normal probability graph paper. The 50 percentile point on the best straight line furnishes the T_m value. As stated above, this method measures only the main DNA species (in this case nuclear).

This procedure also permits a convenient calculation of 2-sigma values (total of mean plus and minus 34% on the graph) which represents (3) twice the standard deviation of the compositional distribution of the nucleotide pairs in the DNA molecules in the sample. Different 2-sigma values reflect a different base distribution in samples having the same T_m value.

Determination of the base composition from buoyant density values. The methods described by Schildkraut et al. (26) and Szybalski (29) were used for density gradient centrifugation of some of the DNA samples. A Spinco model E ultracentrifuge equipped with a scanner and multiplexer unit was used for this work. Saturated stock solution of CsCl in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.5, was mixed with sample DNA, reference *Micrococcus lysodeikticus* DNA, and water to achieve a final density of 1.700 g/cc for all of the runs. Cesium chloride, code 62, was obtained from the Rare Earth Division of American Potash and Chemical Corp., West Chicago, Ill. *M. lysodeikticus* DNA was obtained from Miles Laboratories, Kankakee, Ill.

RESULTS

Isolation of DNA in a highly polymerized form was easily realized for the majority of the strains. Poor yields and lack of spoolable DNA were noted for *K. aestuarii, K. africanus, K. delphensis,* and *K. veronae,* when fresh cells were used. However, by starting with an acetone powder of these species (see Materials and Methods) satisfactory results were obtained.

All of the DNA samples extracted by the modified Marmur technique (16) were characterized by monophasic temperature-absorbancy curves, confirming that by this isolation procedure negligible or at most very minor DNA components of mitochondrial origin contaminated the nuclear DNA. The DNA samples from acetone-precipitated cells, which contain somewhat higher levels of mitochondrial DNA, sometimes showed a slight rise in optical density during melting before the rise in optical density occurred due to melting of the nuclear DNA. In such cases the base line of optical density was taken after the initial rise subsided. The results, based on two procedures for determining T_m values and in several cases on buoyant density measurements, are presented in Table 2, which also lists literature values for base composition of those species for which such values have been published. The hyperchromic shifts of the

DNA samples which were subjected to melting varied between 34 and 40%. Table 3 shows the GC percentages of the various type cultures of the *Kluyveromyces* species in increasing order of their GC content.

DISCUSSION

The GC contents reported in Table 2 of species for which literature values are available agree quite well in most instances. Based on our experience the accuracy of the melting procedure spans a range of about 1% GC, and the buoyant density values are estimated to be accurate to within about 0.5% GC.

Whether or not the somewhat lower GC values of the two Louisiana strains of *K. drosophilarum* (UCD 71-45 and 71-50), obtained

from marine marshland grass, is significant in comparison to the California strain from *Drosophila* (CBS 2105), must await homology studies on the DNA species from these strains.

The original diagnosis of *K. lactis* indicated that maltose was assimilated by strains of this species (12). More recently maltose-negative strains have been isolated and described. Van der Walt (33) has listed this property as variable (+ or −) in the current diagnosis of this species. In our study two maltose-negative strains were included (CBS 2621 and CBS 4574), both of which revealed approximately a 1% lower GC content than the two maltose-positive strains. Here too evaluation of the significance of this relatively small difference in GC content must await DNA homology studies.

TABLE 2. *DNA base composition of 18 species, including strains, of the genus Kluyveromyces*

