HYBRIDIZATION STUDIES WITHIN THE GENUS *KLUYVEROMYCES* VAN DER WALT EMEND. VAN DER WALT

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ABSTRACT

Hybridization studies based on the prototrophic selection technique, involving the use of auxotrophic mutants of strains of all accepted species of the genus *Kluyveromyces*, are reported. Two main groups of mutually interfertile taxa were established within the genus. The first group comprises *Kluyveromyces bulgaricus*, *Kluyveromyces cicerisporus*, *Kluyveromyces dobzhanskii*, *Kluyveromyces drosophilarum*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces phaseolosporus*, *Kluyveromyces vanudenii* and *Kluyveromyces wikenii*. The second group consists of *Kluyveromyces dobzhanskii*, *Kluyveromyces drosophilarum*, *Kluyveromyces lactis*, *Kluyveromyces vanudenii* and *Kluyveromyces wickerhamii*. Hybrids were also detected in crosses involving *Kluyveromyces drosophilarum* and *Kluyveromyces waltii* as well as *Kluyveromyces marxianus* and *Kluyveromyces thermotolerans*.

In terms of the concept of the biological species and in compliance with the requirements of the International Code of Botanical Nomenclature, taxa which hybridize with *Kluyveromyces marxianus* and form fertile recombinants at frequencies observed in intraspecific crosses, are accepted as varieties of *Kluyveromyces marxianus*.

Hybridization was observed between *Kluyveromyces marxianus* var. *lactis* and the presumed imperfect forms of some *Kluyveromyces* species, namely *Candida kefyr*, *Candida macedoniensis* and *Torulopsis sphaerica*.

Recombination was not detected in crosses involving *Kluyveromyces marxianus* var. *marxianus* and representatives of other yeast genera, i.e. *Pichia*, *Saccharomyces*, *Torulaspora* and *Zygosaccharomyces*.

Conclusions regarding the relationship between members of the genus *Kluyveromyces*, reached on the basis of this investigation are compared with those reported by other workers, who based their investigations on phenotypic characteristics as well as on the determinations of mol % G+C and DNA-DNA homology studies.

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1. INTRODUCTION

1.1 HISTORY OF THE GENUS *KLUYVEROMYCES*

The genus *Kluyveromyces* was introduced by van der Walt (1956a) to classify an undescribed, budding, fermentative yeast, *Kluyveromyces polysporus*, which formed large asci containing up to seventy or more reniform to long-oval spores. The genus was named in honour of Prof Albert J. Kluyver. Van der Walt (1956a) diagnosed the genus as:

Kluyveromyces van der Walt 1956

"Cells oval to long-oval. Vegetative reproduction by multilateral budding. A pseudomycelium may be formed. An isogamous or heterogamous conjugation generally precedes ascus formation. Asci may also be formed without immediately preceding conjugation. The large asci are multispored. Ascospores long-oval to reniform. In liquid media a pellicle may be formed. Fermentation. Nitrate not assimilated."

In the same year van der Walt (1956b) isolated and described a second multispored species forming 1-16 spores per ascus, which he assigned to the genus as *Kluyveromyces africanus*. Nine years later, van der Walt (1965) amended the genus *Kluyveromyces*, which resulted in the inclusion of several yeast species of uncertain taxonomic position. Before the formal diagnosis and the demarcation of the amended genus *Kluyveromyces* is presented a short review of the controversial approaches to the classification of some species forming reniform ascospores, included by van der

Walt in the genus, is appropriate.

Approximately two years before van der Walt introduced the genus *Kluyveromyces*, Kudriawzew (1954) in his revision of the classification of *Saccharomyces macedoniensis* Diddens et Lodder and *Saccharomyces fragilis* Jörgensen, stressed that these two species differed significantly from what he considered to be the "true" *Saccharomyces* species - i.e. based on *Saccharomyces cerevisiae* Hansen - in both morphological and physiological properties. These two species unlike the species of *Saccharomyces sensu stricto* formed bean-shaped ascospores in fragile asci which ruptured easily at maturity. In contrast to *Saccharomyces sensu stricto* these two species were found to be weak producers of ethanol forming only 4-4,5% ethanol in media of high sugar content. On the basis of these differences, Kudriawzew (1954) assigned *Sacch. macedoniensis* and *Sacch. fragilis* to a new genus *Fabospora* as *Fabospora macedoniensis* (Diddens et Lodder) Kudriawzew and *Fabospora fragilis* (Jörgensen) Kudriawzew. On the same grounds Kudriawzew (1954) transferred *Saccharomyces marxianus* Hansen and a new isolate from an exudate of oak, to a second new genus *Zygofabospora* as *Zygofabospora marxiana* (Hansen) Kudriawzew and *Zygofabospora krassilnikovii* Kudriawzew, respectively. In his monograph of 1954 Kudriawzew omitted to give a formal latin diagnosis of the two genera. This was, however, corrected in the German edition of his work in 1960, in which these genera were cited as:

Fabospora Kudriawzew 1960

"*Saccharomycetaceae* which in old cultures form four-spored asci parthenogenically. Spores, bean-shaped, smooth, hyaline, after germination give rise to a haploid generation. Membranes of asci fragile. Some cells are converted into arthrospores. Good oxidation of carbohydrates.

Fermentation weak."

Zygofabospora Kudriawzew 1960

"*Saccharomycetaceae* which in old cultures give rise to vegetative growth by copulation of pairs of cells and by the subsequent formation of up to four spores in zygotes. Spores bean-shaped, smooth, hyaline, after germination give rise to a haploid generation. Membranes of zygotes and asci fragile."

At approximately the same time Wickerham (1955) and Wickerham & Burton (1956a, b) arrived at a similar conclusion to that of Kudriawzew, namely that the genus *Saccharomyces* (Meyen) Reess, as defined by Lodder & Kreger-van Rij (1952a), was heterogenous and that the yeast species forming reniform ascospores were not in fact representatives of this genus. *Saccharomyces lactis* Dombrowski, *Sacch. fragilis* Jörgensen and *Sacch. marxianus* Hansen, in Wickerham's opinion, constituted an alien group not related to *Sacch. cerevisiae* Hansen. These three species as well as *Saccharomyces dobzhanskii* Shehata, Mrak et Phaff were reported by Wickerham (1955) and Wickerham & Burton (1956a, b) to form hybrids. The other properties differentiating them from *Saccharomyces* included the rupturing of asci directly on the medium on which they were produced, the utilization of a greater number of carbon sources, production of marked amounts of ethyl acetate and formation of red pigment, probably pulcherrimin. On the basis of these differences Wickerham (1955) and Wickerham & Burton (1956a, b) proposed to assign these species to the anticipated genus *Dekkeromyces*. Although Wickerham & Burton (1956a, b) gave a detailed description of the envisaged genus and discussed the lines of presumed evolutionary development of the species assigned to *Dekkeromyces*, no formal diagnosis or designation of the type species was submitted. As the

generic name *Dekkeromyces* does not meet the requirements of the International Code of Botanical Nomenclature (Art. 36 and 41, Stafleu *et al.*, 1972), all binomial citations involving this generic name are *nomena illegitima*. It should be emphasised that the envisaged genus *Dekkeromyces* comprised the four species which Kudriawzew (1954, 1960) assigned to *Fabospora* and *Zygofabospora*.

Novák & Zsolt in 1961 proposed a new system for the yeast classification and formally introduced a new family of *Fabosporaceae* for the yeasts forming reniform ascospores citing it as:

Fabosporaceae Novák et Zsolt 1961

"Budding cells, in some species pseudomycelium or true mycelium, respectively. No arthrospores. Holozygotic ascus formation. Spores reniform or oblong-ovoid, smooth."

Three genera were assigned to this family, namely:

Guilliermondella Nadson et Krassilnikov

Kluyveromyces van der Walt

Dekkeromyces Wickerham et Burton

Novak & Zsolt (1961) introduced ten new combinations in the genus *Dekkeromyces*, which in terms of Art. 36 and 43 of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972), are all illegitimate.

Boidin, Abadie, Jacob & Pignal (1962) revising the classification of the budding yeast species forming reniform ascospores, proposed to assign these species to the amended genus *Guilliermondella* Nadson et Krassilnikov, based on the type species *Guilliermondella selenospora*

Nadson et Krassilnikov. The amended genus was cited as:

Guilliermondella (Nadson et Krassilnikov) emend. Boidin, Abadie, Jacob et Pignal 1962

syn.: *Fabospora* Kudriawzew 1960 (type: *F. macedoniensis*)

Zygofabospora Kudriawzew 1960 (type: *Z. marxiana*)

"Budding yeasts which form asci which open easily. Spores, crescent-shaped, kidney-shaped (or bean-shaped) exceptionally spherical with a thin, smooth, non-amyloid wall. Ability to ferment glucose, very often also galactose, sucrose and raffinose, rarely maltose and lactose. Assimilation of numerous carbon substances other than sugars, namely glycerol, very often ethanol, pyruvate, lactate and succinate. No assimilation of nitrate or (with exception) growth without vitamins. Often an isogamous or heterogamous conjugation. True mycelium or (and) pseudomycelium may or may not be formed. A pellicle may or may not be formed on assimilation media. Several representatives form a brown-red pigment under certain conditions."

On the basis of their amendment Boidin *et al.* (1962) introduced nine new combinations in *Guilliermondella*. The inclusion of *G. selenospora* together with other budding yeasts to the same family of the *Fabosporaceae* by Novák & Zsolt (1961) or to the single genus *Guilliermondella* as proposed by Boidin *et al.* (1962) was questioned by Kreger-van Rij & van der Walt (1963). These authors observed fundamental differences in the ultrastructure of the falcate ascospores of *G. selenospora* and reniform ascospores of *Sacch. fragilis*. In addition, Kreger-van Rij & van der Walt (1963) stressed the fact that the fragility of mature asci was also observed in species unrelated to either *G. selenospora* or *Sacch. fragilis*. This

supported their contention that *G. selenospora* should be excluded from both the family of *Fabosporaceae* and the genus *Guilliermondella*.

Van der Walt (1965) pointed out that the genus *Kluyveromyces* was differentiated from the genera *Fabospora* Kudriawzew, *Zygofabospora* Kudriawzew and *Guilliermondella* (Nadson et Krassilnikov) emend. Boidin *et al.* (with the exception of *G. selenospora*) mainly on the basis of the multispored asci observed in the type species, i.e. *K. polysporus* and in *K. africanus*. As the formation of multispored asci is believed to be due to the non-synchronous, supernumerary mitotic division of the haploid nuclei (Roberts & van der Walt, 1959) and since the presence of more than four spores per ascus was not restricted to *Kluyveromyces* but was also observed in *Sacch. marxianus* (Guilliermond & Negroni, 1929) as well as in *Sacch. cerevisiae*, *Pichia membranaefaciens*, *Pichia pseudopolymorpha* and *Pichia terricola* (Lindegren & Lindegren, 1953; Santa Maria, 1959; 1966), van der Walt (1965) amended the genus *Kluyveromyces* to include species forming 1-4 spores per ascus. This emendation resulted in the genera *Fabospora* and *Zygofabospora* becoming synonymous with the name *Kluyveromyces*.

The genus was then cited as:

Kluyveromyces van der Walt emend. van der Walt 1965

"Vegetative reproduction solely by budding. Cells spheroidal, ellipsoidal, cylindrical or elongate. Pseudomycelium may be formed. True mycelium absent. Asci rupture on maturation liberating the ascospores which tend to agglutinate. Ascospores crescentiform, reniform, oblong with obtuse ends, spheroidal or prolate-ellipsoidal. Number of ascospores per ascus ranges from one to numerous. Ascospores smooth. Interfertility within certain affinities. Metabolism oxidative and fermentative. External vitamin source required. Nitrate not utilized. Red,

noncarotenoid pigment(s) may be formed."

Van der Walt cited 12 new combinations in the amended genus *Kluyveromyces*. These combinations were, however, only validated by him in 1971.

In the second edition of The Yeasts edited by Lodder, van der Walt (1970a) retained the genus with the addition of four species, namely *Kluyveromyces bulgaricus*, *Kluyveromyces cicerisporus*, *Kluyveromyces veronae* (syn. *Kluyveromyces thermotolerans* see Yarrow 1972) and *Kluyveromyces wikenii*.

With the speciation within the genus based on generally employed morphological and physiological criteria, the following 20 taxa listed in alphabetical order are presently accepted in the genus *Kluyveromyces*:

Kluyveromyces aestuarii (Fell) van der Walt 1961

Kluyveromyces africanus van der Walt 1956b

Kluyveromyces blattae Henninger et Windisch 1976

Kluyveromyces bulgaricus (Santa Maria) van der Walt 1956

Kluyveromyces cicerisporus van der Walt, Nel et van Kerken 1966

Kluyveromyces delphensis (van der Walt et Tscheuschner) van der Walt 1956

Kluyveromyces dobzhanskii (Shehata, Mrak et Phaff) van der Walt 1955.

Kluyveromyces drosophilarum (Shehata, Mrak et Phaff) van der Walt 1955

Kluyveromyces fragilis (Jörgensen) van der Walt 1909

Kluyveromyces lactis (Dombrowski) van der Walt 1910

Kluyveromyces lodderi (van der Walt et Tscheuschner) van der Walt 1957

Kluyveromyces marxianus (Hansen) van der Walt 1888

Kluyveromyces phaffii (van der Walt) van der Walt 1963

Kluyveromyces phaseolosporus (Shehata, Mrak et Phaff) van der Walt 1955

Kluyveromyces polysporus van der Walt 1956a

Kluyveromyces thermotolerans (Philippov) Yarrow 1932

Kluyveromyces vanudenii (van der Walt et Nel) van der Walt 1963

Kluyveromyces waltii Kodama 1974

Kluyveromyces wickerhamii (Phaff, Miller et Shifrine) van der Walt 1956

Kluyveromyces wikenii van der Walt, Nel et van Kerken 1966.

1.2 CONTROVERSY OVER THE AMENDED GENUS *KLUYVEROMYCES*

The emendation of the genus *Kluyveromyces* (van der Walt, 1965) has not, however, terminated the controversy over the demarcation within this taxonomic area. Taxonomists raised various objections and suggested numerous criteria for establishing the affinity between the taxa assigned

to this genus as well as between *Kluyveromyces* and presumably related genera.

Santa Maria & Sanchez (1970) on the basis of the comparative study of various physiological properties proposed to exclude six species from the genus *Kluyveromyces*, namely *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. phaseolosporus* and *K. wickerhamii* and to transfer them to the reinstated genus *Dekkeromyces*. However, as Santa Maria & Sanchez (1970) failed to validate the genus in terms of the requirements of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972), the new combinations proposed by them are illegitimate.

Nakase & Komagata (1971) as well as Poncet & Fiol (1972) on the basis of DNA composition studies basically subscribed to the retention of the genus in its present form as demarcated by van der Walt. On the other hand, Kocková-Kratochvílová *et al.* (1972) applying numerical analysis supported the recommendation of Santa Maria & Sanchez (1970) to retain the multispored species in the genus *Kluyveromyces* with the concomitant transfer of the remaining species into *Dekkeromyces*. Campbell (1972), also on the basis of numerical analysis of serological, morphological and physiological properties, proposed to incorporate *Kluyveromyces* into the genus *Saccharomyces*. Poncet (1973) suggested a reduction in the number of species from eighteen to seven, with the recognition of two main groups in the genus.

The criteria applied by other workers in their attempt to elucidate the interspecific and intergeneric affinities of the strains assigned to this taxonomic area included the following: antigenic properties (Tsuchiya *et al.*, 1965; Segal *et al.*, 1975); immunochemical and

physical properties of the cell-wall mannans (Spencer & Gorin, 1969; Šandula *et al.*, 1974); structure of the cell wall (Bastide *et al.*, 1975); cellular lipid composition (Kaneko *et al.*, 1976); intracellular enzymes and vitamin requirements (Fiol, 1967, 1972, 1973, 1975; Fiol & Poncet, 1971); coenzyme Q-system (Yamada *et al.*, 1976); DNA base composition (Meyer & Phaff, 1970; Martini *et al.*, 1972) and DNA-DNA homologies (Bicknell & Douglas, 1970; Martini, 1973; Martini & Phaff, 1973; Groot *et al.*, 1975).

Other criteria include: spectral requirements for photoreactivation of UV-inactivated cells (Sarachek & Bish, 1975); production of extracellular ribonucleases (Burt & Cazin, 1976) as well as physical and immunological properties of exo-β-glucanases (Lachance & Phaff, 1977).

Despite their diversity, these numerous attempts have one common factor, namely a search for suitable criteria for establishing the parameters of a species and of a genus. It is interesting to note that with the exclusion of the DNA base composition and DNA-DNA homology studies all these attempts were based on phenotypic characteristics of the yeast species. It can also be observed that all these workers with the exception of Fiol & Poncet (1971), who reported on hybridization between two *Kluyveromyces* species and also Herman (1970a, b), who studied the sex-specific growth responses in *Kluyveromyces*, did not consider the far-reaching statement of Wickerham & Burton (1956a) that in this taxonomic area hybridization should be employed "for determining whether two closely related taxons should be considered as varieties of a species or as two independent species."

The correct evaluation of the suitability of various criteria employed in taxonomy requires a clear definition of the basic unit which

such criteria intend to demarcate. Such a basic unit in taxonomy is a species.

1.3 CONCEPT OF A SPECIES

The term "species" so universally used by workers in various disciplines of biology has many, not infrequently contradictory meanings and remains "one of the most poorly understood of all basic units of biological organization" (Grant, 1971a). Considering the fact that the only tangible unit in taxonomy is an individual, or in yeast taxonomy a strain, a species is to a certain degree an abstract concept (Bradley & Bond, 1974). Different branches of biology arrive at a definition of a species in their distinctive and frequently divergent ways. This term generally denotes a group comprising similar and closely related organisms, designated by a common name. The assessment of the similarity and relatedness is frequently very difficult and in the majority of biological sciences, including zymology, is based on numerous and diverse criteria. Since the choice of these criteria depends to a greater or lesser extent on the individual preferences or insights of taxonomists, the results obtained cannot lead to unanimous taxonomic conclusions.

John Ray, a 17th century English naturalist, was one of the first to propose that species should be recognized on the basis of the structural differences transmitted from generation to generation. He also proposed a formal arrangement of species. The binomial system used (with some modifications) in modern taxonomy was developed by Carolus Linnaeus (1758). In the opinion of Linnaeus each species, being the result of a separate act of creation, was fixed and did not undergo any changes. Such a "static" concept of the species can result in regarding

each single individual organism (corresponding to a strain in zymology) as an entire species.

Jean Lamarck's observations on the gradual changes to which all species are subjected (Darwin, 1878) called for a more flexible and dynamic concept of the species. The necessity to alter the concept of the species became even greater with the publication in 1859 of Charles Darwin's "The origin of species" and the rediscovery in 1900 of Gregor Mendel's pioneering work on heredity (Hayes, 1968a ; Rothwell, 1976a). The concept of the species should therefore accommodate both the genetics of the organisms studied as well as the evolutionary changes to which these organisms were, and are presently being subjected.

The three main concepts of the species currently recognized are:

taxonomic species (1.3.1);

biological species (1.3.2);

evolutionary species (1.3.3).

1.3.1 Taxonomic species

A taxonomic species bearing a certain resemblance to the Linne'an typological species as well as to the morphological species, is delimited on the basis of phenotypic differences between individuals. This delimitation is based on the differences in morphological and physiological properties.

In the yeast domain the concept of the taxonomic species can be traced to Hansen's (1888) pioneering studies. Hansen sought to distinguish yeast strains on the basis of

their cultural and morphological properties as well as their ability to ferment different sugars. Until 1970 this concept of the yeast species was, with a few exceptions (e.g. genus *Hansenula* Wickerham & Burton, 1962; Wickerham, 1966, 1969), adopted by taxonomists.

It should be stressed that the expression of certain morphological or physiological properties might be due to the actions of a single gene. The delimitation of taxa in terms of the taxonomic concept of the species is at times dubious and some taxa defined as separate species, have been found to differ basically in the utilization of a single carbon source or in the rate of fermentation of a single sugar (e.g. *K. bulgaricus* and *K. cicerisporus* as well as *K. fragilis* and *K. marxianus*, respectively).

The reliance on the morphological properties in the delimitation of species became debatable with the recovery by Ditlevsen (1944) of a heterozygous strain of *Saccharomyces italicus* with a gene for elongated cells being dominant over a gene for short-cell formation. Furthermore, Lindegren & Lindegren (1951) showed that in *Sacch. cerevisiae* cell size was proportional to the degree of ploidy. The applicability of the taxonomic species based on physiological criteria ultimately broke down when Scheda (1966) and Scheda & Yarrow (1966, 1968) investigated the instability of the fermentation and assimilation patterns in *Saccharomyces sensu stricto*, *Saccharomycopsis vini* and *Candida vanriji* and found that these criteria were subject to mutational changes. Scheda & Yarrow

(1966) with some justification concluded that: "We can assume that every physiological property of a yeast belonging to any genus may be unstable under certain conditions".

The concept of the taxonomic species permits, however, a relatively quick and easy way of identifying yeast strains.

1.3.2 Biological species

The early naturalists arrived at the concept of the biological species by basing it on the belief that "like begets its own like". For a detailed account of the historical development of this concept the monograph of Grant (1971b) should be consulted.

At present, the biological species is understood to be an isolated system of interbreeding populations or the sum total of interbreeding individuals (Mayr, 1940). The concept of a biological species, in which a sexual process constitutes a *sine qua non* refers only to biparental organisms. This concept, which is based on sexual recognition and readiness to exchange genetic material emphasises the similarities between populations instead of stressing their differences. It also underlines the mechanisms of reproductive isolation by which the integrity of the species is maintained. Since the reproductive isolation can be affected not only by true genetic incompatibility but also by mechanical, geographic or ecological isolation, a taxonomist, in order to understand the nature of the species, is required to study all these

aspects in the life of the chosen group of organisms.

In the yeast domain the concept of the biological species stems from the genetic studies of Winge & Lautsen (1939a) on *Saccharomyces*. These authors maintained that:

> "(1) two types generally ought to be reckoned to the same species when hybridizing and their hybrid being fully fertile, whereas
>
> (2) two types would be regarded as specifically different when they are unable to hybridize or their hybrid shows reduced fertility".

While the introduction of interfertility as a criterion for speciation received little attention in the following years, Wickerham's studies on the genus *Hansenula* summarized in 1969 refocussed the attention on the application of interfertility as a primary criterion for speciation.

Because of the ubiquity of most yeast genera, geographical dispersion does not seem to constitute an isolating mechanism. Consequently, it may be concluded that in the yeast domain biological species are separated mainly on the basis of genetic incompatibility.

It is apparent that the application of the biological concept of the species might be impracticable in the yeast genera in which sexuality has not been detected. The

growing list of imperfect taxa in which the sexual process has been demonstrated (e.g. *Rhodosporidium*, Banno, 1967; *Filobasidium*, Olive, 1968; *Leucosporidium*, Fell *et al.*, 1969; *Filobasidiella*, Kwon-Chung, 1975; *Stephanoascus*, Smith *et al.*, 1976) is, however, encouraging in this respect.

It should be noted that the delimitation of taxa in terms of the taxonomic and biological species is not always exclusive. In fact, in many groups such taxa appear to be synonymous (e.g. *Hansenula anomala* and *Hansenula cifferii*).

1.3.3 Evolutionary species

An evolutionary species, as defined by Simpson (1961), "is a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own evolutionary role and tendencies". Such a lineage possesses its own particular ecological niche and undergoes evolutionary changes during the course of its history. The evolutionary concept of the species embraces both biparental and uniparental organisms and can be considered to be more universal than the biological species (Grant, 1971a).

The evolutionary species embraces the biological species as is evident from the definition of a species given by Dobzhansky (1951): "The species is not a static unit, but a stage in the process of evolutionary divergence. Species are formed when a once actually or potentially interbreeding array of Mendelian populations becomes segregated in two or more reproductively isolated arrays. Species

are, accordingly, groups of populations the gene exchange between which is limited or prevented in nature by one, or by a combination of several, reproductive isolating mechanisms." As pointed out by Lamanna and Mallette (1953) this concept stresses: firstly, the importance of studying differences between populations of individuals and not between individuals; secondly, it focusses the attention on the gap preventing interbreeding rather than on the differences traditionally sought in separating species; thirdly, it underlines the genetic compatibility allowing the exchange of genetic material between populations and lastly, it introduces a dynamic aspect to the concept of a species in accepting it as groups of populations constantly undergoing evolutionary changes.

In relating the concept of the evolutionary species to the yeast domain it must be stressed that the lack of confirmed fossil records makes it difficult to apply this concept to the yeasts. Consequently, taxonomists working in this area have to rely on presumed phylogenies based on extant taxa. A model of such phylogenetic studies was submitted by Wickerham for the genus *Hansenula* (1969). He considered the ecological studies to be the principal ingredients of taxonomy and the sexuality to constitute "the backbone of phylogeny", which in his opinion was "the only effective basis for classification".

Wickerham also emphasised the dynamic aspect of taxonomy - its ever changing relationships requiring

continuous remodification of classification.

From the preceding considerations the following salient points emerge:

(i) taxonomic species on the basis of its manifested shortcomings should, where possible, be abandoned;

(ii) evolutionary or natural species is difficult to apply in yeast taxonomy, due to the lack of confirmed fossil records;

(iii) biological species can be profitably adopted in the demarcation of species in perfect yeast genera.

The acceptance of the concept of the biological species results in recognizing interfertility as a cardinal criterion for speciation.

1.4 INTERFERTILITY

The interfertility existing between organisms implies not only their genetic relatedness but also and primarily the ability to exchange genetic material, which results in the formation of fertile zygotes capable of vegetative as well as sexual reproduction. Interfertility might also be interpreted as an ability to overcome mechanisms isolating the populations of organisms. The isolating mechanisms can be divided into two main groups: prezygotic mechanisms preventing the formation of a zygote and postzygotic mechanisms operating against the independent multiplication of the offspring resulting from the zygote formation.

The isolating mechanisms can also be visualized in the form of barriers preventing:

(i)	transfer of genetic material	prezygotic isolating mechanisms
(ii)	recombination of genetic material	
(iii)	phenotypic expression of combined genomes	postzygotic isolating mechanisms
(iv)	further multiplication	

The process in which the barriers separating mating cells are overcome is referred to as the process of conjugation or mating and constitutes a *sine qua non* of the interfertility studies. Hybridization experiments defining the area in which interfertility might be operative constitute the first step of such studies.

Preparatory stages preceding the act of conjugation, the actual fusion of independent cells followed by karyogamy and meiosis, as well as the subsequent formation of ascospores, are considered to be under the control of the mating type locus (*mat* locus) (Fogel & Mortimer, 1971; Crandall *et al.*, 1977). This complex process will be discussed in terms of the functions controlled by the *mat* locus during haploid (1.5) and diploid (1.6) phases of the life-cycle of the yeast cell. Factors affecting conjugation will be discussed in a separate section (1.7).

There exist comparatively few studies concerning hybridization and the conjugation process in the genus *Kluyveromyces* (Wickerham, 1955; Wickerham & Burton, 1952, 1956a, b; Herman, 1970a, b; Herman & Roman, 1966; Fiol & Poncet, 1971; Mas *et al.*, 1974; Morgan *et al.*, 1977; Allmark *et al.*, 1978). Only limited information is available on the

genetics of these organisms (Tingle *et al.*, 1968; Sanders *et al.*, 1974; Penman & Duffus, 1975; Algeri *et al.*, 1977). However, since the main stages in the conjugation process in yeasts are basically similar (Fowell, 1969; Mortimer & Hawthorne, 1969; Crandall *et al.*, 1977) the ensuing sections are based on extensive studies involving three yeast species, namely *Sacch. cerevisiae*, *Schizosaccharomyces pombe* and *Hansenula wingei*.

1.5 FUNCTIONS CONTROLLED BY THE MATING-TYPE LOCUS DURING HAPLOPHASE

The mating type locus (*mat* locus) is believed to comprise a cluster of genes, both regulatory and structural, which govern the expression of haploid and diploid functions in yeast cells (Friis & Roman, 1968; Fogel & Mortimer, 1971; MacKay & Manney, 1974a; Crandall *et al.*, 1977).

The following functions are controlled by this locus during the haplophase:

determination of the mating type specificity (1.5.1);

production of agglutination factors and diffusible sex factors (1.5.2);

morphological changes occurring during conjugation (1.5.3), including:

dissolution of the cell wall (1.5.3.1);

plasmogamy (cytoplasmic fusion) (1.5.3.2);

karyogamy (fusion of nuclei) (1.5.3.3);

zygote formation and zygotic budding (1.5.3.4);

nucleic acid and protein metabolism during conjugation (1.5.4).

1.5.1 Determination of the mating type specificity

Winge & Lautsen (1939b) discovered a gene determining mating type or compatibility in *Saccharomycodes ludwigii*. The existence of the mating type specificity for *Sacch. cerevisiae* was demonstrated four years later by Lindegren & Lindegren (1943b) and for *Schiz. pombe* 11 years later by Leupold (1950).

It has since been established that some yeast species are strictly heterothallic, e.g. *H. wingei* (Wickerham, 1956, 1969), some exclusively homothallic, e.g. the species of the genus *Kluyveromyces* except for *K. lactis*, (Wickerham & Burton, 1952), while others can exhibit both forms of thallism, e.g. *Sacch. cerevisiae* (Winge, 1935; Lindegren & Lindegren, 1943b; Ahmad, 1953) *Schiz. pombe* (Leupold, 1950, 1955, 1956, 1958; Gutz & Doe, 1973a, 1975) and *Saccharomyces chevalieri* (Takahashi & Ikeda, 1959). It also appears that some haploid yeasts possess genes for both mating types but only one is expressed, e.g. *Schiz. pombe* (Gutz *et al.*, 1975; Egel, 1976). Furthermore, evidence is accumulating that the mating type locus comprises more than two genes (Takahashi, 1958; Harashima *et al.*, 1974) and that it can undergo mutation in respect of the mating ability or specificity (Takahashi, 1958, 1961, 1964; Takahashi & Ikeda, 1959; Hawthorne, 1963; Takano & Oshima, 1967, 1970a, b; Friis & Roman, 1968; Fogel & Mortimer, 1971; Oshima & Takano, 1971; 1972; MacKay *et al.*, 1972; Gutz & Doe, 1973b; Harashima *et al.*, 1974;

MacKay & Manney, 1974a, b; Blamire, 1975; Blamire & Melnick, 1975; Hopper & Hall, 1975a, b; Egel, 1976; Hicks & Herskowitz, 1976a).

According to the experimental evidence obtained by Stahl (1978) in his study on the protoplasts of *Saccharomycopsis lipolytica*, mating type alleles control only the initial steps in conjugation, namely cell recognition and cell agglutination, but do not appear to mediate in karyogamy and meiosis. It is presently believed (Crandall *et al.*, 1977) that the presence of a complex genetic structure in the homothallic yeasts enable them to modify or diversify their sexual functions, offering them higher chances for survival. A homothallic system might therefore be considered to be evolutionarily superior to the more primitive heterothallic system.

1.5.2 Production of agglutination factors and diffusible sex factors

Although sexual agglutination has not, as yet, been observed in the genus *Kluyveromyces* it nevertheless constitutes an important stage in the process of conjugation and will be briefly discussed.

Two types of sexual agglutination are recognized in yeasts:

(a) constitutive, when cells agglutinate almost immediately after coming into contact, e.g. *H. wingei* and *Citeromyces matritensis* (Wickerham, 1956, 1958); and

(b) inducible, requiring prolonged contact between yeast cells, lasting at least one generation, before agglutination can be demonstrated, e.g. *Schiz. pombe* (Egel, 1971), *Sacch. cerevisiae* (Sakai & Yanagishima, 1972).

The agglutination factors are responsible for the recognition of the opposite sex and for the formation of complexes between the cell walls which, in effect, initiates the process of cell merging (Sakai & Yanagishima, 1971; Sakurai *et al.*, 1975; Shimoda & Yanagishima, 1975). It is believed that in *Sacch. cerevisiae* and *Schiz. pombe* the agglutination factors are situated on the fimbriae extending from the cell-wall (Yoo *et al.*, 1971; Day *et al.*, 1975; Poon & Day, 1975; Calleja *et al.*, 1976) and that these surface organelles might "serve to transmit mating type-specific signals during induction of mating" (Crandall *et al.*, 1977).

No sex-specific agglutination was observed in the experiments involving protoplasts of complementary haploid strains of *Sacch. cerevisiae* (Svoboda, 1976), which constitutes additional proof for the association of agglutination factors with the cell wall. Shimoda & Yanagishima (1975) were able to isolate the sex-specific agglutination factors from the cell wall of mating types of *Sacch. cerevisiae* and Shimoda *et al.* (1975) suggested that the protease-susceptible agglutination factors might be a glycoprotein, similar to the agglutinative factors from *H. wingei* (Taylor, 1964; Sakai & Yanagishima, 1972). The mechanism of sexual agglutination was extensively studied

by Taylor (1964) and Taylor & Orton (1968) and the factors affecting agglutination by Calleja (1974).

The agglutination reactions differ in their intensity depending on the type of yeasts, e.g. they are much weaker in *Sacch. cerevisiae* than in *H. wingei* (Crandall & Brock, 1968). Moreover the agglutination might be nonspecific since large clumps of cells can be observed within cultures of the same mating type (Biliński *et al.*, 1975). Evidence that the sexual agglutination may require specific metal ions, such as cupric ions for *Sacch. cerevisiae* and vanadium ions for *H. wingei*, was obtained by Duntze *et al.* (1973) and Crandall & Caulton (1975), respectively. A marked enhancement in β-glucanase activity during the early stages of cell agglutination has been reported for *Sacch. cerevisiae* and *Schiz. pombe* by Shimoda & Yanagishima (1971) and Fleet & Phaff (1974), respectively.

Diffusible sex factors (pheromones) are the chemical compounds produced by cells of one mating type capable of inducing a sexual response in the opposite mating type (Karlson & Lüscher, 1959; Hicks & Herskowitz, 1976b; Crandall *et al.*, 1977). Such a sexual response might be manifested by an increase in cell volume and a change in cell shape (Yanagishima, 1969a, b; Osumi *et al.*, 1974), induction of agglutinability in opposite mating-type cells (Sakai & Yanagishima, 1972; Sakurai *et al.*, 1974; Yanagishima *et al.*, 1974), retardation of budding and DNA synthesis (Biliński *et al.*, 1974; Wilkinson & Pringle, 1974), as well as in sex-specific

orientation in bud formation (Herman, 1971b). Work by Egel & Egel-Mitani (1974) established some evidence for the existence of diffusible sex factors in *Schiz. pombe*, but their presence was not detected in *H. wingei* (Crandall *et al.*, 1977).

A detailed discussion concerning the isolation, chemical composition and the influence of diffusible sex factors on yeast cells was presented by Crandall *et al.* (1977).

1.5.3 Morphological changes observed during conjugation

The first noticeable morphological change occurring when cells of opposite mating types of *Sacch. cerevisiae* are brought into contact is a pronounced increase in the cell volume, which can be up to 30-40% (Yanagishima, 1969a, b) and an alteration in cell shape (Osumi *et al.*, 1974; Crandall *et al.*, 1977) resulting in the formation of elongated forms called shmoos (Levi, 1956).

Before the constituents of cells undergoing conjugation can merge the mechanical barriers separating them, namely cell walls and cytoplasmic membranes (plasmolemmas), must be overcome.

1.5.3.1 Dissolution of the cell wall

Evidence from electron microscopic studies of *Schizosaccharomyces octosporus* (Conti & Naylor, 1960) and *Sacch. cerevisiae* (Osumi *et al.*, 1974) indicates that, in the initial phase of cell conjugation, a thinning of the electron

transparent layer of cell walls takes place in the region towards which the nuclei migrate. A rapid increase in the cellular autolytic activity occurring at this stage of conjugation has been reported by Shimoda & Yanagishima (1972) for *Sacch. cerevisiae* and by Kröning & Egel (1974) for *Schiz. pombe*.

The electron-dense material collecting at the site of the partially dissolved cell walls might be interpreted as a kind of "cementing substance produced in response to the cell-to-cell contact" (Osumi *et al.*, 1974). The cell wall in the region of contact undergoes further degradation, starting from the central point of contact and gradually becoming completely dissolved (Conti & Naylor, 1960), while the localized extensions of the cell walls in the proximity of the site of contact lead to the formation of protuberances. Fusion of the protuberances results in the formation of conjugation tubes (Conti & Brock, 1965; Osumi *et al.*, 1974) which involves an increase in the activity of both degradative and synthetic enzymes (Brock, 1965; Nisizawa & Hashimoto, 1970). These changes in the cell wall structure are accompanied by the fusion of cytoplasmic membranes, followed by their fragmentation and subsequent formation of a coiled

membrane structure in the cytoplasm (Osumi *et al.*, 1974). Changes in the morphology of mitochondria occurring simultaneously with the disintegration of the cytoplasmic membranes were observed by Smith *et al.* (1972).

The vital role played by the cell wall in conjugation was revealed in studies of the mating reaction in yeast protoplasts. Svoboda (1976) observed neither pairing nor fusion between protoplasts of complementary haploid strains of *Sacch. cerevisiae* until the regeneration of the cell wall was completed. Induced fusion of yeast protoplasts was, however, reported for *Sacch. cerevisiae* by van Solingen & van der Plaat (1977), for *Saccharomycopsis lipolytica* by Stahl (1978) and for *K. lactis* by Morgan *et al.* (1977) and Allmark *et al.* (1978). In these cases the presence of polyethylene glycol was found necessary before the conjugation of protoplasts could be observed.

1.5.3.2 Plasmogamy (cytoplasmic fusion)

The movement of cytoplasm and cytoplasmic organelles begins when the cell wall and cytoplasmic membrane are only partially dissolved. Proliferation of endoplasmic reticulum (Osumi *et al.*, 1974) and pronounced morphological changes in mitochondria such as elongation and

de-differentiation (Smith *et al.*, 1972) were observed during the early stages of cytoplasmic mixing. With the movement of the cytoplasm, nuclear migration occurs and at the early phase of cytoplasmic fusion a transient state of heterokaryosis is observed. The duration of the heterokaryon state is not known, but it probably varies according to the yeast species. The heterokaryon state usually ends with the formation of the first zygotic bud (Crandall *et al.*, 1977). Cytoplasmic fusion taking place in the zygote is usually immediately followed by the fusion of nuclei.

