Taxonomic Revision of the Yeast Genus *Kluyveromyces* by Nuclear Deoxyribonucleic Acid Reassociation

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Deoxyribonucleic acid relatedness among the type strains of all taxa and known anamorphs assigned to the yeast genus *Kluyveromyces* was assessed by the optical reassociation technique. Three groups of species related at the 95% level or higher were found: (i) *K. lactis* including *K. drosophilum*, *K. phaseolosporus*, *K. vanudeni*, and the anamorph *Candida sphaerica*; (ii) *K. marxianus* with *K. bulgaricus*, *K. cicerisorum*, *K. fragilis*, *K. wickerhamii*, and the anamorphs *Candida kefyr*, *Candida macdoniensis*, and *Candida pseudotropicalis*; (iii) *K. thermotolerans* with *K. veronae* and the anamorph *Candida datilia*. The remaining species, including the recently described *K. blattae* and *K. wallii*, are not related to each other or to the members of the above three groups. The nomen nudum *K. celllobiovorus* is not conspecific with any of the species of the genus. The species assignment obtained by nuclear deoxyribonucleic acid reassociation does not entirely conform with the previously proposed reorganization of the genus *Kluyveromyces* (J.-P. van der Walt and E. Johannsen, p. 224-251, in N. J. W. Kreger-van Rij, ed., *The Yeasts. A Taxonomic Study*, 1984).

The genus *Kluyveromyces* was established in 1956 by van der Walt (52) to classify the newly isolated, budding, fermentative yeast species *K. polysporus*, which produces large, multispored ascus containing as many as 70 or more reinform to long oval spores. In the same year, an additional species, *K. africana*, forming up to 16 spores per ascus, was included in the genus by van der Walt (53). The salient property of the asci of these two species was their early evanescence at maturity, to release the spores.

In 1965, the diagnosis of the genus was emended by van der Walt (54) on the assumption that the species with multisporous asci represented only a separate line of development in relation to other species which normally form only four spores. The above approach was based on a series of controversial taxonomic treatments of those yeast species that form kidney-shaped spores (5, 18, 19, 34, 60). Van der Walt included in *Kluyveromyces* all the species formerly classified in the genera *Fahospora*, *Zygofahospora* Kudriavzev (19), *Dekkeromyces* nomen nudum Wickerham and Burton (59), and *Guilliermondella* (5). Later, Santamaria and Sanchez (41) proposed to assign the nomen nudum *Dekkeromyces* to those species of the genus that are unable to form multisporous asci (*K. dobzhanskii*, *K. drosophilum*, *K. fragilis*, *K. lactis*, *K. phaseolosporus*, and *K. wickerhamii*). Van der Walt (55) applied the overall criteria that had suggested the earlier emendation and assigned to the genus 19 species including several recently described new taxa.

Several subsequent approaches to the taxonomy of the genus *Kluyveromyces* were attempted, such as those based on vitamin requirements (9), electrophoretic isoenzyme patterns (45, 46), structure of exo-β-glucanases (23), chemical and immunochromatographic studies of cell wall components (7, 13, 31, 40, 51), numerical taxonomy (6, 35), ultrastructure of the ascospore wall (2), type of coenzyme Q (62), interfertility studies (14, 15), and nuclear deoxyribonucleic acid (DNA) base composition (28, 33, 36).

Since Bicknell and Douglas (4) first showed that the DNAs of *K. marxianus* and *K. fragilis* shared approximately 93% of their base sequences, DNA base sequence relatedness between some other species of the genus (10, 28) has been investigated extensively.

In a recent monograph on yeast taxonomy, van der Walt and Johannsen (57) established the overall composition of the genus on the basis of the results of interfertility studies (14, 15). Since their proposals do not entirely agree with previous DNA-DNA reassociation studies (26; H. J. Phaff, M. A. Lachance, and H. L. Presley, Abstr. 12th Int. Congr. Microbiol. 1978, S27.3, p. 38), we decided to reinvestigate genetic relatedness within the genus, including all newly described species, using the spectrophotometric technique of Seidler and Mandel (45) and Seidler et al. (43) as described by Kurtzman et al. (22).