Organisms	$T_m{}^a$ with standard deviation[b]	%GC	$T_m{}^a$ with standard deviation[c]	%GC	%GC[d]	%GC from the literature[e] (references in parentheses)
K. aestuarii	85.5 ± 0.1	39.5	85.5 ± 0.7	39.5		
K. africanus	84.7 ± 0.3	37.5	84.8 ± 0.3	37.8	38.6	
K. bulgaricus	86.8 ± 0.1	42.6	86.6 ± 0.4	42.2		
K. cicerisporus	87.1 ± 0.3	43.4	87.1 ± 0.3	43.4		
K. delphensis	85.7 ± 0.3	40.0	85.6 ± 0.4	39.6	39.9	
K. dobzhanskii	87.3 ± 0.1	43.9	87.1 ± 0.4	43.4	42.6	42.0 (1); 43.0 (17)
K. drosophilarum CBS 2105	86.1 ± 0.3	40.9	85.9 ± 0.2	40.5		40.0 (21)
K. drosophilarum UCD 71-45					39.6	
K. drosophilarum UCD 71-50					38.9	
K. fragilis CBS 397					41.3	41.0 (1); 41.0 (24); 41.6 (11); 42.0 (27); 40.0, 40.5, 40.7 (21)
K. fragilis UCD 61-293	85.8 ± 0.3	40.2			41.0	
K. lactis CBS 683					41.4	41.0, 41.0 (1); 40.0, 39.3 (21)
K. lactis C-21	86.1 ± 0.3	40.9	86.3 ± 0.6	41.4		
K. lactis CBS 2621					40.0	
K. lactis CBS 4574					40.0	
K. lodderi	84.8 ± 0.1	37.8	84.7 ± 0.2	37.5		
K. marxianus	85.9 ± 0.6	40.5	85.5 ± 0.5	39.4		40.0 (1); 41.0 (21)
K. phaffii	84.6 ± 0.1	37.4	84.6 ± 0.1	37.4		
K. phaseolosporus	86.0 ± 0.5	40.7	86.1 ± 0.6	40.9		
K. polysporus	84.1 ± 0.2	36.1	83.7 ± 0.1	35.1	34.7	34.4 (17); 33.4 (21)
K. vanudenii	86.2 ± 0.2	41.2	86.6 ± 0.5	42.2		
K. veronae CBS 2803					47.4	44.4, 45.9 (21)
K. veronae UCD 61-518					46.7	
K. veronae UCD 68-118					46.3	
K. wickerhamii UCD 61-346					40.9	
K. wickerhamii UCD 67-539					40.6	
K. wickerhamii UCD 68-821C					40.6	
K. wickerhamii CBS 2745					41.5	40.0 (1)
K. wikenii	87.0 ± 0.3	43.1	87.0 ± 0.3	43.1		

[a] Mean value of at least five determinations.
[b] After Marmur and Doty (14).
[c] After Knittel et al. (8).
[d] Based on buoyant density measurements.
[e] All literature values quoted are based on T_m determinations.

TABLE 3. *DNA base composition of the type strains of the various Kluyveromyces species in increasing order*[a]

Organisms	%GC
Group I	
K. polysporus	35.3
K. phaffii	37.4
K. lodderi	37.7
K. africanus	38.0
Group IIA	
K. aestuarii	39.5
K. delphensis	39.8
K. marxianus	40.0
K. drosophilarum	40.7
K. phaseolosporus	40.8
K. fragilis	41.3
K. lactis	41.4
K. wickerhamii	41.5
K. vanudenii	41.7
Group IIB	
K. bulgaricus	42.4
K. wikenii	43.1
K. dobzhanskii	43.3
K. cicerisporus	43.4
K. veronae	47.4

[a] The values are averages based on footnotes *b, c,* and *d* of Table 2, insofar as applicable.

The overall range of GC contents for species of the genus is summarized in Table 3. The values are based on the average figures for the type strains of each species based on columns 2, 3, and 4 (as far as available) in Table 2. It appears that on the basis of GC contents the multispored species *K. polysporus* and *K. africanus* cannot be separated from the remaining four-spored species. This finding does not support the proposal (25) to place the four-spored species in a separate genus *Dekkeromyces*. On the other end of the range, however, *K. veronae* is separated from the rest of the species by a significant gap in GC level. In view of the conjugation behavior of cells prior to sporulation, van der Walt (33) stated that he placed this species only provisionally and for purely practical reasons in the genus *Kluyveromyces*. Our work has now revealed in addition the high GC content of the nuclear DNA in *K. veronae*. The two California strains showed a somewhat lower GC content than the type strain which was isolated in Italy. Two different strains studied in Japan (21) by the melting procedure gave the lowest values. Possible causes may include taxonomic criteria of the strains used and procedural or equipment