1.5.3.3 Karyogamy (fusion of nuclei)

At an early stage of karyogamy nuclei migrating towards the region of contact between conjugating cells, become slightly elongated. The initial point of contact between merging nuclei increases in size and karyogamy is accomplished by the breaking down and subsequent rejoining of the nuclear membranes (Osumi *et al.*, 1974). More detailed observations on the mechanism of nuclear fusion in *Sacch. cerevisiae* were conducted by Byers and Goetsch (1973). The newly formed diploid nucleus will either proliferate mitotically or will undergo meiosis, depending

on the experimental conditions prevailing.

During the processes of plasmogamy and karyogamy rejoining of the cytoplasmic membrane and of the cell wall of the zygote is initiated. However, even when these two processes are completed, the scars in the cell wall at the region of the initial contact between conjugating cells remain visible (Osumi *et al.*, 1974).

1.5.3.4 Zygote formation

The newly formed zygotes usually have a typical dumb-bell shape. T-shaped zygotes are observed when conjugation is initiated at the long side of a cell. The process of zygote formation has been studied in both non-synchronized (Crandall *et al.*, 1974) and synchronized cultures (Sena *et al.*, 1973, 1975, 1976; Biliński *et al.*, 1975). Synchronization of the culture is a prerequisite to studies of biochemical and kinetic changes occurring during the mating process and in subsequent zygote formation.

The efficiency of zygote formation depends upon many genetic factors existing in the particular yeast, as well as on the media and growth conditions employed (Egel, 1971; Crandall *et al.*, 1977). The initiation of zygote formation in *Sacch. cerevisiae* was observed by Sena *et al.* (1975, 1976) within the first 60 min of bringing

opposite mating types into contact but only after four and a half hours by Biliński *et al.* (1975), who used a slightly different technique for synchronous zygote production. The number of zygotes subsequently formed is reported to increase almost exponentially (Sena *et al.*, 1976). The reported frequencies of zygote formation vary from 30-40% (Biliński *et al.*, 1975), 30-50% (Egel, 1971), 60% (Sena *et al.*, 1975), 70-80% (Conti & Brock, 1965) to over 95% (Mortimer, 1955) depending on the organism, technique and the composition of the medium.

In *Sacch. cerevisiae* budding was observed to be initiated within approximately 30 min after zygote formation (Sena *et al.*, 1976). The first zygotic bud is usually formed at a central part of the conjugation tube or at the junction of mating cells in the absence of a conjugation tube, presumably because the cell wall in this region is very thin (Brock, 1961; Byers & Goetsch, 1973; Waxman *et al.*, 1973). Only a small number of zygotes has been observed to form a first bud at one or other of the poles (Crandall *et al.*, 1977). It has been established that in *H. wingei* and *Sacch. cerevisiae* the first zygotic buds possess diploid nuclei (Brock, 1961; Byers & Goetsch, 1973;

Sena *et al.*, 1976), while in *Schiz. pombe* a diploid culture is formed only under special conditions (Crandall *et al.*, 1977). The diploid nuclei of the first zygotic buds were found to contain genetic material derived from both parental nuclei. However, the distribution of cytoplasmic genetic constituents between the zygote and its bud was found to be highly heterogeneous (Birky, 1974; Dujon *et al.*, 1974; Strausberg & Perlman, 1974), and this could be a result of cytoplasmic mixing, mitochondrial dissolution and/or fusion, as well as mitochondrial (*mt*) DNA mixing and packaging. After the formation of the first zygotic bud the endoplasmic reticulum and mitochondria revert to their original appearance (Smith *et al.*, 1972; Osumi *et al.*, 1974).

1.5.4 Nucleic acid and protein metabolism during conjugation

Relatively little is known about the biochemical reactions taking place during conjugation. The increase in cell volume observed at the initial stage of conjugation (1.5.3) could, in part, be due to intensive protein and RNA synthesis (Biliński *et al.*, 1975).

Duplication of both nuclear and *mt* DNA has been studied more extensively. Investigations by Sena *et al.* (1973, 1976) on mating in *Sacch. cerevisiae* revealed that the total DNA

content per parental cell reached a level of approximately 16 femtogram (fg)* per parental pair during cell pairing and the initial stage of zygote formation. With the initiation of the first zygotic bud the amount of DNA began to increase and continued to increase during zygote maturation to reach a level of 75 fg of DNA per parental cell by the time at least one mature bud had formed. Further studies with ^{15}N-labelled parental cells mated in ^{14}N-medium demonstrated differences in the replication of nuclear and *mt* DNA. Nuclear DNA synthesis was discontinued during the first two hours of mating and re-initiated approximately two and a half hours later, with the simultaneous formation of a zygotic bud. Replication of *mt* DNA continued throughout the mating reaction and subsequent zygote formation and maturation. Both types of DNA reached a maximum level during zygote maturation, approximately five and a half hours after the initiation of mating. Analyses of the DNA content of the first zygotic buds revealed the level of both nuclear and *mt* DNA to be diploid. Considering the differences in the mode of replication of both types of DNA Sena *et al.* (1976) concluded that the mechanisms controlling the replication of the two types of DNA were different.

1.6 FUNCTIONS CONTROLLED BY THE MATING-TYPE LOCUS DURING DIPLOPHASE

The functions of the *mat* locus, at the stage when the conjugating cells reach the diploid state, focus primarily on the promotion of meiosis

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*1 fg = 10^{-15} g

and sporulation with the simultaneous suppression of haploid functions. The morphological and physiological changes accompanying the closely interlinked processes of meiosis and sporulation have been extensively studied by numerous geneticists and cytologists and will be reviewed in the following order:

suppression of haploid functions (1.6.1);

morphological changes occurring during the initial stages of meiosis (1.6.2);

chromosomal behaviour during meiosis (1.6.3);

morphological changes occurring during sporulation (1.6.4);

physiological changes observed during meiosis and sporulation (1.6.5).

1.6.1 Suppression of haploid functions

The suppression of haploid functions is basically expressed by the inhibition of conjugation. In *H. wingei* a most conspicuous function of the *mat* locus genes in the diploid state is the cessation of the production of agglutination factors (Crandall & Brock, 1968)*. Rare cases of agglutination occurring between induced diploids do not result in the formation of tetraploid zygotes, since further steps in conjugation are arrested.

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*These factors can, however, be induced in diploids under special physiological conditions (Crandall & Caulton, 1975).

In *Sacch. cerevisiae*, the composition of the *mat* locus in diploid cells significantly influences the mating ability. Thus, in heterozygous diploids mating is generally inhibited (Haber, 1974; Crandall *et al.*, 1977), whereas the mating ability of homozygous diploids seems to be the same as in corresponding haploid types (Roman & Sands, 1953; Mortimer, 1958; Duntze *et al.*, 1970).

In *Schiz. pombe*, meiosis and sporulation occur almost immediately after the fusion of nuclei and therefore the diploid cells are encountered only sporadically. Infrequent formation of triploid and even tetraploid zygotes in this species has, however, been observed by Leupold (1956) and Crandall *et al.* (1977).

1.6.2 Morphological changes occurring during the initial stages of meiosis

The first morphological changes observed in diploid cells at the beginning of meiosis and sporulation are an increase in cell volume (Pontefract & Miller, 1962; Croes, 1967) and an enhanced movement of the cytoplasm (Hagedorn, 1964). The large number of ribosomes present in diploid vegetative cells rapidly decreases with the initiation of sporulation (Mundkur, 1961). Fragmentation of vacuoles with a simultaneous increase in numbers of small granules is also observed at this stage (Miller *et al.*, 1963; Svihla *et al.*, 1964; Croes, 1967). The granules increase both in number and size and finally condense into a compact mass situated in the centre of the

cell (Fowell, 1969). Some granules are considered to be mitochondria (Brandt, 1941) while others appear to constitute reserves of glycogen and fat (Pontefract & Miller, 1962; Miller *et al.*, 1963).

The diploid nucleus situated in the dense, granular mass enlarges, becomes elongated and less compact and subsequently assumes a shape similar to a dumb-bell. The central, elongated part of the nucleus becomes disrupted with the formation of two daughter nuclei, which shortly afterwards divide again, forming four smaller nuclei in the final phase (Winge & Roberts, 1954; Pontefract & Miller, 1962; Fowell, 1969).

A more detailed description of the nuclear changes taking place during meiosis and sporulation was provided by Sherman & Roman (1963); Moens (1971, 1973); Moens & Rapport (1971); Roth & Fogel (1971); Guth *et al.* (1972) and Moens *et al.* (1977). During the first six hours after the transfer of cells onto sporulation media, diploid nuclei showed a single plaque and a nucleolus. Within the next two hours, synaptonemal complexes are observed to aggregate into a single polycomplex body. At the same time the spindle plaque starts replicating. During the ninth hour the plaques move apart and form a short spindle. No free pairs of homologous chromosomes are observed at this stage. The spindle becomes more elongated and both plaques duplicate. The nuclear changes described so far are considered to correspond to the chromosomal

changes observed during the first meiotic division (*cf.* chromosomal behaviour 1.6.3.1).

The initiation of a second meiotic division probably takes place when two plaques of each pair become separated and form new spindles. The walls of the future ascospores begin to develop in the vicinity of these four plaques. Newly formed spindles elongate and the simultaneous distribution of cytoplasm, mitochondria and nuclei into four clusters occurs. The second meiotic division is accomplished when the nuclear material is distributed between the four developing ascospores.

1.6.3 Chromosomal behaviour during meiosis

Meiosis in yeasts follows the same general pattern as that observed in higher eukaryotic organisms (Fowell, 1969; Fogel & Mortimer, 1971; Baker *et al.*, 1976). The following description of the main stages and substages distinguishable in meiosis, is based on the account given by Hayes (1968b) and Rothwell (1976b). A schematic representation of the events taking place in meiosis at the chromosome level is given in Fig. 1.

1.6.3.1 First meiotic division

At the beginning of prophase I, i.e. during leptonema, the chromosomes of the diploid (zygotic) cell appear as thin, elongated threads or chromonemata, with more intensely staining areas called chromomeres.

First meiotic division

Prophase I:

leptonema	-	elongation of chromosomes
zygonema	-	stage of active pairing of homologous chromosomes (pairing of synapsis)
pachynema	-	doubling of chromosomes
diplonema	-	separation of the chromatids (crossing over, chiasma formation)
diakinesis	-	contraction of chromosomes and their separation

Metaphase I - spindle formation

Anaphase I - migration of chromosomes towards opposite poles of the cell

Telophase I - reappearance of nucleolus and nuclear membranes

Interphase

Second meiotic division

Prophase II

Metaphase II

Anaphase II

Telophase II

FIG. 1 A SCHEMATIC REPRESENTATION OF EVENTS TAKING PLACE IN MEIOSIS AT THE CHROMOSOMAL LEVEL

During zygonema homologous chromosomes become attracted towards one another, migrate in the direction of their partners (even over large distances in the nucleus) and finally are observed to lie together with corresponding alleles on both chromosomes being intimately associated. Such paired chromosomes are referred to as bivalents or synaptonemal complexes. After zygonema bivalents become shorter and conspicuously thicker. This change in chromosomal morphology initiates the next stage, pachynema, during which doubling of each chromosome, but not their centromeres, takes place. At this stage complexes of tetrads, consisting of two chromatid pairs each, are observed. At diplonema, an event opposite to the pairing of homologous chromosomes occurs, namely the chromosomes start moving apart with the force of "repulsion" being the strongest at the region of the centromeres. The separation of the chromatids is not always complete and in certain regions they remain in contact. These regions are named chiasmata and are areas in which an exchange of chromosomal segments takes place. This process of reciprocal exchange of allelic genes between chromatids is referred to as crossing over or recombination. If recombination takes place between genetically identical

sister chromatids the outcome of the process is undetectable. When non-sister chromatids recombine new gene complexes are produced resulting in the formation of new genetic combinations.

Holliday (1968, 1974, 1977) proposed that a specific filamentous or fibrillar DNA-binding protein may be involved in the pairing of homologous chromosomes. According to his hypothesis this protein could possess one or two binding sites. With the condensation of the protein attached to a specific base sequence, identical on both homologues, the chromosomes are brought into proximity - a distance of about 100 nm, i.e. the width observed in the synaptonemal complexes. This initial point of cohesion can spread by a "zipper"-like mechanism in either direction along the adjacent chromosomes. Such an interaction between homologous chromosomes can occur over considerable distances. The initially fragile structure of recombining naked DNA molecules would be converted into a visible and strong chiasma at a later stage.

Two types of genetic recombination can be encountered during meiosis. In reciprocal recombination, the exchange of allelic genes

occurs between widely spaced markers; in non-reciprocal recombination a small fragment of genetic material is transferred from one chromatid to the other without the exchange of outside markers (Holliday, 1977). The latter type of recombination can only be detected by applying fine structure and/or tetrad - or half tetrad - analyses and is considered to be a gene conversion. In yeasts, recombination is associated with gene conversion (Fogel & Mortimer, 1971; Hurst *et al.*, 1972). The process of recombination is of the utmost importance in nature as well as in artificially induced hybridization, ensuring great genetic diversity.

Allelic genes can enter into new combinations in one or more regions of the adjoining chromosomes. When more than one cross occurs between the homologous chromatids the phenomenon of interference may be encountered. Three types of interference are known: chiasma interference, localized negative interference and chromatid interference. A detailed account of these three types of interference is given by Hayes (1968c).

The next stage of prophase I, referred to as diakinesis, is characterized by a marked contraction of chromosomes, complete separation of

bivalents and changes in their distribution in the nucleus. At the end of prophase I the nuclear membrane dissolves and the nucleolus disappears.

During metaphase I a structure known as the spindle (consisting of fibres, denser than the cytoplasm, radiating from opposite poles of the cell) appears, and the bivalents, still sharing the same centromere, arrange themselves in the central part of the spindle, called the equatorial plate. Centromeres of homologous bivalents become attached to the spindle fibres and point towards opposite poles of the spindle.

In anaphase I movement of the homologous bivalents to opposite poles of the spindle occurs.

Telophase I is characterized by the reappearance of the nuclear membrane and nucleolus.

At the end of the first meiotic division, each half of the cell possesses a haploid number of bivalents but a diploid number of chromatids (some of them being recombinants in one or more parts). It should be stressed that the parental chromosomes are distributed totally at random.

1.6.3.2 Second meiotic division

After a short interphase the second meiotic division begins during which the true reduction in the number of chromosomes is effected.

In prophase II no duplication of chromosomes occurs, the nuclear membrane disintegrates and the nucleolus vanishes. At metaphase II bivalents again migrate to the equatorial plate.

With the division of centromeres, anaphase II is initiated and the final separation of sister chromatids is achieved. The single chromosomes (chromatids) of each bivalent pair travel again to the opposite poles of the spindle.

In telophase II, four nuclei appear each with a haploid number of chromosomes.

Thus, at the end of the meiotic process in zygotic cells, four nuclei are present, each with a single set of randomly distributed chromosomes. If the process of recombination had occurred, these nuclei would possess new combinations of genes not observed in parental cells.

1.6.4 Morphological changes occurring during sporulation

The initiation of ascospore formation can be observed during the second meiotic division of the chromosomes (1.6.3.2)

The contours of spore boundaries surrounding the dividing nuclei become conspicuous and spore walls start appearing. The outer part of the wall consists of a dense layer with a rough surface, while the less dense inner wall constitutes the new vegetative cell wall. A considerable amount of residual cytoplasm, i.e. epiplasm, containing lipids, glycogen and mitochondria surrounds the immature ascospores (Hashimoto *et al.*, 1958; Marquardt, 1963; Fowell, 1969). During ascospore maturation the epiplasm disappears. It is probably used for the building of the outer spore wall and may serve as food reserves in the spore (Marquardt, 1963).

1.6.5 Physiological changes occurring during meiosis and sporulation

Meiosis and sporulation in yeasts are aerobic processes (Fogel & Mortimer, 1971). The specific oxygen requirement was, however, found to be restricted to the period between the fourth and seventh hour after the transfer of diploid cells into sporulation media (Croes, 1967). Sporulation, as a rule, occurs under near-starvation conditions during which cells cannot proliferate vegetatively (Fowell, 1969). Since the individual requirements for optimum sporulation vary from species to species, a variety of presporulation and sporulation media has been devised (Fowell, 1969; van der Walt, 1970b). Factors affecting sporulation in yeasts have been extensively discussed by Fowell (1969).

Sporulating cells of *Sacch. cerevisiae* are characterized by an increased activity of proteases (Chen & Miller,

1968) and ribonucleases (Tsuboi & Yanagishima, 1976). Cellular permeability is reduced thus affecting the uptake of adenine and amino acids (Mills, 1972).

After transfer of yeast cultures into sporulation media, taken as time zero, the following changes occur:

(i) DNA synthesis in *Sacch. cerevisiae* is initiated within the first four hours with an increase in the DNA content of approximately 50% taking place eight to eleven hours later (Croes, 1967; Roth & Lusnak, 1970);

(ii) synthesis of protein was observed by Esposito *et al.* (1969) to have two maxima, one at approximately six and the second at approximately 24 h; the peak periods of RNA synthesis were reported by Mills (1972) to occur at about four and nine hours. However, due to the extensive turnover of both protein and RNA, the contents of these two constituents are reduced to their initial levels within 20-25 h (Croes, 1967; Esposito *et al.*, 1969; Mills, 1972; Haber & Halvorson, 1975);

(iii) lipid synthesis during sporulation shows fluctuation during the first 24 h, having two maxima, one at approximately ten hours and the second at approximately 24 h (Henry & Halvorson, 1973);

(iv) a substantial increase in carbohydrate content, which can account for at least two-thirds of

the total increase in cell mass during sporulation, takes place within the first ten hours (Kane & Roth, 1974);

(v) the synthesis of glycogen is initiated after five hours, while the glycogen degradation was observed to accompany the maturation of ascospores and occurred after 20 h (Pontefract & Miller, 1962; Kane & Roth, 1974).

The period of time elapsing between the transfer of diploid cultures into sporulation media and the appearance of mature ascospores is usually 24-30 h. However, immature ascospores were observed in *Sacch. cerevisiae* within ten hours (Moens, 1971). This implies that all the events on the chromosomal and nuclear level, (e.g. duplication of chromosomes, formation of synaptonemal complexes, recombination, division of nuclei) as well as the complex process of ascospore formation and maturation must be completed within this relatively short time. These processes are accompanied by a pronounced increase in all cell activities, but especially in protein, DNA, RNA, lipid and carbohydrate syntheses.

1.7 FACTORS AFFECTING CONJUGATION

It should be emphasized that the successful completion of the conjugation process depends not only on genetic compatibility of the cells but also on their physiological state as well as on the external conditions. The following factors were found to affect the process of conjugation:

growth phase of cells (1.7.1);

cell ratio and cell concentration (1.7.2);

composition of the medium (1.7.3);

aeration (1.7.4);

inhibitors of conjugation (1.7.5);

stimulators of conjugation (1.7.6).

1.7.1 Growth phase of cells

It has been reported that yeast cells mate most readily while in the G1 phase of their cycle, i.e. when they possess a single set of chromosomes and are not in the process of replication. In other words, the G1 phase is achieved when the cells have completed the first round of DNA synthesis (Campbell, 1973; Hartwell, 1973, 1974; Sena *et al.*, 1973, 1975; Crandall *et al.*, 1977). When non-synchronized cultures are used this phase is generally considered to be equivalent to non-budding, stationary-phase cultures.

1.7.2 Cell ratio and cell concentration

The highest percentage of zygotes in *Sacch. cerevisiae* was obtained when cells of opposite mating types were present in equal numbers and the cell concentration did not exceed 10^8 cells/ml (Biliński *et al.*, 1973, 1975; Sena *et al.*, 1973, 1975). Similar recommendations were given by Brock (1961) and Crandall & Brock (1968) for *H. wingei*.

1.7.3 Composition of the medium

Whether the mating process takes place in an artificial medium or in natural ecological surroundings, nutrients available to the conjugating cells must correspond to their specific requirements.

It is known that media devoid of a nitrogen source, salts, trace elements and vitamins (Brock, 1961; Crandall & Brock, 1968) or containing growth-limiting concentrations of nutrients (Herman, 1971a) favour the process of conjugation in *H. wingei*. In *Schiz. pombe* starvation for nitrogen, but not for sugar, vitamins, salts, amino acids or nucleotides creates conditions favouring conjugation (Crandall *et al.*, 1977). In the case of *Sacch. cerevisiae* complex media were observed to enhance mating (Lindegren & Lindegren, 1944; Pomper & Burkholder, 1949; Jakob, 1962; Haefner, 1965; Fowell, 1969; Biliński *et al.*, 1973). Malt extract agar was successfully employed by Wickerham & Burton (1956a, b) in the hybridization of some *Kluyveromyces* species. The concentration of the carbon source as well as the pH of the medium can also influence mating efficiency and zygote formation (Egel, 1971; Biliński *et al.*, 1973; Lee *et al.*, 1975; Crandall *et al.*, 1977).

1.7.4 Aeration

Aeration is required for zygote formation in *H. wingei* (Brock, 1961; Crandall & Brock, 1968) and *Schiz. pombe* (Calleja & Johnson, 1971; Egel, 1971; Calleja, 1973).

Although aeration ehances zygote formation in *Sacch. cerevisiae* (Jakob, 1962; Sena *et al.*, 1975) it was not found to be essential for conjugation itself, since successful mating experiments between respiratory-deficient strains of this species were reported by Ephrussi & Hottinguer (1951) and Jakob (1962).

Mas *et al.* (1974) reported that an increase in the partial pressure of oxygen significantly enhances the conjugation frequency in *K. lactis*.

1.7.5 Inhibitors of conjugation

Temperatures above 31 ^{o}C and UV-irradiation of cells prior to conjugation were found to retard the process of conjugation (Brock, 1961).

The following chemical substances were reported to have an inhibitory effect on conjugation in yeasts: respiratory poisons, such as potassium cyanide and sodium arsenite, proteolytic enzymes, detergents, organic solvents, fluorophenylalanine, ethionine, dinitrophenol, sodium oxide as well as the antibiotics, cycloheximide, nystatin, neomycin and puromycin (Hunt & Carpenter, 1963; Calleja, 1973). In some cases conjugation was inhibited by ammonium salts, amino acids or nucleotides (Crandall *et al.*, 1977). Cell fusion in *Sacch. cerevisiae* was inhibited by 2-deoxyglucose (Shimoda & Yanagishima, 1974) but conjugation in *Schiz. pombe* was not affected by this compound (Kröning & Egel, 1974). The inhibitory effect of chloramphenicol and polymyxin on conjugation

varies depending on the stage at which these antibiotics were introduced into the mating cells of *Schiz. pombe* (Calleja, 1973).

1.7.6 Stimulators of conjugation

Ergosterol and its precursor squalene were reported by Mas *et al.* (1974) to stimulate mating process in *K. lactis*. Polyethylene glycol (1.5.3.1) was also found to induce fusion of protoplasts in *Sacch. cerevisiae* (van Solingen & van der Plaat, 1977), *Saccharomycopsis lipolytica* (Stahl, 1978) and *K. lactis* (Morgan *et al.*, 1977 and Allmark *et al.*, 1978).

1.8 AIM OF THE STUDY AND CHOICE OF METHODS

The aim of this study is to establish the existence of genetic relatedness between the species of the genus *Kluyveromyces*, expressed as their ability to hybridize. The study, which is based on hybridization experiments involving representative strains of all the species currently assigned to this genus, provides the basis for more detailed genetic investigation into the fertility of the hybrid progeny. The outcome of these investigations could lead to the resetting of the limits of the species within the genus and to assess the validity of regarding certain asporogenous yeast taxa as constituting the imperfect states of some members of the genus.

The generally employed methods in hybridization studies are based on two principles. The first involves micromanipulation and is based on pairing of either single ascospores (Winge & Lautsen, 1938) or mating of single haploid cells (Chen, 1950). The other approach involves the mass mating of prototrophic cultures as described by Lindegren & Lindegren (1943a)

or the mass mating of auxotrophic mutants followed by the selective recovery of prototrophic recombinants, a technique introduced by Pomper & Burkholder (1949).

Considerable problems are encountered while using hybridization techniques based on micromanipulation. Apart from being time consuming these methods pose additional difficulties with regard to *Kluyveromyces* species. Firstly, the ascosporal viability in this genus as reported by Wickerham (1955) is low. Secondly, the single cell mating developed for heterothallic organisms suffers from an inherent defect, namely that the paired vegetative cells of homothallic species may not conjugate immediately after being brought into contact with one another. Moreover, in some species the selection of haploid cells becomes difficult when the vegetative phase consists of a mixture of ploidies or when it is stabilized as a diplophase.

On the other hand, the mass mating of prototrophic cultures can only be applied while dealing with heterothallic species or with homothallic species possessing complementary assimilation patterns. In the latter case the non-hybrid progeny have to be separated from the true recombinants, by a laborious process of "diluting out" as applied by Wickerham & Burton (1956b). Since all the *Kluyveromyces* species except for *K. lactis* (Wickerham & Burton, 1952) are homothallic, and since not all of them possess complementary assimilative properties, this procedure could not be consistently applied.

Consequently, the prototrophic selection technique (Pomper & Burkholder, 1949) involving the use of complementary auxotrophic mutants was employed. According to Pomper & Burkholder (1949) this method ensures the recovery of prototrophic recombinants arising from heterothallic conjugation.

Auxotrophic mutants used in this work were induced with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Nitroso compounds, of which MNNG is a representative, constitute a group of highly potent chemical mutagens. MNNG was used for the first time by Mandell & Greenberg (1960) to induce mutants in bacteria. Mori (1972) used MNNG as a mutagenic agent for the induction of auxotrophic mutants in *Saccharomyces rouxii*. Subsequently MNNG was used to obtain auxotrophic mutants in *K. lactis* (Del Giudice & Puglisi, 1974; Allmark *et al.*, 1978) and in *K. fragilis* and *K. lactis* (Whittaker & Leach, 1977).

Drake (1969), reviewing the mutagenic mechanism of various mutagens, concluded that MNNG mutagenizes the replication point of DNA, which results in the formation of transitions and transversions but not in the formation of frameshift mutations. Similarly, Auerbach (1976) was of the opinion that MNNG seems to act directly on DNA at the replication fork. This action might be facilitated by special conditions such as strand-separation, which makes the nucleotide bases more accessible to MNNG.

The MNNG-induced mutants used in this study were selected for auxotrophic requirements, which in the case of *Sacch. cerevisiae* are controlled by genes known to be positioned on chromosomes and which segregate according to Mendelian principles (Pomper & Burkholder, 1949; Sherman & Lawrence, 1974). It can therefore be assumed that, if prototrophy in the recovered recombinants is restored, karyogamy and the exchange of genetic material between the parental strains involving either the independent assortment of chromosomes or a crossing over between homologous chromosomes, had taken place. The possibility that prototrophy was restored without fusion of the parental nuclei, and that the recombinant progeny existed in a dikaryon phase, was excluded by nuclear staining of confirmed hybrid strains grown on minimal media.

2. MATERIALS

2.1 PROTOTROPHIC YEAST STRAINS USED IN THE INVESTIGATION

The prototrophic yeast strains studied in this work and used for the induction of auxotrophic mutants were obtained from the Yeast Collections of the Centraalbureau voor Schimmelcultures (CBS) in Delft (Netherlands), Deutsche Mikroorganismen Sammlung (DMS) Institut für Gärungsgewerbe und Biotechnologie in Berlin (West Germany), University of California, Davis (UD), College of Agricultural and Environmental Science, Dept. of Food Science and Technology (U.S.A.) and the Council for Scientific and Industrial Research (CSIR) in Pretoria (South Africa).

The importance of employing actively sporulating strains in hybridization studies has been stressed by Fowell (1969). Consequently the yeast strains used in the present study were carefully selected and only strains which displayed adequate sporulation were used in the hybridization experiments and for the induction of the auxotrophic mutants. For this reason, the neotype strain of *K. marxianus* CBS 712, which has been in cultivation for at least 57 years and which shows reduced sexual activity and impaired ability to sporulate could not be employed in this study (see Results 4.2.3, Table 7). Paucity or absence of ascospores observed in the type strains of *K. africanus*, *K. bulgaricus*, *K. drosophilarum*, *K. fragilis*, *K. polysporus* and *K. thermotolerans* also eliminated these strains from the present investigation. Consequently more recent isolates or strains other than type, displaying adequate sporulation were used.

Generally each species of the genus *Kluyveromyces* was represented by a single strain. However, in certain instances more than one strain was

employed. In the case of *K. aestuarii* two strains which differed in their ability to assimilate maltose were used. In order to exclude a possible influence of the mating type specificity on the outcome of the hybridization experiments three haploid, non-sporulating strains of *K. lactis*, representative of two mating types were employed. Due to the importance and possible taxonomic implications of the results obtained in crosses involving *K. marxianus*, three strains of this species were used in the study. In the case of *K. wickerhamii* the type strain was used in interspecific experiments involving the remaining species of the genus and three additional strains were crossed with the strains of *K. marxianus* only.

The strains used are alphabetically listed and the information concerning their origin and number is given in Tables 1, 2 and 3.

2.2 MEDIA

The media used in the course of this investigation are described below. All the media were sterilized by autoclaving at 103,4 kPa gauge pressure (which at the altitude of Pretoria corresponds to 118,5 °C), for 15 min, unless stated otherwise. Whenever considered pertinent, the type of glassware used is indicated. Nucleopore polycarbonate membranes 0,2 µm pore size (autoclaved) were used for filter sterilization.

General-purpose growth media

2.2.1 Yeast extract-malt extract (YM) agar (Wickerham, 1951):

3 g yeast extract (Difco), 3 g malt extract (Oxoid), 5 g peptone (Difco), 10 g glucose (Merck) and 15 g agar (Difco) were dissolved in 1 ℓ distilled water. The pH of the medium ranged between pH 5 and pH 6.

2.2.2 Yeast extract-malt extract (YM) broth (Wickerham, 1951):

The composition of this medium is identical to YM agar with the omission of 1,5% *m*/v agar.

Sporulation media

All the sporulation media were dispensed into tubes (10 ml) and slanted after sterilization.

2.2.3 Fowell's acetate agar (Fowell, 1952):

5 g sodium acetate trihydrate (Merck) was dissolved in 1 ℓ distilled water, the pH of the solution was adjusted to pH 6,5-7,0 and 15 g agar (Difco) was added to this solution. Before slanting, 2 drops of the stock vitamin solution (2.2.10) were aseptically introduced into each tube containing the sterile medium.

2.2.4 Gorodkowa agar (modified) (van der Walt, 1970b):

1 g glucose (Merck), 10 g peptone (Difco), 5 g sodium chloride (Merck) and 15 g agar (Difco) were dissolved by steaming in 1 ℓ of tap water and sterilized.

2.2.5 Malt extract (ME) agar (Wickerham, 1951):

12 g agar (Difco) was dissolved in 400 ml distilled water by steaming. Malt extract (Oxoid) (20 g) was dissolved in the hot agar solution.

2.2.6 McClary's acetate agar (McClary *et al.*, 1959):

1 g glucose (Merck), 1,8 g potassium chloride (Merck),

2,5 g yeast extract (Difco), 8,2 g sodium acetate trihydrate (Merck) and 15 g agar (Difco) were dissolved in 1 ℓ distilled water.

2.2.7 V8 agar (Wickerham, 1946; van der Walt, 1970b):

Commercial blend of vegetable juice (V8 Campbell Soup Co., U.S.A.) was diluted 1:1 with distilled water, pH was adjusted with 1 N KOH to pH 5,7-5,9 and 1,5% *m*/v agar (Difco) was added.

Identification media

All the compounds used for the preparation of the liquid assimilation and fermentation media were produced by Merck.

2.2.8 Liquid assimilation media (Wickerham, 1946, 1951; Wickerham & Burton, 1948; van der Walt, 1970b).

These media were prepared as described by van der Walt (1970b).

2.2.8.1 Carbon assimilation media: 6,7 g of Bacto-yeast nitrogen base (Difco) was dissolved in 100 ml distilled water (tenfold concentration) and the requisite amount of the carbon compound (containing the equivalent amount of carbon as in 5 g glucose) was introduced. The solution was sterilized by filtration and aseptically dispensed as 0,5 ml aliquots into cotton-plugged test tubes with 4,5 ml sterile distilled water. The final concentration of the carbon source was 0,5% *m*/v except for raffinose which was 1% *m*/v.

Media with the following carbon sources were prepared: glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol, galactitol, D-glucitol, D-mannitol, α-methyl-D-glucoside, salicin, DL-lactic acid, citric acid and inositol.

2.2.8.2 Nitrogen assimilation media: 11,7 g Bacto-yeast carbon base (Difco) and the requisite amount of a nitrogen source were dissolved in 100 ml of distilled water (tenfold concentration). As a nitrogen source 0,78 g potassium nitrate, 0,26 g sodium nitrite or 0,64 g ethylamine hydrochloride was used. The medium was filter-sterilized and aseptically dispensed as 0,5 ml aliquots into cotton-plugged test tubes with 4,5 ml sterile, distilled water.

2.2.9 Fermentation media:

These media were prepared as described by van der Walt (1970b) by dissolving sugars in the diluted 1:10 yeast autolysate. Glucose, galactose, lactose, maltose and sucrose were prepared as 2% *m*/v solutions. For raffinose a 4% *m*/v solution was used.

Sugar solutions were dispensed into test tubes with Durham insert tubes and sterilized by autoclaving at 68,9 kPa gauge pressure (which at the altitude of Pretoria corresponds to 112,5 °C), for 15 min.

2.2.10 Stock vitamin solution - 100-fold concentration (van der Walt & van Kerken, 1961):

0,2 mg biotin (Fisher Scientific Company), 40 mg calcium pantothenate (Merck), 0,2 mg folic acid (BDH), 200 mg inositol (Merck), 40 mg niacin (Merck), 20 mg *p*-amino-benzoic acid (Merck), 40 mg pyridoxine hydrochloride (Merck), 20 mg riboflavin (Merck) and 100 mg thiamin (Merck) were dissolved in 1 ℓ distilled water and filter-sterilized. After dispensing into sterile tubes the solution was frozen and kept at -15 °C.

Minimal media

2.2.11 Minimal agar medium:

This medium consisted of Wickerham's (1951) nitrogen base with the omission of amino acids and glucose but supplemented with 1,5% *m*/v washed agar (Lodder & Kreger-van Rij, 1952b) and 1,0% *m*/v of the filter-sterilized carbohydrate source. In the case of raffinose a concentration of 2,0% *m*/v was used.

The medium was prepared by mixing aseptically 10 ml of solution A, 10 ml of solution B, 0,1 ml of solution C and 1 ml of solution D with 78,9 ml of sterile, melted 1,5% washed agar. The compositions of solutions A, B, C and

D were as follows:

Solution A - 10% *m*/v of a carbon source (20% in the case of raffinose).

Solution B - 10-fold concentration of the salts : 50 g ammonium sulphate, 8,5 g monobasic potassium phosphate, 1,5 g dibasic potassium phosphate, 5 g magnesium sulphate, 1 g sodium chloride and 1 g calcium chloride were dissolved in 1 ℓ distilled water. The solution was sterilized by autoclaving.

Solution C - 1000-fold concentration of trace elements : 0,5 g boric acid, 0,04 g copper sulphate, 0,1 g potassium iodide, 0,2 g ferric chloride, 0,4 g manganese sulphate, 0,2 g sodium molybdate and 0,4 g zinc sulphate were dissolved in 1 ℓ distilled water. The solution was sterilized by autoclaving.

Solution D - stock vitamin solution (van der Walt & van Kerken, 1961) : detailed composition 2.2.10.

2.2.12 Liquid minimal glucose medium:

The composition of this medium was identical to minimal medium 2.2.11, with the omission of 1,5% *m*/v agar.

2.2.13 Starvation medium:

This medium consisted of the same ingredients as the minimal agar medium but with the omission of 1,5% *m*/v washed agar, 1,0% *m*/v glucose and vitamins.

The medium was prepared by introducing 10 ml of solution B and 0,1 ml of solution C (2.2.11) into 89 ml of distilled water and sterilized by autoclaving.

Media in which mutagenesis was carried out

2.2.14 Medium used for the induction of auxotrophic mutants by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and UV-irradiation:

Acetate buffer 0,2 M, pH 5,5 was prepared by mixing 68 ml of 0,2 M acetic acid with 432 ml of 0,2 M sodium acetate and diluting the mixture to 1 ℓ with distilled water. The pH of the solution was adjusted to pH 5,5.

2.2.15 Medium used for the induction of auxotrophic mutants with ethyl-methanesulfonate (EMS):

Phosphate buffer 0,2 M, pH 8,0 was prepared by mixing 5,3 ml of 0,2 M solution of monobasic sodium phosphate with 94,7 ml of 0,2 M solution of dibasic sodium phosphate and diluting the mixture to 200 ml with distilled water. The pH of the solution was adjusted to pH 8,0.

The same buffer was used to terminate the mutagenesis with nitrous acid.

2.2.16 Medium used for the induction of auxotrophic mutants with nitrous acid (NA):

Citrate-phosphate buffer 0,1 M, pH 4,0 was prepared by mixing 30,7 ml of 0,1 M solution of citric acid with 19,3 ml of 0,2 M solution of dibasic sodium phosphate and diluting the mixture to 200 ml with distilled water. The pH of the solution was adjusted to pH 4,0.

2.2.17 Medium used for the induction of mutants by X-ray irradiation:

This medium consisted of 0,05 M solution of monobasic potassium phosphate, pH 4,0.

2.2.18 Malt extract (ME) (Lodder & Kreger-van Rij, 1952b):

To prepare 10% *m*/v ME, 10 g malt extract (Oxoid) was dissolved in 100 ml distilled water by heating. After cooling, the pH of the extract was adjusted to pH 5,4.

Substrates used for storage of the yeast strains

2.2.19 Silica gel (Merck):

Approximately 2 g of silica gel 60, 70-230 mesh was introduced into glass screw-cap tubes (17 x 60 mm) plugged with cotton-wool and dry-heat sterilized for 2 h at 160 ^{o}C. The caps were sterilized separately with propylene oxide (Merck) for 7 days.

2.2.20 Evaporated, unsweetened milk (Carnation brand) was distributed in 4 ml volumes into cotton-plugged sterile

test tubes and sterilized by autoclaving.