(A preliminary report of part of this work has been presented [58].)

**MATERIALS AND METHODS**

**Microorganisms.** Thirty six strains representative of the 21 species or varieties included in the genus *Kluyveromyces* by van der Walt and Johannsen (57), as well as a few species of the genus *Candida* sensu Meyer et al. (30) considered to represent the anamorphs (imperfect forms) of some species of the genus, were investigated. Strain designations are given in the Table 1. For clarity, we adopted the nomenclature of van der Walt (55); the correspondence with the latest revision of the genus also is shown in Table 1.

**DNA purification and reassociation reactions.** DNA extraction and purification were done by combining the procedures of Marmur (24) and Bernardi et al. (3) as described by Price et al. (37). Absorbance ratios at 260/280 nm and 230/260 nm were used to assess DNA purity together with thermal melting profiles. The kinetics of nuclear DNA reassociation were followed spectrophotometrically by using the method of Seidler and Mandel (44) and Seidler et al. (43), as modified by Kurtzman et al. (21).

**Determination of $T_m$.** The thermal denaturation temperature ($T_m$) was determined by the method of Marmur and Doty (25) with a Gifford model 2500 automatic recording spectrophotometer equipped with a model 2527 Thermo-Programmer. The samples contained 25 μg of DNA per ml. A standard preparation of *Candida parapsilosis* CBS 604...
TABLE 1. DNA base composition of the type strains of the species of the genus Kluyveromyces

