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

ALESSANDRO MARTINI,¹ HERM J. PHAFF, AND STEPHEN A. DOUGLASS

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

Received for publication 19 April 1972

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 ascis containing as many as seventy or more reniform to long oval spores. A second species, *K. africanaus*, 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 multisporated 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 ascus 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 multisporated asci (*K. dozhanokii*, *K. lactis*, *K. drosophilaram*, *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.

¹Present address: Istituto di Microbiologia Agraria e Tecnica, University of Perugia, Italy.
Kluuyveromyces aestuarii (Fell) van der Walt 1961
K. africanaus van der Walt 1956
K. bulgaricus (Santa Maria) van der Walt 1956
K. cicerisporus van der Walt, Nel et van Kerken 1966
K. delphensis (van der Walt et Tscheuschner) van der Walt 1956
K. dobzhanskii (Shehata, Mrak et Phaff) van der Walt 1955
K. drosophilum (Shehata, Mrak et Phaff) van der Walt 1955
K. drosophilum
K. fragilis (Jørgensen) van der Walt 1909
K. fragilis
K. lactis (Dombrowski) van der Walt 1910
K. lactis
K. lactis (Dombrowski) van der Walt (synon. Sacch. so-
ciass Ramirez)
K. lodderi (van der Walt et Tscheuschner) van der Walt 1957
K. marxianus (Hansen) van der Walt 1888
K. phaffii (van der Walt) van der Walt 1963
K. phaseolosporus (Shehata, Mrak et Phaff) van der Walt 1955
K. polysporus van der Walt 1956
K. vanadenii (van der Walt et Nel) van der Walt 1963
K. veronae (Lodder et Kreger-van Rij) van der Walt 1952
K. veronae
K. veronae
K. wickerhamii (Phaff, Miller et Shifrine) van der Walt 1956
K. wickerhamii
K. wickerhamii
K. wickerhamii van der Walt, Nel et van Kerken 1966

<table>
<thead>
<tr>
<th>Name</th>
<th>Culture number and source</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. africanaus</td>
<td>UCD 61-29, CBS 4438*</td>
</tr>
<tr>
<td>K. bulgaricus</td>
<td>UCD 57-16, CBS 2517*</td>
</tr>
<tr>
<td>K. cicerisporus</td>
<td>UCD 71-13, CBS 2762*</td>
</tr>
<tr>
<td>K. delphensis</td>
<td>UCD 71-14, CBS 4857*</td>
</tr>
<tr>
<td>K. dobzhanskii</td>
<td>UCD 56-2, CBS 2170*</td>
</tr>
<tr>
<td>K. drosophilum</td>
<td>UCD 50-45, CBS 2104*</td>
</tr>
<tr>
<td>K. drosophilum</td>
<td>UCD 51-130, CBS 2105*</td>
</tr>
<tr>
<td>K. fragilis</td>
<td>UCD 71-45, Meyers et al.</td>
</tr>
<tr>
<td>K. fragilis</td>
<td>UCD 71-50, Meyers et al.</td>
</tr>
<tr>
<td>K. fragilis</td>
<td>UCD 71-58, CBS 397*</td>
</tr>
<tr>
<td>K. lactis (Dombrowski)</td>
<td>UCD 61-293*, —</td>
</tr>
<tr>
<td>K. lactis</td>
<td>UCD 71-59, CBS 683*</td>
</tr>
<tr>
<td>K. lactis</td>
<td>UCD C-21*, —</td>
</tr>
<tr>
<td>K. lactis</td>
<td>UCD 71-73, CBS 2621</td>
</tr>
<tr>
<td>K. lactis (Dombrowski)</td>
<td>UCD 70-2, CBS 4574</td>
</tr>
<tr>
<td>K. lodderi (van der Walt et Tscheuschner)</td>
<td>UCD 70-3, CBS 2757*</td>
</tr>
<tr>
<td>K. marxianus (Hansen)</td>
<td>UCD 55-82, CBS 712*</td>
</tr>
<tr>
<td>K. phaffii (van der Walt)</td>
<td>UCD 70-5, CBS 4417*</td>
</tr>
<tr>
<td>K. phaseolosporus (Shehata, Mrak et Phaff)</td>
<td>UCD 50-80, CBS 2103*</td>
</tr>
<tr>
<td>K. polysporus</td>
<td>UCD 57-17, CBS 2163*</td>
</tr>
<tr>
<td>K. vanadenii (van der Walt et Nel)</td>
<td>UCD 70-4, CBS 4372*</td>
</tr>
<tr>
<td>K. veronae (Lodder et Kreger-van Rij)</td>
<td>UCD 55-41, CBS 2803*</td>
</tr>
<tr>
<td>K. veronae</td>
<td>UCD 61-518*, —</td>
</tr>
<tr>
<td>K. veronae</td>
<td>UCD 68-118*, —</td>
</tr>
<tr>
<td>K. wickerhamii (Phaff, Miller et Shifrine)</td>
<td>UCD 54-210, CBS 2745*</td>
</tr>
<tr>
<td>K. wickerhamii</td>
<td>UCD 61-346*, —</td>
</tr>
<tr>
<td>K. wickerhamii</td>
<td>UCD 67-539*, —</td>
</tr>
<tr>
<td>K. wickerhamii</td>
<td>UCD 68-821*C, —</td>
</tr>
<tr>
<td>K. wickerhamii van der Walt, Nel et van Kerken 1966</td>
<td></td>
</tr>
</tbody>
</table>