variation. In any event there does appear to be more variation between *K. veronae* strains with respect to GC levels than is the case for the other species of which a number of strains have been studied. Reasonable agreement between our values for three strains suggests that *K. veronae*, in spite of its relatively high GC content, be retained tentatively in the genus *Kluyveromyces* until future studies will resolve its taxonomic position. For the present, van der Walt's grouping (5) could be improved by moving *K. veronae* from Group 5 (Table 4) to a monotypic sixth group. Group 6 would then be characterized by spheroidal ascospores, a GC content of approximately 46 to 47% GC, a requirement for biotin and inositol, and sensitivity to cycloheximide.

With this exclusion the genus is relatively homogeneous with respect to GC range, spanning from 35.3 to 43.4% GC, a narrower range than is found for most genera of the *Hemiascomycetes* (18–21). Two groups of species can be recognized (Table 3). Group I includes those with the lowest GC content (35.3–38.0%). Among these four species *K. polysporus* shows a significantly lower GC level. The remaining species (*K. veronae* excepted) are placed in Group II. Subgroup IIA lists those with GC levels around 40 to 41%, whereas the GC percentage in subgroup IIB is around 43%. Van der Walt (33), on the basis of number and shape of the ascospores and on the basis of assimilatory and fermentative properties, suggested arrangement of the species in five groups as shown in Table 4. Starting with the column headed by %GC and going to the right we have entered a number of additional properties of the various species. Van der Walt (33) considers the species of the first two groups as the most primitive in the genus. Those in the second group were separated because of the multispored asci which they produce. It seems to us that although the majority of the five species in these two groups have a relatively low GC content, they constitute a rather heterogeneous group on the basis of the various properties listed. *K. delphensis*, on the basis of GC content, seems to fit better with the species of groups 3 and 4 which van der Walt separated on the basis of disaccharide splitting ability. Since disaccharide utilization may be controlled by a single gene, species separated by such a criterion (commonly done in yeast taxonomy) could have extremely similar genomes. The data in Table 4 indicate that the species in van der Walt's groups 3 and 4 show two levels of GC content but form otherwise a fairly homogeneous group.

Table 4. *Characteristics of the species of Kluyveromyces*

Organisms	Groupings proposed by van der Walt (5)	%GC[a]	2-Sigma values[b]	No. of positive properties[c]	Vitamins required[d]	Ethylamine assimilation[e]	Mannan p.m.r. spectrum groups[f]	Cycloheximide resistance[g]
1st line of development, ascospores reniform								
K. delphensis	1st Group	40.0	7.45	6	1,5	+ slow	II	+
K. phaffii		37.4	4.20	5	1,4,6	–	—[h]	–
K. lodderi		37.8	4.00	12	1	+ slow	VI	+
K. africanus	2nd Group	37.5	3.77	5	3,5,6	–	XVII	low or –
K. polysporus		36.1	5.00	10	6	–	V	low or –
K. wickerhamii	3rd group	41.5	—	17	1,2	+	II	+
K. marxianus		40.5	6.00	23	1,2	+	III	+
K. fragilis		41.3	—	23	1,2,4,5	+	III	+
K. phaseolosporus	4th Group	40.7	3.54	19	1,2	+	XII	+
K. drosophilarum		40.9	4.86	23	1,2	+	V	+
K. dobzhanskii		43.9	4.53	25	1,2	+	V	+
2nd line of development ascospores spherical								
K. wikenii	5th Group	43.1	4.60	16	1,2	+	—	+
K. veronae		49.5	—	18	1,3	+	II	–
K. vanudenii		41.2	3.60	24	1,2	+	—	+
K. cicerisporus		43.4	4.17	24	1,2	+	—	+
K. bulgaricus		42.6	4.58	19	1,2	+	—	+
K. aestuarii		39.5	4.72	21	1,2	+	ᵥ	–
K. lactis		41.4	—	25	1,2	+	V	+

[a] From Table 2; calculated according to Marmur and Doty (14), except for *K. fragilis, K. lactis,* and *K. wickerhamii* whose values are derived from buoyant density values.