2.2.21 Skim-milk was distributed in 4 ml volumes into cotton-plugged sterile test tubes and steamed for 20 min on three consecutive days.

2.2.22 Whatman filter paper No 41 was cut into approximately 10 x 20 mm pieces, wrapped in aluminium foil and sterilized by autoclaving.

2.3 REAGENTS

All the reagents enumerated below except for Glusulase (2.3.3), liquid paraffin (2.3.5) and the reagents used for nuclear staining (2.3.11 - 2.3.16) were sterilized by filtration through autoclaved Nucleopore polycarbonate membranes (0,2 µm pore size).

2.3.1 Amino acids used for the characterization of auxotrophic mutants:

L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-leucine, L-isoleucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan and L-valine (all Merck).

The amino acids used for the preparation of amino acid pools (Holliday, 1956) were dissolved in hot distilled water in quantities of 100 mg per 10 ml. The filter-sterilized amino acids were introduced into basal and assimilation media at a final concentration of 20 mg/ℓ, except for L-isoleucine, L-leucine, L-lysine and L-tyrosine

which were used at a final concentration of 30 mg/ℓ, L-phenylalanine - 50 mg/ℓ, L-aspartic acid, L-glutamic acid, L-valine - 100 mg/ℓ, L-threonine - 200 mg/ℓ and L-serine 375 mg/ℓ (Sherman & Lawrence, 1974).

The glassware and filter holders used for the preparation of amino acid solutions were washed in 0,01 N HCl and repeatedly rinsed in distilled water before sterilization. For filter sterilization of small volumes of amino acids or nucleic acid bases sterile, disposable syringes were used.

2.3.2 Ethyl-methanesulfonate (EMS) (Sigma):

1-5% v/v solutions of EMS in 0,2 M phosphate buffer pH 8,0 were prepared.

2.3.3 Glusulase (Endo Laboratories):

A preparation of the intestinal juice of the snail *Helix pomatia* containing a mixture of enzymes including β-glucuronidase and sulfatase is marketed in sterile ampoules.

2.3.4 ICR-191G:

A sample of this mutagen was kindly supplied by Dr H. Creech of the Institute of Cancer, Philadelphia, U.S.A. ICR-191G (1 mg) was dissolved in 10 ml of distilled water.

2.3.5 Liquid paraffin (Merck) was dry-heat sterilized at 160 ^{o}C for 1 h.

2.3.6 Nitrous acid (NA) solutions:

0,02 M - 0,2 M solutions of nitrous acid were prepared as 0,013 M - 0,067 M solutions of sodium nitrite in 0,1 M citrate-phosphate buffer pH 4,0 (2.2.16).

2.3.7 N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (Sigma):

2,5 or 10 mg of MNNG was introduced into 5 ml of double distilled water and dissolved with the aid of a magnetic stirrer.

2.3.8 Nucleic acid bases:

The following nucleic acid bases were used at a final concentration of 50 mg/ℓ: adenine (Merck), guanine sulphate (Sigma), thymine (Merck) and uracil (Sigma).

The glassware and filter holders were cleaned as described in 2.3.1.

2.3.9 Nystatin (Sigma):

5 mg of nystatin was dissolved in 5 ml absolute methanol (Merck).

2.3.10 Sodium thiosulphate (Merck):

6% *m*/v solution of sodium thiosulphate was prepared.

Reagents used for nuclear staining

2.3.11 Meyer's albumin:

Equal weights of the white of an egg and glycerol (Merck) were mixed with 1 g sodium benzoate (Merck), shaken

vigorously for 10 min with glass beads and filtered through a filter paper. The reagent was maintained at 4 °C.

2.3.12 Helly's fixative:

Solution A - 30 g of potassium chromate (Merck) and 50 g of mercury chloride (Merck) were dissolved in 1 000 ml distilled water;

solution B - 40% commercial formalin (B. Owen Jones Ltd) maintained over calcium carbonate;

60 ml of solution A were thoroughly mixed with 3,0 ml of solution B just before use.

2.3.13 Ethanol solution 70% v/v:

700 ml of absolute ethanol (Merck) were diluted with 300 ml of distilled water.

2.3.14 1N hydrochloric acid:

1N HCl "Titrisol" (Merck) was diluted to a final volume of 1 ℓ.

2.3.15 Phosphate solution pH 6,9:

1 g of dibasic potassium phosphate (Merck) was dissolved in 1 000 ml distilled water. The solution was adjusted to pH 6,9.

2.3.16 Giemsa's solution:

0,75 ml of Giemsa's solution (Merck) was diluted in 62,5 ml of phosphate solution pH 6,9 (2.3.15).

3. METHODS

3.1 MAINTENANCE OF CULTURES

In order to determine whether or not the genus *Kluyveromyces* might possibly be subject to the type of mutation observed among the *Saccharomyces sensu stricto* affecting the fermentative and assimilatory properties (Scheda, 1966; Scheda & Yarrow, 1966, 1968; van der Walt, 1970c), the physiological characteristics of all the strains studied were reconfirmed according to the procedure described by van der Walt (1970b).

Stock cultures of prototrophic and auxotrophic strains were stored on YM agar slants (2.2.1), on sterile silica gel (2.2.19) as described by Gutz *et al.* (1974), and on sterile filter paper (2.2.22) according to the method recommended by the Division of Medical Physics, Donner Laboratory, University of California, Berkeley, California, U.S.A. (personal communication). Suspensions of the abundantly sporulating yeast cultures, containing approximately 10^9-10^{10} cells/ml were prepared in sterile evaporated milk (2.2.20) or sterile skim-milk (2.2.21), transferred onto sterile silica gel and sterile filter paper, respectively, and maintained in a desiccator over self-indicating silica gel. All the cultures were stored at 7 °C.

Routine transfers were made onto YM agar slants and incubated at 28 °C.

Before use all prototrophic cultures employed for the induction of auxotrophic mutants and in hybridization experiments were brought to a state of active sporulation by heat-treatment at 57 °C, as described by

Wickerham & Burton (1954). Abundantly sporulating single colonies were selected and cloned by replating on YM agar.

3.2 INDUCTION OF AUXOTROPHIC MUTANTS

Numerous mutagenic agents have been employed for the induction of auxotrophic mutants in yeasts (Mortimer & Manney, 1971). A series of exploratory experiments was consequently carried out in order to assess the suitability of the most commonly used agents for the genus *Kluyveromyces*.

In this series of experiments the following mutagenic agents were employed:

Physical agents

X-ray irradiation (3.2.1);

Ultraviolet irradiation (3.2.2).

Chemical agents

Ethyl-methanesulfonate (EMS) (3.2.3);

ICR-191G (3.2.4);

Nitrous acid (NA) (3.2.5);

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (3.2.6).

In all cases 24 hour-old cultures grown on YM agar slants (2.2.1) at 28 °C were used as inocula for the propagation of prototrophic cultures prior to mutagenesis. Shake cultures of prototrophic strains were propagated in Erlenmeyer flasks with YM broth (2.2.2) as a medium. The cultures were incubated for 16-24 h, at 28 °C on a New Brunswick Scientific gyrotory shaker, model G53, adjusted to 180-200 rev/min and a

stroke of 50 mm.

The harvested cells were washed twice by centrifugation with sterile, distilled water and suspended to the required concentration in an appropriate buffer.

3.2.1 X-ray irradiation experiments were carried out according to the method described by Beam *et al.* (1954). The experimental conditions were as follows: 0,1 ml aliquots of the yeast suspension in sterile 0,05 M potassium phosphate pH 4,0 (2.2.17) containing approximately 2 x 10^3 cells/ml were uniformly spread on YM agar plates (2.2.1), placed 100 mm from the tube target and irradiated for 2-120 sec, with the X-ray source (Siemens Dermipan) operating at 50 kV and 25 mA, the dose rate being 340 R/sec. The plates were incubated for 3 days at 28 ^{o}C.

3.2.2 Ultraviolet irradiation experiments were carried out according to the procedure described by Parker & Mattoon (1969). The experimental conditions were as follows: 0,1 ml aliquots of the yeast suspension in sterile 0,2 M acetate buffer pH 5,5 (2.2.14) containing approximately 10^4 cells/ml were uniformly spread on YM agar plates (2.2.1), placed 200 mm from the Phillips TUV 6 W germicidal lamps (radiating most of the energy at 253,7 nm) and irradiated for 0,5-10 min. The plates were then incubated for 3 days at 28 ^{o}C.

3.2.3 The induction of auxotrophic mutants with EMS (2.3.2) was based on the method described by Lindegren *et*

al. (1965). The experimental conditions were as follows: the yeast suspensions in sterile 0,2 M phosphate buffer pH 8,0 (2.2.15) containing approximately 10^8 cells/ml were treated with 1-5% v/v solutions of EMS in the same buffer for 30-120 min, at 30 °C. The EMS was subsequently inactivated by diluting the reacting suspensions in sterile 6% *m*/v sodium thiosulphate (2.3.10) in the ratio of 1:50 (Bautz & Freese, 1960). The inactivated suspensions were serially diluted in sterile distilled water, plated on YM agar (2.2.1) and incubated for 3-5 days at 28 °C.

3.2.4 For the induction of auxotrophic mutants with ICR-191G (2.3.4) the method described by Brusick (1970) was used. The experimental conditions were as follows: starved yeast cells suspended in sterile distilled water at a concentration of approximately 10^6 cells/ml were treated with ICR-191G at a final concentration of 10 µg/ml for 30-60 min, at 30 °C. The experiments were carried out under red light to minimize photo-inactivation of the mutagen. The suspensions were subsequently serially diluted in sterile distilled water, plated on YM agar (2.2.1) and incubated for 3-5 days at 28 °C.

3.2.5 The induction of auxotrophic mutants with NA (2.3.6) was based on the method of Sarachek & Bish (1976). The experimental procedure was as follows: yeast suspensions in sterile 0,1 M citrate-phosphate buffer pH 4,0 (2.2.16) containing approximately 10^8 cells/ml were treated with 0,02 M - 0,1 M nitrous acid solutions for

20-60 min, at 25 °C. The treatment was terminated by diluting the reacting suspensions in sterile 0,2 M phosphate buffer pH 8,0 (2.2.15) in the ratio of 1:10. The suspensions were subsequently serially diluted in sterile distilled water, plated on YM agar (2.2.1) and incubated for 3-5 days at 28 °C.

3.2.6 The induction of auxotrophic mutants with MNNG (2.3.7) was based on the methods of Mori (1972) and of Del Giudice & Puglisi (1974). The detailed description of this method is presented in the next section (3.2.7).

These exploratory experiments established that the MNNG technique was superior for the induction of stable auxotrophic mutants in *Kluyveromyces* strains and this method was therefore adopted throughout the study.

3.2.7 Finally adopted procedure

A loopful of prototrophic yeast strains grown on YM agar (2.2.1) for 24 h at 28 °C was inoculated into 50 ml of YM broth (2.2.2) in a 250 ml Erlenmeyer flask. Cultures were incubated on a New Brunswick Scientific gyrotory shaker, model G53, (adjusted to 180-200 rev/min and a stroke of 50 mm) for 20 h at 28 °C. The broth-grown cells were then harvested and washed twice by centrifugation with sterile, distilled water. A concentration of approximately 10^8 cells/ml was prepared by resuspending the pellet of cells in 20 ml of sterile 0,2 M acetate buffer pH 5,5 (2.2.14). A sample (2,5 ml) of this washed suspension was transferred to a sterile 25 ml Erlenmeyer flask to which 1,5 ml of sterile

acetate buffer pH 5,5 and 1 ml of the sterile stock MNNG solution (2.3.7) were added. The final concentration of MNNG was 100 µg/ml.

The Erlenmeyer flask was transferred to a Getriebefett Centroplex Extraleicht reciprocal shaker (adjusted to 80 strokes/min and a stroke length of 50 mm), placed in a water bath at 30 °C and maintained there for 30-120 min. The MNNG-treated cells were recovered by filtration through a sterile, Nucleopore polycarbonate membrane (1,0 µm pore size) and washed with 200 ml of sterile distilled water.

In order to reduce the non-mutant population, the MNNG-treated cells were subjected to treatment with nystatin (Snow, 1966). The filter membrane with the layer of washed cells was aseptically transferred to a 250 ml Erlenmeyer flask containing 20 ml of YM broth and placed on the gyrotory shaker for 20 h at 28 °C. The cells were then harvested, washed twice by centrifugation with sterile distilled water, resuspended in 20 ml of starvation medium (2.2.13) in a 250 ml Erlenmeyer flask and replaced on the shaker. After a 24-hour starvation period 5 ml of minimal glucose medium (2.2.12) was added to the yeast cells and the flasks were replaced on the shaker at 28 °C. After 6 h incubation, 0,2 ml of a stock nystatin solution (2.3.9) was introduced and the culture shaken for a further 60 min at 28 °C.

Nystatin-treated cells were serially diluted in sterile distilled water and 0,1 ml aliquots spread on YM agar. A minimum of five plates per dilution were prepared and the plates were incubated for 2-3 days at 28 °C.

If a mutant with more than one nutritional requirement was sought, the additional marker was introduced by subjecting an auxotrophic mutant with a single nutritional requirement to a second mutagenesis. The procedure followed was identical to the one described above.

While this procedure gave satisfactory results with the majority of the strains studied, higher concentrations of MNNG (up to 400 µg/ml) were required for the induction of stable auxotrophic mutants in *K. aestuarii*, *K. fragilis* and *K. thermotolerans*.

3.3 ISOLATION AND CHARACTERIZATION OF AUXOTROPHIC MUTANTS

YM agar (2.2.1) plates spread with the mutagen-treated cells were incubated for 3-5 days at 28 °C. Plates with a suitable number of colonies (up to 200/plate) were replica-plated onto minimal glucose agar (2.2.11) and incubated for 48 h at 28 °C. The presumptive auxotrophic colonies were isolated, purified on YM agar and their auxotrophy confirmed. Stable mutants were characterized for their nucleic acid base and amino acid requirements by the auxanographic technique using minimal glucose agar medium (van der Walt, 1970b). Individual crystalline amino acids (2.3.1) or nucleic acid bases (2.3.8) were applied locally. Zones of growth surrounding the sites of application were taken as being indicative of the

requirement for the specific amino acid or nucleic acid base.

Characterization of the auxotrophic mutants with more than one nutritional requirement was carried out in a similar manner. A sterile solution of nucleic acid base or amino acid required by a parental mutant strain was introduced into minimal glucose agar in the concentration indicated in 2.3.1 or 2.3.8, before the routine auxanographic technique was applied. On three occasions mutants with more than one nutritional requirement were isolated after a single treatment with MNNG. In such cases the nutritional requirements were established by using pools of amino acids and nucleic acid bases instead of individual compounds. The amino acid and purine/pyrimidine pools were prepared according to Holliday (1956).

Selected auxotrophic mutant strains were re-examined for their fermentative and assimilatory properties as well as their ability to sporulate. The carbon assimilation media (2.2.8.1) were supplemented according to the amino acid or nucleic acid base requirements of the tested auxotrophic mutants.

3.4 HYBRIDIZATION EXPERIMENTS

Representatives of all the *Kluyveromyces* species, listed in Table 1, were crossed in all possible combinations with a minimum of two crosses per parental pair. In intergeneric hybridization experiments parental strains were crossed as indicated in Tables 35-39. The mass mating technique described by Lindegren & Lindegren (1943a) and Wickerham & Burton (1956a, b) was employed throughout this study.

Loopfuls of 24 hour-old parental strains grown on YM agar slants (2.2.1) at 28 °C were transferred to the surface of a freshly prepared

2% ME agar slant (2.2.5), thoroughly mixed and spread. The mated cultures were incubated at 28 °C and serially transferred onto fresh 2% ME agar slants at 7-day intervals over a period of 4 weeks, according to the procedure described by Wickerham & Burton (1956a, b). Although such a long incubation period allows for the sporulation and subsequent proliferation of the initially formed hybrids and also increases the chances of back mutations, it nonetheless increases the probability of detecting slowly formed hybrids or hybrids formed in low numbers only (see Results 4.2.1 and Table 5).

After 7 days' incubation the growth from the slant of the final transfer of the mated cultures was collected in 2,5 ml of sterile tap water, washed by centrifugation and resuspended in 2,0 ml of sterile tap water. The suspension containing approximately 10^8-10^9 cells/ml was enumerated in triplicate by serial dilution and plating. The total viable cell count of the mated parent cultures was determined on YM agar (2.2.1) plates. For the enumeration of recombinants the prototrophic selection technique of Pomper & Burkholder (1949) was employed using a recovery medium consisting of a minimal agar (2.2.11) with a carbon source which permitted the development of the anticipated hybrids only. Carbon sources utilized latently in the liquid assimilation media were not employed in the minimal agar media as this would require prolonged incubation of the agar plates and their ensuing dehydration. For this reason D-ribose, utilized by *K. blattae* after 10-12 days' incubation, could not be used in the recovery medium although it would have constituted a convenient marker as only three other species of the genus, namely *K. cicerisporus*, *K. fragilis* and *K. marxianus* are known to assimilate this pentose.

Plates were incubated at 28 °C. Colonies on YM agar were enumerated after 3 days and on minimal recovery agar after 7 and 14 days.

Controls of auxotrophic mutant parent strains accompanying each cross were similarly cultivated on 2% ME agar (2.2.5) slants with serial transfers at 7 day intervals. During the 4 week period of incubation the controls were checked on minimal glucose agar (2.2.11) for possible back mutation. The 7 day-old growth of the final transfer of the mutant parent strain was collected in 2,5 ml of sterile tap water, washed by centrifugation and resuspended in 2,0 ml of sterile tap water. This undiluted suspension was enumerated for possible back mutation by plating 4 aliquots of 0,1 ml each on the minimal agar (2.2.11) containing the carbon source utilized by the mutant strain and also employed for the recovery of the anticipated recombinants. The total viable count was obtained on YM agar plates incubated at 28 °C. Colonies on YM agar were counted after 3 days and on minimal agar after 7 and 14 days. Back mutation if not detected in 0,4 ml of the undiluted suspension was anticipated to be lower than 3 per 10^8 cells of the mated population. Experiments in which back mutation of the auxotrophic mutant strain occurred during or after 4 weeks' incubation were discarded.

3.5 ISOLATION AND CHARACTERIZATION OF HYBRIDS

A minimum of three colonies developing from the highest dilution in which recombinants were detected were cloned by replating on YM agar (2.2.1). The pure cultures were characterized in respect of their prototrophy, physiological properties and their ability to sporulate. To exclude the possibility that the hybrid progeny existed in a dikaryon state, nuclear staining of vegetative cells was carried out.

3.5.1 Confirmation of physiological properties and prototrophy

Oxidative and fermentative utilization of carbon sources by the presumed hybrids was confirmed according to the procedure recommended by van der Walt (1970b) using the identification media (2.2.8 and 2.2.9) described in Chapter 2. Utilization of galactose, maltose, lactose, raffinose, trehalose and sucrose was tested routinely. Whenever pertinent, the utilization of additional carbon sources was tested.

Prototrophy of hybrids was confirmed on the minimal glucose agar (2.2.11).

3.5.2 Confirmation of hybrid fertility

Ascospore formation of the presumed hybrid strains was examined on YM agar (2.2.1), Fowell's acetate agar (2.2.3), Gorodkowa agar (2.2.4), ME agar (2.2.5), McClary's acetate agar (2.2.6) and V8 agar (2.2.7) and checked by microscopic examination of both water mounts and stained preparations. The shape and number of ascospores formed per ascus was noted for each presumed hybrid strain.

Ascosporal viability was confirmed by heat-treatment (3.5.2.1), the ultrasonic-paraffin technique (3.5.2.2) and in some cases by micromanipulation (3.5.2.3). The sporulating hybrid strains grown on 2% ME agar (2.2.5) at 28 ^{o}C for 3-4 days were used for the isolation of ascospores.

3.5.2.1 The heat-treatment was carried out according to the method of Wickerham & Burton (1954) in a water bath at 57 °C. Samples were withdrawn at 1 min intervals for up to 20 min, plated on YM agar (2.2.1) and incubated at 28 °C.

3.5.2.2 The ultrasonic-paraffin technique described by Siddiqi (1971) comprises the enzymatic liberation of ascospores (Bevan & Woods, 1963), separation of haploid ascospores with simultaneous killing of diploid cells by sonication (Siddiqi, 1971) and selective isolation of ascospores from the vegetative phase in paraffin (Emeis & Gutz, 1958).

Abundantly sporulating hybrid strains were suspended in 5 ml of 1:1, 1:2 or 1:3 dilutions of snail enzyme (Glusulase 2.3.3) and vigorously shaken on a Griffin & George Ltd microid flask shaker, in 25 ml Erlenmeyer flasks at 28 °C for 0,5-1 h. Due to the significant differences in the number of spontaneously liberated ascospores and the solubility of the ascus wall, optimum conditions for the enzymatic treatment had to be established individually for each hybrid strain investigated. The Glusulase-treated cultures were washed twice by centrifugation and resuspended in 10 ml of sterile distilled water. The suspensions in 25 ml beakers were placed in

an ice bath and sonicated with an M.S.E. 100 W Ultrasonic Disintegrator (Measuring & Scientific Equipment, Ltd., England). The titanium probe of 19 mm diameter and velocity ratio 3,6:1 was inserted to a depth of *ca* 5 mm in the suspension containing cells and ascospores. An amplitude of 7 µm (peak to peak) was applied for a period of 4-8 min. The sonicated cells were washed again by centrifugation, resuspended in 5 ml of sterile distilled water and transferred to sterile, 25 ml Erlenmeyer flasks. Sterile liquid paraffin (2.3.5), 2 ml was added and the flasks were placed on a Griffin & George Ltd microid flask shaker, shaken vigorously for 20 min to enable the transfer of lipophilic ascospores into the dispersed paraffin phase. The Erlenmeyer flasks were left standing for 15 min to allow separation of the paraffin phase. The paraffin layer was subsequently transferred with a sterile Pasteur pipette into a sterile centrifuge tube. The paraffin phase was washed twice by centrifugation with distilled water. The total number of ascospores in the paraffin phase was estimated by a direct count in a haemacytometer. The number of viable ascospores was obtained by plating 0,1 ml volumes of the undiluted suspension and serial dilutions in paraffin on

YM agar (2.2.1). Plates were incubated at 28 °C. Microscopic observations were carried out after each step of this procedure.

3.5.2.3 Isolation of single ascospores by micromanipulation was carried out according to the Carlsberg technique (Winge, 1935; Winge & Roberts, 1958; Sherman, 1975) using a Zeiss micromanipulator. Single ascospores were transferred to droplets of 10% ME (2.2.18) and incubated for 24 h at 28 °C in a moist chamber. The resulting microcolonies were transferred into 10 ml of 10% ME and after propagation at 28 °C were subsequently cloned on YM agar.

3.5.3 Nuclear staining of the vegetative phase

The staining of nuclei in the 8 hour-old hybrid strains grown on minimal glucose agar (2.2.11) and YM agar (2.2.1) was carried out according to the method described by Robinow (1961). The yeast cell suspensions, applied to the albumin-coated slides (2.3.11), were fixed in Helly's fixative (2.3.12) for 10 min and washed three times in 70% v/v ethanol (2.3.13). Preparations were hydrolysed in 1N HCl (2.3.14) at 60 °C for 5-6,5 min. After repeated washing in phosphate solution pH 6,9 (2.3.15) the preparations were stained with Giemsa's solution (2.3.16) for 15-20 min at room temperature. Preparations were examined microscopically under oil.

4. RESULTS

The abbreviations used in Tables 4-41, 43, 45-47 to denote the amino acid and nucleic acid base requirements of the auxotrophic mutants are in accordance with the nomenclature proposed by Sherman & Lawrence (1974). The symbols ARA, CEL, GAL, LAC, MAL, RAF, RIB, SOR, SUC and TRE denote the oxidative utilization of L-arabinose, cellobiose, galactose, lactose, maltose, raffinose, D-ribose, L-sorbose, sucrose and trehalose, respectively. The symbols ara, cel, gal, lac, mal, raf, rib, sor, suc and tre denote the absence of oxidative utilization of L-arabinose, cellobiose, galactose, lactose, maltose, raffinose, D-ribose, L-sorbose, sucrose and trehalose, respectively. Since glucose is utilized by all the yeast species studied the notation of this property has been omitted. The symbols Ren and Sph denote the formation of reniform and spheroidal ascospores, respectively. In the case of *K. lactis* the symbol Sph refers to the shape of ascospores formed when sexually compatible mating types are brought into contact.

4.1 AUXOTROPHIC MUTANTS EMPLOYED IN THE HYBRIDIZATION EXPERIMENTS

During the course of these investigations 376 auxotrophic mutants with a single nutritional requirement and 57 auxotrophic mutants with more than one nutritional requirement, were isolated and characterized. Out of this group, 95 stable auxotrophic mutants were selected and used in the hybridization experiments. The nutritional requirements of these auxotrophic mutants as well as their pertinent physiological and morphological characteristics are listed in Table 4.

4.2 PRELIMINARY EXPERIMENTS

4.2.1 Experiments to establish the effect of time of contact between mated cultures on the number of recombinants recovered

In order to ensure the recovery of slowly formed hybrids, the effect of the duration of incubation of the mated cultures on the number of recombinants recovered was investigated. In this series of preliminary experiments the recombinants were enumerated twice - the first time seven days after mating of parental strains and the second time seven days after the final transfer of the mated cultures. Therefore, in the latter case, the mated cultures were in contact for four weeks, having been transferred onto fresh medium every seven days.

The results of the experiments involving auxotrophic mutants of *K. lactis* CBS 683 and some other species of the genus *Kluyveromyces* are presented in Table 5. One week after mating, recombinants were absent in crosses involving both auxotrophic mutants of *K. lactis* CBS 683 and *K. cicerisporus* CBS 4857 and only a low number of recombinants was found in the experiments in which *K. lactis* CBS 683 arg was crossed with *K. fragilis* CBS 1556, *K. marxianus* CBS 6556 and CBS 6923 as well as in the cross between *K. lactis* CBS 683 trp and *K. wikenii* CBS 5671. However, in all these cases the recombinants were present in the final serial transfer (i.e. after the mated cultures had been in contact for 28 days) at frequencies of 10^3-$10^6/10^8$ cells of the mated populations (c.m.p.).

These results therefore stress the necessity for prolonged incubation of the mated cultures and justify the method employed throughout the study (3.4).

4.2.2 Experiments to establish recombination levels in intraspecific crosses involving strains of *K. marxianus* and strains of *K. lactis*

The aim of this series of exploratory experiments was to establish a level at which interspecific recombinants in the genus *Kluyveromyces* could occur. Since this genus comprises both homothallic and heterothallic species, intraspecific crosses involving auxotrophic mutants of homothallic and heterothallic strains were made. Three strains of *K. marxianus* and three strains of *K. lactis* were employed in these experiments. The number of prototrophic, intraspecific recombinants was established in the final transfer after one week's incubation. The results of these experiments are presented in Table 6.

In the case of *K. marxianus* prototrophic recombinants were recovered in each of the 11 crosses. The frequency of recombination varied from 10^3-$10^6/10^8$ c.m.p. The recombinant colonies were not pigmented.

Prototrophic recombinants observed in crosses involving opposite mating types of *K. lactis* were formed at frequencies between 10^5-$10^7/10^8$ c.m.p. The sporulating recombinant colonies were pigmented.

The number of zygotes and asci formed by the mated, non-sporulating, prototrophic strains of *K. lactis* obtained

by a direct count in a haemacytometer was of the same order as the number of prototrophic recombinant colonies detected on minimal agar. This agreement is considered to constitute an additional proof of recombination between mated strains and demonstrates the suitability of the adopted technique.

4.2.3 Experiments involving the type strain of *K. marxianus* CBS 712

The aim of these experiments was to establish the suitability of employing the non-sporulating type strain of *K. marxianus* in the hybridization studies. Auxotrophic mutants of three strains of *K. lactis* were mated with a prototrophic strain of *K. marxianus* CBS 712. The results of these experiments are given in Table 7.

Recombinants were recovered in only two out of six crosses at frequencies of 10^3-$10^4/10^8$ c.m.p. The recombinant progeny utilized both lactose and maltose. Predominantly stoutly reniform and occasionally ellipsoidal ascospores were detected in some, but not all, of the recombinant progeny. The sporulation was extremely sparse.

As the reduced sexual activity of the type strain of *K. marxianus* influenced both the frequency of recombination with *K. lactis* and the sporulation ability of the recovered recombinants, it became apparent that this strain could not confidently be used in further hybridization studies.

4.3 INTERSPECIFIC HYBRIDIZATION EXPERIMENTS WITHIN THE GENUS *KLUYVEROMYCES*

The results of the hybridization studies in which auxotrophic mutants of 25 *Kluyveromyces* strains were crossed with the remaining species of the genus, are presented in Tables 8-33. In the comments concerning confirmation of recombinant formation in the crosses studied, only pertinent differential properties of the parental strains are indicated. (Additional physiological characteristics of the strains under investigation are listed in Table 4). The results are presented in alphabetical order.

4.3.1 Crosses involving *Kluyveromyces aestuarii* and the remaining species of the genus

The results of the experiments involving auxotrophic mutants of two strains of *K. aestuarii*, namely CBS 4438 and CBS 4904, which differ in their ability to assimilate maltose, are given in Tables 8 and 9.

As is apparent from the results presented in Tables 8 and 9, recombinants between *K. aestuarii* and the remaining species of the genus were not detected in 92 experiments.

4.3.2 Crosses involving *Kluyveromyces africanus* and the remaining species of the genus

The results of these experiments are listed in Table 10.

No recombinants were detected between *K. africanus* CBS 2654 and the remaining species of the genus in 60 hybridization experiments.

4.3.3 Crosses involving *Kluyveromyces blattae* and the remaining species of the genus

The results of these experiments are presented in Table 11.

No recombinants were recovered between *K. blattae* and the remaining species of the genus in 55 hybridization experiments.

4.3.4 Crosses involving *Kluyveromyces bulgaricus* and the remaining species of the genus

The results of 57 experiments are given in Table 12.

Crosses involving *K. bulgaricus* sor cel rib x *K. cicerisporus* SOR CEL RIB

In these crosses the recombinant frequencies ranged from 10^3-$10^6/10^8$ c.m.p. The recombinant colonies were not pigmented, formed spheroidal ascospores and utilized lactose, cellobiose, L-sorbose and D-ribose.

Crosses involving *K. bulgaricus* mal cel LAC Sph x *K. dobzhanskii* MAL CEL lac Ren, *K. bulgaricus* mal cel LAC Sph x *K. drosophilarum* MAL CEL lac Ren and *K. bulgaricus* mal cel LAC x *K. vanudenii* MAL CEL lac

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were observed. Recombinant progeny utilized lactose, maltose and cellobiose. In crosses involving *K. bulgaricus* x *K. dobzhanskii* and *K. bulgaricus* x *K. drosophilarum*, additional proof of the existence of recombination was obtained on the basis of the observed diversity

in shape and size of the ascospores. The recombinant colonies were pigmented.

It should be noted that no recombinants were recovered from crosses involving auxotrophic mutants of *K. bulgaricus* and the prototrophic strain of *K. drosophilarum*. The failure to recover recombinants in these two crosses is difficult to explain and must presumably be due to the incompatibility of the two parent strains.

Crosses involving *K. bulgaricus* cel Sph x *K. fragilis* CEL Ren and *K. bulgaricus* cel Sph x *K. marxianus* CEL Ren

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were observed. Recombinant progeny utilized lactose and cellobiose and was characterized by a variation in ascosporal shape and size. The recombinant colonies, except for those recovered from the medium containing cellobiose as a carbon source, were not pigmented.

Crosses involving *K. bulgaricus* mal cel x *K. lactis* MAL CEL

Recombinant frequencies in these crosses involving haploid, non-sporulating mating types of *K. lactis*, ranged from 10^5-$10^6/10^8$ c.m.p. Recovery of the self-sporulating hybrid progeny which utilized lactose, maltose and cellobiose constituted proof of the existence of recombination. The ascospores of the recombinant progeny were spheroidal. Recombinant colonies were pigmented.

Crosses involving *K. bulgaricus* sor cel LAC Sph x *K. phaseolosporus* SOR CEL lac Ren

Out of three crosses involving auxotrophic mutants of *K. bulgaricus* and the prototrophic strain of *K. phaseolosporus*, only one yielded recombinants at a frequency of $10^2/10^8$ c.m.p. The recombinant progeny utilizing lactose, L-sorbose and cellobiose was characterized by variation in ascosporal shape with the ascospores predominantly stoutly reniform to ellipsoidal. The recombinant colonies were lightly pigmented.

Crosses involving *K. bulgaricus* LAC x *K. wikenii* lac

The frequency of recombination observed between these two strains was low and ranged from 10^2-$10^3/10^8$ c.m.p. Since the physiological activity of *K. wikenii* is rather limited and because both strains form spheroidal ascospores, the recovery of recombinant progeny with combined parental phenotypes was not possible. Recombinant colonies were not pigmented.

No recombinants were observed in the remaining 28 crosses involving *K. bulgaricus* and the other species of the genus.

4.3.5 Crosses involving *Kluyveromyces cicerisporus* and the remaining species of the genus

The results of 48 experiments are presented in Table 13.

Crosses involving *K. cicerisporus* SOR CEL RIB x *K. bulgaricus* sor cel rib

In these crosses the recombinant frequencies ranged from 10^3-$10^6/10^8$ c.m.p. Physiological and morphological similarities between these two species, as well as a lack of differential characteristics, made confirmation of the existence of recombination between them very difficult. The recombinant colonies were not pigmented and utilized cellobiose, L-sorbose and D-ribose as well as lactose.

Crosses involving *K. cicerisporus* mal LAC Sph x *K. dobzhanskii* MAL lac Ren, *K. cicerisporus* mal LAC Sph x *K. drosophilarum* MAL lac Ren and *K. cicerisporus* mal LAC x *K. vanudenii* MAL lac

In these crosses recombinant frequencies varied between 10^3-$10^5/10^8$ c.m.p. The hybrid progeny recovered from these crosses utilized both lactose and maltose. In crosses involving *K. cicerisporus* x *K. dobzhanskii* and *K. cicerisporus* x *K. drosophilarum*, further proof of the existence of recombination was obtained on the basis of the observed heterogeneity in the shape and size of the ascospores of the hybrid progeny. The recombinant colonies were pigmented.

Crosses involving *K. cicerisporus* Sph x *K. fragilis* Ren and *K. cicerisporus* Sph x *K. marxianus* Ren

In these crosses recombinant frequencies of 10^2-$10^5/10^8$ c.m.p. were observed. Variation in the recombinant progeny in respect of ascosporal shape and size constituted a basis for the confirmation of recombination between these physiologically similar species. The recombinant colonies

were not pigmented.

Crosses involving *K. cicerisporus* mal x *K. lactis* MAL

Recombinant frequencies in these crosses involving haploid, non-sporulating mating types of *K. lactis* varied from 10^4-$10^6/10^8$ c.m.p. The recovery of self-sporulating progeny which utilized both lactose and maltose and formed spheroidal ascospores, constituted proof of the existence of recombination. Recombinant colonies were pigmented.

Crosses involving *K. cicerisporus* LAC Sph x *K. phaseolosporus* lac Ren

Both crosses involving auxotrophic mutants of *K. cicerisporus* and the prototrophic strain of *K. phaseolosporus* yielded recombinants at a frequency of $10^3/10^8$ c.m.p. The lactose utilizing recombinant progeny displayed wide variation in ascosporal shape, with ascospores ranging from spheroidal and ellipsoidal to stoutly reniform and reniform. The dimensions of hybrid ascospores also varied. The recombinant colonies were strongly pigmented.

Crosses involving *K. cicerisporus* LAC x *K. wikenii* lac

In both crosses recombinants were formed at a frequency of $10^3/10^8$ c.m.p. Since physiological activity of *K. wikenii* is rather limited, and because both strains form spheroidal ascospores, the recovery of recombinant progeny with combined parental phenotypes was not possible. Recombinant colonies were lightly pigmented.

No recombinants were detected in the remaining 23 crosses involving *K. cicerisporus* and the other species of the genus.

4.3.6 Crosses involving *Kluyveromyces delphensis* and the remaining species of the genus

The results of the experiments are presented in Table 14.

No recombinants were detected in crosses between *K. delphensis* and the other species of the genus in 54 hybridization experiments.

4.3.7 Crosses involving *Kluyveromyces dobzhanskii* and the remaining species of the genus

The results of 51 hybridization experiments are given in Table 15.

Crosses involving *K. dobzhanskii* MAL CEL lac Ren x *K. bulgaricus* mal cel LAC Sph, *K. dobzhanskii* MAL lac Ren x *K. cicerisporus* mal LAC Sph, *K. dobzhanskii* MAL lac x *K. fragilis* mal LAC and *K. dobzhanskii* MAL lac x *K. marxianus* mal LAC

Recombinants in these crosses were formed at frequencies of 10^3-$10^6/10^8$ c.m.p. The hybrid progeny recovered from all these crosses utilized both maltose and lactose. In crosses between *K. dobzhanskii* x *K. bulgaricus* and *K. dobzhanskii* x *K. cicerisporus*, variation in ascosporal shape and size was observed. In addition, the hybrid progeny in the cross *K. dobzhanskii* x *K. fragilis* fermented both maltose and lactose, although the latter property is absent in *K. dobzhanskii* and the

former absent in *K. fragilis*. The recombinant colonies were lightly to strongly pigmented.

Crosses involving *K. dobzhanskii* x *K. drosophilarum* and *K. dobzhanskii* MAL x *K. phaseolosporus* mal

In these crosses recombinant frequencies varied between 10^4-$10^5/10^8$ c.m.p. Confirmation of recombination between these three physiologically and morphologically closely related strains was difficult due to the lack of differential characteristics. The hybrid progeny utilized maltose and formed reniform ascospores of variable size. The recombinant colonies were strongly pigmented.

Crosses involving *K. dobzhanskii* lac Ren x *K. lactis* LAC Sph

In these crosses recombination frequencies of 10^4-$10^5/10^8$ c.m.p. were observed. Verification of recombination in these crosses involving haploid, non-sporulating mating types of *K. lactis* was based on the recovery of self-sporulating progeny which utilized both maltose and lactose and which formed spheroidal, ellipsoidal and stoutly reniform ascospores. The recombinant colonies were pigmented.