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

Type culture.
Isolated from spartina grass in the coastal marshlands of Louisiana.
Isolated from Drosophila melanogaster in California.
Isolated from unpasteurized sour milk, California.
Isolated from crop contents Drosophila melanogaster, Lake Berryessa, California.
Isolated from a prune conveyor belt, Yuba City, California.
Isolated from Salix slime exudate, Sacramento River, California.
Isolated from Acer slime exudate, Morioka, Japan.
Isolated from Betula slime exudate, British Columbia, Canada.

One of these was originally described as Saccharomyces sociassii (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. drosophilum had been previously isolated only from crop contents of Drosophila in California, two strains were included that were recently isolated from spartina grass (Spartina alterni-

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 Erlen-
meier flasks) on a rotary shaker at room tempera-
ture. Late logarithmic-phase cells were harvested by centrifugation and washed three times with a sa-
lime 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. aestivalii, K. africanaus, 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). 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 plotting 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. aestivalii, K. africanaus, 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. drosophilbarum (UCD 71-45 and 71-50), obtained from marine marshland grass, is significant in comparison to the California strain from Drosohpila (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>
<thead>
<tr>
<th>Organisms</th>
<th>%GC</th>
<th>%GC</th>
<th>%GC from the literature&lt;sup&gt;c&lt;/sup&gt; (references in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. aestuarii</td>
<td>85.5 ± 0.1</td>
<td>39.5 ± 0.7</td>
<td>39.5</td>
</tr>
<tr>
<td>K. africanaus</td>
<td>84.7 ± 0.3</td>
<td>37.5 ± 0.3</td>
<td>37.8, 38.6</td>
</tr>
<tr>
<td>K. bulgaricus</td>
<td>86.8 ± 0.1</td>
<td>42.6 ± 0.4</td>
<td>42.1</td>
</tr>
<tr>
<td>K. cicerisporus</td>
<td>87.1 ± 0.3</td>
<td>43.4 ± 0.3</td>
<td>43.4</td>
</tr>
<tr>
<td>K. delphensis</td>
<td>85.7 ± 0.3</td>
<td>40.0 ± 0.4</td>
<td>39.6, 39.9</td>
</tr>
<tr>
<td>K. dozhanskii</td>
<td>87.3 ± 0.1</td>
<td>43.9 ± 0.4</td>
<td>43.4, 42.6</td>
</tr>
<tr>
<td>K. drosophilbarum CBS 2105</td>
<td>86.1 ± 0.3</td>
<td>40.9 ± 0.2</td>
<td>40.5, 42.6</td>
</tr>
<tr>
<td>K. drosophilbarum UCD 71-45</td>
<td>88.4 ± 0.1</td>
<td>38.9 ± 0.3</td>
<td>39.6</td>
</tr>
<tr>
<td>K. drosophilbarum UCD 71-50</td>
<td>88.4 ± 0.0</td>
<td>38.9 ± 0.2</td>
<td>40.0 (1)</td>
</tr>
<tr>
<td>K. fragilis CBS 397</td>
<td>85.8 ± 0.3</td>
<td>40.2 ± 0.3</td>
<td>41.0</td>
</tr>
<tr>
<td>K. lactis CBS 683</td>
<td>86.1 ± 0.3</td>
<td>40.9 ± 0.6</td>
<td>41.4, 41.0, 41.0 (1); 40.0, 39.3 (21)</td>