<table>
<thead>
<tr>
<th>Designation of van der Walt (1970)*</th>
<th>CBS strain no.</th>
<th>Designation of van der Walt and Johannsen (1984)</th>
<th>mol% G+C based on:</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. aestivalii</td>
<td>4438T</td>
<td>K. aestivalii</td>
<td>36 (36)-39 (27)</td>
</tr>
<tr>
<td>K. africanus</td>
<td>2517T</td>
<td>K. africanus</td>
<td>36 (36)-38 (27)</td>
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<tr>
<td></td>
<td>6284T</td>
<td>K. blattae</td>
<td>39 (27)-38 (11)</td>
</tr>
<tr>
<td></td>
<td>7153T</td>
<td>K. cellohiavorus nomen nudum*</td>
<td>30 (29)-42 (3)</td>
</tr>
<tr>
<td>K. delphensis</td>
<td>2170T</td>
<td>K. delphensis</td>
<td>40 (27)-37 (11)</td>
</tr>
<tr>
<td>K. dobzhanski</td>
<td>2104T</td>
<td>K. marxianus var. dobzhanski</td>
<td>43 (29)-42 (4)</td>
</tr>
<tr>
<td>K. lodderi</td>
<td>2757T</td>
<td>K. lodderi</td>
<td>42 (36)-43 (27)</td>
</tr>
<tr>
<td>K. phaffii</td>
<td>4417T</td>
<td>K. phaffii</td>
<td>34 (36)-37 (27)</td>
</tr>
<tr>
<td>K. polysporus</td>
<td>2163T</td>
<td>K. polysporus</td>
<td>36 (36)-37 (27)</td>
</tr>
<tr>
<td></td>
<td>6430T</td>
<td>K. wilenii</td>
<td>35 (33)-34 (36)</td>
</tr>
<tr>
<td>K. wilenii</td>
<td>2745T</td>
<td>K. wikenii</td>
<td>35 (35)-35 (27)</td>
</tr>
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<td>K. lactis group</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>K. lactis</td>
<td>683T</td>
<td>K. marxianus var. lactis</td>
<td>41 (4)-40 (33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41 (27)-40 (11)</td>
</tr>
<tr>
<td>K. drosophiliform</td>
<td>2105T</td>
<td>K. marxianus var. drosophiliform</td>
<td>41 (36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40 (4)-40 (11)</td>
</tr>
<tr>
<td>K. phaseolosporus</td>
<td>2103T</td>
<td>K. marxianus var. phaseolosporus</td>
<td>41 (36)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>40 (11)</td>
</tr>
<tr>
<td>K. vanudeni</td>
<td>4372T</td>
<td>K. marxianus var. vanudeni</td>
<td>38 (36)-42 (27)</td>
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<td>T. sphaerica</td>
<td>141T</td>
<td>K. marxianus var. lactis</td>
<td>40 (11)</td>
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<td>K. marxianus group</td>
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<td></td>
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<td>K. marxianus</td>
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<td>K. marxianus var. marxianus</td>
<td>39 (36)-39 (27)</td>
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<td></td>
<td>41 (11)</td>
</tr>
<tr>
<td>K. bulgaricus</td>
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<td>K. marxianus var. bulgaricus</td>
<td>34 (36)-42 (27)</td>
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<td></td>
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<td></td>
<td>41 (11)</td>
</tr>
<tr>
<td>K. cicerisporus</td>
<td>4857T</td>
<td>K. marxianus var. cicerisporus</td>
<td>39 (36)-43 (27)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>41 (11)</td>
</tr>
<tr>
<td>K. fragilis</td>
<td>397T</td>
<td>K. marxianus var. fragilis</td>
<td>41 (39)-42 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41 (27, 11)</td>
</tr>
<tr>
<td>K. wilenii</td>
<td>5671T</td>
<td>K. marxianus var. wilenii</td>
<td>35 (36)-43 (27)</td>
</tr>
<tr>
<td>C. kefir</td>
<td>1970T</td>
<td>K. marxianus var. kefir</td>
<td>40 (33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41 (11)</td>
</tr>
<tr>
<td>C. macedoniensis</td>
<td>600T</td>
<td>K. marxianus var. macedoniensis</td>
<td>40 (33)</td>
</tr>
<tr>
<td>C. pseudotropicalis</td>
<td>607T</td>
<td>K. marxianus var. pseudotropicalis</td>
<td>41d</td>
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<td>K. thermotolerans group</td>
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</tr>
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<td>K. thermotolerans</td>
<td>6340T</td>
<td>K. thermotolerans</td>
<td>46 (11)</td>
</tr>
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<td>K. veronae</td>
<td>2803T</td>
<td>K. thermotolerans</td>
<td>44 (36)-47 (27)</td>
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<tr>
<td>T. dattila</td>
<td>137T</td>
<td>K. thermotolerans</td>
<td>46 (11)</td>
</tr>
</tbody>
</table>

* Groups determined in the present study.
* CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. T, Type strain.
* References are in parentheses.
* Based on our own results.
* See reference 32.

DNA (Tm = 85.9°C) was included in every determination as a control.

RESULTS

The type strains of the 21 species included in the genus Kluyveromyces sensu van der Walt (55), together with the nuclear DNA base composition, are given in Table 1. The guanine-plus-cytosine (G+C) contents of the more recently described species were determined by the melting point procedure of Marmur and Doty (25) as described by Meyer and Phaff (29).

Kluyveromyces species K. marxianus, K. bulgaricus, K. cicerisporus, K. fragilis, and K. wikenii and three species of the genus Candida, C. keifer, C. macedoniensis, and C. pseudotropicalis, which are considered anamorphs of K. fragilis and K. marxianus (55), were related to K. marxianus at the 93% level or higher. All remaining species included in the genus showed less than 34% reassociation with K. marxianus CBS 712T: K. aestivalii (4%), K. africanus (12%), K. blattae (7%), K. delphensis (18%), K. dobzhanski (10%), K. drosophiliform (22%), K. lactis (15 to 18%), K. lodderi (14%), K. phaffii (31%), K. phaseolosporus (11%), K. polysporus (8%), K. thermotolerans (10%), K. vanudeni (24%), K. veronae (34%), K. wikenii (21%), K. wikenii (11%), and Torulopsis sphaerica (21%). Thus, they appeared to be only distantly related to the K. marxianus group. Type strains of K. drosophiliform, K. phaseolosporus, K. vanudeni, and Candida (Torulopsis) sphaerica were closely related to K. lactis at the 85 to 104% homology level.