Crosses involving *K. dobzhanskii* Ren x *K. vanudenii* Sph and *K. dobzhanskii* MAL CEL Ren x *K. wikenii* mal cel Sph

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were obtained. The recovery of maltose utilizing recombinant progeny displaying variation in ascosporal size and shape constituted proof of recombination. The recombinant colonies were strongly pigmented.

Crosses involving *K. dobzhanskii* MAL lac RAF x *K. wickerhamii* mal LAC raf

Recombinant frequencies in these crosses ranged between 10^3-$10^5/10^8$ c.m.p. The recombinant progeny were phenotypically unstable, easily segregating to parental forms. Confirmation of the existence of recombination was based on the recovery of hybrid progeny utilizing both maltose and lactose. Utilization of raffinose, a property lacking in *K. wickerhamii*, was not confirmed in 18 recombinant isolates. Reniform ascospores of variable size were observed in recombinant progeny. The hybrid colonies were pigmented.

Recombinant formation was not observed in the remaining 19 crosses involving *K. dobzhanskii* and the other species of the genus.

4.3.8 Crosses involving *Kluyveromyces drosophilarum* and the remaining species of the genus

The results of 54 hybridization experiments are shown in Table 16.

Crosses involving *K. drosophilarum* MAL CEL lac Ren x *K. bulgaricus* mal cel LAC Sph, *K. drosophilarum* MAL lac Ren x *K. cicerisporus* mal LAC Sph, *K. drosophilarum* MAL lac x *K. fragilis* mal LAC and *K. drosophilarum* MAL lac x *K. marxianus* mal LAC

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were observed. Recombinant progeny recovered from these crosses assimilated both maltose and lactose. In the crosses between *K. drosophilarum* x *K. bulgaricus*

and *K. drosophilarum* x *K. cicerisporus* diversity in ascosporal shape and size was observed. The recombinant colonies were pigmented.

Crosses involving *K. drosophilarum* x *K. dobzhanskii* and *K. drosophilarum* MAL x *K. phaseolosporus* mal

In these crosses recombinant frequencies were high and ranged from 10^5-$10^6/10^8$ c.m.p. Verification of the existence of recombination between these physiologically and morphologically closely related strains was difficult due to the lack of differential characteristics. The hybrid progeny utilized maltose and formed reniform ascospores of various sizes. The recombinant colonies were very strongly pigmented.

Crosses involving *K. drosophilarum* lac Ren x *K. lactis* LAC Sph

Recombinant frequencies of 10^4-$10^5/10^8$ c.m.p. were observed. In these crosses, which involved haploid, non-sporulating mating types of *K. lactis*, recombinant progeny were self-sporulating and utilized maltose and lactose. The ascospores were spheroidal, ellipsoidal and stoutly reniform. The recombinant colonies were pigmented.

Crosses involving *K. drosophilarum* Ren x *K. vanudenii* Sph and *K. drosophilarum* MAL CEL Ren x *K. wikenii* mal cel Sph

High recombinant frequencies of the order of 10^5-$10^6/10^8$ c.m.p. were observed in these crosses. The recovery of maltose utilizing recombinant progeny, exhibiting

great variety in ascosporal shape and size, constituted a basis for the confirmation of recombination. The recombinant colonies were strongly pigmented.

Crosses involving *K. drosophilarum* GAL MAL Ren x *K. waltii* gal mal Sph

Recombinant frequencies of 10^5-$10^6/10^8$ c.m.p. were detected in two out of four crosses involving auxotrophic mutants of *K. drosophilarum* and the prototrophic strain of *K. waltii*. The recombinant progeny utilized maltose and galactose and formed predominantly spheroidal to ellipsoidal ascospores. Stoutly reniform ascospores were observed only occasionally. The recombinant progeny were not found to be osmophilic in that they did not grow in the presence of 50% glucose. Recombinant colonies were lightly pigmented.

Crosses involving *K. drosophilarum* MAL lac RAF x *K. wickerhamii* mal LAC raf

In these crosses recombinant frequencies varied between 10^3-$10^5/10^8$ c.m.p. The recovered hybrid progeny utilized maltose, lactose and raffinose and formed reniform ascospores of variable size. Ellipsoidal or elongated ascospores were occasionally encountered. The recombinant strains were phenotypically stable. The hybrid colonies were pigmented.

No recombinants were detected in the remaining 19 crosses involving *K. drosophilarum* and the other species of the genus.

4.3.9 Crosses involving *Kluyveromyces fragilis* and the remaining species of the genus

The results of 47 experiments are given in Table 17.

Crosses involving *K. fragilis* CEL Ren x *K. bulgaricus* cel Sph and *K. fragilis* Ren x *K. cicerisporus* Sph

The recombinant frequencies in these crosses varied from 10^3-$10^5/10^8$ c.m.p. The hybrid progeny utilized cellobiose and lactose and produced spheroidal to stoutly reniform ascospores. The recombinant colonies were not pigmented.

Crosses involving *K. fragilis* mal LAC x *K. dobzhanskii* MAL lac, *K. fragilis* mal LAC x *K. drosophilarum* MAL lac, *K. fragilis* mal LAC Ren x *K. vanudenii* MAL lac Sph

In these crosses recombinant frequencies ranged from 10^3-$10^5/10^8$ c.m.p. The recombinant progeny recovered from these crosses utilized both maltose and lactose. Hybrids derived from the cross *K. fragilis* x *K. vanudenii* also displayed a variation in ascosporal shape and size. Recombinant colonies were pigmented.

Crosses involving *K. fragilis* mal Ren x *K. lactis* MAL Sph

Recombinant frequencies of 10^5-$10^6/10^8$ c.m.p. were observed. Confirmation of recombination in these crosses involving haploid, non-sporulating mating types of *K. lactis* was based on the recovery of self-sporulating recombinant progeny which utilized both lactose and maltose and formed predominantly reniform and occasionally spheroidal to ellipsoidal ascospores. The recombinant colonies were

pigmented in the crosses involving *K. lactis* CBS 2359. Pigmentation was absent in the recombinant colonies involving *K. lactis* CBS 683.

Crosses involving *K. fragilis* x *K. marxianus*

In these crosses recombination frequencies of 10^3-$10^4/10^8$ c.m.p. were found. Since *K. fragilis* and *K. marxianus* are differentiated only by the rate of lactose fermentation, confirmation of the hybridization based on the recovery of recombinant progeny with combined parental phenotypes was not possible. Recombinant colonies were not pigmented.

Crosses involving *K. fragilis* tre LAC x *K. phaseolosporus* TRE lac

In these crosses recombinants were formed at frequencies of 10^4-$10^5/10^8$ c.m.p. Recombinant progeny utilized both trehalose and lactose and formed reniform ascospores of variable size. Recombinants were unstable, easily segregating to parental forms. Recombinant colonies were pigmented.

Crosses involving *K. fragilis* LAC Ren x *K. wikenii* lac Sph

Recombination frequencies in these crosses varied from 10^3-$10^4/10^8$ c.m.p. Confirmation of hybridization was based on the diversity in ascosporal shape and size observed in the recombinant, lactose utilizing progeny. The recombinant colonies were lightly pigmented.

Recombination was not observed in the remaining 22 crosses involving *K. fragilis* and other species of the genus.

4.3.10 Crosses involving *Kluyveromyces lactis* and the remaining species of the genus

Auxotrophic mutants of three haploid strains of *K. lactis*, namely CBS 683, CBS 2359 and CBS 6315 were used in the hybridization experiments. The results are presented in Tables 18-20. The hybridization experiments involving *K. lactis* (CBS 683 and CBS 2359) and other species of the genus in which recombinant formation was detected, will be discussed jointly.

Crosses involving *K. lactis* MAL CEL x *K. bulgaricus* mal cel and *K. lactis* MAL x *K. cicerisporus* mal

In these crosses recombinants were formed at frequencies of 10^4-$10^6/10^8$ c.m.p. Verification of hybridization was based on the recovery of self-sporulating recombinant progeny utilizing lactose, maltose and cellobiose and forming spheroidal ascospores. Sporulation was abundant. Recombinant colonies were pigmented.

Crosses involving *K. lactis* LAC Sph x *K. dobzhanskii* lac Ren, *K. lactis* LAC Sph x *K. drosophilarum* lac Ren and *K. lactis* MAL LAC Sph x *K. phaseolosporus* mal lac Ren

Recombinant frequencies in these crosses ranged from 10^4-$10^6/10^8$ c.m.p. The self-sporulating recombinant progeny utilized maltose as well as lactose and formed predominantly reniform to ellipsoidal and spheroidal ascospores.

Hybrid progeny in the first cross fermented both lactose and maltose whereas the former property is absent in *K. dobzhanskii* and the latter in *K. lactis*. The recombinant colonies were invariably pigmented.

Crosses involving *K. lactis* MAL Sph x *K. fragilis* mal Ren and *K. lactis* MAL Sph x *K. marxianus* mal Ren

Recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were observed. The recovered self-sporulating recombinant progeny utilized both maltose and lactose. Ascospores were produced abundantly and were predominantly stoutly reniform and occasionally ellipsoidal or spheroidal. The recombinant colonies were pigmented.

Crosses involving *K. lactis* LAC x *K. vanudenii* lac and *K. lactis* MAL LAC x *K. wikenii* mal lac

In these crosses recombinants were observed at frequencies of 10^3-$10^6/10^8$ c.m.p. Confirmation of hybridization in these crosses stemmed from the recovery of self-sporulating recombinant progeny which utilized both maltose and lactose. Hybrid ascospores were spheroidal. The recombinant colonies were pigmented.

Crosses involving *K. lactis* MAL RAF Sph x *K. wickerhamii* mal raf Ren

Recombinant colonies were detected in crosses involving *K. lactis* CBS 683 at frequencies of 10^3-$10^5/10^8$ c.m.p. and in one cross, involving *K. lactis* CBS 2359, at the low level of $78/10^8$ c.m.p. The recombinant progeny utilized

maltose, lactose and raffinose and sporulated abundantly. Heterogeneity in ascosporal shape was very pronounced. Reniform, stoutly reniform, ellipsoidal and spheroidal ascospores were observed. The recombinant colonies were pigmented. No recombinants were observed in the remaining two crosses involving auxotrophic mutants of *K. lactis* CBS 2359 and *K. lactis* CBS 6315 and the prototrophic strain of *K. wickerhamii*.

No recombinants were detected in crosses in which auxotrophic mutants of *K. lactis* were hybridized with *K. aestuarii*, *K. africanus*, *K. blattae*, *K. delphensis*, *K. lodderi*, *K. phaffii*, *K. polysporus*, *K. thermotolerans* and *K. waltii*. These strains, as well as the strain of *K. wickerhamii*, were repeatedly crossed with the auxotrophic mutants of the third strain of *K. lactis* CBS 6315, which does not utilize maltose. The results of these experiments are given in Table 20. No recombinants were detected in these 12 crosses.

4.3.11 Crosses involving *Kluyveromyces lodderi* and the remaining species of the genus

The results of these experiments are presented in Table 21.

No recombinants were recovered between *K. lodderi* and the remaining species of the genus in 59 hybridization experiments.

4.3.12 Crosses involving *Kluyveromyces marxianus* and the remaining species of the genus

Three strains of *K. marxianus* were used in the hybridization experiments. Due to the possible taxonomic implications of the outcome of these studies and due to the fact that the type strain, *K. marxianus* CBS 712 could not confidently be employed in these experiments (see Results 4.2.3), a minimum of five crosses involving auxotrophic mutants of *K. marxianus* CBS 6556 and *K. marxianus* CBS 6923 were made for each parental pair. The results of 142 hybridization experiments are given in Tables 22 and 23.

Crosses involving *K. marxianus* CEL Ren x *K. bulgaricus* cel Sph, *K. marxianus* Ren x *K. cicerisporus* Sph

In these crosses recombinant frequencies of 10^2-$10^6/10^8$ c.m.p. were found. The recombinant progeny were characterized by a marked variation in shape and size of the ascospores. Some recombinant asci contained both reniform and spheroidal to ellipsoidal ascospores. The recombinant colonies, except for those recovered from the medium containing cellobiose as a carbon source, were not pigmented.

Crosses involving *K. marxianus* mal LAC x *K. dobzhanskii* MAL lac and *K. marxianus* mal LAC x *K. drosophilarum* MAL lac

Recombinant frequencies in these crosses ranged from 10^4-$10^6/10^8$ c.m.p. The recombinant progeny utilized both maltose and lactose. Sporulation was abundant and reniform to stoutly reniform ascospores, frequently larger than those

of the parental strains, were observed. The recombinant colonies were strongly pigmented.

Crosses involving *K. marxianus* x *K. fragilis*

In these crosses recombination frequencies ranged from 10^3-$10^4/10^8$ c.m.p. Since *K. marxianus* and *K. fragilis* differ only in the rate of lactose fermentation, additional confirmation of hybridization by the recovery of progeny with combined parental phenotypes was not possible. Recombinant colonies were not pigmented.

Crosses involving *K. marxianus* mal Ren x *K. lactis* MAL Sph

Recombination frequencies in these crosses varied between 10^3-$10^6/10^8$ c.m.p. The hybrid progeny recovered from these crosses involving haploid, non-sporulating mating types of *K. lactis* was self-sporulating, utilized both lactose and maltose and formed predominantly reniform ascospores. Spheroidal and ellipsoidal ascospores were also observed. Recombinant colonies were pigmented.

Crosses involving *K. marxianus* tre LAC x *K. phaseolosporus* TRE lac

In these crosses recombination frequencies of 10^2-$10^4/10^8$ c.m.p. were observed. The hybrid progeny utilized both trehalose and lactose. Reniform ascospores of variable dimensions were observed. Recombinant colonies were pigmented.

Crosses involving *K. marxianus* mal LAC Ren x *K. vanudenii* MAL lac Sph

Recombination frequencies in these crosses ranged between 10^5-$10^6/10^8$ c.m.p. The recombinant progeny utilized both maltose and lactose and displayed diversity in ascosporal shape and size. The recombinant colonies were strongly pigmented.

Crosses involving *K. marxianus* LAC Ren x *K. wikenii* lac Sph

In these crosses recombinant frequencies of 10^4-$10^6/10^8$ c.m.p. were found. The recombinant progeny were characterized by a marked variation in ascosporal shape and size and utilized lactose and trehalose. The latter property is lacking in both parents. The recombinant colonies were pigmented.

Crosses involving *K. marxianus* mal LAC Ren x *K. thermotolerans* MAL lac Sph

The detection of recombinant formation in these crosses was difficult. Recombinants were detected in only three out of eight crosses between auxotrophic mutants of the two strains of *K. marxianus* and the prototrophic strain of *K. thermotolerans*. Recombination frequencies in these crosses were low, ranging from 40-58/10^8 c.m.p. Out of 21 presumptive recombinants brought into pure culture, only three were found to possess combined parental phenotypic characteristics. These recombinants assimilated both maltose and lactose and formed reniform to ellipsoidal and

spheroidal ascospores of various sizes. The recombinant colonies were lightly pigmented. One of these confirmed hybrids was deposited with the Centraalbureau voor Schimmelcultures in Delft as CBS 6925 for reference purposes.

No recombinants were detected in four additional crosses involving two mutant strains of *K. thermotolerans* and two prototrophic strains of *K. marxianus*.

Crosses involving *K. marxianus* RAF x *K. wickerhamii* raf

Out of 10 crosses involving auxotrophic mutants of the three strains of *K. marxianus* and the type strain of *K. wickerhamii* only three pigmented presumptive recombinant colonies were detected on minimal raffinose agar. However, the physiological properties of these colonies proved to be unstable and on purification yielded only parental types. No recombinants were detected in 10 additional crosses involving auxotrophic mutants of both parents. Since *K. wickerhamii* was found to give fertile, confirmed hybrids with *K. dobzhanskii*, *K. drosophilarum*, *K. lactis* CBS 683 and CBS 2359 and *K. vanudenii*, three more strains of this species were used in additional hybridization experiments, results of which are given in Table 24.

No recombinants were detected in the additional 24 experiments involving eight auxotrophic mutants of the three strains of *K. marxianus* and three prototrophic strains of *K. wickerhamii*.

Crosses involving *K. marxianus* in which recombinants were not detected

No recombinants were detected in 10^8 cells of the mated populations in crosses involving auxotrophic mutants of *K. marxianus* and strains of *K. aestuarii*, *K. africanus*, *K. blattae*, *K. delphensis*, *K. lodderi*, *K. phaffii*, *K. polysporus* and *K. waltii*. These strains as well as *K. thermotolerans* and the type strain of *K. wickerhamii* were repeatedly hybridized with the auxotrophic mutants of the third strain of *K. marxianus* CSIR Y293. The results of these experiments are presented in Table 25.

Cross involving *K. marxianus* mal LAC Ren x *K. thermotolerans* MAL lac Sph

Recombinants were detected in only one out of three crosses involving auxotrophic mutants of *K. marxianus* and the prototrophic strain of *K. thermotolerans*. Recombination frequency was low, being $42/10^8$ c.m.p. The recombinant progeny were characterized by the utilization of maltose and lactose and formation of reniform, ellipsoidal and spheroidal ascospores. No recombinants were observed in the remaining two crosses involving auxotrophic mutants of *K. thermotolerans* and the prototrophic strain of *K. marxianus*. The recombinant colonies were lightly pigmented.

No recombinants were detected in the remaining 24 crosses involving *K. marxianus* CSIR Y293 and other species of the genus.

4.3.13 Crosses involving *Kluyveromyces phaffii* and the remaining species of the genus

The results of these experiments are presented in Table 26.

No recombinants were detected between *K. phaffii* and the other species of the genus in 60 hybridization experiments.

4.3.14 Crosses involving *Kluyveromyces phaseolosporus* and the remaining species of the genus

The results of 60 experiments are given in Table 27.

Crosses involving *K. phaseolosporus* SOR CEL lac Ren x *K. bulgaricus* sor cel LAC Sph

Recombinants were detected in only one out of three crosses between auxotrophic mutants of *K. bulgaricus* and the prototrophic strain of *K. phaseolosporus*. The recombination frequency was low, namely $10^2/10^8$ c.m.p. The recombinant progeny utilized L-sorbose, cellobiose and lactose. Ascospores varied in their shape and size. The recombinant colonies were lightly pigmented.

Crosses involving *K. phaseolosporus* lac Ren x *K. cicerisporus* LAC Sph

Recombinants in these crosses were observed at a frequency of $10^3/10^8$ c.m.p. The lactose utilizing recombinant progeny were characterized by a marked diversity of ascospores. The recombinant colonies were strongly pigmented.

Crosses involving *K. phaseolosporus* mal x *K. dobzhanskii* MAL and *K. phaseolosporus* mal x *K. drosophilarum* MAL

In these crosses recombinant frequencies of 10^4-$10^6/10^8$ c.m.p. were found. These crosses, involving physiologically and morphologically closely related strains, yielded strongly pigmented hybrid progeny utilizing maltose. Due to the lack of differential characteristics, confirmation of combined phenotypic properties in recombinant progeny was not possible. Variation in ascosporal size was observed in the recombinant progeny.

Crosses involving *K. phaseolosporus* TRE lac x *K. fragilis* tre LAC and *K. phaseolosporus* TRE lac x *K. marxianus* tre LAC

Recombination frequencies in these crosses varied from 10^2-$10^5/10^8$ c.m.p. The hybrid progeny recovered from these crosses utilized both lactose and trehalose and formed reniform to stoutly reniform ascospores of variable dimensions. Recombinants of the first cross were unstable, easily segregating into parental forms. Recombinant colonies were pigmented.

Crosses involving *K. phaseolosporus* mal lac Ren x *K. lactis* MAL LAC Sph

In these crosses involving non-sporulating mating types of *K. lactis* recombinants were formed at frequencies of 10^4-$10^6/10^8$ c.m.p. The self-sporulating progeny utilized maltose and lactose and formed predominantly small, reniform

and only occasionally spheroidal ascospores. Recombinant colonies were pigmented.

Crosses involving *K. phaseolosporus* mal Ren x *K. vanudenii* MAL Sph

Recombinant frequencies in these crosses ranged from 10^5-$10^6/10^8$ c.m.p. The recovered hybrid progeny utilized maltose and displayed variation in ascosporal shape and size. Recombinant colonies were strongly pigmented.

Crosses involving *K. phaseolosporus* SOR CEL TRE Ren x *K. wikenii* sor cel tre Sph

Only two out of ten crosses involving these physiologically related species yielded recombinants at frequencies of 10^3-$10^5/10^8$ c.m.p. The recombinant progeny utilized L-sorbose, cellobiose and trehalose and formed predominantly spheroidal to ellipsoidal and only occasionally stoutly reniform ascospores. Recombinant colonies were lightly pigmented.

Recombinants were not observed in the remaining 30 crosses involving *K. phaseolosporus* and the other species of the genus.

4.3.15 Crosses involving *Kluyveromyces polysporus* and the remaining species of the genus

The results of the experiments are listed in Table 28.

Recombinants were not detected in 62 hybridization experiments involving *K. polysporus* and the remaining species

of the genus.

4.3.16 Crosses involving *Kluyveromyces thermotolerans* and the remaining species of the genus

The results of the experiments are presented in Table 29.

Crosses involving *K. thermotolerans* MAL lac Sph x *K. marxianus* mal LAC Ren

Proof of the existence of hybridization between these two species was based on the recovery of recombinant progeny utilizing both maltose and lactose and forming reniform, stoutly reniform, ellipsoidal and spheroidal ascospores. Recombination frequencies in these crosses were low and ranged from 40-58/10^8 c.m.p. In only three out of 10 crosses involving auxotrophic mutants of three strains of *K. marxianus* were recombinants detected. The recombinant colonies were lightly pigmented. Out of 21 presumptive recombinants brought into pure culture, only three were found to possess combined, parental phenotypic properties. One of these confirmed hybrids was deposited with the Centraalbureau voor Schimmelcultures in Delft as CBS 6925 for reference purposes.

Recombinants were not detected in six additional crosses involving two mutant strains of *K. thermotolerans* and three prototrophic strains of *K. marxianus*.

Recombinants were not detected in the remaining 64 crosses involving *K. thermotolerans* and other species of the genus.

4.3.17 Crosses involving *Kluyveromyces vanudenii* and the remaining species of the genus

The results of 52 hybridization experiments are given in Table 30.

Crosses involving *K. vanudenii* MAL CEL lac x *K. bulgaricus* mal cel LAC and *K. vanudenii* MAL lac x *K. cicerisporus* mal LAC

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were found. The recovered hybrid progeny utilized both maltose and lactose and formed spheroidal to ellipsoidal ascospores. Recombinant colonies were pigmented.

Crosses involving *K. vanudenii* Sph x *K. dobzhanskii* Ren, *K. vanudenii* Sph x *K. drosophilarum* Ren and *K. vanudenii* MAL Sph x *K. phaseolosporus* mal Ren

Recombination frequencies in these crosses ranged from 10^4-$10^6/10^8$ c.m.p. Maltose utilizing recombinant progeny formed spheroidal, ellipsoidal to stoutly reniform and reniform ascospores. Recombinant colonies were invariably strongly pigmented.

Crosses involving *K. vanudenii* MAL lac Sph x *K. fragilis* mal LAC Ren and *K. vanudenii* MAL lac Sph x *K. marxianus* mal LAC Ren

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were observed. The hybrid progeny utilized both maltose and lactose. Ascospores displayed strong diversity in their shape and size. Recombinant colonies were strongly pigmented.

Crosses involving *K. vanudenii* lac x *K. lactis* LAC

Recombination frequencies of 10^4-$10^6/10^8$ c.m.p. were observed in these crosses involving non-sporulating, haploid mating types of *K. lactis*. The self-sporulating hybrid progeny utilized maltose and lactose. The abundantly formed ascospores were spheroidal or ellipsoidal. Recombinant colonies were pigmented.

Crosses involving *K. vanudenii* MAL lac RAF Sph x *K. wickerhamii* mal LAC raf Ren

Recombination frequencies in these crosses ranged between 10^3-$10^5/10^8$ c.m.p. The recombinant progeny utilizing maltose, lactose and raffinose formed predominantly reniform to spheroidal and ellipsoidal ascospores. Recombinant colonies were pigmented.

Crosses involving *K. vanudenii* CEL MAL x *K. wikenii* cel mal

High recombination frequencies of $10^6/10^8$ c.m.p. were observed in crosses involving these closely related species. Due to the absence of differential characteristics, recovery of recombinant progeny with combined parental phenotypes was not possible. Strongly pigmented recombinant colonies were observed.

Recombinants were not detected in the remaining 21 crosses involving *K. vanudenii* and other species of the genus.

4.3.18 Crosses involving *Kluyveromyces waltii* and the remaining species of the genus

The results of 58 experiments are listed in Table 31.

Crosses involving *K. waltii* gal mal Sph x *K. drosophilarum* GAL MAL Ren

Recombinants were detected in two out of four crosses involving the prototrophic strain of *K. waltii* and auxotrophic mutants of *K. drosophilarum*. Recombination frequencies were high and ranged from 10^5-$10^6/10^8$ c.m.p. The recombinant progeny utilizing maltose and galactose formed predominantly spheroidal to ellipsoidal ascospores. Stoutly reniform ascospores were occasionally observed. The ability of *K. waltii* to grow in the presence of 50% glucose was not observed in the hybrid. Recombinant colonies were lightly pigmented.

Recombinant colonies were not detected in the remaining 54 crosses between *K. waltii* and the other species of the genus.

4.3.19 Crosses involving *Kluyveromyces wickerhamii* and the remaining species of the genus

The results of 72 experiments are presented in Table 32.

Crosses involving *K. wickerhamii* mal LAC raf x *K. dobzhanskii* MAL lac RAF and *K. wickerhamii* mal LAC raf x *K. drosophilarum* MAL lac RAF

In these crosses recombination frequencies of 10^3-$10^5/10^8$ c.m.p. were found. Recombinants recovered from the first cross were phenotypically unstable and segregated into parental forms.

Utilization of maltose and lactose was confirmed in this cross. Recombinants recovered from the second cross appeared to be stable and the utilization of maltose, lactose and raffinose was readily confirmed. Reniform to stoutly reniform ascospores of variable size were observed in hybrid progeny. Recombinant colonies were pigmented.

Crosses involving *K. wickerhamii* mal raf Ren x *K. lactis* MAL RAF Sph

Recombination frequencies in these crosses ranged from 78 to $10^5/10^8$ c.m.p. It should be stressed that the recombinant formation was observed in only three out of five crosses involving *K. wickerhamii* and non-sporulating mating types of *K. lactis*. Low recombination frequency was observed in a cross involving *K. lactis* CBS 2359. The self-sporulating hybrid progeny utilized maltose, lactose and raffinose and formed stoutly reniform and spheroidal to ellipsoidal ascospores. Recombinant colonies were pigmented.

Crosses involving *K. wickerhamii* mal LAC raf Ren x *K. vanudenii* MAL lac RAF Sph

In these crosses recombinant frequencies of 10^3-$10^5/10^8$ c.m.p. were observed. The recombinant progeny utilized maltose, lactose and raffinose and formed ascospores of variable size and shape. Recombinant colonies were pigmented.

Recombinants were not observed in the remaining 58 crosses involving *K. wickerhamii* and the other species of the genus.

4.3.20 Crosses involving *Kluyveromyces wikenii* and the remaining species of the genus

The results of 63 hybridization experiments are given in Table 33.

Crosses involving *K. wikenii* lac x *K. bulgaricus* LAC and *K. wikenii* lac x *K. cicerisporus* LAC

In these crosses recombination frequencies were low and ranged from 10^2-$10^3/10^8$ c.m.p. Due to the lack of differential characteristics, recovery of recombinant progeny with combined parental phenotypes was not possible. Recombinant colonies were not pigmented in the first cross and slightly pigmented in the second.

Crosses involving *K. wikenii* mal cel Sph x *K. dobzhanskii* MAL CEL Ren and *K. wikenii* mal cel Sph x *K. drosophilarum* MAL CEL Ren

High recombinant frequencies of the order of 10^3-$10^6/10^8$ c.m.p. were observed in these crosses. The recombinant progeny utilized maltose and cellobiose and formed spheroidal, ellipsoidal and reniform ascospores. Recombinant colonies were strongly pigmented.

Crosses involving *K. wikenii* lac Sph x *K. fragilis* LAC Ren and *K. wikenii* lac Sph x *K. marxianus* LAC Ren

Recombinant frequencies in these crosses varied from 10^3-$10^6/10^8$ c.m.p. The lactose utilizing recombinant progeny exhibited heterogeneity in ascosporal shape and size. Hybrids obtained from the second cross also utilized trehalose, a property lacking in both parental strains. The recombinant

colonies were pigmented.

Crosses involving *K. wikenii* mal lac x *K. lactis* MAL LAC

In these crosses recombinant frequencies of 10^3-$10^6/10^8$ c.m.p. were found. The self-sporulating recombinant progeny utilized both maltose and lactose. Ascospores were spheroidal to ellipsoidal. Recombinant colonies were pigmented.

Crosses involving *K. wikenii* sor cel tre Sph x *K. phaseolosporus* SOR CEL TRE Ren

Recombinants were detected in only two out of ten crosses involving these closely physiologically related species. The recombination frequencies ranged from 10^3-$10^5/10^8$ c.m.p. The recombinant progeny utilized L-sorbose, cellobiose and trehalose and formed predominantly spheroidal to ellipsoidal and only occasionally stoutly reniform ascospores. Recombinant colonies were lightly pigmented.

Crosses involving *K. wikenii* cel mal x *K. vanudenii* CEL MAL

High recombination frequencies of $10^6/10^8$ c.m.p. were observed in these crosses. Recovery of recombinant progeny with combined parental phenotypes was not possible, due to the absence of differential characteristics. Recombinant colonies were strongly pigmented.

No recombinants were observed in the remaining 31 crosses involving *K. wikenii* and the other species of the genus.

4.4 HYBRIDIZATION EXPERIMENTS INVOLVING AN UNDESCRIBED REPRESENTATIVE OF *KLUYVEROMYCES* AND SPECIES OF THE GENUS *KLUYVEROMYCES*

An undescribed representative of *Kluyveromyces* was isolated by Prof S. Windisch of the Institut für Gärungsgewerbe und Biotechnologie in Berlin in 1976 from fruit-curd-feed manufacture and submitted to the Microbiology Research Group of the CSIR for an opinion. This strain, labelled DSM 70885, is phenotypically distinct from all hitherto described species. Its properties are as follows:

Fermentation:

Glucose	+	Maltose	-
Galactose	+	Lactose	-
Sucrose	+	Raffinose	+

Assimilation of carbon compounds:

Glucose	+	D-ribose	-
Galactose	+	L-rhamnose	-
L-sorbose	-	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	-	Erythritol	-
Cellobiose	-	Ribitol	-
Trehalose	+s	Galactitol	-
Lactose	-	D-glucitol	-
Melibiose	-	D-mannitol	-
Raffinose	+	α-Methyl-D-glucoside	-
Melezitose	-	Salicin	-
Inulin	+	DL-Lactic acid	+

Soluble starch	-	Succinic acid	+
D-xylose	+	Citric acid	-
L-arabinose	+	Inositol	-
D-arabinose	-	Potassium gluconate	-

Utilization of nitrogen sources:

Potassium nitrate	-
Potassium nitrite	-
Ethylamine	+

Growth in the presence of 100 p.p.m. cycloheximide: +

Growth in vitamin-free medium: -

Growth in osmotic medium: +ws

Growth at 37 ^{o}C: +

Pseudomycelium is abundantly produced both aerobically and anaerobically. Spheroidal to ellipsoidal ascospores, some with an indentation, 1-2 per ascus were observed on YM agar.

Auxotrophic mutants were induced in this strain and hybridization experiments involving DSM 70885 and the remaining species of the genus were carried out. The results of these experiments are given in Table 34.

Crosses involving DSM 70885 lac x *K. bulgaricus* LAC and DSM 70885 cel lac x *K. cicerisporus* CEL LAC

Recombination frequencies in these crosses varied between 10^{3}-$10^{6}/10^{8}$ c.m.p. Lactose utilizing hybrid progeny were characterized by unusually large asci and ascospores. Ascospores were predominantly spheroidal to ellipsoidal and some of them were indented. Recombinant colonies were not

pigmented.

Crosses involving DSM 70885 mal Sph x *K. dobzhanskii* MAL Ren and DSM 70885 mal Sph x *K. drosophilarum* MAL Ren

In these crosses recombination frequencies of 10^2-$10^5/10^8$ c.m.p. were observed. The maltose utilizing recombinant progeny displayed pronounced heterogeneity in ascosporal size and shape. Large ascospores - crescentiform, reniform, stoutly reniform, ellipsoidal to spheroidal - were observed. Many irregular-shaped vegetative cells were also encountered. Recombinant colonies were strongly pigmented.

Crosses involving DSM 70885 lac Sph x *K. fragilis* LAC Ren and DSM 70885 lac Sph x *K. marxianus* LAC Ren

Recombination frequencies in these crosses ranged from 10^2-$10^5/10^8$ c.m.p. The recombinant progeny utilized lactose and formed large reniform, stoutly reniform, ellipsoidal and spheroidal ascospores. Some vegetative cells were aberrant to amoeboid in shape. Recombinant colonies were not pigmented.

Crosses involving DSM 70885 mal lac x *K. lactis* MAL lac

Recombinant frequencies in these crosses involving non-sporulating mating types of *K. lactis* ranged from 10^2-$10^6/10^8$ c.m.p. The self-sporulating recombinant progeny utilized both maltose and lactose and formed large, long-oval to ellipsoidal ascospores, some of which possessed an indentation. Vegetative cells of abnormal shape were also encountered. Recombinant colonies were lightly pigmented.

Crosses involving DSM 70885 mal x *K. vanudenii* MAL

In both crosses high recombinant frequencies of 10^5-$10^6/10^8$ c.m.p. were obtained. Recombinant progeny utilizing maltose were characterized by the presence of exceptionally large, spheroidal and ellipsoidal ascospores, some of which possessed a distinct indentation. Recombinant colonies were strongly pigmented.

No recombinants were recovered in crosses involving strain DSM 70885 and *K. aestuarii*, *K. africanus*, *K. blattae*, *K. delphensis*, *K. lodderi*, *K. phaffii*, *K. phaseolosporus*, *K. polysporus*, *K. thermotolerans*, *K. waltii*, *K. wickerhamii* and *K. wikenii*.

4.5 HYBRIDIZATION EXPERIMENTS INVOLVING *KLUYVEROMYCES LACTIS* AND PRESUMPTIVE IMPERFECT FORMS OF SOME *KLUYVEROMYCES* SPECIES

The results of these experiments are presented in Table 35.

The non-sporulating strains of *K. lactis* were chosen for these experiments as the recovery of sporulating progeny constitutes an easily detectable proof of recombination.

Cross involving *K. lactis* MAL x *C. kefyr*

In only one out of four crosses involving auxotrophic mutants of non-sporulating mating types of *K. lactis* and *C. kefyr* were recombinants detected at a frequency of $10^5/10^8$ c.m.p. The recombinant progeny utilized maltose and lactose and formed predominantly reniform and occasionally

spheroidal to ellipsoidal ascospores. Recombinant colonies were lightly pigmented.

Crosses involving *K. lactis* MAL x *C. macedoniensis*

In these crosses involving auxotrophic mutants of non-sporulating mating types of *K. lactis* and a presumptive imperfect form of *K. marxianus*, recombinant frequencies ranged from 10^4-$10^5/10^8$ c.m.p. The self-sporulating recombinant progeny utilized maltose and lactose. The ascospores were predominantly reniform and stoutly reniform and occasionally spheroidal to ellipsoidal. Recombinant colonies were pigmented.

Crosses involving *K. lactis* x *T. sphaerica*

Since *T. sphærica* is considered to be the imperfect form of *K. lactis*, the recovery of recombinant progeny with combined parental phenotypes was not possible. Recombinant frequencies of 10^4-$10^7/10^8$ c.m.p. were observed. The self-sporulating progeny utilized maltose and lactose and formed spheroidal ascospores.

Recombinants were not detected in four crosses involving auxotrophic mutants of *K. lactis* and the type strain of *C. pseudotropicalis*.

4.6 HYBRIDIZATION EXPERIMENTS INVOLVING *KLUYVEROMYCES MARXIANUS* AND REPRESENTATIVES OF OTHER ASCOGENOUS SPECIES

The results of these experiments are presented in Tables 36-38.

No recombinants were detected in 65 crosses involving auxotrophic mutants of three strains of *K. marxianus* and prototrophic strains of representatives of other ascogenous species.

4.7 HYBRIDIZATION EXPERIMENTS INVOLVING *KLUYVEROMYCES THERMOTOLERANS* AND *SACCHAROMYCES BAYANUS*

The results of these experiments are shown in Table 39.

In these crosses involving auxotrophic mutants of *K. thermotolerans* and two strains of physiologically closely related *Sacch. bayanus*, no recombinants were detected.

4.8 SPORULATION OF HYBRID PROGENY AND VIABILITY OF HYBRID ASCOSPORES

Ascospore formation in the recovered, cloned recombinants was as a rule abundant and easily detectable on YM agar. In some cases, for example, in crosses of *K. bulgaricus* x *K. phaseolosporus*, *K. dobzhanskii* x *K. wikenii*, *K. lactis* CBS 683 x *K. wickerhamii* and *K. cicerisporus* x DSM 70885, sporulation was more abundant on 2% ME agar, V8 agar and McClary's acetate agar.

In crosses involving parental strains characterized by the formation of spheroidal and reniform ascospores, the hybrid ascospores showed considerable diversity in shape and size. Intermediate forms, i.e. large, stoutly reniform, or large ellipsoidal ascospores as well as long oval spores with a lateral indentation were regularly encountered. Reniform and spheroidal ascospores were not infrequently observed in a single ascus.

Viability of hybrid ascospores was confirmed by the heat-treatment technique and by the ultrasonic-paraffin method, as described in Methods (*cf.* 3.5.2.1 and 3.5.2.2). The assimilatory properties of the recombinant progeny and the ability to sporulate were confirmed for each hybrid in at least two colonies developing from single ascospores on YM agar plates.