</tr>
<tr>
<td>K. lactis C-21</td>
<td>86.1 ± 0.3</td>
<td>40.9 ± 0.6</td>
<td>41.4</td>
</tr>
<tr>
<td>K. lactis CBS 2621</td>
<td>86.1 ± 0.3</td>
<td>40.9 ± 0.6</td>
<td>41.4</td>
</tr>
<tr>
<td>K. lactis CBS 4574</td>
<td>86.1 ± 0.3</td>
<td>40.9 ± 0.6</td>
<td>41.4</td>
</tr>
<tr>
<td>K. loddii</td>
<td>84.8 ± 0.1</td>
<td>37.8 ± 0.2</td>
<td>37.5</td>
</tr>
<tr>
<td>K. marxianus</td>
<td>85.9 ± 0.6</td>
<td>40.5 ± 0.5</td>
<td>39.4</td>
</tr>
<tr>
<td>K. phaffii</td>
<td>84.6 ± 0.1</td>
<td>37.4 ± 0.1</td>
<td>37.4</td>
</tr>
<tr>
<td>K. phaseolosporus</td>
<td>86.0 ± 0.5</td>
<td>40.7 ± 0.6</td>
<td>40.9</td>
</tr>
<tr>
<td>K. polyosporus</td>
<td>84.1 ± 0.2</td>
<td>36.1 ± 0.1</td>
<td>35.1</td>
</tr>
<tr>
<td>K. vanudeni</td>
<td>86.2 ± 0.2</td>
<td>41.2 ± 0.5</td>
<td>42.2</td>
</tr>
<tr>
<td>K. veronae CBS 2035</td>
<td>87.0 ± 0.3</td>
<td>43.1 ± 0.3</td>
<td>43.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value of at least five determinations.
<sup>b</sup> After Marmur and Doty (14).
<sup>c</sup> After Knittel et al. (8).
<sup>d</sup> Based on buoyant density measurements.
<sup>e</sup> All literature values quoted are based on T<sub>m</sub> determinations.
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 *Kluveromyces*. 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 *Kluveromyces* 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 *Hemiascomyces* (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 ascii 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 3. DNA base composition of the type strains of the various *Kluveromyces* species in increasing order

<table>
<thead>
<tr>
<th>Organisms</th>
<th>%GC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
</tr>
<tr>
<td><em>K. polysporus</em></td>
<td>35.3</td>
</tr>
<tr>
<td><em>K. phaffii</em></td>
<td>37.4</td>
</tr>
<tr>
<td><em>K. lodderi</em></td>
<td>37.7</td>
</tr>
<tr>
<td><em>K. africanus</em></td>
<td>38.0</td>
</tr>
<tr>
<td><strong>Group IIA</strong></td>
<td></td>
</tr>
<tr>
<td><em>K. aettuarii</em></td>
<td>39.5</td>
</tr>
<tr>
<td><em>K. delphensis</em></td>
<td>39.8</td>
</tr>
<tr>
<td><em>K. marxianus</em></td>
<td>40.0</td>
</tr>
<tr>
<td><em>K. drosophilum</em></td>
<td>40.7</td>
</tr>
<tr>
<td><em>K. phaseolosporus</em></td>
<td>40.8</td>
</tr>
<tr>
<td><em>K. fragilis</em></td>
<td>41.3</td>
</tr>
<tr>
<td><em>K. lactis</em></td>
<td>41.4</td>
</tr>
<tr>
<td><em>K. wickerhamii</em></td>
<td>41.5</td>
</tr>
<tr>
<td><em>K. vanudenii</em></td>
<td>41.7</td>
</tr>
<tr>
<td><strong>Group IIB</strong></td>
<td></td>
</tr>
<tr>
<td><em>K. bulgaricus</em></td>
<td>42.4</td>
</tr>
<tr>
<td><em>K. wikenii</em></td>
<td>43.1</td>
</tr>
<tr>
<td><em>K. dobzhanski</em></td>
<td>43.3</td>
</tr>
<tr>
<td><em>K. cicerisporus</em></td>
<td>43.4</td>
</tr>
<tr>
<td><em>K. veronae</em></td>
<td>47.4</td>
</tr>
</tbody>
</table>