Sequence comparisons of the reference strain K. lactis CBS 683T with the species of the K. marxianus group showed less than 20% complementarity. The same overall situation of very low relatedness was also observed in relation to other species of the genus such as K. aestivalii (20%), K. africanus (10%), K. delphensis (14%), and K. dobzhanski (20%).

The type strains of K. thermotolerans and K. veronae, and of Candida dattila, which is considered to be their imperfect counterpart, showed >94% homology. Little or no homology was shown between the type strains of K. aestivalii and K. vanudeni (19%); K. africanus and K. polysporus (4%); K. blattae and K. lodderi (18%) or K. phaffii (2%) or K. polysporus (0%); K. cellohiavorus and K. delphensis (0%) or
K. wickerhamii (8%); K. delphensis and K. lodderi (26%) or K. phaffii (10%); K. delphensis and K. polysporus (13%) or K. wickerhamii (11%); K. dozhanskii and K. wickerhamii (13%); K. phaffii and K. polysporus (10%) or K. wickerhamii (9%); K. waltii and K. cellobiovorus (7%) or K. dozhanskii (2%).

**DISCUSSION**


Our results are in agreement with those of Bicknell and Douglas (3), who used both 25S ribosomal DNA-ribonucleic acid (RNA) competition reactions and DNA-DNA reassociation with labeled DNA to show that the nuclear DNAs of *K. fragilis* and *K. marxianus* have a high degree of relative homology, while *K. lactis* is not related to *K. marxianus*. Martini and Phaff (27) used a simple spectrophotometric procedure proposed earlier for the investigation of genetic relatedness in bacteria. They confirmed the high level of sequence complementarity between *K. fragilis* and *K. marxianus* and the very low complementarity between *K. lactis* and *K. marxianus* (ca. 6%). Low DNA relatedness was also found between *K. dozhanskii* and *K. fragilis* or *K. wickerhamii* and *K. lactis*.

A more detailed study by Martini (26) further elucidated the relationships among some of the lactose-fermenting taxa of the genus, showing three groups characterized by a high degree of intragroup relative homology (>90%) comprising (i) *K. lactis* and *K. vanudeni*, (ii) *K. marxianus* and *K. fragilis*, and (iii) *K. bulgaricus*, *K. cicerisporus*, and *K. wikenii*. The overall taxonomic situation of the lactose-assimilating and/or -fermenting species and anamorphs of *Kluyveromyces* spp. was recently reviewed by Vaughan Martini and Martini (58), who found a high level of complementarity (>90%) between the species of the group *K. marxianus-K. fragilis* and those of the group *K. bulgaricus-K. cicerisporus-K. wikenii*.

The new information derived from our present data is the high level of complementarity (>90%) found when the DNA of *K. marxianus* was reassociliated with those of *K. bulgaricus*, *K. cicerisporus*, and *K. wikenii*. These last results are in agreement with those obtained by DNA-DNA reassociation in a solution with iodinated DNA (Phaff et al., 12th Int. Congr. Microbiol.).

A recent study of eight species of *Kluyveromyces* by Fiol and Poncet (10), carried out by using hybridization in liquid media with 32P-labeled DNA (applied earlier to yeasts by Price et al. [37]), confirms the high level of DNA relatedness found in previous investigations between *K. marxianus* and *K. fragilis* or *K. cicerisporus*, but also reports a high relatedness value between *K. marxianus* and *K. dozhanskii* (87.0%) as well as between *K. marxianus* and *K. lactis* (84.5%). These last two observations are at variance with the homology values obtained by other investigators for the same strains (4, 27) or results reported here.