Approximate quantitative results, based on the isolation of ascospores by the ultrasonic-paraffin technique, of the ascosporal viability for the hybrids formed by *K. marxianus* and other members of the genus as well as the hybrids recovered from the crosses of *K. dobzhanskii* x *K. fragilis*, *K. drosophilarum* x *K. waltii* and *K. vanudenii* x *K. wickerhamii*, are presented in Table 40. In addition, the viability of ascospores formed by the hybrids recovered from crosses involving *K. marxianus* x *K. lactis* and *K. marxianus* x *K. thermotolerans* was confirmed by the isolation of single ascospores (*cf.* Methods 3.5.2.3). These determinations were kindly carried out by Dr J.P. van der Walt. Ascospores isolated by this technique from the first hybrid, i.e. *K. marxianus* CBS 6923 x *K. lactis* CBS 2354 arg ura showed 60% viability and from the second hybrid, i.e. *K. marxianus* CBS 6923 ade x *K. thermotolerans* CBS 6924 showed 50% viability.

4.9 NUCLEAR STAINING OF THE VEGETATIVE PHASE OF HYBRID PROGENY

In order to exclude the possibility that prototrophy in the recovered recombinant progeny was restored as a result of the formation of a stable, complementary dikaryon state, the nuclear status of the vegetative phase of the hybrid strains was examined by nuclear staining (*cf.* Methods 3.5.3).

The following hybrid strains with combined parental phenotypes were examined: *K. marxianus* x *K. cicerisporus*, *K. marxianus* x *K. bulgaricus*, *K. marxianus* x *K. dobzhanskii*, *K. marxianus* x *K. drosophilarum*, *K. marxianus* x *K. fragilis*, *K. marxianus* x *K. lactis*, *K. marxianus* x *K. phaseolosporus*, *K. marxianus* x *K. thermotolerans*, *K. marxianus* x *K. vanudenii*, *K. marxianus* x *K. wikenii*, *K. dobzhanskii* x *K. fragilis*, *K. dobzhanskii* x *K. wickerhamii*, *K. drosophilarum* x *K. waltii* and *K. vanudenii* x *K. wickerhamii*.

In all preparations of these hybrid strains the uninucleate state prevailed. The binucleate state was only observed in cells which were in the process of budding. In one of the hybrids, namely *K. marxianus* x *K. dobzhanskii*, which sporulated within eight hours of cultivation on the minimal agar medium, multinucleate cells as well as uninucleate ascospores were observed.

5. DISCUSSION OF THE RESULTS

Before the results obtained in this investigation are discussed, a short evaluation of the suitability of the technique employed will be given.

5.1 EVALUATION OF THE TECHNIQUE EMPLOYED

The prototrophic selection technique of Pomper & Burkholder (1949), employed in this investigation was reported by Fowell (1969), Mortimer & Hawthorne (1969) and Sherman & Lawrence (1974), to be one of the most convenient methods of isolating hybrid clones among biotypes of *Sacch. cerevisiae*. Its successful application for genetic studies is largely due to the fact that in these strains the vegetative and ascigeric phases can be stabilized by the use of suitable media. This advantage allows for the isolation of the first filial generation in a cloned condition permitting further genetic studies. The complete stabilization of the vegetative and ascigeric phases may, however, not be possible in other yeast genera, e.g. *Kluyveromyces*.

The suitability of the technique for the detection of hybrids formed by members of the genus *Kluyveromyces* was confirmed in this study, as illustrated by the results reported in Chapter 4. The method has the distinct advantage of detecting recombination between yeast strains at very low frequencies, not easily achieved by the mating of either single ascospores or individual vegetative cells. This is demonstrated by the successful cross involving *K. thermotolerans* x *K. marxianus* in which the observed recombination frequencies ranged from 40-58/10^8 c.m.p. (*cf.* Table 29).

Irrespective of this advantage, the use of auxotrophic mutants for hybridization purposes has certain limitations.

5.2 LIMITATIONS OF THE TECHNIQUE

Limitations of the technique employed will be discussed in the following order:

stability of auxotrophic mutants (5.2.1);

mutations affecting the mating-type locus (5.2.2);

position of the nutritional markers (5.2.3).

5.2.1 Stability of auxotrophic mutants

The method requires the isolation and characterization of an appreciable number of auxotrophic mutants before suitably stable mutant strains are recovered. This is reflected by the fact that out of 433 recovered mutants only 95 (approximately 20%) proved stable for the duration of the hybridization studies. The presence of a second nutritional requirement did not increase the stability of the auxotrophic mutants since mutants with more than one marker were frequently observed to revert to prototrophic conditions as easily as those characterized by a single nutritional requirement. This confirms an observation of Kimball (1970) who reported that the MNNG-induced lesions in DNA appear to be easily repaired and only those which occur near the DNA replication point result in the formation of stable mutants.

The auxotrophic mutants of the haploid strains of *K. lactis* were found to be the most stable. One of the mutants of *K. lactis* CBS 2359, requiring arginine and uracil, did not revert to the prototrophic state during the three years since its isolation. Some mutants of *K. aestuarii* CBS 4904, *K. marxianus* CBS 6923, *K. polysporus*, *K. thermotolerans* and *K. vanudenii* were stable for two years.

A strict control of the stability of the auxotrophic mutants is imperative for the successful application of this technique.

5.2.2 Mutations affecting the mating-type locus

The mating ability of yeast strains is under the control of the mating-type locus. As MNNG can induce more than one mutation, the MNNG-treatment aimed at the introduction of nutritional markers might have induced mutations in the *mat* locus, thus affecting the ability of the mutant strain to conjugate. Consequently the mating ability of auxotrophic mutants could be either enhanced or reduced.

Fabre & Roman (1977) reporting on the influence of UV and X-ray irradiation on *Sacch. cerevisiae* observed that the irradiation can have two effects, of which "one enlarges the pool of cells able to recombine, and the other introduces in the chromosomes prerecombinational lesions that promote recombination in the competent cells." These authors also concluded that the ability of yeast cells to recombine depends on an inducible mechanism and that "lesions in the DNA

appear to be responsible for the induction of the recombinational ability." Although the conclusions of Fabre & Roman (1977) refer to intraspecific recombination, the possibility exists that some of the MNNG-induced auxotrophic mutants employed in the present investigation might possess a de-repressed or enhanced mating ability not expressed in the prototrophic state of the parental strains.

The impairment of the mating ability of the mutant induced by the MNNG-treatment was probably observed during this investigation. A mutant of *K. marxianus* CBS 6923 labelled M160, requiring adenine, was crossed with the remaining species of the genus. As will be observed from the results presented in Table 41, no recombinants were detected in any of the 21 crosses. The remaining mutants of this strain of *K. marxianus*, including another also requiring adenine, as well as the mutants of the second strain of *K. marxianus* i.e. CBS 6556 nevertheless readily hybridized with *K. cicerisporus*, *K. bulgaricus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. phaseolosporus*, *K. vanudenii* and *K. wikenii* (*cf.* Tables 22 and 23). The failure of the mutant M160 to hybridize with other members of the genus *Kluyveromyces* might also be due to the unsuitable position of its nutritional marker on a chromosome.

5.2.3 Position of the nutritional markers

If the restoration of the prototrophic condition during recombination requires the formation of chiasmata between homologous chromosomes of mated strains, the position

of the nutritional marker might affect hybrid formation. According to Hayes (1968c) chiasmata are seldom formed in the vicinity of centromeres. Consequently, the loci in these regions of chromosomes show a low degree of recombination. If the nutritional marker is positioned too close to the centromere the exchange of such a part of a chromosome during crossing over might be prevented.

The genetic map of *Saccharomyces*, presented by Sherman & Lawrence (1974) reveals nine loci controlling the requirement for adenine, with loci ade 1 and ade 6 placed close to the centromeres of chromosomes I and VII, respectively. Therefore it is possible that the mutant M160 requiring adenine could have undergone mutation at one of the loci which do not recombine during crossing over.

The possibility that other mutants used in this study might possess similar, unfavourably situated markers, cannot be excluded.

To summarize, the successful application of the method requires:

(i) the availability of nutritionally-characterized auxotrophic mutants, which are stable at least for the duration of the hybridization experiments;

(ii) auxotrophic mutants must not undergo mutation impairing the locus mediating the mating reaction;

(iii) the position of the nutritional markers must be such that it will not prevent or restrict their recombination during crossing over.

In view of these eventualities more than one mutant of a parent strain should be used for each cross studied and the parental pairs which did not yield recombinants in the present investigation should not be regarded as being ultimately incapable of hybridization.

Before the areas of hybridization in the genus *Kluyveromyces*, as established under the prevailing experimental conditions are defined, the quantitative aspect of the results as well as the phenotypic characteristics of the recombinant progeny recovered, will be discussed.

5.3 DETECTION OF RECOMBINATION AND VARIATION IN THE RECOMBINATION FREQUENCIES

The results of 861 hybridization experiments are summarized in Table 42.

The detection of recombinants in 203 interspecific crosses within the genus *Kluyveromyces* involving 51 parental pairs, which constitutes the core of this investigation, will be discussed in greater detail.

It was observed that the recombination frequencies in crosses involving various auxotrophic mutants of the same parental strains were subject to some variation. Considering the complexity of the hybridization process and of the mechanism involved in the restoration of the prototrophic condition, a variation in the recombination frequencies is to be anticipated. The range of such anticipated variation is apparent from the

intraspecific crosses involving auxotrophic mutants of three homothallic strains of *K. marxianus* and three heterothallic strains of *K. lactis* (*cf.* Results 4.2.2 and Table 6).

On the basis of these results and in view of the fact that the majority of species of the genus *Kluyveromyces* are homothallic, the recombination frequency of 10^3 or higher/10^8 c.m.p., as observed in these intraspecific crosses, was accepted as indicative of genetic relatedness at a species level.

The positive results obtained in this investigation, i.e. crosses in which recombinants were detected, can be divided into four categories:

(i) crosses in which recombinants were observed at frequencies of 10^3 or higher/10^8 c.m.p. in all tested combinations of parental strains (5.3.1);

(ii) crosses in which recombinants were observed in all tested combinations of parental strains, but occasionally present at frequencies lower than $10^3/10^8$ c.m.p. (5.3.2);

(iii) crosses in which recombinants were detected only in some of the parental combinations at frequencies of 10^3 or higher/10^8 c.m.p. (5.3.3);

(iv) crosses in which recombinants were detected only occasionally and at low frequencies (5.3.4).

5.3.1 To the first category belong crosses in which all auxotrophic mutants of one parent strain yielded recombinant colonies at frequencies of 10^3 or higher/10^8 c.m.p.

when hybridized with prototrophic or reciprocally marked auxotrophic strains of the other parent. Such readily detectable recombination was observed in the majority of hybridizing taxa. Crosses involving *K. marxianus* CBS 6923 and *K. bulgaricus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. vanudenii* as well as *K. wikenii* (*cf.* Table 23) constitute examples of the results placed in this category.

5.3.2 Three examples, i.e. crosses involving *K. bulgaricus* x *K. wikenii*, *K. marxianus* CBS 6923 x *K. cicerisporus* and *K. marxianus* CBS 6923 x *K. phaseolosporus*, illustrate the crosses belonging to the second category. In all three cases the recombinant colonies in one parental cross were present at a frequency of $10^2/10^8$ c.m.p., although the remaining parental combinations yielded recombinants at frequencies of 10^3 or $10^4/10^8$ c.m.p. (*cf.* Tables 12 and 23).

5.3.3 High frequencies of recombinants, formed in only some combinations of the parental strains, were observed in the following three cases:

Crosses involving *K. bulgaricus* x *K. drosophilarum*

In two crosses involving auxotrophic mutants of *K. bulgaricus* and the prototrophic strain of *K. drosophilarum* recombinants were not detected. However, in the reverse crosses, involving auxotrophic mutants of *K. drosophilarum* and the prototrophic strain of *K. bulgaricus* high recombination frequencies of 10^5-$10^6/10^8$ c.m.p. were observed

(*cf.* Table 12). The failure of the auxotrophic mutants of *K. bulgaricus* to form recombinants with *K. drosophilarum* cannot be attributed to their reduced mating ability or to the position of the nutritional marker, (i.e. to its proximity to the centromere). This is evident from the fact that the same mutants readily crossed with *K. cicerisporus*, *K. dobzhanskii*, *K. fragilis*, *K. marxianus* CBS 6556 and CBS 6923, *K. vanudenii* and *K. wikenii*.

Crosses involving *K. phaseolosporus* x *K. wikenii*

Recombinants were detected in only two out of ten crosses involving these physiologically related species. The observed recombination frequencies were high and ranged from 10^3-$10^5/10^8$ c.m.p. (*cf.* Table 27). It should be noted that both of these crosses involved the same uracil-requiring mutant of *K. wikenii*. The fact that the recombinant progeny displayed a variation in ascosporal shape and utilized L-sorbose, cellobiose and trehalose, properties absent in *K. wikenii*, confirmed the hybrid nature of the recovered recombinants.

Crosses involving *K. waltii* x *K. drosophilarum*

Two out of four crosses involving auxotrophic mutants of *K. drosophilarum* and the prototrophic strain of *K. waltii* yielded recombinants at high frequencies of 10^5-$10^6/10^8$ c.m.p. (*cf.* Table 31). Considering the differences in physiological and morphological properties between these two taxa as well as the difference in mol % G+C (38,5-40,9% for

K. drosophilarum, and 45,6% for *K. waltii*, see Table 44), the detection of interfertility in these crosses was unexpected.

5.3.4 To the last category are assigned crosses in which recombinant colonies were detected only occasionally or were formed at low frequencies. The following examples illustrate results of this type:

Crosses involving *K. bulgaricus* x *K. phaseolosporus*

Only one out of three crosses involving auxotrophic mutants of *K. bulgaricus* and the prototrophic strain of *K. phaseolosporus* yielded recombinants at a frequency of $10^2/10^8$ c.m.p. (*cf.* Table 12). Since the other two mutants of *K. bulgaricus* formed recombinants with *K. cicerisporus*, *K. dobzhanskii*, *K. fragilis*, *K. marxianus* CBS 6556 and CBS 6923, *K. vanudenii* and *K. wikenii*, their failure to hybridize with *K. phaseolosporus* does not seem likely to be due to the position of the nutritional marker or to reduced mating ability.

Crosses involving *K. lactis* CBS 2359 x *K. wickerhamii*

In one of the two crosses involving *K. lactis* CBS 2359 and a type strain of *K. wickerhamii*, recombinants were detected but at a low frequency, namely $78/10^8$ c.m.p. (*cf.* Table 19). It is surprising that in this cross involving auxotrophic mutants of both parental strains, interfertility was detected, while in the other cross involving the same mutant of *K. lactis* and a prototrophic strain of *K. wickerhamii* no recombinants were observed. It should be stressed

that both crosses involving *K. lactis* CBS 683 and *K. wickerhamii* yielded recombinants at frequencies of 10^3-$10^5/10^8$ c.m.p.

Crosses involving *K. marxianus* x *K. thermotolerans*

In only three out of eleven crosses involving auxotrophic mutants of the three strains of *K. marxianus* and the prototrophic strain of *K. thermotolerans* were recombinants detected (*cf.* Tables 23 and 25). Six additional crosses between auxotrophic mutants of *K. thermotolerans* and prototrophic strains of *K. marxianus*, failed to yield detectable recombinants (*cf.* Table 29). Since no recombinants were detected in 32 crosses involving the same mutants of *K. thermotolerans* and other species of the genus the possibility that these mutants possessed reduced mating ability or that the position of the nutritional markers in these mutants was unfavourable, cannot be excluded. Hybrid formation between *K. marxianus* and *K. thermotolerans* is surprising since these two taxa are reported to differ in their mol % G+C content by as much as 5% (see Table 44, Phaff, 1978).

To summarize the remarks concerning the four categories of positive results, the following should be emphasized:

(i) in crosses assigned to the first three categories the hybrid formation, verified by the recovery of recombinant progeny possessing phenotypic properties of both parents and forming viable ascospores, cannot be doubted;

(ii) the variation in recombinant frequencies as observed in the second and third categories is probably due to a variable degree of compatibility between hybridized strains, caused either by the position of nutritional markers in the auxotrophic mutants or by a possible impairment of their mating ability. It should be stressed that recombination frequencies lower than those observed in intraspecific crosses were detected in only six cases;

(iii) as the recombination frequencies observed in the crosses assigned to the fourth category are appreciably lower than those observed in intraspecific crosses, it may be concluded that such low recombination frequencies might not be indicative of the relatedness at a species level.

5.4 MORPHOLOGICAL CHARACTERISTICS OF RECOMBINANT COLONIES AND CELLULAR MORPHOLOGY OF RECOMBINANT PROGENY

Recombinant colonies as observed on minimal agar showed a great diversity in respect of their size, surface, margin, elevation, consistency and pigmentation as well as in the presence or absence of lawns of microcolonies formed in their proximity. Cellular morphology of the recombinant strains was likewise subject to variation in respect of the size and shape of both vegetative cells and ascospores. The observed differences in both colonial and cellular morphology constitute useful characteristics of the recombinant progeny and will be briefly discussed.

The size of recombinant colonies varied from 0,5-3,0 mm in diameter

with the majority of recombinants forming colonies with diameters between 1,5-2,0 mm. The surface of recombinant colonies varied from smooth and shiny, and smooth and dull, to rough, rugose or crateriform, frequently changing on prolonged incubation. The margins of the majority of recombinant colonies were entire and smooth and only occasionally lobate to irregular. Most of the recombinant colonies were convex, some of them were flat and only on one occasion were crater-like colonies encountered (in the cross between *K. marxianus* CBS 6923 x DSM 70885). As a rule recombinant colonies were butyrous in consistency, but occasionally coherent to friable colonies were encountered.

Pigmentation of recombinant colonies was subject to variation. Recombinant colonies obtained from intraspecific crosses involving auxotrophic mutants of *K. marxianus* were white to cream-white without any brown to maroon pigment being detected. Non-pigmented recombinant colonies were also observed in crosses involving *K. bulgaricus* x *K. cicerisporus* as well as *K. marxianus* x *K. fragilis*. Considering the physiological and morphological closeness of these two pairs, these crosses might actually be regarded as involving strains of the same species.

The majority of interspecific crosses, however, yielded pigmented recombinant colonies with colour ranging from beige, light brown or pale pink to maroon, presumably due to the formation of pulcherrimin (van der Walt, 1952). It should be noted, however, that not all interspecific crosses were characterized by pigment formation on minimal agar. Thus pigment formation was not observed in crosses involving *K. bulgaricus* x *K. wikenii*, *K. fragilis* x *K. bulgaricus*, *K. fragilis* x *K. cicerisporus*, *K. marxianus* x *K. bulgaricus* and *K. marxianus* x *K. cicerisporus*.

Pigmentation of recombinant colonies was also observed by Wickerham & Burton (1956a, b) in hybrids obtained from crosses involving *K. lactis* x *K. marxianus* and *K. fragilis* x *K. dobzhanskii*. The antibiotic activity of the pigment reported by these authors was not observed in this investigation, with the exception of a cross involving *K. lactis* CBS 2359 x *K. wikenii*. In this cross recombinant colonies on minimal agar were surrounded by a 0,5-1,0 mm zone of inhibition.

Lawn formation in the vicinity of recombinant colonies was probably induced by cross feeding and due to the excretion by the hybrid colony of:

(i) nutritional factors required by the auxotrophic mutants employed in the cross;

(ii) enzymes hydrolyzing the disaccharide employed for the differential recovery of hybrids.

Such lawns were absent in all intraspecific crosses and in crosses involving physiologically similar taxa such as *K. bulgaricus* x *K. cicerisporus*, *K. marxianus* x *K. fragilis* or *K. dobzhanskii* x *K. drosophilarum*. Similarly, lawns were not encountered in crosses involving either *K. bulgaricus* or *K. cicerisporus* and other lactose utilizing species such as *K. fragilis*, *K. lactis* and *K. marxianus*.

The production of pigment and the formation of lawns of microcolonies in the proximity of recombinant colonies were not invariably concomitant. For instance, the pigmented recombinant colonies derived from crosses involving *K. lactis* CBS 683 and *K. bulgaricus*, *K. cicerisporus* as well as *K. marxianus* CBS 6923 were not surrounded by such lawns.

Recombinant strains recovered and examined in this study frequently consisted of cells with dimensions appreciably larger than those observed in either parent. Some hybrid strains were characterized by an enhancement of zygote and ascospore formation as compared to the parental strains. This was particularly marked in crosses of *K. bulgaricus* x *K. fragilis*, *K. dobzhanskii* x *K. wikenii* and *K. marxianus* x *K. vanudenii*. This phenomenon was interpreted as an enhanced sexual vigour of hybrid strains.

Diversity in ascosporal shape and size observed in the recombinant progeny in this investigation (*cf.* Results 4.8) was previously reported by Wickerham & Burton (1956a, b) for hybrids recovered from crosses involving *K. marxianus* x *K. lactis* as well as *K. dobzhanskii* x *K. fragilis*. The presence of spheroidal and reniform ascospores in a single ascus, observed by Lindegren (1949) in an infertile hybrid obtained from crossing *K. fragilis* with *Sacch. cerevisiae*, was also encountered among some hybrid strains recovered in this investigation.

5.5 SEGREGATION OF MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE RECOMBINANT PROGENY

On replating, recombinant colonies isolated from minimal agar not infrequently produced two or sometimes three morphologically distinct types of colonies. Cloned recombinant colonies differed in their size and pigmentation as well as in their consistency and type of surface.

The physiological properties of cloned recombinant colonies with combined parental characteristics were as a rule stable. However, in some cases further segregation of physiological characteristics and the recovery of parental forms was observed. Crosses involving *K. phaseolosporus* x *K. bulgaricus*, *K. phaseolosporus* x *K. cicerisporus*, *K. phaseolosporus* x *K. fragilis*, *K. wickerhamii* x *K. dobzhanskii* and *K. wickerhamii* x

K. lactis CBS 683 and CBS 2359 constitute examples of such instability among recombinants.

The segregation of morphological and physiological properties of the recombinant progeny was not studied quantitatively, as

(i) the confirmed first filial generation (F_1) could not be recovered (see 5.7.2); and

(ii) such detailed genetic investigation falls beyond the scope of this taxonomic study.

During the present study it was also noted that prolonged storage of stock recombinant strains maintained as slant cultures, affected some of their fermentative properties. For instance, the recombinants obtained from crosses of *K. fragilis* x *K. dobzhanskii*, *K. marxianus* CBS 6923 x *K. dobzhanskii* and *K. marxianus* CBS 6923 x *K. thermotolerans* upon isolation possessed combined parental phenotypes reflected by their ability to ferment both lactose and maltose. The latter property was, however, absent after one year of storage on YM agar at 7 °C. The oxidative utilization of maltose in all three cases during this period nevertheless remained unchanged. The stability of the oxidative utilization of carbon compounds in the recombinant progeny unquestionably establishes this property as the most reliable criterion for the confirmation of interfertility.

5.6 CRITERION FOR INTERFERTILITY

On the basis of the results obtained in the present investigation and under the prevailing experimental conditions, two ascogenous yeast strains are considered to be interfertile when:

(i) they form recombinants at frequencies of 10^3 or higher/10^8 c.m.p. in at least two different parental combinations;

(ii) the recombinants possess combined parental phenotypes; and

(iii) the recombinant progeny is fertile and produces viable ascospores.

5.7 FERTILITY OF THE RECOMBINANT PROGENY

Fertility of the recombinant progeny implies that such progeny is fully capable of sexual reproduction expressed by the formation of viable ascospores and the capability of filial generations to hybridize.

5.7.1 Ascosporal viability

The formation of viable ascospores was confirmed in all the recovered recombinant strains (*cf.* Results 4.8). As becomes evident from Table 40 the viability of hybrid ascospores was subject to some variation and ranged from 4,0-49,2%. The low viability of ascospores recovered from the cross involving *K. marxianus* and *K. fragilis* (4,0%) is in agreement with the observation of Lindegren (1949), who found the viability of ascospores of *K. fragilis* to be exceptionally low. It should, however, be stressed that the low viability of hybrid ascospores, as reported in this investigation might be due to the harshness of the ultrasonic-paraffin method. This is evident from the fact that higher percentages of viable ascospores were obtained when hybrid ascospores were isolated by micromanipulation. The percentages of viable ascospores recovered

from hybrids of *K. marxianus* x *K. lactis* and *K. marxianus* x *K. thermotolerans*, based on the ultrasonic-paraffin technique, were 30,8% and 18,5%, respectively, while the percentages of viable ascospores isolated from the same hybrid strains by micromanipulation were 60% and 50%, respectively.

5.7.2 Fertility of the recombinant progeny

The recombinant colonies of *Kluyveromyces* - unlike the biotypes of *Sacch. cerevisiae* - sporulated directly and abundantly on the minimal recovery medium as well as on media considered suitable for the maintenance of the stable vegetative phase. In view of the rapidity of the sexual cycle and early release of ascospores in *Kluyveromyces*, these recombinant colonies developing on the minimal recovery agar plates in all probability consisted of a mixture of filial generations.

Due to the inability to recover authentic or confirmed representatives of the first filial (F_1) generation, confirmation of interfertility between recombinant progeny can be based on further hybridization experiments involving:

(i) auxotrophic mutant strain of a reference *Kluyveromyces* species and auxotrophic mutant of a hybrid strain, e.g. *K. lactis* x (*K. marxianus* x *K. thermotolerans*);

(ii) auxotrophic mutants of hybrid strains derived from different parental strains of the same species, e.g. (*K. marxianus* CBS 6923 x *K. lactis*

CBS 2359) x (*K. marxianus* CBS 6556 x *K. lactis* CBS 2359);

(iii) auxotrophic mutants of hybrid strains derived from various hybrid progeny, e.g. (*K. marxianus* x *K. bulgaricus*) x (*K. drosophilarum* x *K. vanudenii*).

Examples of such complex crosses between filial generations are given in Table 43.

Auxotrophic mutants of the hybrid strains were obtained by subjecting the hybrid progeny obtained from single ascospore isolates to the treatment with MNNG, as described in Methods (3.2.7). The recombinants in crosses listed in Table 43 were enumerated seven days after mating of respective parental strains.

In the first two crosses involving an auxotrophic mutant of *K. lactis* CBS 2359 and auxotrophic mutants of the hybrid obtained from crossing *K. marxianus* with *K. thermotolerans*, the recombinant frequencies were of the order of $10^3/10^8$ c.m.p. In the remaining four crosses involving auxotrophic mutants of hybrid strains recombinant frequencies were in the range of 10^2-$10^5/10^8$ c.m.p. Recombinant progeny sporulated abundantly.

On the basis of these results it can be concluded that the filial generations of the crosses cited in Table 43 are, in fact, capable of further hybridization. The recovery of sporulating progeny from the cross involving *K. marxianus* x *K. bulgaricus* and *K. drosophilarum* x *K. vanudenii*

provides a persuasive evidence that the mutual interfertility among these taxa is operative.

5.8 AREAS OF INTERFERTILITY DETECTED WITHIN THE GENUS *KLUYVEROMYCES*

The results obtained in this work clearly established that in the genus *Kluyveromyces*, hybridization is operative among a large group of taxa - a situation anticipated by Wickerham (1955) and Wickerham & Burton (1956a). For the sake of convenience these results will be discussed in terms of the presumed phylogenetic development as proposed by Wickerham & Burton (1956a, b) and van der Walt (1970a).

5.8.1 Interfertility among taxa forming reniform ascospores

The first basic line of presumed phylogenetic development comprises taxa forming crescentiform or reniform ascospores. The detection under the experimental conditions of interfertility between taxa of this group is illustrated in Fig. 2.

As becomes apparent from Fig. 2 interfertility was not detected within the so-called "primitive" group of taxa comprising *K. africanus*, *K. delphensis*, *K. lodderi*, *K. phaffii* and *K. polysporus*. Recombinants were not observed in 40 crosses involving all possible combinations of these taxa (*cf.* Tables 10, 14, 21, 26 and 28).

Interfertility was found to be operative among the physiologically more advanced organisms of this line. Two partially overlapping groups of mutually interfertile taxa

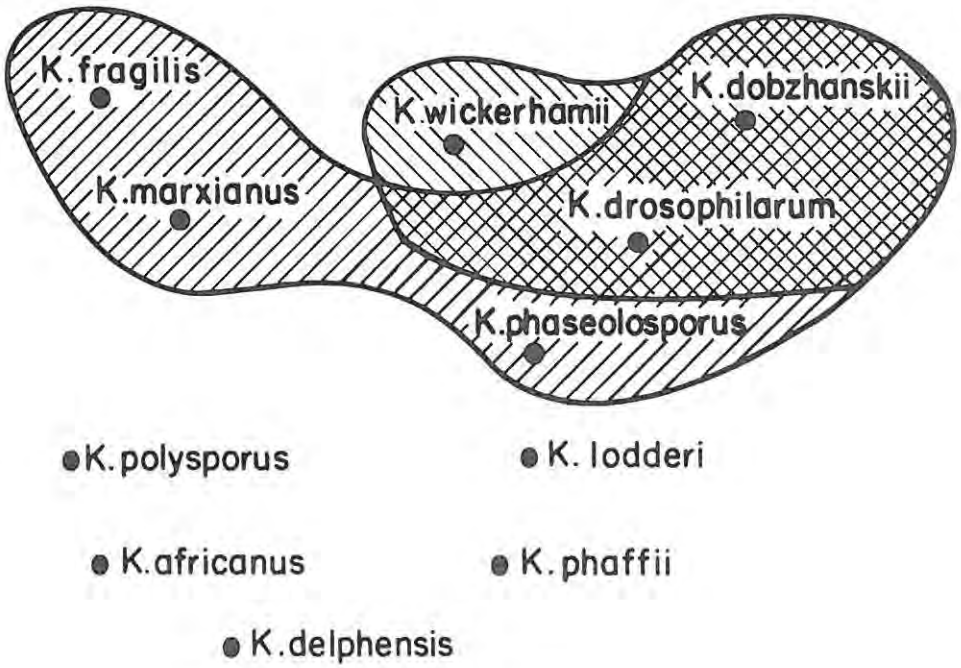

FIG. 2 TWO GROUPS OF RECOMBINING SPECIES WHICH FORM RENIFORM ASCOSPORES

were established. The first comprises *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis* and *K. marxianus* as well as *K. phaseolosporus* (*cf.* Tables 15, 16, 17, 22, 23 and 27). The second interbreeding group consists of *K. wickerhamii*, *K. dobzhanskii* and *K. drosophilarum* (*cf.* Table 32). It is surprising that the lactose utilizing *K. wickerhamii* was found to be genetically more closely related to *K. dobzhanskii* and *K. drosophilarum* (maltose utilizing taxa) than to *K. fragilis* and *K. marxianus* (taxa utilizing lactose). This is evident from the observation that interfertility was not detected in 46 crosses involving four strains of *K. wickerhamii*, three strains of *K. marxianus* and one strain of *K. fragilis* (*cf.* Tables 17, 22-25 and 32).

These two groups of interbreeding taxa may be regarded as constituting two syngameons. The term syngameon was introduced by Lotsy (1925, 1931) for naturally hybridizing taxa of higher plants.

5.8.2 Interfertility among taxa forming spheroidal ascospores

The second line of presumed phylogenetic development comprises taxa forming spheroidal to ellipsoidal ascospores. As becomes apparent from Fig. 3 a single group of interbreeding taxa was established within this basic line of development. This group comprises *K. bulgaricus*, *K. cicerisporus*, *K. lactis*, *K. vanudenii* and *K. wikenii*, the latter presumably constituting the least advanced element within this syngameon (*cf.* Tables 12, 13, 18, 19, 30 and 33).

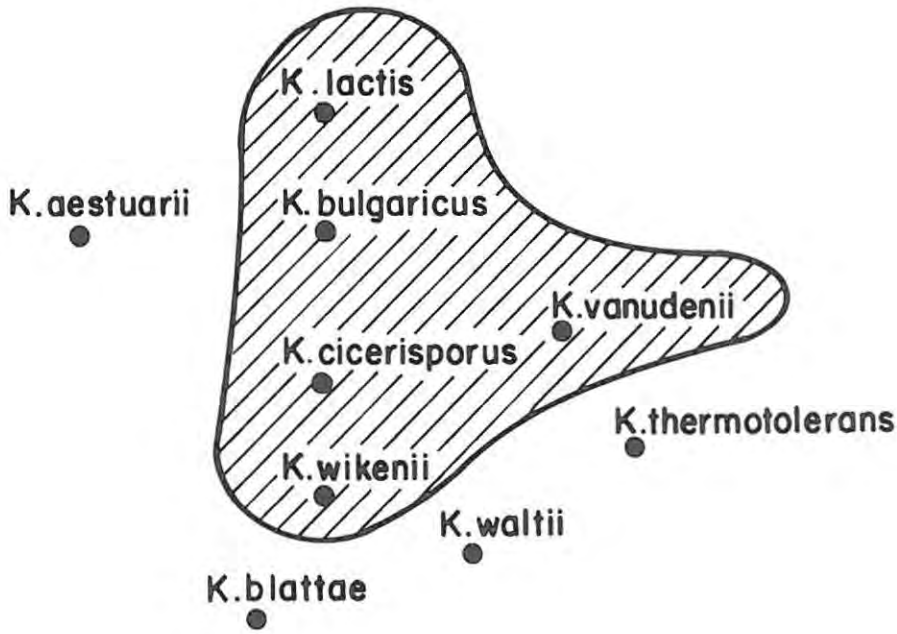

FIG. 3 GROUP OF RECOMBINING SPECIES WHICH FORM SPHEROIDAL ASCOSPORES

It should be noted that although *K. aestuarii* resembles *K. lactis* physiologically, it appears to be genetically distinct since no recombinants were detected in 13 crosses involving auxotrophic mutants of two strains of *K. aestuarii* and three strains of *K. lactis* (*cf.* Tables 8 and 9). Interfertility was also absent between two osmophilic taxa, namely *K. waltii* and *K. thermotolerans* (*cf.* Table 31). *K. blattae*, hitherto the most primitive species forming spheroidal ascospores, was not found to be interfertile with any of the remaining taxa belonging to this line of development (*cf.* Table 11).

5.8.3 Interfertility between taxa forming reniform ascospores and taxa forming spheroidal ascospores

A graphic illustration of interfertility detected between taxa presently assigned to the genus *Kluyveromyces* based on the results obtained in this investigation is provided in Fig. 4.

The full lines in Fig. 4 represent parental combinations in which recombination frequencies equal to or higher than $10^3/10^8$ c.m.p. were observed in at least two crosses. The broken lines represent crosses in which recombination frequencies lower than $10^3/10^8$ c.m.p. were encountered. Taxa on the right hand side of the diagram are characterized by the formation of spheroidal or ellipsoidal ascospores and those on the left hand side by the formation of reniform or crescentiform ascospores.

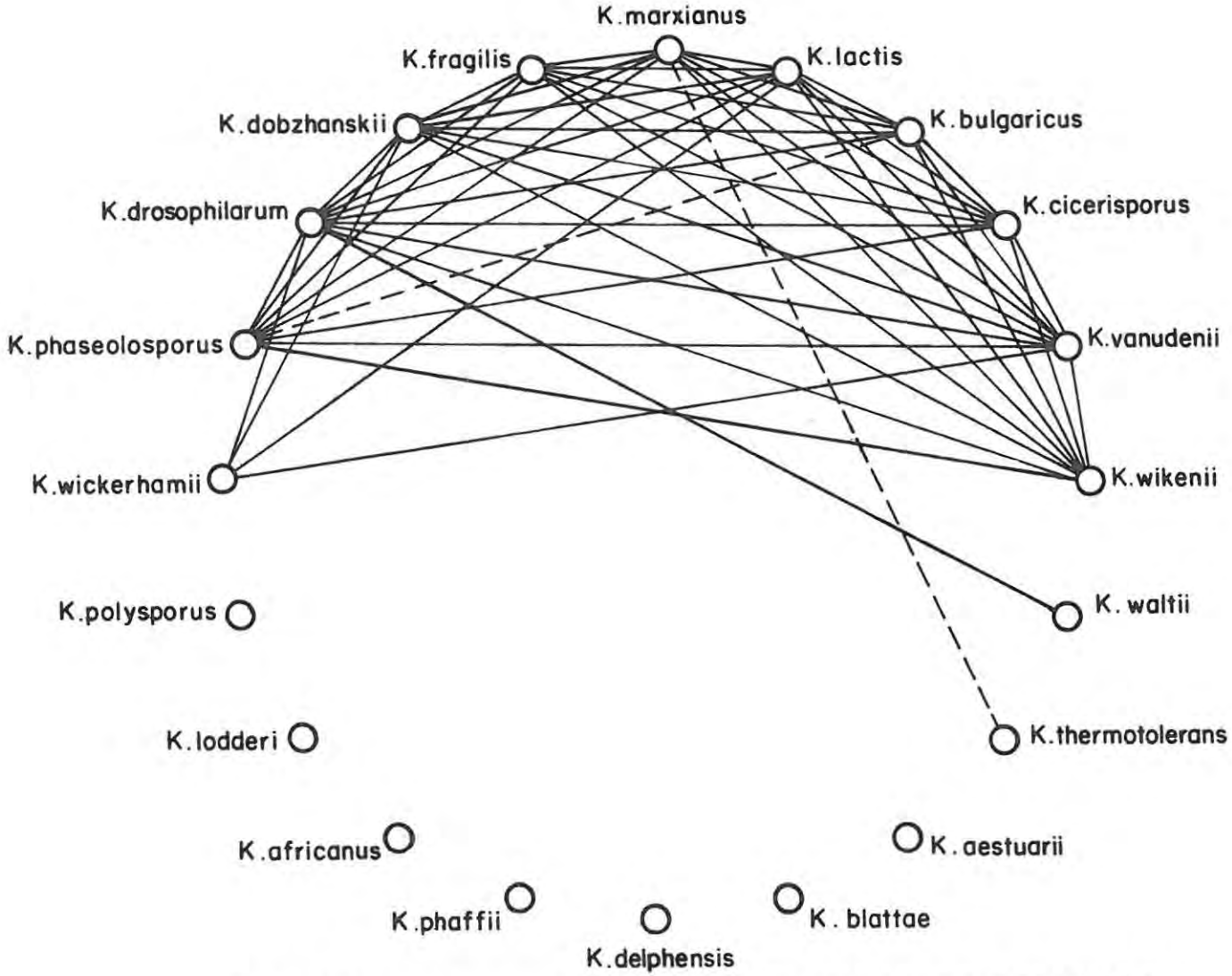

FIG.4 RECOMBINATION IN THE GENUS KLUYVEROMYCES

From this diagram it is apparent that recombination between taxa forming spheroidal ascospores and those forming reniform ascospores is easily achieved. Moreover, this recombination is most pronounced among the presumed more evolved taxa, capable of utilizing a large number of polyalcohols and di- and trisaccharides.