[b] Calculated graphically after Knittel et al. (8).

[c] Number of biochemical properties reported as positive by van der Walt (33).

[d] According to Fiol (5) and data from our laboratory: 1, biotin; 2 nicotinamide; 3, *meso*-inositol; 4, pantothenate; 5, pyridoxine; 6, thiamine.

[e] After van der Walt (33).

[f] Proton magnetic resonance (p.m.r.) spectrum groups established by Gorin and Spencer (6).

[g] + denotes that good growth occurs in the presence of 100 mg of cycloheximide/liter.

[h] Data not available.

If, as suggested above, *K. veronae* is eliminated from the fifth group (species with spherical spores), the remaining organisms also form a rather homogenous group, with the possible exception of *K. aestuarii* which has a lower %GC and is cycloheximide-sensitive. As far as they have been determined, the proton magnetic resonance spectra of the cell wall mannans (6) seem to correlate poorly with any of van der Walt's groupings or with GC levels (Table 4). Whether the spore shape of the species in group 5 is highly significant seems doubtful in view of the fertile hybrids prepared by Wickerham and Burton (34) between *K. lactis* and *K. marxianus* (syn. *Zygosaccharomyces ashbyi*).

Several of the species of *Kluyveromyces* appear to be very similar and differ only in a single fermentative or assimilatory reaction on a particular sugar. For example, *K. bulgaricus* differs from *K. cicerisporus* only by the positive cellobiose assimilation of the latter. *K. wikenii* differs from *K. bulgaricus* only by its inability to assimilate lactose. The GC contents of the DNA of these species are the same within experimental error, as are also the 2-sigma values. Bicknell and Douglas (1) have already demonstrated that *K. marxianus* and *K. fragilis*, which differ in the fermentation of lactose (weak or negative in the former and positive in the latter), show a high degree of DNA-DNA homology and may therefore be

considered varieties of the same species. In a subsequent paper we intend to present data on DNA-DNA homology between the various species in the hope of establishing the species concept in the genus on a more solid basis.