Price et al. (37), using the same methodology to compare the genomes of different yeast genera, obtained much more defined results with low and high homology values ranging from 1.6 to 23.9% and from 80 to 99.9%, respectively. The lack of low DNA-DNA relatedness values and the inability to discriminate with certainty between related and nonrelated species may be due to the exceedingly low specific activity (<3.500 cpm/μg) of the labeled DNA samples used by Fiol and Poncet (10) in their investigation.

The nuclear DNA-DNA affinities within the genus *Kluyveromyces* were recently investigated by Fison et al. (11), using the method of hybridization in liquid media (37). Their results indicate the presence of two groups of highly related species: one includes *K. marxianus*, *K. bulgaricus*, *K. cicerisporus*, *K. fragilis*, *K. wikenii*, *C. kefy*, and *C. pseudotropicalis*; and the other includes *K. lactis*, *K. drosophilum*, *K. phaseolosporus*, and *K. vanudeni*.

With the sole exception of the study of Fiol and Poncet (10), investigations of the nuclear acid homologies within *Kluyveromyces* spp. by methods as different as 25S ribosomal RNA-DNA competition reactions and DNA-DNA renaturation on nitrocellulose filters (4), optical reassociation under less controlled (26) and stringent conditions (see above), and hybridization in liquid media followed by hydroxylapatite fractionation (11), yielded very similar results. Most of these studies were not restricted to the type strains, thus allowing a wider and clearer definition and delimitation of the species within the genus.

Nearly all the studies of the genus *Kluyveromyces* by different authors and with different methods of comparison of informational macromolecules produced convincing evidence of the general taxonomic composition and provided support for the molecular approach to yeast taxonomy.

**Comparison of DNA reassociation with other taxonomic criteria.** (i) DNA base composition. DNA base composition is commonly considered to play an exclusionary role in bacterial taxonomy (48). In yeasts, as in bacteria, a 1 to 1.5% divergence in base composition roughly indicates that two isolates do not share similar polynucleotide sequences (20, 37).

Most of the G+C values were obtained by the thermal denaturation method of Marmur and Doty (25), which is considered less precise than the buoyant density procedure of Schildkraut et al. (42), because of the interference of mitochondrial DNA and the presence of other impurities, such as RNA and carbohydrates. Purity of DNA samples may account for the intraspecific variability encountered within the *K. lactis* group (range, 40 to 42 mol%) and *K. marxianus* group (39 to 43 mol%) as defined in our study. The values reported by Poncet and Fiol (36) are consistently and significantly lower than those of all other investigations. The mean base compositions of the remaining species cover a wide range (31 to 47 mol% G+C), indicating a definite genetic heterogeneity within the genus.

Buoyant density base composition values of the homologous species in the *K. lactis* and *K. marxianus* groups are characterized by intraspecific variability well within the proposed 1.0 to 1.5% exclusionary difference in nuclear base
composition (20, 37). Unfortunately, not all laboratories have access to the necessary equipment for buoyant density determination.

(ii) Numerical taxonomy. The relationships indicated by numerical analyses (6, 35) of the species included in the genus *Kluyveromyces* (34) differ from the DNA relationships indicated here. Both numerical classifications included the species *K. africanaus*, *K. delphensis*, *K. loddieri*, *K. phaffii*, and *K. polysporus* in the same cluster. According to our DNA study they are clearly separated since they share less than 20% of their nucleotide sequences. On the other hand, 12 species (*K. aestuarii*, *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanski*, *K. drosophilaram*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudeni*, *K. wickerhamii*, and *K. wikenii*) were grouped by Campbell (6) and Poncet (35), while sequence data delineated two only distantly related groups of closely related species: (i) *K. marxianus*, *K. fragilis*, *K. cicerisporus*, *K. bulgaricus*, and *K. wikenii*; and (ii) *K. lactis*, *K. drosophilaram*, *K. phaseolosporus*, and *K. vanudeni*. The remaining species (*K. aestuarii*, *K. dobzhanski*, and *K. wickerhamii*) showed very little interspecific kinship or conspecificity with any member of the above two groups.