From Fig. 4 two large syngameons are detectable. The first comprises nine mutually interfertile taxa: *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. vanudenii* and *K. wikenii* (*cf.* Tables 12, 13, 15, 16, 17, 18, 19, 22, 23, 30 and 33). Since *K. phaseolosporus* was found to form recombinants with *K. bulgaricus* in only one cross and at a low frequency (*cf.* Table 27), it cannot be regarded as being fully interfertile with *K. bulgaricus*. *K. phaseolosporus* was, however, found to be interfertile with the remaining members of this group, including *K. cicerisporus*. It should be stressed that this syngameous group encompasses the redefined genus *Dekkeromyces*, as proposed by Santa Maria & Sanchez (1970).

Although *K. wickerhamii* failed to form recombinants with *K. bulgaricus*, *K. cicerisporus*, *K. fragilis*, *K. marxianus*, *K. phaseolosporus* and *K. wikenii* it formed a second syngameous group with *K. dobzhanskii*, *K. drosophilarum*, *K. lactis* and *K. vanudenii* (*cf.* Table 32).

Apart from these large interbreeding groups genetic

relatedness was detected between two additional pairs of taxa. These are *K. thermotolerans* and *K. marxianus* as well as *K. waltii* and *K. drosophilarum* (*cf.* Tables 29 and 31). As these respective pairs of taxa differ appreciably in their phenotypic characteristics recombinant formation between them was unexpected.

Another salient feature of the results presented in Fig. 4 is the fact that the physiologically similar "primitive" taxa such as *K. africanus*, *K. blattae*, *K. lodderi*, *K. phaffii* and *K. polysporus* failed to hybridize.

K. aestuarii appears to be unique in that although it represents an advanced element of the genus, it failed to hybridize with either taxa forming spheroidal ascospores or taxa forming reniform ascospores (*cf.* Tables 8 and 9). *K. aestuarii* emerges as genetically isolated and probably represents an evolutionary stage of the genus adapted to marine environments.

It should be emphasized that the failure to detect interfertility between parental pairs in fact implies that recombinants (if formed) in such crosses were not detected in 10^8 c.m.p./ml and provides no absolute assurance that the hybridized taxa are not in fact interfertile. The failure to detect recombinants could be due to

(i) true genetic incompatibility of the parental strains;

(ii) experimental conditions under which hybridization was carried out (*cf.* Introduction 1.7);

(iii) limitations of the prototrophic selection technique (*cf.* Discussion of the results 5.2).

The designation of the apparentaly genetically isolated taxa, based on the results of this study, should therefore not be regarded as final.

5.9 HYBRIDIZATION EXPERIMENTS INVOLVING AN UNDESCRIBED REPRESENTATIVE OF *KLUYVEROMYCES* AND SPECIES OF THE GENUS *KLUYVEROMYCES*

Hybridization experiments involving strain DSM 70885 (*cf.* Results 4.4, Table 34) constitute an example of how the relatedness between undescribed, phenotypically distinct representatives of the genus *Kluyveromyces* and taxa presently assigned to the genus could be established. Strain DSM 70885 was found to form recombinants with *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. marxianus* and *K. vanudenii*. In terms of the biological concept of the species and in accordance with the requirements of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972) strain DSM 70885 can be regarded as a variety of *K. marxianus* (see Taxonomic Implications, 7).

5.10 GENETIC RELATEDNESS BETWEEN *KLUYVEROMYCES* AND SOME OTHER YEAST GENERA

On the basis of their physiological characteristics a number of asporogenous strains was considered to constitute imperfect forms of some

Kluyveromyces species. The existence of interfertility was investigated between *K. lactis* and *C. kefyr*, *C. macedoniensis*, *C. pseudotropicalis* as well as *T. sphaerica* (*cf.* Table 35). Sporulating recombinants were recovered from one cross between *K. lactis* x *C. kefyr*, from four crosses between *K. lactis* x *C. macedoniensis* and from six crosses between *K. lactis* x *T. sphaerica*, which confirms the assumption that these so-called imperfect taxa are in fact non-sporulating representatives of *Kluyveromyces*.

On the assumption that *C. pseudotropicalis* is representative of an imperfect state of the genus *Kluyveromyces*, the failure to detect recombinants between this species and *K. lactis* might be due to:

(i) reduced sexual activity of *C. pseudotropicalis*, being a result of prolonged maintenance on artificial media;

(ii) *C. pseudotropicalis* representing a mutant with impairment at the *mat* locus;

(iii) *C. pseudotropicalis* representing a higher form of ploidy incapable of mating.

Recombinants were not detected in 65 crosses involving auxotrophic mutants of the three strains of *K. marxianus* and prototrophic strains of the representatives of some ascogenous genera, i.e. *Pichia etchellsii*, *Sacch. cerevisiae*, *Sacch. chevalieri*, *Sacch. kloeckerianus*, *Sacch. montanus*, *Torulaspora rosei* and *Zygosacch. rouxii* (*cf.* Tables 36-38). Recombinants were also absent in four crosses between physiologically similar *K. thermotolerans* and *Sacch. bayanus* (*cf.* Table 39).

The apparent inability of the above listed strains to hybridize with the representatives of the genus *Kluyveromyces* suggests their genetic distinctiveness and underlines the specificity of the prototrophic selection technique.

6. GENERAL DISCUSSION

6.1 INTRODUCTORY REMARKS

Earlier investigations on hybridization between *Kluyveromyces* species established the existence of interfertility between *K. lactis* and *K. marxianus*, *K. fragilis* and *K. dobzhanskii* (Wickerham & Burton, 1956a, b), *K. lactis* and *K. vanudenii* (van der Walt & Nel, 1963), as well as between *K. lactis* and *K. phaseolosporus* (van der Walt, 1970a). These results were confirmed in the present study (*cf.* Tables 17-19).

Contrary to Herman (1970a), who observed a positive, sex-specific growth response between *K. aestuarii* and *K. lactis*, no recombinants were recovered in 13 hybridization experiments involving five strains of these two species (*cf.* Tables 8 and 9). Likewise, recombination was not observed in crosses involving auxotrophic mutants of *K. aestuarii* and the prototrophic strain of *K. wikenii* (*cf.* Tables 8 and 9) although Fiol & Poncet (1971) and Fiol (1973) reported hybridization between these two taxa.

In retrospect, two main approaches can be observed among other investigators studying the relationship between taxa assigned to this taxonomic area. The first relies on phenotypic properties, which constitute the basis for speciation in terms of the concept of the taxonomic species. The second approach, being an attempt to break away from this concept of the species, is based on the molecular characteristics of the yeast genome, with particular reference to the nuclear DNA base composition and DNA-DNA homology.

The conclusions reached in the present study will be compared to those obtained by the investigators, who applied other criteria aimed at establishing relatedness between *Kluyveromyces* species and will be discussed in the following order:

numerical taxonomy (6.2);

antigenic properties (6.3);

proton magnetic resonance spectra of mannans and mannose-containing polysaccharides (6.4);

vitamin requirements and intracellular enzymes (6.5);

exo-β-glucanases (6.6);

coenzyme Q-system (6.7);

other criteria based on phenotypic properties (6.8);

DNA base composition determinations (6.9);

nuclear DNA-DNA homologies (or DNA-DNA renaturation) (6.10).

6.2 NUMERICAL TAXONOMY

Numerical taxonomy relies on computer-based assessments of similarities or differences in conventional morphological and physiological properties which reflect only a part of the yeast genome in the various taxa. It should be stressed that this procedure makes no reference to the presence or absence of interfertility between taxa. Conclusions based on high matching coefficients, without reference to sexual recognition between organisms, may be misleading as already pointed out by van der Walt

& Liebenberg (1973). Reservations regarding the use of numerical taxonomy for classification purposes were likewise expressed by Price *et al.* (1977).

Kocková-Kratochvilová *et al.* (1972) on the basis of the study of 43 strains representing *Kluyveromyces* species observed the heterogeneity of the genus. The authors tested each strain for 70 properties and analysed their results by the taxonomic distance and matching coefficient. Kocková-Kratochvilová *et al.* (1972) accepted the existence of 5 phenons within the genus and considered *K. thermotolerans* to constitute a separate and unrelated taxon. To the first phenon the authors assigned *K. africanus*, *K. delphensis*, *K. lodderi*, *K. phaffii* and *K. polysporus*; the second comprised *K. dobzhanskii*, *K. drosophilarum*, *K. phaseolosporus* and *K. wickerhamii*; in the third phenon the authors placed *K. lactis*; the fourth phenon included *K. marxianus* and the fifth *K. cicerisporus*, *K. fragilis* and *K. wikenii*. The first phenon was found to be uniform and sufficiently different from the remaining clusters to justify its separation and retention as a single genus. The authors supported Santa Maria & Sanchez's (1970) proposal of reintroducing the genus *Dekkeromyces* to accommodate the remaining four clusters into which other species of the genus were assigned.

Kocková-Kratochvilová *et al.* concluded that the presumed imperfect forms of some *Kluyveromyces* species, namely *C. pseudotropicalis* and *T. sphaerica* formed separate clusters, unrelated to the remaining *Kluyveromyces* species.

The experimental evidence obtained in the present work confirmed the distinctiveness of the so-called "primitive" species from other species of the genus, the close relatedness of the remaining species clustered in

phenons 2-5 (*cf.* Fig. 4) and the lack of relatedness between *K. lactis* and *C. pseudotropicalis* (*cf.* Table 35). In contrast to the opinion held by Kocková-Kratochvilová *et al.* (1972), *K. thermotolerans* was found to be related to *K. marxianus* (*cf.* Table 29), and *C. sphaerica* to *K. lactis* (*cf.* Table 35).

Campbell (1972), also on the basis of the numerical analysis of 58 characters, established the existence of three groups of species within the genus *Kluyveromyces* and stressed the relatedness of these three groups to the genus *Saccharomyces*. The first group, i.e. the *K. lactis* group consisted of 12 species, namely: *K. aestuarii*, *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudenii*, *K. wickerhamii* and *K. wikenii*; the second was represented by a single species, *K. thermotolerans* and the third group comprised the five "primitive" species, namely: *K. africanus*, *K. delphensis*, *K. lodderi*, *K. phaffii* and *K. polysporus*. It should be emphasized that the first group, with the exception of *K. aestuarii*, comprises all the species found in the present investigation to hybridize with *K. lactis* (*cf.* Tables 18 and 19).

Furthermore, Campbell (1972) proposed that the following *Kluyveromyces* species should be considered synonymous:

(i) *K. africanus* and *K. phaffii*;

(ii) *K. dobzhanskii* and *K. drosophilarum*;

(iii) *K. marxianus*, *K. cicerisporus* and *K. fragilis*;

(iv) *K. phaseolosporus*, *K. bulgaricus* and *K. wikenii*;

(v) *K. polysporus* and *K. lodderi*.

Although fertile recombinants were recovered from crosses involving species of the second, third and fourth groups (*cf.* Fig. 4), the existence of genetic relatedness was not confirmed between *K. africanus* and *K. phaffii*, as well as between *K. polysporus* and *K. lodderi* (*cf.* Tables 10 and 28, respectively).

The matching coefficient of 85%, observed by Campbell (1972), between *K. thermotolerans* and *Sacch. bayanus* was considered by him to constitute proof of high degree of relatedness between these species. However, no recombinants were detected in crosses involving auxotrophic mutants of *K. thermotolerans* and *Sacch. bayanus* (*cf.* Table 39), although the method employed, despite its limitations, was sensitive enough to detect a low recombinant frequency in crosses between *K. marxianus* and *K. thermotolerans*.

On the basis of a high matching coefficient (70%), Campbell (1972) proposed that the genera *Saccharomyces* and *Kluyveromyces* should be regarded as a single genus, *Saccharomyces*. In the present investigation no recombinants were observed in 33 crosses involving three strains of *K. marxianus* and four representative species of the genus *Saccharomyces* (*cf.* Tables 36-38).

Poncet (1973) applying a factor analysis method evaluated the affinities among species of the genus *Kluyveromyces*. Her conclusions, which resulted in the recognition of two basic groups within the genus, are basically supported by the experimental evidence obtained in this investigation. Poncet's first group, comprising the five "primitive" species, corresponds to the group within which interbreeding was not detected. To the second group Poncet assigned the following species: *K. aestuarii*,

K. bulgaricus, *K. cicerisporus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudenii*, *K. wickerhamii* and *K. wikenii*, stressing close relatedness between *K. wikenii* and *K. bulgaricus*; *K. fragilis*, *K. marxianus* and *K. cicerisporus*; *K. phaseolosporus* and *K. wickerhamii*; *K. lactis*, *K. vanudenii* and *K. aestuarii*, as well as between *K. drosophilarum* and *K. dobzhanskii*. On the basis of hybridization experiments, genetic relatedness between *K. phaseolosporus* and *K. wickerhamii* (*cf.* Table 27), *K. aestuarii* and *K. lactis*, as well as *K. aestuarii* and *K. vanudenii* (*cf.* Tables 8 and 9) was not established. Species of the second group, with the exclusion of *K. aestuarii* and *K. wickerhamii*, were assigned in the present work to the single species *K. marxianus* (see Taxonomic Implications, 7).

6.3 ANTIGENIC PROPERTIES

Antigenic structure of the yeast cell wall, similarly to the conventional morphological and physiological properties, is a phenotypic characteristic reflecting only a part of the yeast genome.

Tsuchiya *et al.* (1965), on the basis of agglutination reactions and cross-absorption experiments, analysed the antigenic structure of *K. fragilis*, *K. marxianus* and *K. thermotolerans* and compared it to the antigenic structure of *Saccharomyces* species. The authors observed that *K. marxianus* and *K. fragilis* were identical serologically and that the difference in the fermentation of lactose did not justify their separation into two independent species. The above observation of close relatedness between these two taxa was confirmed in the present investigation and the specific name of *K. fragilis* passed into synonymy with *K. marxianus* (see Taxonomic Implications, 7). The strains of *K. fragilis* and *K. marxianus* were agglutinated by unabsorbed anti-*Candida albicans* antiserum and by monospecific

antisera homologous to antigens 8, 10, 28, 31 and *a*. Tsuchiya *et al.* (1965) also reported that on the basis of the thermostable antigens *K. thermotolerans* was closely related to *Saccharomyces carlsbergensis* and *Saccharomyces elegans*.

In the light of the recombination observed between *K. marxianus* and *K. thermotolerans* in the present study, it is interesting to note that the analysis of the antigenic structure of *K. thermotolerans* and *K. marxianus* revealed that these species possess three identical antigens, namely 8, 10 and 28.

Campbell (1968, 1971), on the basis of the serological properties, divided the *Saccharomyces* species into four antigenic groups. Three species currently assigned to the genus *Kluyveromyces* were included in his study. *K. thermotolerans* was assigned to the serological group A together with *Sacch. cerevisiae*, *Sacch. carlsbergensis*, *Saccharomyces acidifaciens* and *Saccharomyces rouxii*.

K. fragilis and *K. marxianus* were considered by Campbell (1971) to be antigenically indistinguishable, and were placed in the serological group B together with *Sacch. cerevisiae* and *Sacch. chevalieri*. On the basis of physiological and morphological properties, as well as on the basis of the results obtained in the present investigation, *K. fragilis* and *K. marxianus* were concluded to be synonymous. The relatedness of *K. marxianus* to *Sacch. cerevisiae* and *Sacch. chevalieri* was, however, not confirmed in the hybridization experiments (*cf.* Tables 36-38).

In his subsequent studies Campbell (1972) investigated antigenic properties of 72 strains representing 18 *Kluyveromyces* species, namely *K. aestuarii*, *K. africanus*, *K. bulgaricus*, *K. cicerisporus*, *K. delphensis*,

K. dobzhanskii, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. lodderi*, *K. marxianus*, *K. phaffii*, *K. phaseolosporus*, *K. polysporus*, *K. thermotolerans*, *K. vanudenii*, *K. wickerhamii* and *K. wikenii*. The same four antigens, A, B, C and D, were found to be present in the species of the genus *Kluyveromyces* and in the genus *Saccharomyces*. In the present work, genetic relatedness expressed as the ability to hybridize, was not detected between the three strains of *K. marxianus* and seven strains representing the genus *Saccharomyces* (*cf.* Tables 36-38).

Immunochemical studies of yeast mannans carried out by Šandula *et al.* (1974) established the existence of close relatedness among some *Kluyveromyces* species as well as the existence of some affinity between *Kluyveromyces* and representative species of the genus *Saccharomyces*. The authors concluded that the wall mannans of *K. fragilis*, *K. marxianus* and *K. lactis* possess identical immunodeterminant groups. This indication of relatedness was fully supported by the results obtained in the present investigation. Šandula *et al.* (1974) have, furthermore, noticed an antigenic relationship between *K. thermotolerans* and *K. fragilis*. A low level of recombination was observed in the present work between *K. marxianus* and *K. thermotolerans*.

Lack of antigenic closeness between *K. fragilis* and *K. polysporus* was confirmed by the negative results of the hybridization experiments.

The readily recovered recombinants from crosses involving *K. fragilis* and *K. drosophilarum* contradict the absence of antigenic relatedness between these two species reported by Šandula *et al.* (1974). Furthermore, these authors stated that *Sacch. chevalieri* possessed a mannan identical to that of *K. fragilis* and *K. marxianus*. Genetic relatedness was, however,

not confirmed in crosses involving *K. marxianus* and *Sacch. chevalieri* (*cf.* Tables 36-38). Likewise, the antigenic closeness between *Sacch. cerevisiae* and *K. marxianus* was not confirmed in the 14 crosses involving three strains of *K. marxianus* and four strains of *Sacch. cerevisiae* (*cf.* Tables 36-38).

As pointed out by Šandula *et al.* (1974) a yeast species can comprise strains differing in their antigenic structure. Segal *et al.* (1975) emphasized the fact that "conclusions regarding antigenic relationships, as considered by specificity of immunoglobulin-class reaction might be different if based on data resulting from intensive or non-intensive immunization".

In view of the reservations expressed by these authors and in view of the discrepancies observed between the conclusions based on the hybridization studies and on the antigenic structure of the yeast cell wall, caution should be exercised in applying the latter criterion for taxonomic purposes.

6.4 PROTON MAGNETIC RESONANCE SPECTRA OF MANNANS AND MANNOSE-CONTAINING POLYSACCHARIDES

Spencer & Gorin (1969) examined the proton magnetic resonance spectra of mannans and mannose-containing polysaccharides as an aid in yeast classification. On the basis of their results the authors divided the representatives of seven yeast genera into thirteen groups. Representatives of the genus *Kluyveromyces* were assigned to seven different groups in the following manner:

(i) *K. delphensis* was placed in group II together with *Sacch. cerevisiae*, *Saccharomyces diastaticus*,

Saccharomyces inusitatus and *Saccharomyces mellis;*

(ii) *K. thermotolerans* and *K. wickerhamii* were assigned to group III together with *Sacch. carlsbergensis, Sacch. bayanus, Saccharomyces carbajali, Saccharomyces fermentati, Saccharomyces florentinus, Saccharomyces fructuum, Saccharomyces heterogenicus, Saccharomyces italicus, Saccharomyces logos, Saccharomyces oviformis, Saccharomyces steineri, Saccharomyces uvarum* and *Saccharomyces willianus;*

(iii) *K. fragilis* and *K. marxianus* were placed in group IV together with *Sacch. chevalieri;*

(iv) *K. aestuarii* and *K. dobzhanskii* were located in group VI together with *Saccharomyces exiguus, Saccharomyces kluyveri* and *Nematospora coryli;*

(v) *K. dobzhanskii* and *K. lactis* constituted group VII;

(vi) *K. phaseolosporus* was placed singly in group IX. Its spectrum contained signals observed in *Schizosaccharomyces malidevorans, Schiz. octosporus, Schiz. pombe, Schizosaccharomyces versatilis* as well as in *Endomycopsis bispora* and *Hansenula canadensis;*

(vii) *K. lodderi* was located in subgroup Xa together with *Saccharomyces dairensis, Saccharomyces delbrueckii* and *Saccharomyces microellipsoides*. The spectrum of *K. lodderi* was found to be similar to the spectra of *Hanseniaspora osmophila, Brettanomyces anomalus*

and *Brettanomyces dublinensis*. *K. polysporus* and *K. africanus* were assigned to subgroup Xc together with *T. sphaerica*.

On the basis of these results it can be concluded that the proton magnetic resonance spectra of polysaccharides of representatives of the *Kluyveromyces* species possess properties observed in the representatives of other yeast genera.

The mutually interfertile group comprising *K. marxianus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis* and *K. phaseolosporus* was spread over four groups. The proposed relatedness between *K. marxianus* and *Sacch. chevalieri* as well as between *K. thermotolerans* and *Sacch. bayanus* was not confirmed in the present investigation (*cf.* Tables 36-38 and Table 39, respectively).

Relatedness between taxa, based on proton magnetic resonance spectra of polysaccharides, does not correlate with the relatedness determined by sexual recognition between taxa. The lack of correlation between proton-magnetic resonance spectra of mannans and mannose-containing polysaccharides and the ability of yeast taxa to hybridize, is not unexpected since the first criterion reflects only some properties of the outer component of the yeast cell wall, while the second manifests the relatedness between yeast taxa expressed as their ability to interbreed.

6.5 VITAMIN REQUIREMENTS AND INTRACELLULAR ENZYMES

Fiol (1967), on the basis of vitamin requirements and the ability to assimilate cellobiose, divided species of the genus *Kluyveromyces* into three groups. The first included two multispored species, i.e. *K.*

africanus and *K. polysporus*, which require various vitamins and which do not assimilate cellobiose. The second group consisted of *K. delphensis*, *K. lodderi* and *K. phaffii*, species requiring biotin and unable to utilize cellobiose.

The third group comprised nine species of the genus, namely *K. aestuarii*, *K. drosophilarum*, *K. dobzhanskii*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudenii* and *K. wickerhamii*, requiring biotin and assimilating cellobiose. In the present investigation recombinants were not observed among the species assigned to the first two groups, while the species assigned to the third group with the exception of *K. aestuarii* were characterized by the ability to hybridize.

Fiol (1972, 1973) extended his studies on the genus *Kluyveromyces* to include the intracellular enzyme systems. He proposed the recognition of two groups within the genus of which the first comprises five "primitive" species and the second, with the exclusion of *K. aestuarii* corresponds to taxa between which hybridization was detected in the present investigation. *K. thermotolerans* was not included in any of these groups. Close affinity observed by Fiol (1973) between *K. fragilis* and *K. lactis*, *K. bulgaricus*, *K. wikenii*, *K. lactis* and *K. vanudenii* as well as between *K. drosophilarum* and *K. dobzhanskii*, was confirmed in the hybridization experiments. Since no recombinants were detected in 44 crosses involving three strains of *K. marxianus* and four strains of *K. wickerhamii* (*cf.* Tables 22-25) it must be concluded that, contrary to Fiol's (1973) opinion, the differences between these two species are more fundamental and are not restricted to the utilization of raffinose.

Fleming & Duerksen (1967) studying β-glucosidases from *K. dobzhanskii* and *K. fragilis* as well as from the hybrid formed by these two yeasts,

concluded that the structural genes for β-glucosidase production, derived from both parents, were present in the hybrid strain. The authors observed that the optimal pH for substrate hydrolysis by the hybrid enzyme fell between the pH values for the enzymes obtained from parental strains and that hybrid β-glucosidase possessed other intermediate characteristics.

In the present investigation *K. dobzhanskii* and *K. fragilis* were observed to form fertile recombinants, which correlates with the observation of Fleming & Duercksen (1967).

6.6 EXO-β-GLUCANASES

Lachance & Phaff (1977) and Phaff *et al.* (1978) evaluated the phylogenetic significance of the yeast enzyme exo-β-glucanase, as applied to the genus *Kluyveromyces*. The authors concluded that on the basis of this criterion they could not support the division of the genus into two developmental lineages based on the shape of ascospores. The glucanase of *K. fragilis* was found to be identical to that of *K. marxianus* and closely related to glucanases derived from three species forming spheroidal ascospores, namely *K. bulgaricus*, *K. cicerisporus* and *K. wikenii*. The relatedness between these species was fully confirmed in the present work on the basis of the existence of interfertility between these taxa. Lachance & Phaff (1977) have also observed differences in the amino acid sequence in the enzyme obtained from *K. fragilis* and other species of the genus.

6.7 COENZYME Q-SYSTEM

On the basis of the results obtained in the study of the coenzyme Q-system (ubiquinone) in 80 *Saccharomyces* and ten *Kluyveromyces* strains, Yamada *et al.* (1976) concluded that the representatives of these two genera,

including *K. thermotolerans*, were characterized without exception by the presence of the coenzyme Q-6 system. The presence of the same coenzyme system was suggestive, in the opinion of the authors, of a certain degree of homogeneity between these genera and might indicate the occurrence of some phylogenetic relationship among the species of *Saccharomyces* and *Kluyveromyces*. However, the presence of the same Co-Q-6 system was observed in some representatives of the genera *Debaryomyces*, *Kloeckera*, *Hanseniaspora* and *Saccharomycodes*. Considering the diversity in morphological and physiological characteristics of these genera, it can be concluded that the presence of the same Co-Q-6 system does not necessarily reflect genuine genetic or phylogenetic relatedness.

6.8 OTHER CRITERIA BASED ON PHENOTYPIC PROPERTIES

The remaining criteria based on phenotypic properties of yeast cells, i.e. spectral requirements for photoreactivation of UV-irradiated cells (Sarachek & Bish, 1975); enzymatic hydrolysis of yeast cell walls (Bastide *et al.*, 1975) and the production of ribonucleases (Burt & Cazin, 1976) were applied only to single representatives of the genus *Kluyveromyces*. On the basis of the limited information the evaluation of the suitability of these criteria for taxonomic purposes is difficult.

6.9 DNA BASE COMPOSITION DETERMINATIONS

DNA base composition determinations were introduced as a criterion aiding systematics of fungi by Belozersky & Spirin (1960), Uryson & Belozersky (1960) and Vanyushin *et al.* (1960). Meyer & Phaff (1969) introduced this criterion for the classification of yeasts. Mean molar percentage of guanine plus cytosine (mol % G+C) was regarded by Stanier (1971) as the roughest genetic measure indicating possible evolutionary

relationships between organisms. Closely related organisms may be anticipated to have similar base compositions. Nevertheless, mol % G+C does not provide information on the base sequence of the nuclear DNA, which determines genetic identity of organisms. Consequently, yeast strains with similar or identical mol % G+C might be incapable of sexual recognition and could constitute different biological species. Acceptable variations in the mol % G+C values corresponding to the separation of fungal taxa at species and genus level, were estimated by Storck & Alexopoulos (1970). These authors defined "a fungal species as a DNA population with a standard deviation of less than 2% GC" while a fungal genus according to them "is characterized by a standard deviation value of less than 10% and on the average close to 3% GC". As observed by Poncet & Fiol (1972), the standard deviation value of mol % G+C (2,87) in the genus *Kluyveromyces* is smaller than within other genera, e.g. *Hansenula* (4,43), *Saccharomyces* (4,75), *Candida* (7,60), *Cryptococcus* (3,69), *Rhodotorula* (7,36) and *Torulopsis* (6,57).

Meyer & Phaff (1970), reviewing the DNA base composition of various yeast genera, noted that the majority of *Kluyveromyces* species possessed mol % G+C in a close range (*cf.* Table 44) except for *K. polysporus* for which this value appeared to be considerably lower. Nakase & Komagata (1971) on the basis of mol % G+C studies subscribed to the emendation of the genus *Kluyveromyces* (van der Walt, 1965), resulting in the exclusion of reniform-spored species from the genus *Saccharomyces*. Similarly, Poncet & Fiol (1972) favoured the retention of the emended genus *Kluyveromyces* as a single taxon, finding the intrageneric variation in the mol % G+C, namely 33,6-44,5% too small to justify the demarcation of two genera. Martini *et al.* (1972), on the basis of the mol % G+C content, could not

separate *K. polysporus* and *K. africanus* from the four-spored species of the genus and, consequently, did not support the division of the genus *Kluyveromyces* proposed by Santa Maria & Sanchez (1970). With the exception of *K. thermotolerans* these authors found the emended genus *Kluyveromyces* to be relatively homogenous in respect of mol % G+C content, with values ranging from 35,3-43,4%.

The application of nuclear DNA base composition analyses for speciation purposes, is restricted by the fact that the values reported by various authors differ appreciably. This is evident from Table 44 in which the results of the four groups of workers are presented. These differences might be accounted for by the various degrees of purity of the analysed DNA and by the different techniques employed for the determination of mean mol % G+C (Price *et al.*, 1978).

Analysing the DNA base composition of some *Kluyveromyces* species, Meyer & Phaff (1970) stressed the closeness in mol % G+C for *K. fragilis* and *K. marxianus* and their respective imperfect forms *C. pseudotropicalis* and *C. macedoniensis*. In the present work, *K. fragilis* and *K. marxianus* were found to be interfertile and the two taxa are considered to be synonymous (see Taxonomic Implications, 7).

On the basis of the DNA base composition, Nakase & Komagata (1971) considered the group comprising the following species: *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. marxianus*, *K. lactis* and *K. wickerhamii* to be homogenous, with the mol % G+C ranging from 39,3-42%. In the present investigation these taxa were found to be mutually interbreeding (*cf.* Fig. 4) except for *K. marxianus* and *K. wickerhamii* as well as *K. fragilis* and *K. wickerhamii*.

Poncet & Fiol (1972) on the basis of the mean molar percentage G+C distinguished two groups in the genus *Kluyveromyces*. The first one correlated with mol % G+C between 33-37% and comprised the so-called "primitive" species, namely: *K. africanus*, *K. delphensis*, *K. lodderi*, *K. phaffii* and *K. polysporus*, while the second group with mol % G+C values ranging from 38-45% included the remaining species of the genus except for *K. aestuarii*, *K. bulgaricus* and *K. wikenii*. Hybridization experiments confirmed the sexual distinctiveness of the first group and of *K. aestuarii* (*cf.* Fig. 4). The existence of interfertility was established among the species assigned by Poncet & Fiol (1972) to the second group with the inclusion of *K. bulgaricus* and *K. wikenii*.

Martini *et al.* (1972) divided the genus *Kluyveromyces* with regard to mol % G+C values into two groups, along the same lines as those proposed by Poncet & Fiol (1972). The first group comprised the "primitive" species with the exception of *K. delphensis*. The second group, with the exclusion of *K. aestuarii* and *K. delphensis*, corresponds to the group of species among which interbreeding was detected in the present work (*cf.* Fig. 4). Martini *et al.* (1972) pointed out that on the basis of morphological and physiological similarities and the closeness of mol % G+C values, *K. cicerisporus* and *K. fragilis* should become varieties of *K. bulgaricus* and *K. marxianus*, respectively, a proposal fully supported by the results of the present investigations (see Taxonomic Implications, 7).

On the basis of the values reported in 1978 by Phaff (personal communication), the mol % G+C of the DNA base composition of *K. marxianus*, as delimited in this study, must be accepted to fall within the range of 39,9-42,6%, showing a variation of 2,7%. In view of this accepted variation, differences in mol % G+C of this order cannot, on their own and

without reference to interfertility, be admitted as criterion for speciation in *Kluyveromyces*.

As *K. thermotolerans* and *K. marxianus* are reported to differ in their mol % G+C by some 5%, the recovery of recombinants with combined parental phenotypes from crosses involving these two species is unexpected.

6.10 NUCLEAR DNA-DNA HOMOLOGIES (OR DNA-DNA RENATURATION)

Polynucleotide sequence relatedness constitutes a more advanced technique in molecular studies aimed at the elucidation of affinities between yeast taxa. Studies of DNA base sequence relatedness are in the opinion of Price *et al.* (1977) "most useful for the delineation of natural yeast species". The results obtained on the basis of this technique relate to the nuclear DNA base sequence but they do not provide information whether the homologous bases represent actively transcribing genes, constituting only a limited portion of the DNA (Kolata, 1975), or whether the homologous bases "consist of inactive genes, regions of DNA used for the control of gene expression, and regions used for chromosome organization" (Kolata, 1975).

Bicknell & Douglas (1970) were the first authors to investigate nuclear DNA-DNA homologies among *Kluyveromyces* species. A high degree of relatedness between *K. fragilis* and *K. marxianus*, corresponding to 93% DNA-DNA homology, was confirmed in the present work by the recovery of recombinants from crosses involving auxotrophic mutants of these two taxa (*cf.* Table 17). Bicknell & Douglas (1970) found zero per cent homology between the DNA of *Sacch. cerevisiae* and the DNA of the representative *Kluyveromyces* species, which tallies with the negative results obtained in crosses involving *K. marxianus* and *Sacch. cerevisiae* (*cf.* Tables 36-38)

and is also in agreement with the results reported by Groot *et al.* (1975) based on nuclear DNA-DNA homology.

Bicknell & Douglas (1970) included in their studies two hybrids obtained from Dr L.J. Wickerham, namely *K. fragilis* x *K. lactis* and *K. fragilis* x *K. dobzhanskii*. Both hybrids showed high degrees of homology with *K. fragilis* (87 and 98%, respectively) and variable degrees of homology with *K. lactis* (85 and 33%, respectively). These authors concluded that the hybrid *K. fragilis* x *K. dobzhanskii* contained the genomes of both parents.

Low degrees of homology were reported by Bicknell & Douglas (1970) between *K. dobzhanskii* and *K. lactis* (30%); *K. dobzhanskii* and *K. fragilis* (27%), as well as *K. lactis* and *K. marxianus* (9%), although in these three cases fertile recombinants were recovered from all crosses involving respective parental strains.

Martini (1973) and Martini & Phaff (1973), in their study on DNA-DNA renaturation, carried out between *Kluyveromyces* strains, accepted degrees of DNA-DNA complementarity of 80-100% as positive homology, indicative of close genetic relatedness and of 0-20% as negative homology, indicative of the absence of relatedness between taxa. Degrees of re-association of 0-30% were considered to indicate nearly complete absence of relatedness. The synonymy of *K. cicerisporus* and *K. bulgaricus*; *K. fragilis* and *K. marxianus*; *K. vanudenii* and *K. lactis*; *K. wikenii* and *K. bulgaricus*, based on DNA-DNA renaturation, reported by Martini (1973), was confirmed in the present work (see Taxonomic Implications, 7). Similarly, results of the hybridization experiments reported in this study, support the conclusion of Phaff *et al.* (1978) that *K. cicerisporus*, *K. bulgaricus*,

K. fragilis, *K. marxianus* and *K. wikenii* should be regarded as synonymous species and that *K. drosophilarum*, *K. lactis*, *K. phaseolosporus* and *K. vanudenii* constitute a group of closely related taxa.

The results obtained in the present work support the observation of Price *et al.* (1977) that the strains between which homology in nuclear base sequence corresponds to 70-100%, can be considered to constitute a single species.

Comparing the conclusions concerning genetic relatedness between various *Kluyveromyces* strains based on DNA-DNA renaturation and on hybridization experiments reported in this study, the following can be observed:

(i) recombinant frequencies of 10^3 or higher/10^8 cells of the mated population (c.m.p.) coupled with the recovery of fertile hybrid progeny correlated, without exception, with degrees of DNA-DNA renaturation equal to or higher than 70% (see Table 45);

(ii) failure to detect recombinants correlated well with degrees of DNA-DNA renaturation below 36% (see Table 46);

(iii) however, it should be stressed that degrees of DNA-DNA renaturation lower than 36% did not invariably preclude the recovery of fertile recombinants with combined parental phenotypes at frequencies appreciably higher than $10^3/10^8$ c.m.p. Twelve such discrepancies, listed in Table 47 can be detected between the results submitted by

Martini (1973) and those obtained in this study.

These discrepancies in genetic relatedness as determined by DNA-DNA renaturation on the one hand and the ability of strains to hybridize on the other, are difficult to explain. It is nevertheless apparent that interbreeding between taxa implies genetic relatedness in terms of the entire, organized yeast genome. In terms of DNA-DNA complementarity, relatedness is reflected by high degrees of *in vitro* renaturation of fragments of denatured DNA (van der Walt & Johannsen, 1978), without taking into account the distributions of actively transcribing genes (Kolata, 1975).

7. TAXONOMIC IMPLICATIONS

Acceptance of the concept of the biological species establishes interfertility between yeast strains as the cardinal criterion for speciation. Consequently, the interfertility observed between certain members of the genus *Kluyveromyces* requires a resetting of the limits of these interbreeding taxa which leads to the restructuring of the genus.

As *K. bulgaricus*, *K. cicerisporus*, *K. dobzhanskii*, *K. drosophilarum*, *K. fragilis*, *K. lactis*, *K. marxianus*, *K. phaseolosporus*, *K. vanudenii* and *K. wikenii* are mutually interfertile and since there is no substantial evidence of their geographical or ecological isolation, these ten taxa must be accepted as representative of a single species (Johannsen & van der Walt, 1978). In uniting these taxa in terms of Art. 57 of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972), the oldest legitimate epithet, i.e. *marxianus*, introduced by Hansen (1888), has to be retained.

These syngameous taxa are, nevertheless, easily differentiated on the basis of their morphological and physiological properties, which appear to be constant and not subject to instability observed among biotypes of *Sacch. cerevisiae* (Scheda, 1966; Scheda & Yarrow, 1966, 1968; Windisch & Neumann, 1972; Yarrow & Nakase, 1975). As phenotypic characteristics are very useful for the construction of routinely employed keys for classification of yeast strains and since well-established specific names undoubtedly convey a certain amount of information, it is proposed that the most characteristic of these taxa should be, in terms of Art. 60 of the International Code of Botanical Nomenclature (Stafleu *et al.*, 1972), relegated to the status

of seven varieties of *K. marxianus* as follows:

Kluyveromyces marxianus (Hansen) van der Walt (1971)

1. *Kluyveromyces marxianus* (Hansen) van der Walt var. *marxianus*.
   Basionym: *Saccharomyces marxianus* Hansen *in* Medd. Carlsberg Lab. 2 : 220. 1888.
   Synonym: *Kluyveromyces fragilis* (Jörgensen) van der Walt (1971).