*The values are averages based on footnotes b, c, and d of Table 2, insofar as applicable.*
### Table 4. Characteristics of the species of Kluyveromyces

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Groupings proposed by van der Walt (5)</th>
<th>%GC</th>
<th>2-Sigma values</th>
<th>No. of positive properties</th>
<th>Vitamins required</th>
<th>Ethyl-amine assimilation</th>
<th>Mannan p.m.r. spectrum groups</th>
<th>Cycloheximide resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st line of development,</td>
<td>K. bulgaricus</td>
<td>1st Group</td>
<td>40.0</td>
<td>7.45</td>
<td>6 1,5</td>
<td>+ slow</td>
<td>II</td>
<td>+</td>
</tr>
<tr>
<td>ascopores reniform</td>
<td>K. phaffii</td>
<td>37.4</td>
<td>4.20</td>
<td>5 1,4,6</td>
<td>-</td>
<td>-</td>
<td>-A</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>K. loddieri</td>
<td>37.8</td>
<td>4.00</td>
<td>12 1</td>
<td>+ slow</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>K. africanaus</td>
<td>2nd Group</td>
<td>37.5</td>
<td>3.77</td>
<td>5 3,5,6</td>
<td>-</td>
<td>-</td>
<td>XVII</td>
<td>low or -</td>
</tr>
<tr>
<td>K. polyosporus</td>
<td></td>
<td>36.1</td>
<td>5.00</td>
<td>10 6</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>low or -</td>
</tr>
<tr>
<td>K. wickerhamii</td>
<td>3rd group</td>
<td>41.5</td>
<td>-</td>
<td>17 1,2</td>
<td>+</td>
<td>II</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. marxianus</td>
<td></td>
<td>40.5</td>
<td>6.00</td>
<td>23 1,2</td>
<td>+</td>
<td>III</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. fragilis</td>
<td></td>
<td>41.3</td>
<td>-</td>
<td>23 1,2,4,5</td>
<td>+</td>
<td>III</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. phaseolosporus</td>
<td>4th Group</td>
<td>40.7</td>
<td>3.54</td>
<td>19 1,2</td>
<td>+</td>
<td>XII</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. drosophilum</td>
<td></td>
<td>40.9</td>
<td>4.86</td>
<td>23 1,2</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K. dozhanskii</td>
<td></td>
<td>43.9</td>
<td>4.53</td>
<td>25 1,2</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2nd line of development,</td>
<td>K. wikenii</td>
<td>5th Group</td>
<td>43.1</td>
<td>4.60</td>
<td>16 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ascopores spherical</td>
<td>K. veronae</td>
<td>49.5</td>
<td>-</td>
<td>18 1,3</td>
<td>+</td>
<td>II</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>K. vanudenii</td>
<td>41.2</td>
<td>3.60</td>
<td>24 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>K. cicerisporus</td>
<td>43.4</td>
<td>4.17</td>
<td>24 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>K. bulgaricus</td>
<td>42.6</td>
<td>4.58</td>
<td>19 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>K. aestuarii</td>
<td>39.5</td>
<td>4.72</td>
<td>21 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>K. lactis</td>
<td>41.4</td>
<td>-</td>
<td>25 1,2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* 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.
* Calculated graphically after Knittel et al. (8).
* Number of biochemical properties reported as positive by van der Walt (33).
* According to Fiol (5) and data from our laboratory: 1, biotin; 2 nicotinamide; 3, meso-inositol; 4, pantothenate; 5, pyridoxine; 6, thiamine.
* After van der Walt (33).
* Proton magnetic resonance (p.m.r.) spectrum groups established by Gorin and Spencer (6).
* + denotes that good growth occurs in the presence of 100 mg of cycloheximide/liter.
* 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 mannan (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.

ACKNOWLEDGMENTS

We are indebted to Edwin G. Krebs for the use of the Model E ultracentrifuge. The technical assistance of Gayle Fuson is gratefully acknowledged.

This investigation was supported by Public Health Service grant GM-16007-03 from the National Institute of General Medical Sciences.

LITERATURE CITED