Unlike bacterial systematics where numerical taxonomy and DNA-based relationships often agree, most of the numerical systems thus far proposed for yeasts show little correlation between the two procedures of classification. As stressed by Kurtzman et al. (20), these inconsistencies probably derive from the low number of phenotypic properties considered (ca. 35 in the case of yeasts) for numerical taxonomy, whereas DNA reassocation studies consider the whole genome, which theoretically compares all the properties of the strains investigated.

(iii) Chemical composition of the cell wall. Gorin and Spencer (13) compared the proton magnetic resonance spectra of the isolated mannans of 13 species of the genus *Kluyveromyces* and assigned them to the following groups with closely similar chemical structures (each also including species previously classified in the genus *Saccharomyces*): (i) *K. delphensis*, *K. veronae*, and *K. wickerhamii*; (ii) *K. fragilis* and *K. marxianus*; (iii) *K. aestuarii*, *K. dobzhanski*, *K. drosophilaram*, *K. lactis*, and *K. polysporus*; (iv) *K. loddieri*; (v) *K. africanaus*; and (vi) *K. phaseolosporus*.

Except for assignment of *K. marxianus* and *K. fragilis* to the same cluster, all the remaining phylogenetic relationships indicated by Gorin and Spencer disagree with the DNA groupings. Apparently, the composition of the cell wall mannans does not reflect the evolutionary affinities within the genus *Kluyveromyces*.

Classifications of yeasts based on cell wall antigentic structures were proposed by Tsuchiya et al. (51), Sandula et al. (40), Careta et al. (7), and Montrocher (31). A consistent feature of these studies was that they all considered only the same restricted and related group of species of the genus: *K. marxianus* and *K. fragilis*, and their proposed anamorphs (*C. pseudotropicalis*, *C. macedoniensis*, and *C. kefyr*). These results are in accord with the findings of this study. Cell wall antigen analyses of the other species of *Kluyveromyces* are not available.

In other genera, Price et al. (37) showed that some yeasts with markedly different cell wall antigentic structures had essentially identical DNA polynucleotide sequences, while the opposite situation was encountered among others.

Many yeast (37) and bacterial (49) taxonomists suggest that the systematic value of serological analyses of the cell wall surface components is often dubious. This conclusion is supported by Ballou (2), who showed that the synthesis of many immunochemical determinants of the mannan molecule is controlled by single, easily mutable genes and by the observation that they may undergo considerable modifications in different culture media (38).

Comparison of electrophoretic studies of isoenzyme patterns. Sidenberg and Lachance analysed seven isoenzymes to assess the taxonomic relationships among type strains of *Kluyveromyces* spp. (45) and among many isolates obtained from natural habitats (46). Their results indicated the following: (i) *K. austearii*, *K. africanaus*, *K. dobzhanski*, *K. loddieri*, *K. phaffii*, *K. polysporus*, *K. thermotolerans*, *K. waltii* and *K. wickerhamii* should be retained as separate species; (ii) *K. drosophilaram*, *K. lactis*, *K. phaseolosporus*, and *K. vanudeni* should be considered conspecific; (iii) *K. bulgaricus*, *K. cicerisporus*, *K. fragilis*, *K. marxianus*, and *K. wickenii* form a cohesive electrophoretic group; and (iv) *K. waltii* has a high degree of electrophoretic similarity to *K. bulgaricus*.