2. *Kluyveromyces marxianus* (Hansen) van der Walt var. *bulgaricus* (Santa Maria) Johannsen et van der Walt *comb. nov.*
   Basionym: *Saccharomyces fragilis* (Jörgensen) var. *bulgaricus* Santa Maria *in* Anales Inst. Nac. Invest. Agric. 8 : 165. 1956.
   Synonyms: *Kluyveromyces bulgaricus* (Santa Maria) van der Walt (1971); *Kluyveromyces cicerisporus* van der Walt, Nel et van Kerken 1966.

3. *Kluyveromyces marxianus* (Hansen) van der Walt var. *dobzhanskii* (Shehata, Mrak et Phaff) Johannsen et van der Walt *stat. nov., comb. nov.*
   Basionym: *Saccharomyces dobzhanskii* Shehata, Mrak et Phaff *in* Mycologia 47 : 805. 1955.
   Synonym: *Kluyveromyces dobzhanskii* (Shehata, Mrak et Phaff) van der Walt (1971).

4. *Kluyveromyces marxianus* (Hansen) van der Walt var. *drosophilarum* (Shehata, Mrak et Phaff) Johannsen et van der Walt *stat. nov., comb. nov.*
   Basionym: *Saccharomyces drosophilarum* Shehata, Mrak et Phaff *in* Mycologia 47 : 804. 1955.
   Synonyms: *Kluyveromyces drosophilarum* (Shehata, Mrak et Phaff) van

der Walt (1971); *Kluyveromyces phaseolosporus* (Shehata, Mrak et Phaff) van der Walt (1971).

5. *Kluyveromyces marxianus* (Hansen) van der Walt var. *lactis* (Dombrowski) Johannsen et van der Walt *stat. nov., comb. nov.*
   Basionym: *Saccharomyces lactis* Dombrowski *in* Zbl. Bakt. Abt II 28 : 366. 1910.
   Synonym: *Kluyveromyces lactis* (Dombrowski) van der Walt (1971).

6. *Kluyveromyces marxianus* (Hansen) van der Walt var. *vanudenii* (van der Walt et Nel) Johannsen et van der Walt *stat. nov., comb. nov.*
   Basionym: *Saccharomyces vanudenii* van der Walt et Nel *in* Mycopathol. Mycol. Appl. 20 : 73. 1963.
   Synonym: *Kluyveromyces vanudenii* (van der Walt et Nel) van der Walt (1971).

7. *Kluyveromyces marxianus* (Hansen) van der Walt var. *wikenii* (van der Walt, Nel et van Kerken) Johannsen et van der Walt *stat. nov., comb. nov.*
   Basionym: *Kluyveromyces wikenii* van der Walt, Nel et van Kerken *in* Antonie van Leeuwenhoek J. Microbiol. Serol. 32 : 393. 1966.

On the basis of the results obtained *K. thermotolerans* appears to show some degree of genetic relatedness to *K. marxianus*. In view of the low recombination frequencies observed in the crosses involving *K. thermotolerans* and *K. marxianus* and the paucity of fertile hybrids with combined parental phenotypes, *K. thermotolerans* is retained as a separate species.

The observed interfertility between *K. waltii* and *K. marxianus* var. *drosophilarum* as well as between *K. wickerhamii* and *K. marxianus* var.

dobzhanskii, *K. marxianus* var. *drosophilarum*, *K. marxianus* var. *lactis* and *K. marxianus* var. *vanudenii* is also interpreted as an expression of limited degrees of relatedness within the genus. As the creation of subvarietal taxa is not considered to be a useful contribution to the taxonomy of the genus *Kluyveromyces*, it is proposed to retain *K. waltii* and *K. wickerhamii* as separate species.

The fact that recombinants were not detected in any crosses involving *K. aestuarii*, *K. africanus*, *K. blattae*, *K. delphensis*, *K. lodderi*, *K. phaffii* and *K. polysporus*, suggests that these taxa constitute sexually isolated and genetically distinct species. In these cases the biological and taxonomic species are synonymous.

On the basis of the results of this study the genus is defined as:

Kluyveromyces van der Walt emend. van der Walt 1965

Vegetative cells spheroidal, ovoid, ellipsoidal, cylindrical to elongate, reproducing by budding.

Asci conjugated or unconjugated, evanescent, uni- to multispored.

Ascospores crescentiform, reniform, oblong with obtuse ends, ellipsoidal or spheroidal, tending to agglutinate.

Fermentation.

Nitrate not utilized as sole source of nitrogen.

Coenzyme Q-6 system present.

External vitamin source required.

Type species of the genus

Kluyveromyces polysporus van der Walt.

Species and varieties accepted in the genus

1. *Kluyveromyces aestuarii* (Fell) van der Walt 1961

2. *Kluyveromyces africanus* van der Walt 1956b

3. *Kluyveromyces blattae* Henninger et Windisch 1976

4. *Kluyveromyces delphensis* (van der Walt et Tscheuschner) van der Walt 1956

5. *Kluyveromyces lodderi* (van der Walt et Tscheuschner) van der Walt 1957

6-12. *Kluyveromyces marxianus* (Hansen) van der Walt 1888

6. *Kluyveromyces marxianus* (Hansen) van der Walt var. *marxianus*

7. *Kluyveromyces marxianus* (Hansen) van der Walt var. *bulgaricus* (Santa Maria) Johannsen et van der Walt 1956

8. *Kluyveromyces marxianus* (Hansen) van der Walt var. *dobzhanskii* (Shehata, Mrak et Phaff) Johannsen et van der Walt 1955

9. *Kluyveromyces marxianus* (Hansen) van der Walt var. *drosophilarum* (Shehata, Mrak et Phaff) Johannsen et van der Walt 1955

10. *Kluyveromyces marxianus* (Hansen) van der Walt var. *lactis* (Dombrowski) Johannsen et van der Walt 1910

11. *Kluyveromyces marxianus* (Hansen) van der Walt var. *vanudenii* (van der Walt et Nel) Johannsen et van der Walt 1963

12. *Kluyveromyces marxianus* (Hansen) van der Walt var. *wikenii* (van der Walt, Nel et van Kerken) Johannsen et van der Walt 1966

13. *Kluyveromyces phaffii* (van der Walt) van der Walt 1963

14. *Kluyveromyces polysporus* van der Walt 1956a

15. *Kluyveromyces thermotolerans* (Philippov) Yarrow 1932

16. *Kluyveromyces waltii* Kodama 1974

17. *Kluyveromyces wickerhamii* (Phaff, Miller et Shifrine) van der Walt 1956

The proposed key to the genus *Kluyveromyces*

1a Asci 1-4 spored 2

b Asci containing more than 4 ascospores 15

2a Lactose assimilated 3

b Lactose not assimilated 7

3a Inulin fermented, occasionally slowly 4

b Inulin not fermented 5

4a Ascospores crescentiform to reniform

K. marxianus var. *marxianus*

b Ascospores spheroidal to ellipsoidal

K. marxianus var. *bulgaricus*

5a Ascospores crescentiform to reniform

K. wickerhamii

b Ascospores spheroidal to ellipsoidal 6

6a Lactose fermented

K. marxianus var. *lactis*

b Lactose not fermented

K. aestuarii

7a Ascospores crescentiform to reniform 8

b Ascospores spheroidal to ellipsoidal 12

8a Maltose fermented

K. marxianus var. *dobzhanskii*

b Maltose not fermented 9

9a Mannitol assimilated

K. marxianus var. *drosophilarum*

b Mannitol not assimilated 10

10a Raffinose assimilated

K. lodderi

b Raffinose not assimilated 11

11a Galactose assimilated

K. phaffii

b Galactose not assimilated

K. delphensis

12a Galactose assimilated 13

b Galactose not assimilated

K. waltii

13a Growth on 50% glucose

K. thermotolerans

b No growth on 50% glucose 14

14a Cellobiose assimilated

K. marxianus var. *vanudenii*

b Cellobiose not assimilated

K. marxianus var. *wikenii*

15a Sucrose assimilated

K. polysporus

b Sucrose not assimilated 16

16a D-ribose assimilated

K. blattae

b D-ribose not assimilated

K. africanus

8. CONCLUSIONS

The following conclusions were reached on the basis of the results obtained in the present investigation:

1. The genus *Kluyveromyces* is characterized by distinct areas in which hybrid formation readily occurs. These areas comprise the physiologically more advanced elements of the genus.

2. The confirmation of interfertility between members of the genus, based on the recovery of fertile hybrid progeny permits speciation in terms of the concept of the biological species.

3. Acceptance of the concept of the biological species necessitates the restructuring of the genus, which results in the recognition of eleven distinct species, i.e. *Kluyveromyces aestuarii*, *Kluyveromyces africanus*, *Kluyveromyces blattae*, *Kluyveromyces delphensis*, *Kluyveromyces lodderi*, *Kluyveromyces phaffii*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Kluyveromyces waltii*, *Kluyveromyces wickerhamii* and *Kluyveromyces marxianus*, the latter comprising seven varieties: *Kluyveromyces marxianus* var. *marxianus*, *Kluyveromyces marxianus* var. *bulgaricus*, *Kluyveromyces marxianus* var. *dobzhanskii*, *Kluyveromyces marxianus* var. *drosophilarum*,

Kluyveromyces marxianus var. *lactis*, *Kluyveromyces marxianus* var. *vanudenii* and *Kluyveromyces marxianus* var. *wikenii*, to which nine interfertile taxa, previously recognized as separate species, are assigned. On the basis of this restructuring the species *Kluyveromyces marxianus* shows appreciable phenotypic diversity.

4. The assumption that *Candida kefyr*, *Candida macedoniensis* and *Torulopsis sphaerica* represent imperfect states of some *Kluyveromyces* is confirmed by the fact that these taxa hybridize with *Kluyveromyces marxianus* var. *lactis*.

5. *Kluyveromyces marxianus* var. *marxianus* is genetically unrelated to *Pichia etchellsii*, *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces kloeckerianus*, *Saccharomyces montanus*, *Torulaspora rosei* and *Zygosaccharomyces rouxii* since recombination was not detected between these taxa.

6. The prototrophic selection technique can probably be applied for speciation purposes to other perfect yeast genera characterized by life cycles involving the fusion of independent cells.

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TABLE 1

PROTOTROPHIC *KLUYVEROMYCES* STRAINS

SPECIES	Strain number	Origin
K. aestuarii (Fell) van der Walt	CBS 4438 T	estuarine mud (Fell, 1961)
	CBS 4904	sea water, van Uden in 1961 (van der Walt, 1970a)
K. africanus van der Walt	CBS 2654	soil from South Africa (van der Walt, 1956b)
K. blattae Henninger et Windisch	CBS 6284=DSM 70876 T	intestinal tract of *Blatta orientalis* (Henninger & Windisch, 1976)
K. bulgaricus (Santa Maria) van der Walt	CBS 5667	South African Sorghum Beer, Dr J.P. van der Walt in 1966
K. cicerisporus van der Walt, Nel et van Kerken	CBS 4857=ATCC 22295 T	dorsal area of a cow (Klokke & Kamp, 1962)
K. delphensis (van der Walt et Tscheuschner) van der Walt	CBS 2170=NRRL Y-2379 T	sugary efflorescence on dried figs (van der Walt & Tscheuschner, 1956)
K. dobzhanskii (Shehata, Mrak et Phaff) van der Walt	CBS 2104=UD 50-45 T	*Drosophila pseudoobscura* (Shehata *et al.*, 1955)
K. drosophilarum (Shehata, Mrak et Phaff) van der Walt	CBS 2896	slime flux of oak, Krassilnikov in 1927 (Kudriawzew, 1960)
K. fragilis (Jörgensen) van der Walt	CBS 1556	yoghurt, Kolthof (date of isolation unknown)

TABLE 1
Continued

SPECIES	Strain number	Origin
K. lactis (Dombrowski) van der Walt, haploid mating type	CBS 683=IFO 1090= NCYC 416 T (mating type a)	gassy cheese (Allen & Thornley, 1929)
	CBS 2359=ATCC 8585=NRRL Y-1140=Laffer's No 61 (mating type α)	creamery (Laffer, 1936)
	CBS 6315=ATCC 8563=NRRL Y-1118 (mating type a)	creamery
K. lodderi (van der Walt et Tscheuschner) van der Walt	CBS 2757 T	soil from South Africa (van der Walt & Tscheuschner, 1957)
K. marxianus (Hansen) van der Walt	CBS 712 T	Schnegg (origin unknown) (van der Walt, 1970a)
	CBS 6556	fermenting maize dough (Herrera *et al.*, 1973)
	CBS 6923	soil from South Africa, Dr J.P. van der Walt in 1975
	CSIR Y293	maize meal, Dr D.B. Scott in 1962
K. phaffii (van der Walt) van der Walt	CBS 4417 T	soil from South Africa (van der Walt, 1963)
K. phaseolosporus (Shehata, Mrak et Phaff) van der Walt	CBS 2103=UD 50-80 T	*Drosophila pseudoobscura* (Shehata *et al.*, 1955)

TABLE 1
Continued

SPECIES	Strain number	Origin
K. polysporus van der Walt	CBS 6899	soil from South Africa, Dr J.P. van der Walt in 1976
K. thermotolerans (Philippov) Yarrow	CBS 6924	soil from South Africa, Dr J.P. van der Walt in 1975
K. vanudenii (van der Walt et Nel) van der Walt	CBS 4372 T	winery installations (van der Walt & Nel, 1963)
K. waltii Kodama	CBS 6430 T	exudate of *Ilex integra* (Kodama, 1974)
K. wickerhamii (Phaff, Miller et Shifrine) van der Walt	CBS 2745 T	*Drosophila montana* and *Drosophila piricola* (Phaff *et al.*, 1956)
	UD 56-40	flux of *Abies* sp., California in 1956, received from Prof H.J. Phaff
	UD 61-346	exudate of *Salix* sp., California in 1961, received from Prof H.J. Phaff
	UD 68-821C	exudate of *Betula* sp., British Columbia in 1968, received from Prof H.J. Phaff
K. wikenii van der Walt, Nel et van Kerken	CBS 5671=ATCC 22296 T	South African Sorghum Beer (van der Walt *et al.*, 1966)
Kluyveromyces strain, Windisch	DSM 70885	fruit-curd-feed manufacture, received from Prof S. Windisch

TABLE 2

PRESUMPTIVE IMPERFECT FORMS OF SOME *KLUYVEROMYCES* SPECIES

SPECIES	Strain number	Origin
Candida kefyr (Beijerinck) van Uden et Buckley	CBS 834=NRRL Y-329 T	kefyr grains (Beijerinck, 1889)
Candida macedoniensis (Castellani et Chalmers) Berkhout	CBS 600 T	Joekes in 1920 (origin unknown) (van Uden & Buckley, 1970)
Candida pseudotropicalis (Castellani) Basgal	CBS 607 T	bronchomycotic sputum (Castellani, 1911)
Torulopsis sphaerica (Hammer et Cordes) Lodder	CBS 141=IFO 0648=NCTC 1303	yeasty cream (Hammer & Cordes, 1920)

TABLE 3

REPRESENTATIVES OF OTHER ASCOGENOUS SPECIES

SPECIES	Strain number	Origin
Pichia etchellsii Kreger-van Rij	CBS 2011=IFO 1283=NRRL Y-7121 T	fermenting cucumber brine (Etchells *et al.*, 1952)
Saccharomyces bayanus Saccardo	CSIR Y119	grape must, Dr J.P. van der Walt in 1956
	CSIR Y120	grape must, Dr J.P. van der Walt in 1956
Saccharomyces cerevisiae Hansen	CBS 432	slime flux (Batchynskaja, 1914)
	CSIR Y2	South African Sorghum Beer, Dr J.P. van der Walt in 1953
	CSIR Y48	South African Sorghum Beer, Dr J.P. van der Walt in 1953
	CSIR Y108	South African Sorghum Beer, Dr J.P. van der Walt in 1953
Saccharomyces chevalieri Guilliermond	CBS 400 T	palm wine (Guilliermond, 1914)
Saccharomyces kloeckerianus van der Walt	CBS 5503	soil, received by the CBS from Dr L.J. Wickerham in 1951
Saccharomyces montanus Phaff, Miller et Shifrine	CBS 4506 T	*Drosophila* sp. (Phaff *et al.*, 1956)

TABLE 3
Continued

SPECIES	Strain number	Origin
Torulaspora rosei Guilliermond	CSIR Y857	soil from South Africa, Dr J.P. van der Walt in 1975
Zygosaccharomyces rouxii (Boutroux) Yarrow	CBS 4838=ATCC 14680=NRRL Y-2548	miso (Wickerham & Burton, 1960)
	CBS 5499=ATCC 22027	sugar from Mauritius, Dr Antoine in 1962 (Kreger-van Rij, 1966)
	CSIR Y849	soil from South Africa, Dr J.P. van der Walt in 1977
	CSIR Y850	soil from South Africa, Dr J.P. van der Walt in 1977

TABLE 4

AUXOTROPHIC MUTANTS EMPLOYED IN THE STUDY WITH THE INDICATION OF PERTINENT PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS AND NUTRITIONAL REQUIREMENTS

STRAIN	Physiological characteristics	Ascosporal shape	Nutritional requirements
K. aestuarii CBS 4438	GAL SUC MAL LAC RAF ara rib	Sph	his
K. aestuarii CBS 4438	GAL SUC MAL LAC RAF ara rib	Sph	ilv
K. aestuarii CBS 4904	GAL SUC mal LAC RAF ara rib	Sph	ade
K. aestuarii CBS 4904	GAL SUC mal LAC RAF ara rib	Sph	his
K. aestuarii CBS 4904	GAL SUC mal LAC RAF ara rib	Sph	met
K. africanus CBS 2654	GAL suc mal lac raf	Ren	ade
K. africanus CBS 2654	GAL suc mal lac raf	Ren	leu
K. africanus CBS 2654	GAL suc mal lac raf	Ren	met
K. africanus CBS 2654	GAL suc mal lac raf	Ren	met trp
K. blattae CBS 6284	GAL suc mal lac raf RIB	Sph	his
K. blattae CBS 6284	GAL suc mal lac raf RIB	Sph	met
K. blattae CBS 6284	GAL suc mal lac raf RIB	Sph	trp
K. bulgaricus CBS 5667	GAL sor SUC mal cel LAC RAF rib	Sph	leu
K. bulgaricus CBS 5667	GAL sor SUC mal cel LAC RAF rib	Sph	lys
K. bulgaricus CBS 5667	GAL sor SUC mal cel LAC RAF rib	Sph	met
K. cicerisporus CBS 4857	GAL SOR SUC mal CEL LAC RAF RIB	Sph	ilv
K. cicerisporus CBS 4857	GAL SOR SUC mal CEL LAC RAF RIB	Sph	leu
K. cicerisporus CBS 4857	GAL SOR SUC mal CEL LAC RAF RIB	Sph	trp
K. delphensis CBS 2170	gal suc mal lac raf	Ren	his
K. delphensis CBS 2170	gal suc mal lac raf	Ren	leu

TABLE 4
Continued

STRAIN	Physiological characteristics	Ascosporal shape	Nutritional requirements
K. dobzhanskii CBS 2104	GAL SUC MAL CEL lac RAF	Ren	ade
K. dobzhanskii CBS 2104	GAL SUC MAL CEL lac RAF	Ren	his
K. drosophilarum CBS 2896	GAL SUC MAL CEL lac RAF	Ren	ade
K. drosophilarum CBS 2896	GAL SUC MAL CEL lac RAF	Ren	lys
K. drosophilarum CBS 2896	GAL SUC MAL CEL lac RAF	Ren	met thr
K. drosophilarum CBS 2896	GAL SUC MAL CEL lac RAF	Ren	trp
K. fragilis CBS 1556	GAL SUC mal CEL tre LAC RAF	Ren	leu
K. fragilis CBS 1556	GAL SUC mal CEL tre LAC RAF	Ren	met
K. lactis CBS 683	GAL SUC MAL CEL LAC RAF	Sph	arg
K. lactis CBS 683	GAL SUC MAL CEL LAC RAF	Sph	lys
K. lactis CBS 683	GAL SUC MAL CEL LAC RAF	Sph	trp
K. lactis CBS 683	GAL SUC MAL CEL LAC RAF	Sph	ura
K. lactis CBS 2359	GAL SUC MAL CEL LAC RAF	Sph	arg
K. lactis CBS 2359	GAL SUC MAL CEL LAC RAF	Sph	arg ura
K. lactis CBS 6315	GAL SUC mal CEL LAC RAF	Sph	ade
K. lactis CBS 6315	GAL SUC mal CEL LAC RAF	Sph	trp
K. lodderi CBS 2757	GAL SUC mal lac RAF	Ren	arg
K. lodderi CBS 2757	GAL SUC mal lac RAF	Ren	asp

TABLE 4
Continued

STRAIN	Physiological characteristics	Ascosporal shape	Nutritional requirements
K. marxianus CBS 6556	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	ade
K. marxianus CBS 6556	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	his
K. marxianus CBS 6556	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	leu pro
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	ade
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	arg
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	asp
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	his
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	leu
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	lys
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	trp
K. marxianus CBS 6923	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	ura
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	ade
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	arg
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	leu
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	lys
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	met
K. marxianus CSIR Y293	GAL SUC mal CEL tre LAC RAF ARA RIB	Ren	trp
K. phaffii CBS 4417	GAL suc mal lac raf	Ren	ade met
K. phaffii CBS 4417	GAL suc mal lac raf	Ren	arg
K. phaffii CBS 4417	GAL suc mal lac raf	Ren	arg his
K. phaffii CBS 4417	GAL suc mal lac raf	Ren	lys

TABLE 4
Continued

STRAIN	Physiological characteristics	Ascosporal shape	Nutritional requirements
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	ade
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	arg
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	asp
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	lys
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	met thr
K. phaseolosporus CBS 2103	GAL SOR SUC mal CEL TRE lac RAF	Ren	trp
K. polysporus CBS 6899	GAL sor SUC mal lac RAF	Ren	ade
K. polysporus CBS 6899	GAL sor SUC mal lac RAF	Ren	lys
K. polysporus CBS 6899	GAL sor SUC mal lac RAF	Ren	met
K. polysporus CBS 6899	GAL sor SUC mal lac RAF	Ren	trp
K. polysporus CBS 6899	GAL sor SUC mal lac RAF	Ren	trp ura
K. thermotolerans CBS 6924	GAL SUC MAL cel lac RAF	Sph	met
K. thermotolerans CBS 6924	GAL SUC MAL cel lac RAF	Sph	thr
K. vanudenii CBS 4372	GAL SUC MAL CEL lac RAF	Sph	his
K. vanudenii CBS 4372	GAL SUC MAL CEL lac RAF	Sph	ilv
K. vanudenii CBS 4372	GAL SUC MAL CEL lac RAF	Sph	leu
K. vanudenii CBS 4372	GAL SUC MAL CEL lac RAF	Sph	lys
K. vanudenii CBS 4372	GAL SUC MAL CEL lac RAF	Sph	ura
K. waltii CBS 6430	gal SUC mal lac RAF	Sph	arg
K. waltii CBS 6430	gal SUC mal lac RAF	Sph	his met

TABLE 4

Continued

STRAIN	Physiological characteristics	Ascosporal shape	Nutritional requirements
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	ade
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	arg lys
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	leu
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	lys
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	trp
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	trp ura
K. wickerhamii CBS 2745	GAL SUC mal CEL LAC raf	Ren	ura
K. wikenii CBS 5671	GAL SUC mal lac RAF	Sph	ade
K. wikenii CBS 5671	GAL SUC mal lac RAF	Sph	his
K. wikenii CBS 5671	GAL SUC mal lac RAF	Sph	lys
K. wikenii CBS 5671	GAL SUC mal lac RAF	Sph	thr
K. wikenii CBS 5671	GAL SUC mal lac RAF	Sph	ura
Strain DSM 70885	GAL SUC mal lac raf ARA	Ren	ade
Strain DSM 70885	GAL SUC mal lac raf ARA	Ren	trp
Torulopsis sphaerica CBS 141	GAL SUC MAL LAC RAF		ade
Torulopsis sphaerica CBS 141	GAL SUC MAL LAC RAF		ilv

TABLE 5

INFLUENCE OF INCUBATION TIME ON THE NUMBER OF RECOVERED RECOMBINANTS IN CROSSES INVOLVING *KLUYVEROMYCES LACTIS* CBS 683 AND SOME OTHER SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Number of recombinants after 7 days' incubation*	Number of recombinants after 28 days' incubation*
K. lactis arg x *K. bulgaricus* CBS 5667	maltose	$1,23 \times 10^2$	$1,66 \times 10^6$
K. lactis trp x *K. bulgaricus* CBS 5667	maltose	$4,15 \times 10^2$	$7,98 \times 10^5$
K. lactis arg x *K. cicerisporus* CBS 4857	maltose	0	$1,95 \times 10^6$
K. lactis trp x *K. cicerisporus* CBS 4857	maltose	0	$8,15 \times 10^4$
K. lactis arg x *K. dobzhanskii* CBS 2104	lactose	$5,67 \times 10^3$	$1,35 \times 10^5$
K. lactis trp x *K. dobzhanskii* CBS 2104	lactose	$5,05 \times 10^4$	$7,37 \times 10^5$
K. lactis arg x *K. drosophilarum* CBS 2896	lactose	$2,84 \times 10^4$	$1,18 \times 10^5$
K. lactis trp x *K. drosophilarum* CBS 2896	lactose	$2,26 \times 10^5$	$3,59 \times 10^5$
K. lactis arg x *K. fragilis* CBS 1556	maltose	34	$1,79 \times 10^6$
K. lactis trp x *K. fragilis* CBS 1556	maltose	$3,61 \times 10^3$	$7,92 \times 10^5$
K. lactis arg x *K. marxianus* CBS 6556	maltose	20	$2,69 \times 10^4$
K. lactis trp x *K. marxianus* CBS 6556	maltose	$3,97 \times 10^3$	$5,00 \times 10^5$
K. lactis arg x *K. marxianus* CBS 6923	maltose	79	$5,32 \times 10^3$
K. lactis trp x *K. marxianus* CBS 6923	maltose	$4,38 \times 10^4$	$1,63 \times 10^6$
K. lactis arg x *K. phaseolosporus* CBS 2103	lactose	$6,22 \times 10^5$	$9,69 \times 10^5$
K. lactis trp x *K. phaseolosporus* CBS 2103	lactose	$3,71 \times 10^5$	$1,31 \times 10^6$
K. lactis arg x *K. vanudenii* CBS 4372	lactose	$2,98 \times 10^5$	$8,73 \times 10^5$
K. lactis trp x *K. vanudenii* CBS 4372	lactose	$6,37 \times 10^5$	$7,51 \times 10^6$
K. lactis arg x *K. wickerhamii* CBS 2745	maltose	$2,56 \times 10^2$	$8,94 \times 10^3$
K. lactis trp x *K. wickerhamii* CBS 2745	maltose	$1,06 \times 10^3$	$1,10 \times 10^5$
K. lactis arg x *K. wikenii* CBS 5671	lactose	$3,38 \times 10^2$	$2,65 \times 10^3$
K. lactis trp x *K. wikenii* CBS 5671	lactose	35	$5,10 \times 10^5$

* Expressed per 10^8 cells of the mated populations

TABLE 6

INTRASPECIFIC CROSSES INVOLVING THREE STRAINS OF *KLUYVEROMYCES MARXIANUS* AND THREE STRAINS OF *KLUYVEROMYCES LACTIS*

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus CBS 6923 lys x *K. marxianus* CBS 6556 his	lactose	1,09	$4,86 \times 10^4$
K. marxianus CBS 6923 lys x *K. marxianus* CBS 6556 leu pro	lactose	0,97	$2,16 \times 10^5$
K. marxianus CBS 6923 trp x *K. marxianus* CBS 6556 his	lactose	1,10	$7,27 \times 10^4$
K. marxianus CBS 6923 trp x *K. marxianus* CBS 6556 leu pro	lactose	0,76	$2,34 \times 10^5$
K. marxianus CBS 6923 lys x *K. marxianus* CSIR Y293 leu	lactose	3,62	$1,60 \times 10^4$
K. marxianus CBS 6923 lys x *K. marxianus* CSIR Y293 trp	lactose	1,57	$1,66 \times 10^3$
K. marxianus CBS 6923 trp x *K. marxianus* CSIR Y293 leu	lactose	1,27	$7,80 \times 10^5$
K. marxianus CBS 6556 ade x *K. marxianus* CSIR Y293 arg	lactose	1,70	$3,88 \times 10^5$
K. marxianus CBS 6556 ade x *K. marxianus* CSIR Y293 lys	lactose	2,51	$1,90 \times 10^4$
K. marxianus CBS 6556 his x *K. marxianus* CSIR Y293 ade	lactose	2,05	$1,37 \times 10^6$
K. marxianus CBS 6556 his x *K. marxianus* CSIR Y293 lys	lactose	1,47	$1,81 \times 10^6$
K. lactis CBS 683 lys x *K. lactis* CBS 2359 arg ura	lactose	1,49	$4,89 \times 10^5$
K. lactis CBS 683 trp x *K. lactis* CBS 2359 arg ura	lactose	2,19	$2,64 \times 10^7$
K. lactis CBS 683 ura x *K. lactis* CBS 2359 arg	lactose	3,18	$6,28 \times 10^5$
K. lactis CBS 2359 arg ura x *K. lactis* CBS 6315 ade	lactose	1,23	$1,63 \times 10^6$
K. lactis CBS 2359 arg ura x *K. lactis* CBS 6315 trp	lactose	1,51	$3,27 \times 10^6$
K. lactis CBS 683 x *K. lactis* CBS 2359		1,72*	$3,82 \times 10^{5*}$
K. lactis CBS 2359 x *K. lactis* CBS 6315		1,34*	$5,30 \times 10^{5*}$

*The results were calculated from a direct count in a haemacytometer. The mated cultures were maintained for 7 days on 2% ME, at 28 °C. The number of recombinants corresponds to the total number of zygotes and asci observed in the suspension.

TABLE 7

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 712
AND THREE STRAINS OF *KLUYVEROMYCES LACTIS*

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus x *K. lactis* CBS 683 arg	maltose	1,07	0
K. marxianus x *K. lactis* CBS 683 lys	maltose	1,54	0
K. marxianus x *K. lactis* CBS 683 trp	maltose	3,71	$1,40 \times 10^4$
K. marxianus x *K. lactis* CBS 2359 arg ura	maltose	1,37	$4,04 \times 10^3$
K. marxianus x *K. lactis* CBS 6315 ade	maltose	1,77	0
K. marxianus x *K. lactis* CBS 6315 trp	maltose	1,79	0

TABLE 8

CROSSES INVOLVING *KLUYVEROMYCES AESTUARII* CBS 4438
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. aestuarii his x *K. africanus* CBS 2654	lactose	1,54	0
K. aestuarii ilv x *K. africanus* CBS 2654	lactose	4,36	0
K. aestuarii his x *K. blattae* CBS 6284	lactose	1,36	0
K. aestuarii ilv x *K. blattae* CBS 6284	lactose	2,22	0
K. aestuarii his x *K. bulgaricus* CBS 5667	maltose	2,62	0
K. aestuarii ilv x *K. bulgaricus* CBS 5667	maltose	2,16	0
K. aestuarii his x *K. cicerisporus* CBS 4857	maltose	3,09	0
K. aestuarii ilv x *K. cicerisporus* CBS 4857	maltose	2,33	0
K. aestuarii ilv x *K. cicerisporus* CBS 4857 trp	maltose	2,20	0
K. aestuarii his x *K. delphensis* CBS 2170	lactose	1,87	0
K. aestuarii ilv x *K. delphensis* CBS 2170	lactose	1,95	0
K. aestuarii his x *K. dobzhanskii* CBS 2104	lactose	3,84	0
K. aestuarii ilv x *K. dobzhanskii* CBS 2104	lactose	5,42	0
K. aestuarii his x *K. drosophilarum* CBS 2896	lactose	3,23	0
K. aestuarii ilv x *K. drosophilarum* CBS 2896	lactose	3,80	0
K. aestuarii his x *K. fragilis* CBS 1556	maltose	1,82	0
K. aestuarii ilv x *K. fragilis* CBS 1556	maltose	2,63	0
K. aestuarii ilv x *K. lactis* CBS 683 arg	lactose	4,35	0
K. aestuarii ilv x *K. lactis* CBS 683 trp	lactose	3,53	0
K. aestuarii his x *K. lactis* CBS 2359 arg	lactose	1,63	0
K. aestuarii ilv x *K. lactis* CBS 2359 arg ura	lactose	2,93	0
K. aestuarii his x *K. lactis* CBS 6315 trp	lactose	1,68	0
K. aestuarii ilv x *K. lactis* CBS 6315 trp	lactose	2,98	0
K. aestuarii his x *K. lodderi* CBS 2757	lactose	1,54	0
K. aestuarii ilv x *K. lodderi* CBS 2757	lactose	4,09	0

TABLE 8
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. aestuarii his x *K. marxianus* CBS 6556	maltose	2,51	0
K. aestuarii ilv x *K. marxianus* CBS 6556	maltose	3,41	0
K. aestuarii ilv x *K. marxianus* CBS 6556 his	maltose	4,36	0
K. aestuarii x *K. marxianus* CBS 6923 lys	L-arabinose	2,61	0
K. aestuarii x *K. marxianus* CBS 6923 trp	D-ribose	2,57	0
K. aestuarii his x *K. marxianus* CBS 6923	maltose	2,92	0
K. aestuarii his x *K. marxianus* CBS 6923 ura	lactose	2,55	0
K. aestuarii ilv x *K. marxianus* CBS 6923	maltose	1,76	0
K. aestuarii ilv x *K. marxianus* CBS 6923 ade	lactose	2,63	0
K. aestuarii ilv x *K. marxianus* CBS 6923 lys	lactose	2,20	0
K. aestuarii x *K. marxianus* CSIR Y293 leu	L-arabinose	2,56	0
K. aestuarii x *K. marxianus* CSIR Y293 trp	D-ribose	2,55	0
K. aestuarii his x *K. phaffii* CBS 4417	lactose	1,59	0
K. aestuarii ilv x *K. phaffii* CBS 4417	lactose	1,60	0
K. aestuarii his x *K. phaseolosporus* CBS 2103	lactose	4,37	0
K. aestuarii ilv x *K. phaseolosporus* CBS 2103	lactose	5,07	0
K. aestuarii his x *K. polysporus* CBS 6899	lactose	1,78	0
K. aestuarii ilv x *K. polysporus* CBS 6899	lactose	1,65	0
K. aestuarii his x *K. thermotolerans* CBS 6924	lactose	1,56	0
K. aestuarii his x *K. thermotolerans* CBS 6924 met	maltose	4,12	0
K. aestuarii ilv x *K. thermotolerans* CBS 6924	lactose	2,48	0
K. aestuarii ilv x *K. thermotolerans* CBS 6924 met	maltose	1,60	0
K. aestuarii his x *K. vanudenii* CBS 4372	lactose	2,08	0
K. aestuarii ilv x *K. vanudenii* CBS 4372	lactose	1,44	0
K. aestuarii his x *K. waltii* CBS 6430	lactose	2,53	0
K. aestuarii ilv x *K. waltii* CBS 6430	lactose	2,43	0

TABLE 8

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. aestuarii his x *K. wickerhamii* CBS 2745	raffinose	6,62	0
K. aestuarii his x *K. wickerhamii* CBS 2745 ura	lactose	4,51	0
K. aestuarii ilv x *K. wickerhamii* CBS 2745	maltose	4,01	0
K. aestuarii ilv x *K. wickerhamii* CBS 2745 trp	lactose	5,81	0
K. aestuarii his x *K. wikenii* CBS 5671	lactose	2,88	0
K. aestuarii ilv x *K. wikenii* CBS 5671	lactose	1,80	0

TABLE 9

CROSSES INVOLVING *KLUYVEROMYCES AESTUARII* CBS 4904
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. aestuarii his x *K. africanus* CBS 2654	lactose	3,06	0
K. aestuarii his x *K. blattae* CBS 6284	lactose	1,14	0
K. aestuarii his x *K. bulgaricus* CBS 5667 lys	lactose	3,23	0
K. aestuarii his x *K. bulgaricus* CBS 5667 met	lactose	2,59	0
K. aestuarii his x *K. cicerisporus* CBS 4857 trp	lactose	1,24	0
K. aestuarii his x *K. delphensis* CBS 2170	lactose	3,17	0
K. aestuarii his x *K. dobzhanskii* CBS 2104	lactose	7,43	0
K. aestuarii his x *K. drosophilarum* CBS 2896	lactose	2,07	0
K. aestuarii his x *K. fragilis* CBS 1556 met	lactose	1,35	0
K. aestuarii his x *K. lactis* CBS 683 arg	lactose	3,18	0
K. aestuarii his x *K. lactis* CBS 683 trp	lactose	3,80	0
K. aestuarii x *K. lactis* CBS 2359 arg ura	maltose	2,16	0
K. aestuarii ade x *K. lactis* CBS 2359 arg ura	lactose	2,93	0
K. aestuarii his x *K. lactis* CBS 2359 arg ura	lactose	2,83	0
K. aestuarii met x *K. lactis* CBS 2359 arg ura	lactose	1,37	0
K. aestuarii his x *K. lactis* CBS 6315 trp	lactose	2,89	0
K. aestuarii his x *K. lodderi* CBS 2757	lactose	9,04	0
K. aestuarii his x *K. marxianus* CBS 6556 ade	lactose	1,47	0
K. aestuarii his x *K. marxianus* CBS 6556 leu pro	lactose	1,29	0
K. aestuarii x *K. marxianus* CBS 6923 leu	D-ribose	2,31	0
K. aestuarii x *K. marxianus* CBS 6923 lys	L-arabinose	2,61	0
K. aestuarii x *K. marxianus* CBS 6923 trp	D-ribose	2,57	0
K. aestuarii his x *K. marxianus* CBS 6923 ade	lactose	1,87	0
K. aestuarii his x *K. marxianus* CBS 6923 leu	lactose	3,98	0
K. aestuarii his x *K. marxianus* CBS 6923 lys	lactose	1,75	0