LITERATURE CITED

1. Bicknell, J. N., and H. C. Douglas. 1970. Nucleic acid homologies among species of *Saccharomyces*. J. Bacteriol. **101**:505–512.
2. Boidin, J., F. Abadie, J. L. Jacob., and M. C. Pignal. 1962. Les levures à spores réiformes. Bull. Soc. Mycol. Fr. **78**:155–203.
3. Doty, P., J. Marmur, and N. Sueoka. 1959. The heterogeneity in properties and functioning of deoxyribonucleic acid. Brookhaven Symp. Biol. **12**:1–6.
4. Dupont, P. F., and L. R. Hedrick. 1971. Deoxyribonucleic acid base composition and numerical taxonomy in the genus *Trichosporon*. J. Gen. Microbiol. **66**:349–359.
5. Fiol, J. B. 1967. Intéret systématique des tests de croissance en milieu déficient en vitamines pour les genres *Kluyveromyces* (v. der Walt) et *Pichia* (hansen). Rev. Mycol. (Paris) **32**:45–55.
6. Gorin, P. A. J., and J. F. T. Spencer. 1970. Proton magnetic resonance spectroscopy—an aid in identification and chemotaxonomy of yeasts. Advan. Appl. Microbiol. **13**:25–89.
7. Hill, L. R. 1966. An index to deoxyribonucleic acid base composition. J. Gen. Microbiol. **44**:419–437.
8. Knittel, M. D., C. H. Black, W. E. Sandine, and Dorothy K. Fraser. 1968. Use of normal probability paper in determining thermal melting values of deoxyribonucleic acid. Can. J. Microbiol. **14**:239–245.
9. Kreger-van Rij, N. J. W., and J. P. van der Walt. 1963. Ascospores of *Endomycopsis selenospora* (Nadson et Krassilnikov) Dekker. Nature (London) **99**:1012–1013.
10. Kudriavzev, V. I. 1960. Die Systematik der Hefen. Akademie Verlag, Berlin.
11. Leth Bak, A., and A. Stenderup. 1969. Deoxyribonucleic homology in yeast. Genetic relatedness within the genus *Candida*. J. Gen. Microbiol. **59**:21–30.
12. Lodder, J., and N. J. W. Kreger-van Rij. 1952. The yeasts—a taxonomic Study. North Holland Publishing Co., Amsterdam.
13. Mandel, M. 1969. New approaches to bacterial taxonomy: perspectives and prospects. Annu. Rev. Microbiol. **23**:239–274.
14. Marmur, J., and P. Doty, 1962. Determination of base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
15. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. Annu. Rev. Microbiol. **17**:329–372.
16. Meyer, S. A., and H. J. Phaff. 1969. Deoxyribonucleic acid base composition in yeasts. J. Bacteriol. **97**:52–56.
17. Meyer, S. A., and H. J. Phaff. 1970. Taxonomic significance of the DNA base composition in yeast, p. 1–29. *In* D. G. Ahearn (ed.), Recent trends in yeast research, vol. 1. Spectrum (Georgia State Univ.), Atlanta.
18. Nakase, T., and K. Komagata. 1970. Significance of DNA base composition in the classification of yeast genus *Pichia*. J. Gen. Appl. Microbiol. **16**:511–521.
19. Nakase, T., and Komagata. 1971. Significance of DNA base composition in the classification of yeast genus *Debaryomyces*. J. Gen. Appl. Microbiol. **17**:43–50.
20. Nakase, T., and K. Komagata. 1971. Further investigation on the DNA base composition of the genus *Hansenula*. J. Gen. Appl. Microbiol. **17**:77–84.
21. Nakase, T., and K. Komagata. 1971. Significance of DNA base composition in the classification of yeast genus *Saccharomyces*. J. Gen. Appl. Microbiol. **17**:227–238.
22. Novak, E. K., and J. Zsolt. 1961. A new system proposed for yeast. Acta Bot. **7**:93–145.
23. Ramirez-Gomez, C. 1954. Notes sur deux nouvelles espèces de levure isolées de divers milieux. Rev. Mycol. (Paris) **19**:98–102.
24. Rost, K., and H. Venner. 1964. Untersuchungen an Nucleinsäuren. X. Isolierung und Untersuchung von Deoxyribonucleinsäure aus Hefen. Z. Physiol. Chem. **339**:230–237.
25. Santa Maria, J., and C. Sanchez. 1970. Significación taxonómica de las propriedades fisiológicas de las especies incluidas en el género "*Kluyveromyces*". Bol. Inst. Nac. Invest. Agron. **62**:23–36.
26. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **45**:430–433.
27. Storck, R. 1966. Nucleotide composition of nucleic acid of fungi. II. Deoxyribonucleic acid. J. Bacteriol. **91**:227–230.
28. Storck, R., and C. J. Alexopoulos. 1970. Deoxyribonucleic acid of fungi. Bacteriol. Rev. **34**:126–154.
29. Szybalski, W. 1968. Use of cesium sulfate for equilibrium density gradient centrifugation, p. 330–360. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. XII. Academic Press Inc., New York.
30. Van der Walt, J. P. 1956. *Kluyveromyces*—a new yeast genus of the *Endomycetales*. Antonie van Leeuwenhoek J. Microbiol. Serol. **22**:265–272.
31. Van der Walt, J. P. 1956. The yeast *Kluyveromyces africanus* nov. spec. and its phylogenetic significance. Antonie van Leeuwenhoek. J. Microbiol. Serol. **22**:321–326.
32. Van der Walt, J. P. 1965. The emendation of the genus *Kluyveromyces* van der Walt. Antonie van Leeuwenhoek. J. Microbiol. Serol. **31**:341–348.
33. Van der Walt, J. P. 1970. *Kluyveromyces* van der Walt emend. van der Walt, p. 316–378. *In* J. Lodder (ed.), The yeasts—a taxonomic study. North Holland Publishing Co., Amsterdam.
34. Wickerham, L. J., and K. A. Burton. 1956. Hybridization studies involving *Saccharomyces lactis* and *Zygosaccharomyces ashbyi*. J. Bacteriol. **71**:290–295.