These conclusions agree well with those obtained by DNA-DNA comparison. The only discrepancy was the similarity of *K. waltii* and *K. bulgaricus* observed by Sidenberg and Lachance in their first investigation (45). However, by comparison of the electrophoretic pattern of *K. waltii* and those of other members of the *K. marxianus* group, Sidenberg and Lachance (46) later concluded that *K. waltii* should be retained as a separate species. Of all the taxonomic systems previously proposed and used, electrophoretic analysis of isoenzyme patterns is the only procedure which provides results comparable with those of genome comparisons.

Comparison of interfertility and DNA sequence complementarity. According to the definition of a biological species (8), the taxa within a perfect yeast genus can be delineated in terms of interfertility (61). Wickerham and Burton (59, 60) obtained fertile hybrids from *K. fragilis* × *K. dobzhanski* and *K. lactis* × *K. marxianus*. These results were confirmed by Johannsen and van der Walt (15), van der Walt and Johannsen (56), and Johannsen (14), who studied interfertility within the genus *Kluyveromyces* by using prototrophic selection among auxotrophic mutants of strains of all accepted species. Two groups (syngameons) of separate, mutually interfertile taxa were thereby established: syngameon 1 comprises *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanski*, *K. drosophilaram*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudeni*, and *K. wikenii*; syngameon 2 comprises *K. dobzhanski*, *K. drosophilaram*, *K. lactis*, *K. vanudeni*, and *K. wickerhamii*. Lower-frequency recombination hybrids were also obtained between *K. drosophilaram* and *K. waltii*, *K. marxianus* and *K. thermotolerans*, *K. bulgaricus* and *K. phaseolosporus*, and *K. phaseolosporus* and *K. wikenii*. These mating compatibility patterns were used to delimit species of *Kluyveromyces* in the latest monograph on yeast taxonomy (57).

DNA homologies support only part of the lineage set forth by van der Walt and Johannsen (57). Strains characterized by recombination frequencies indicating mating compatibility consistently showed high levels of DNA homology (>80%); while a high level of interfertility was found in 11 crosses between strains showing degrees of DNA-DNA homology lower than 20%. Since genetic relatedness in terms of interfertility implies genetic compatibility with respect to the entire organized genome, it appears difficult to accept the simultaneous presence of *K. lactis*, *K. vanudeni*, and *K. drosophilaram* in two different interfertility groups (syngameons 1 and 2). For example, the same yeast cannot
possibly be interfertile with both the species of the syngameon 1 and those of syngameon 2 which appear to be interrelated by a consistently low (less than 10 × 10^6) degree of interfertility.

An analogous situation was found by Kurtzman et al. (21) between Issatchenkia scutulata var. scutulata and I. scutulata var. exigua, where 25% DNA homology corresponds to a positive intervarietal mating with 2 to 3% viable ascospores. These authors concluded that "failure to mate or to produce progeny may not mean lack of relatedness because of the many factors affecting mating and subsequent development of the sexual spores."

In our opinion, the capability of genetic exchange may imply compatibility of only a small portion of the entire genome. There is indirect evidence for this interpretation in the observation of Spencer et al. (47) that interspecific as well as intergeneric protoplast fusion apparently occurs, even though the resulting hybrids maintain over 90% of one predominating parental genome, while only negligible amounts of genetic material (i.e., the recombinant phenotypic character) are exchanged. Furthermore, preliminary results of systematic tetrad analysis coupled with DNA reassociation experiments (Vaughan Martini et al., unpublished data) indicate that in F1 as well as in F2 generation hybrids of K. thermotolerans and K. marxianus, the genome of the latter species always predominates at greater than 85% homology levels.

ACKNOWLEDGMENTS

We are indebted to Massimo Bellini for invaluable technical assistance. We also thank H. J. Phaff of the University of California at Davis for helpful criticism.

LITERATURE CITED


VOL. 37, 1987

TAXONOMIC REVISION OF KLUYVEROMYCES