TABLE 9

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. aestuarii x *K. marxianus* CSIR Y293 leu	D-ribose	2,27	0
K. aestuarii x *K. marxianus* CSIR Y293 trp	L-arabinose	2,00	0
K. aestuarii his x *K. phaffii* CBS 4417	lactose	2,78	0
K. aestuarii his x *K. phaseolosporus* CBS 2103	lactose	4,94	0
K. aestuarii his x *K. polysporus* CBS 6899	lactose	4,51	0
K. aestuarii his x *K. thermotolerans* CBS 6924	lactose	3,83	0
K. aestuarii his x *K. vanudenii* CBS 4372	lactose	1,98	0
K. aestuarii his x *K. waltii* CBS 6430	lactose	2,54	0
K. aestuarii his x *K. wickerhamii* CBS 2745	raffinose	4,27	0
K. aestuarii his x *K. wikenii* CBS 5671	lactose	2,43	0

TABLE 10

CROSSES INVOLVING *KLUYVEROMYCES AFRICANUS* CBS 2654 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. africanus x *K. aestuarii* CBS 4438 his	lactose	1,54	0
K. africanus x *K. aestuarii* CBS 4438 ilv	lactose	4,36	0
K. africanus x *K. aestuarii* CBS 4904 his	lactose	3,06	0
K. africanus ade x *K. blattae* CBS 6284 his	galactose	3,33	0
K. africanus ade x *K. blattae* CBS 6284 met	galactose	2,63	0
K. africanus ade x *K. blattae* CBS 6284 trp	galactose	2,69	0
K. africanus met x *K. blattae* CBS 6284 his	galactose	3,28	0
K. africanus met x *K. blattae* CBS 6284 trp	galactose	3,36	0
K. africanus x *K. bulgaricus* CBS 5667 lys	lactose	2,62	0
K. africanus x *K. bulgaricus* CBS 5667 met	lactose	4,31	0
K. africanus x *K. cicerisporus* CBS 4857 ilv	lactose	2,66	0
K. africanus x *K. cicerisporus* CBS 4857 trp	lactose	4,14	0
K. africanus ade x *K. delphensis* CBS 2170 his	galactose	3,23	0
K. africanus met x *K. delphensis* CBS 2170	galactose	3,05	0
K. africanus met trp x *K. delphensis* CBS 2170	galactose	4,54	0
K. africanus x *K. dobzhanskii* CBS 2104 ade	maltose	3,42	0
K. africanus x *K. dobzhanskii* CBS 2104 his	maltose	5,91	0
K. africanus x *K. drosophilarum* CBS 2896 ade	maltose	4,73	0
K. africanus x *K. drosophilarum* CBS 2896 lys	maltose	2,43	0
K. africanus x *K. fragilis* CBS 1556 leu	lactose	4,55	0
K. africanus x *K. fragilis* CBS 1556 met	lactose	1,26	0
K. africanus x *K. lactis* CBS 683 arg	lactose	4,44	0
K. africanus x *K. lactis* CBS 683 trp	lactose	4,30	0
K. africanus x *K. lactis* CBS 2359 arg ura	lactose	3,27	0
K. africanus x *K. lactis* CBS 6315 ade	lactose	2,98	0

TABLE 10
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. africanus x *K. lodderi* CBS 2757 arg	raffinose	1,87	0
K. africanus x *K. lodderi* CBS 2757 asp	raffinose	1,37	0
K. africanus ade x *K. lodderi* CBS 2757 arg	raffinose	1,29	0
K. africanus x *K. marxianus* CBS 6556 his	lactose	4,88	0
K. africanus x *K. marxianus* CBS 6556 leu pro	lactose	5,90	0
K. africanus x *K. marxianus* CBS 6923 ade	lactose	1,99	0
K. africanus x *K. marxianus* CBS 6923 arg	lactose	1,60	0
K. africanus x *K. marxianus* CBS 6923 lys	lactose	2,25	0
K. africanus x *K. marxianus* CBS 6923 trp	lactose	1,72	0
K. africanus x *K. marxianus* CSIR Y293 leu	lactose	1,45	0
K. africanus x *K. marxianus* CSIR Y293 met	lactose	2,73	0
K. africanus ade x *K. phaffii* CBS 4417 arg his	galactose	1,51	0
K. africanus leu x *K. phaffii* CBS 4417 ade met	glucose	1,89	0
K. africanus met x *K. phaffii* CBS 4417 arg	galactose	1,78	0
K. africanus met trp x *K. phaffii* CBS 4417 lys	galactose	1,29	0
K. africanus x *K. phaseolosporus* CBS 2103 arg	raffinose	1,45	0
K. africanus x *K. phaseolosporus* CBS 2103 trp	raffinose	3,32	0
K. africanus x *K. polysporus* CBS 6899 ade	raffinose	1,60	0
K. africanus x *K. polysporus* CBS 6899 met	raffinose	1,49	0
K. africanus met x *K. polysporus* CBS 6899 ade	raffinose	1,69	0
K. africanus met x *K. polysporus* CBS 6899 trp	raffinose	2,32	0
K. africanus met trp x *K. polysporus* CBS 6899 ade	raffinose	1,64	0
K. africanus x *K. thermotolerans* CBS 6924 met	maltose	6,70	0
K. africanus x *K. thermotolerans* CBS 6924 thr	maltose	1,57	0

TABLE 10
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. africanus x *K. vanudenii* CBS 4372 his	maltose	1,73	0
K. africanus x *K. vanudenii* CBS 4372 lys	maltose	2,48	0
K. africanus ade x *K. waltii* CBS 6430	galactose	3,85	0
K. africanus ade x *K. waltii* CBS 6430 arg	galactose	4,12	0
K. africanus met x *K. waltii* CBS 6430	galactose	3,28	0
K. africanus x *K. wickerhamii* CBS 2745 ade	lactose	7,94	0
K. africanus x *K. wickerhamii* CBS 2745 trp ura	lactose	3,31	0
K. africanus x *K. wikenii* CBS 5671 his	raffinose	3,07	0
K. africanus x *K. wikenii* CBS 5671 lys	raffinose	4,06	0
K. africanus x *K. wikenii* CBS 5671 thr	raffinose	3,18	0
K. africanus x *K. wikenii* CBS 5671 ura	raffinose	2,14	0

TABLE 11

CROSSES INVOLVING *KLUYVEROMYCES BLATTAE* CBS 6284
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. blattae x *K. aestuarii* CBS 4438 his	lactose	1,36	0
K. blattae x *K. aestuarii* CBS 4438 ilv	lactose	2,22	0
K. blattae x *K. aestuarii* CBS 4904 his	lactose	1,14	0
K. blattae his x *K. africanus* CBS 2654 ade	galactose	3,33	0
K. blattae his x *K. africanus* CBS 2654 met	galactose	3,28	0
K. blattae met x *K. africanus* CBS 2654 ade	galactose	2,63	0
K. blattae trp x *K. africanus* CBS 2654 ade	galactose	2,69	0
K. blattae trp x *K. africanus* CBS 2654 met	galactose	3,36	0
K. blattae x *K. bulgaricus* CBS 5667 lys	lactose	1,51	0
K. blattae x *K. bulgaricus* CBS 5667 met	lactose	1,04	0
K. blattae x *K. cicerisporus* CBS 4857 ilv	lactose	1,41	0
K. blattae x *K. cicerisporus* CBS 4857 trp	lactose	1,67	0
K. blattae his x *K. delphensis* CBS 2170	galactose	3,79	0
K. blattae met x *K. delphensis* CBS 2170	galactose	2,84	0
K. blattae x *K. dobzhanskii* CBS 2104 ade	maltose	5,41	0
K. blattae x *K. dobzhanskii* CBS 2104 his	maltose	1,91	0
K. blattae x *K. drosophilarum* CBS 2896 ade	maltose	5,24	0
K. blattae x *K. drosophilarum* CBS 2896 trp	maltose	3,54	0
K. blattae x *K. fragilis* CBS 1556 leu	lactose	1,38	0
K. blattae x *K. fragilis* CBS 1556 met	lactose	1,14	0
K. blattae x *K. lactis* CBS 683 arg	lactose	3,12	0
K. blattae x *K. lactis* CBS 683 trp	lactose	2,72	0
K. blattae x *K. lactis* CBS 2359 arg ura	lactose	1,61	0
K. blattae x *K. lactis* CBS 6315 ade	lactose	1,85	0
K. blattae x *K. lodderi* CBS 2757 arg	sucrose	1,41	0
K. blattae x *K. lodderi* CBS 2757 asp	sucrose	1,84	0

TABLE 11
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. blattae x *K. marxianus* CBS 6556 his	lactose	1,62	0
K. blattae x *K. marxianus* CBS 6556 leu pro	lactose	1,26	0
K. blattae x *K. marxianus* CBS 6923 leu	lactose	1,48	0
K. blattae x *K. marxianus* CBS 6923 lys	lactose	1,12	0
K. blattae x *K. marxianus* CBS 6923 trp	lactose	1,52	0
K. blattae x *K. marxianus* CBS 6923 ura	lactose	2,02	0
K. blattae x *K. marxianus* CSIR Y293 leu	lactose	1,41	0
K. blattae x *K. marxianus* CSIR Y293 trp	lactose	2,81	0
K. blattae his x *K. phaffii* CBS 4417 ade met	galactose	1,08	0
K. blattae his x *K. phaffii* CBS 4417 arg	galactose	1,39	0
K. blattae his x *K. phaffii* CBS 4417 lys	galactose	1,38	0
K. blattae met x *K. phaffii* CBS 4417 lys	galactose	1,79	0
K. blattae x *K. phaseolosporus* CBS 2103 arg	sucrose	2,51	0
K. blattae x *K. phaseolosporus* CBS 2103 lys	sucrose	2,09	0
K. blattae x *K. polysporus* CBS 6899 ade	sucrose	2,41	0
K. blattae x *K. polysporus* CBS 6899 lys	sucrose	3,15	0
K. blattae x *K. polysporus* CBS 6899 trp	sucrose	3,23	0
K. blattae x *K. thermotolerans* CBS 6924 met	maltose	2,00	0
K. blattae x *K. thermotolerans* CBS 6924 thr	maltose	2,34	0
K. blattae x *K. vanudenii* CBS 4372 his	maltose	2,91	0
K. blattae x *K. vanudenii* CBS 4372 lys	maltose	1,79	0
K. blattae x *K. waltii* CBS 6430 arg	sucrose	1,72	0
K. blattae x *K. waltii* CBS 6430 his met	sucrose	1,47	0
K. blattae his x *K. waltii* CBS 6430	galactose	1,67	0
K. blattae met x *K. waltii* CBS 6430	galactose	2,27	0

TABLE II

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. blattae x *K. wickerhamii* CBS 2745 leu	lactose	4,44	0
K. blattae x *K. wickerhamii* CBS 2745 lys	lactose	1,25	0
K. blattae x *K. wikenii* CBS 5671 ade	sucrose	1,47	0
K. blattae x *K. wikenii* CBS 5671 his	sucrose	1,12	0

TABLE 12

CROSSES INVOLVING *KLUYVEROMYCES BULGARICUS* CBS 5667 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. bulgaricus x *K. aestuarii* CBS 4438 his	maltose	2,62	0
K. bulgaricus x *K. aestuarii* CBS 4438 ilv	maltose	2,16	0
K. bulgaricus lys x *K. aestuarii* CBS 4904 his	lactose	3,23	0
K. bulgaricus met x *K. aestuarii* CBS 4904 his	lactose	2,59	0
K. bulgaricus lys x *K. africanus* CBS 2654	lactose	2,62	0
K. bulgaricus met x *K. africanus* CBS 2654	lactose	4,31	0
K. bulgaricus lys x *K. blattae* CBS 6284	lactose	1,51	0
K. bulgaricus met x *K. blattae* CBS 6284	lactose	1,04	0
K. bulgaricus lys x *K. cicerisporus* CBS 4857 ilv	lactose	1,17	$5,64 \times 10^5$
K. bulgaricus lys x *K. cicerisporus* CBS 4857 trp	lactose	3,88	$7,76 \times 10^3$
K. bulgaricus met x *K. cicerisporus* CBS 4857 ilv	lactose	0,78	$4,90 \times 10^6$
K. bulgaricus lys x *K. delphensis* CBS 2170	lactose	3,38	0
K. bulgaricus met x *K. delphensis* CBS 2170	lactose	2,86	0
K. bulgaricus x *K. dobzhanskii* CBS 2104 ade	maltose	3,38	$1,83 \times 10^5$
K. bulgaricus x *K. dobzhanskii* CBS 2104 his	maltose	1,87	$2,73 \times 10^3$
K. bulgaricus lys x *K. dobzhanskii* CBS 2104	lactose	3,18	$7,20 \times 10^4$
K. bulgaricus met x *K. dobzhanskii* CBS 2104	lactose	1,25	$9,84 \times 10^5$
K. bulgaricus x *K. drosophilarum* CBS 2896 met thr	maltose	1,38	$1,52 \times 10^6$
K. bulgaricus x *K. drosophilarum* CBS 2896 trp	maltose	1,65	$1,05 \times 10^5$
K. bulgaricus lys x *K. drosophilarum* CBS 2896	lactose	1,01	0
K. bulgaricus met x *K. drosophilarum* CBS 2896	lactose	1,41	0
K. bulgaricus lys x *K. fragilis* CBS 1556 leu	lactose	1,45	$1,20 \times 10^5$
K. bulgaricus met x *K. fragilis* CBS 1556 leu	lactose	0,66	$5,56 \times 10^5$
K. bulgaricus x *K. lactis* CBS 683 arg	maltose	1,85	$1,66 \times 10^6$
K. bulgaricus x *K. lactis* CBS 683 trp	maltose	3,37	$7,98 \times 10^5$
K. bulgaricus x *K. lactis* CBS 2359 arg ura	maltose	4,51	$1,02 \times 10^6$

TABLE 12
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. bulgaricus lys x *K. lodderi* CBS 2757	lactose	2,41	0
K. bulgaricus met x *K. lodderi* CBS 2757	lactose	1,77	0
K. bulgaricus lys x *K. marxianus* CBS 6556 leu pro	lactose	1,41	$5,59 \times 10^6$
K. bulgaricus met x *K. marxianus* CBS 6556 his	lactose	1,79	$2,84 \times 10^6$
K. bulgaricus x *K. marxianus* CBS 6923 ade	cellobiose	1,15	$8,96 \times 10^4$
K. bulgaricus x *K. marxianus* CBS 6923 his	cellobiose	1,58	$5,79 \times 10^4$
K. bulgaricus x *K. marxianus* CBS 6923 ura	cellobiose	1,49	$2,45 \times 10^4$
K. bulgaricus lys x *K. marxianus* CBS 6923 trp	lactose	2,06	$3,20 \times 10^3$
K. bulgaricus met x *K. marxianus* CBS 6923 lys	lactose	1,07	$5,30 \times 10^3$
K. bulgaricus lys x *K. phaffii* CBS 4417	lactose	1,59	0
K. bulgaricus met x *K. phaffii* CBS 4417	lactose	1,22	0
K. bulgaricus leu x *K. phaseolosporus* CBS 2103	lactose	3,44	$1,37 \times 10^2$
K. bulgaricus lys x *K. phaseolosporus* CBS 2103	lactose	1,46	0
K. bulgaricus met x *K. phaseolosporus* CBS 2103	lactose	4,29	0
K. bulgaricus lys x *K. polysporus* CBS 6899	lactose	2,60	0
K. bulgaricus met x *K. polysporus* CBS 6899	lactose	3,49	0
K. bulgaricus x *K. thermotolerans* CBS 6924 met	maltose	3,17	0
K. bulgaricus x *K. thermotolerans* CBS 6924 thr	maltose	1,39	0
K. bulgaricus leu x *K. thermotolerans* CBS 6924	lactose	1,59	0
K. bulgaricus lys x *K. thermotolerans* CBS 6924	lactose	1,36	0
K. bulgaricus met x *K. thermotolerans* CBS 6924	lactose	1,54	0
K. bulgaricus x *K. vanudenii* CBS 4372 his	maltose	1,92	$3,65 \times 10^6$
K. bulgaricus x *K. vanudenii* CBS 4372 lys	maltose	1,52	$1,19 \times 10^6$
K. bulgaricus met x *K. vanudenii* CBS 4372	lactose	1,00	$2,20 \times 10^5$

TABLE 12

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. bulgaricus lys x *K. waltii* CBS 6430	lactose	1,67	0
K. bulgaricus met x *K. waltii* CBS 6430	lactose	2,54	0
K. bulgaricus x *K. wickerhamii* CBS 2745 trp	cellobiose	1,70	0
K. bulgaricus lys x *K. wickerhamii* CBS 2745	raffinose	4,03	0
K. bulgaricus met x *K. wickerhamii* CBS 2745	raffinose	4,10	0
K. bulgaricus lys x *K. wikenii* CBS 5671	lactose	2,53	$5,93 \times 10^3$
K. bulgaricus met x *K. wikenii* CBS 5671	lactose	1,90	$7,89 \times 10^2$

TABLE 13

CROSSES INVOLVING *KLUYVEROMYCES CICERISPORUS* CBS 4857 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. cicerisporus x *K. aestuarii* CBS 4438 his	maltose	3,09	0
K. cicerisporus x *K. aestuarii* CBS 4438 ilv	maltose	2,33	0
K. cicerisporus trp x *K. aestuarii* CBS 4438 ilv	maltose	2,20	0
K. cicerisporus trp x *K. aestuarii* CBS 4904 his	lactose	1,24	0
K. cicerisporus ilv x *K. africanus* CBS 2654	lactose	2,66	0
K. cicerisporus trp x *K. africanus* CBS 2654	lactose	4,14	0
K. cicerisporus ilv x *K. blattae* CBS 6284	lactose	1,41	0
K. cicerisporus trp x *K. blattae* CBS 6284	lactose	1,67	0
K. cicerisporus ilv x *K. bulgaricus* CBS 5667 lys	lactose	1,17	$5,64 \times 10^5$
K. cicerisporus ilv x *K. bulgaricus* CBS 5667 met	lactose	0,78	$4,90 \times 10^6$
K. cicerisporus trp x *K. bulgaricus* CBS 5667 lys	lactose	3,88	$7,76 \times 10^3$
K. cicerisporus ilv x *K. delphensis* CBS 2170	lactose	5,92	0
K. cicerisporus trp x *K. delphensis* CBS 2170	lactose	4,69	0
K. cicerisporus x *K. dobzhanskii* CBS 2104 ade	maltose	5,32	$4,44 \times 10^4$
K. cicerisporus ilv x *K. dobzhanskii* CBS 2104	lactose	4,75	$2,88 \times 10^4$
K. cicerisporus trp x *K. dobzhanskii* CBS 2104	lactose	3,16	$8,77 \times 10^5$
K. cicerisporus x *K. drosophilarum* CBS 2896 met thr	maltose	1,04	$1,12 \times 10^4$
K. cicerisporus ilv x *K. drosophilarum* CBS 2896	lactose	3,37	$2,89 \times 10^3$
K. cicerisporus ilv x *K. fragilis* CBS 1556 met	lactose	1,16	$2,05 \times 10^3$
K. cicerisporus trp x *K. fragilis* CBS 1556 leu	lactose	1,59	$4,34 \times 10^3$
K. cicerisporus x *K. lactis* CBS 683 arg	maltose	1,38	$8,15 \times 10^4$
K. cicerisporus x *K. lactis* CBS 683 trp	maltose	4,37	$5,46 \times 10^6$
K. cicerisporus x *K. lactis* CBS 2359 arg ura	maltose	2,39	$8,67 \times 10^6$
K. cicerisporus ilv x *K. lodderi* CBS 2757	lactose	1,42	0
K. cicerisporus trp x *K. lodderi* CBS 2757	lactose	4,21	0

TABLE 13
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. cicerisporus ilv x *K. marxianus* CBS 6556 his	lactose	0,88	$3,32 \times 10^5$
K. cicerisporus trp x *K. marxianus* CBS 6556 leu pro	lactose	1,52	$5,46 \times 10^4$
K. cicerisporus ilv x *K. marxianus* CBS 6923 trp	lactose	2,61	$8,85 \times 10^2$
K. cicerisporus leu x *K. marxianus* CBS 6923 trp	lactose	2,51	$4,78 \times 10^3$
K. cicerisporus trp x *K. marxianus* CBS 6923 lys	lactose	2,67	$2,30 \times 10^3$
K. cicerisporus ilv x *K. phaffii* CBS 4417	lactose	1,05	0
K. cicerisporus trp x *K. phaffii* CBS 4417	lactose	2,13	0
K. cicerisporus ilv x *K. phaseolosporus* CBS 2103	lactose	4,16	$1,03 \times 10^3$
K. cicerisporus trp x *K. phaseolosporus* CBS 2103	lactose	3,80	$2,13 \times 10^3$
K. cicerisporus ilv x *K. polysporus* CBS 6899	lactose	1,04	0
K. cicerisporus trp x *K. polysporus* CBS 6899	lactose	1,28	0
K. cicerisporus x *K. thermotolerans* CBS 6924 thr	maltose	1,85	0
K. cicerisporus ilv x *K. thermotolerans* CBS 6924	lactose	5,22	0
K. cicerisporus trp x *K. thermotolerans* CBS 6924	lactose	2,70	0
K. cicerisporus x *K. vanudenii* CBS 4372 his	maltose	1,64	$1,31 \times 10^5$
K. cicerisporus ilv x *K. vanudenii* CBS 4372	lactose	2,98	$1,28 \times 10^3$
K. cicerisporus trp x *K. vanudenii* CBS 4372	lactose	1,58	$1,01 \times 10^3$
K. cicerisporus ilv x *K. waltii* CBS 6430	lactose	2,17	0
K. cicerisporus trp x *K. waltii* CBS 6430	lactose	3,06	0
K. cicerisporus ilv x *K. wickerhamii* CBS 2745	raffinose	4,32	0
K. cicerisporus trp x *K. wickerhamii* CBS 2745	raffinose	4,89	0
K. cicerisporus ilv x *K. wikenii* CBS 5671	lactose	5,33	$5,23 \times 10^3$
K. cicerisporus trp x *K. wikenii* CBS 5671	lactose	1,49	$8,05 \times 10^3$

TABLE 14

CROSSES INVOLVING *KLUYVEROMYCES DELPHENSIS* CBS 2170
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count x10^8 per ml	Number of recombinants/10^8 mated cell population
K. delphensis x *K. aestuarii* CBS 4438 his	lactose	1,87	0
K. delphensis x *K. aestuarii* CBS 4438 ilv	lactose	1,95	0
K. delphensis x *K. aestuarii* CBS 4904 his	lactose	3,17	0
K. delphensis his x *K. africanus* CBS 2654 ade	galactose	3,23	0
K. delphensis x *K. africanus* CBS 2654 met	galactose	3,05	0
K. delphensis x *K. africanus* CBS 2654 met trp	galactose	4,54	0
K. delphensis x *K. blattae* CBS 6284 his	galactose	3,79	0
K. delphensis x *K. blattae* CBS 6284 met	galactose	2,84	0
K. delphensis x *K. bulgaricus* CBS 5667 lys	lactose	3,38	0
K. delphensis x *K. bulgaricus* CBS 5667 met	lactose	2,86	0
K. delphensis x *K. cicerisporus* CBS 4857 ilv	lactose	5,92	0
K. delphensis x *K. cicerisporus* CBS 4857 trp	lactose	4,69	0
K. delphensis x *K. dobzhanskii* CBS 2104 ade	maltose	3,98	0
K. delphensis x *K. dobzhanskii* CBS 2104 his	maltose	6,11	0
K. delphensis x *K. drosophilarum* CBS 2896 ade	maltose	1,46	0
K. delphensis x *K. drosophilarum* CBS 2896 trp	maltose	1,77	0
K. delphensis x *K. fragilis* CBS 1556 leu	lactose	2,46	0
K. delphensis x *K. fragilis* CBS 1556 met	lactose	3,07	0
K. delphensis x *K. lactis* CBS 683 arg	lactose	6,10	0
K. delphensis x *K. lactis* CBS 683 trp	lactose	3,82	0
K. delphensis x *K. lactis* CBS 2359 arg ura	lactose	6,85	0
K. delphensis x *K. lactis* CBS 6315 ade	lactose	3,61	0
K. delphensis x *K. lodderi* CBS 2757 arg	raffinose	1,18	0
K. delphensis x *K. lodderi* CBS 2757 asp	raffinose	6,38	0
K. delphensis his x *K. lodderi* CBS 2757 arg	galactose	2,10	0
K. delphensis leu x *K. lodderi* CBS 2757 arg	galactose	3,73	0

TABLE 14
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. delphensis x *K. marxianus* CBS 6556 his	lactose	6,61	0
K. delphensis x *K. marxianus* CBS 6556 leu pro	lactose	2,15	0
K. delphensis x *K. marxianus* CBS 6923 ade	lactose	2,55	0
K. delphensis x *K. marxianus* CBS 6923 arg	lactose	3,74	0
K. delphensis x *K. marxianus* CBS 6923 his	lactose	1,39	0
K. delphensis x *K. marxianus* CBS 6923 trp	lactose	3,95	0
K. delphensis x *K. marxianus* CSIR Y293 met	lactose	4,11	0
K. delphensis x *K. marxianus* CSIR Y293 trp	lactose	3,63	0
K. delphensis x *K. phaffii* CBS 4417 ade met	galactose	5,03	0
K. delphensis x *K. phaffii* CBS 4417 arg	galactose	3,74	0
K. delphensis his x *K. phaffii* CBS 4417 arg	galactose	1,18	0
K. delphensis his x *K. phaffii* CBS 4417 lys	galactose	1,21	0
K. delphensis x *K. phaseolosporus* CBS 2103 arg	galactose	1,16	0
K. delphensis x *K. phaseolosporus* CBS 2103 asp	galactose	2,69	0
K. delphensis x *K. polysporus* CBS 6899 ade	galactose	4,11	0
K. delphensis x *K. polysporus* CBS 6899 trp ura	galactose	4,49	0
K. delphensis his x *K. polysporus* CBS 6899 lys	galactose	4,17	0
K. delphensis his x *K. polysporus* CBS 6899 trp	galactose	4,20	0
K. delphensis x *K. thermotolerans* CBS 6924 met	maltose	1,70	0
K. delphensis x *K. thermotolerans* CBS 6924 thr	maltose	4,57	0
K. delphensis x *K. vanudenii* CBS 4372 his	maltose	1,90	0
K. delphensis x *K. vanudenii* CBS 4372 lys	maltose	2,99	0
K. delphensis x *K. waltii* CBS 6430 arg	sucrose	4,59	0
K. delphensis x *K. waltii* CBS 6430 his met	sucrose	3,65	0

TABLE 14
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. delphensis x *K. wickerhamii* CBS 2745 lys	lactose	2,34	0
K. delphensis x *K. wickerhamii* CBS 2745 trp ura	lactose	2,28	0
K. delphensis x *K. wikenii* CBS 5671 thr	raffinose	1,80	0
K. delphensis x *K. wikenii* CBS 5671 ura	raffinose	4,18	0

TABLE 15

CROSSES INVOLVING *KLUYVEROMYCES DOBZHANSKII* CBS 2104 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. dobzhanskii x *K. aestuarii* CBS 4438 his	lactose	3,84	0
K. dobzhanskii x *K. aestuarii* CBS 4438 ilv	lactose	5,42	0
K. dobzhanskii x *K. aestuarii* CBS 4904 his	lactose	7,43	0
K. dobzhanskii ade x *K. africanus* CBS 2654	maltose	3,42	0
K. dobzhanskii his x *K. africanus* CBS 2654	maltose	5,91	0
K. dobzhanskii ade x *K. blattae* CBS 6284	maltose	5,41	0
K. dobzhanskii his x *K. blattae* CBS 6284	maltose	1,91	0
K. dobzhanskii x *K. bulgaricus* CBS 5667 lys	lactose	3,18	$7,20 \times 10^4$
K. dobzhanskii x *K. bulgaricus* CBS 5667 met	lactose	1,25	$9,84 \times 10^5$
K. dobzhanskii ade x *K. bulgaricus* CBS 5667	maltose	3,38	$1,83 \times 10^5$
K. dobzhanskii his x *K. bulgaricus* CBS 5667	maltose	1,87	$2,73 \times 10^3$
K. dobzhanskii x *K. cicerisporus* CBS 4857 ilv	lactose	4,75	$2,88 \times 10^4$
K. dobzhanskii x *K. cicerisporus* CBS 4857 trp	lactose	3,16	$8,77 \times 10^5$
K. dobzhanskii ade x *K. cicerisporus* CBS 4857	maltose	5,32	$4,44 \times 10^4$
K. dobzhanskii ade x *K. delphensis* CBS 2170	maltose	3,98	0
K. dobzhanskii his x *K. delphensis* CBS 2170	maltose	6,11	0
K. dobzhanskii ade x *K. drosophilarum* CBS 2896 lys	maltose	0,82	$2,78 \times 10^5$
K. dobzhanskii ade x *K. drosophilarum* CBS 2896 trp	maltose	4,25	$8,94 \times 10^5$
K. dobzhanskii his x *K. drosophilarum* CBS 2896 lys	maltose	2,33	$2,80 \times 10^5$
K. dobzhanskii x *K. fragilis* CBS 1556 met	lactose	3,34	$9,58 \times 10^4$
K. dobzhanskii ade x *K. fragilis* CBS 1556	maltose	2,83	$2,61 \times 10^3$
K. dobzhanskii his x *K. fragilis* CBS 1556	maltose	2,66	$1,88 \times 10^3$
K. dobzhanskii x *K. lactis* CBS 683 arg	lactose	3,35	$1,35 \times 10^5$
K. dobzhanskii x *K. lactis* CBS 683 trp	lactose	1,56	$7,37 \times 10^5$
K. dobzhanskii x *K. lactis* CBS 2359 arg ura	lactose	1,07	$1,68 \times 10^4$

TABLE 15
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. dobzhanskii ade x *K. lodderi* CBS 2757	maltose	4,02	0
K. dobzhanskii his x *K. lodderi* CBS 2757	maltose	2,86	0
K. dobzhanskii x *K. marxianus* CBS 6556 ade	lactose	3,39	$1,33 \times 10^6$
K. dobzhanskii x *K. marxianus* CBS 6556 leu pro	lactose	1,23	$7,32 \times 10^4$
K. dobzhanskii x *K. marxianus* CBS 6923 asp	lactose	2,80	$7,57 \times 10^5$
K. dobzhanskii x *K. marxianus* CBS 6923 his	lactose	1,27	$7,24 \times 10^4$
K. dobzhanskii his x *K. marxianus* CBS 6923	maltose	3,25	$4,80 \times 10^4$
K. dobzhanskii his x *K. marxianus* CBS 6923 ade	lactose	3,05	$2,58 \times 10^6$
K. dobzhanskii ade x *K. phaffii* CBS 4417	maltose	3,33	0
K. dobzhanskii his x *K. phaffii* CBS 4417	maltose	2,31	0
K. dobzhanskii ade x *K. phaseolosporus* CBS 2103	maltose	2,22	$2,29 \times 10^5$
K. dobzhanskii his x *K. phaseolosporus* CBS 2103	maltose	3,15	$1,90 \times 10^4$
K. dobzhanskii ade x *K. polysporus* CBS 6899	maltose	3,32	0
K. dobzhanskii his x *K. polysporus* CBS 6899	maltose	2,99	0
K. dobzhanskii ade x *K. thermotolerans* CBS 6924 met	maltose	5,48	0
K. dobzhanskii his x *K. thermotolerans* CBS 6924 thr	maltose	6,24	0
K. dobzhanskii ade x *K. vanudenii* CBS 4372 his	maltose	4,74	$1,81 \times 10^5$
K. dobzhanskii ade x *K. vanudenii* CBS 4372 lys	maltose	3,71	$3,49 \times 10^5$
K. dobzhanskii his x *K. vanudenii* CBS 4372 ilv	maltose	1,35	$2,99 \times 10^4$
K. dobzhanskii ade x *K. waltii* CBS 6430	maltose	4,51	0
K. dobzhanskii his x *K. waltii* CBS 6430	maltose	5,48	0
K. dobzhanskii x *K. wickerhamii* CBS 2745 trp	lactose	2,69	$4,16 \times 10^4$
K. dobzhanskii ade x *K. wickerhamii* CBS 2745	maltose	4,62	$7,88 \times 10^5$
K. dobzhanskii his x *K. wickerhamii* CBS 2745	maltose	4,05	$2,19 \times 10^3$
K. dobzhanskii ade x *K. wikenii* CBS 5671	maltose	3,11	$1,47 \times 10^6$
K. dobzhanskii his x *K. wikenii* CBS 5671	maltose	3,45	$5,41 \times 10^3$

TABLE 16

CROSSES INVOLVING *KLUYVEROMYCES DROSOPHILARUM* CBS 2896 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. drosophilarum x *K. aestuarii* CBS 4438 his	lactose	3,23	0
K. drosophilarum x *K. aestuarii* CBS 4438 ilv	lactose	3,80	0
K. drosophilarum x *K. aestuarii* CBS 4904 his	lactose	2,07	0
K. drosophilarum ade x *K. africanus* CBS 2654	maltose	4,73	0
K. drosophilarum lys x *K. africanus* CBS 2654	maltose	2,43	0
K. drosophilarum ade x *K. blattae* CBS 6284	maltose	5,24	0
K. drosophilarum trp x *K. blattae* CBS 6284	maltose	3,54	0
K. drosophilarum x *K. bulgaricus* CBS 5667 lys	lactose	1,01	0
K. drosophilarum x *K. bulgaricus* CBS 5667 met	lactose	1,41	0
K. drosophilarum met thr x *K. bulgaricus* CBS 5667	maltose	1,38	$1,52 \times 10^6$
K. drosophilarum trp x *K. bulgaricus* CBS 5667	maltose	1,65	$1,05 \times 10^5$
K. drosophilarum x *K. cicerisporus* CBS 4857 ilv	lactose	3,37	$2,89 \times 10^3$
K. drosophilarum met thr x *K. cicerisporus* CBS 4857	maltose	1,04	$1,12 \times 10^4$
K. drosophilarum ade x *K. delphensis* CBS 2170	maltose	1,46	0
K. drosophilarum trp x *K. delphensis* CBS 2170	maltose	1,77	0
K. drosophilarum lys x *K. dobzhanskii* CBS 2104 ade	maltose	0,82	$2,78 \times 10^5$
K. drosophilarum lys x *K. dobzhanskii* CBS 2104 his	maltose	2,33	$2,80 \times 10^5$
K. drosophilarum trp x *K. dobzhanskii* CBS 2104 ade	maltose	4,25	$8,94 \times 10^5$
K. drosophilarum x *K. fragilis* CBS 1556 leu	lactose	4,83	$3,89 \times 10^5$
K. drosophilarum x *K. fragilis* CBS 1556 met	lactose	1,93	$1,14 \times 10^3$
K. drosophilarum trp x *K. fragilis* CBS 1556	maltose	1,83	$5,02 \times 10^5$
K. drosophilarum x *K. lactis* CBS 683 arg	lactose	2,33	$1,18 \times 10^5$
K. drosophilarum x *K. lactis* CBS 683 trp	lactose	5,48	$3,59 \times 10^5$
K. drosophilarum x *K. lactis* CBS 2359 arg ura	lactose	3,28	$4,02 \times 10^4$

TABLE 16
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. drosophilarum ade x *K. lodderi* CBS 2757	maltose	3,09	0
K. drosophilarum met thr x *K. lodderi* CBS 2757	maltose	2,12	0
K. drosophilarum x *K. marxianus* CBS 6556 his	lactose	1,53	$5,23 \times 10^6$
K. drosophilarum x *K. marxianus* CBS 6556 leu pro	lactose	1,10	$1,26 \times 10^6$
K. drosophilarum x *K. marxianus* CBS 6923 ade	lactose	3,19	$1,38 \times 10^4$
K. drosophilarum x *K. marxianus* CBS 6923 his	lactose	0,65	$1,15 \times 10^6$
K. drosophilarum x *K. marxianus* CBS 6923 lys	lactose	0,75	$1,19 \times 10^3$
K. drosophilarum ade x *K. phaffii* CBS 4417	maltose	5,05	0
K. drosophilarum trp x *K. phaffii* CBS 4417	maltose	1,11	0
K. drosophilarum ade x *K. phaseolosporus* CBS 2103	maltose	2,67	$5,66 \times 10^6$
K. drosophilarum lys x *K. phaseolosporus* CBS 2103	maltose	2,90	$1,58 \times 10^6$
K. drosophilarum trp x *K. phaseolosporus* CBS 2103	maltose	2,34	$4,83 \times 10^6$
K. drosophilarum ade x *K. polysporus* CBS 6899	maltose	3,56	0
K. drosophilarum met thr x *K. polysporus* CBS 6899	maltose	5,71	0
K. drosophilarum lys x *K. thermotolerans* CBS 6924 met	maltose	1,57	0
K. drosophilarum trp x *K. thermotolerans* CBS 6924 thr	maltose	1,32	0
K. drosophilarum ade x *K. vanudenii* CBS 4372 ura	maltose	2,36	$7,03 \times 10^5$
K. drosophilarum met thr x *K. vanudenii* CBS 4372 his	maltose	0,87	$4,04 \times 10^6$
K. drosophilarum trp x *K. vanudenii* CBS 4372 his	maltose	2,34	$7,61 \times 10^6$
K. drosophilarum ade x *K. waltii* CBS 6430	maltose	1,78	0
K. drosophilarum lys x *K. waltii* CBS 6430	maltose	2,21	0
K. drosophilarum met thr x *K. waltii* CBS 6430	maltose	1,62	$2,11 \times 10^6$
K. drosophilarum trp x *K. waltii* CBS 6430	maltose	1,36	$1,47 \times 10^5$

TABLE 16

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. drosophilarum x *K. wickerhamii* CBS 2745 trp	lactose	0,93	$2,83 \times 10^3$
K. drosophilarum ade x *K. wickerhamii* CBS 2745	maltose	4,90	$6,65 \times 10^5$
K. drosophilarum trp x *K. wickerhamii* CBS 2745	maltose	4,62	$2,06 \times 10^5$
K. drosophilarum ade x *K. wikenii* CBS 5671	maltose	1,27	$5,59 \times 10^6$
K. drosophilarum ade x *K. wikenii* CBS 5671 ura	maltose	0,96	$2,00 \times 10^5$
K. drosophilarum met thr x *K. wikenii* CBS 5671	maltose	1,45	$4,48 \times 10^6$
K. drosophilarum trp x *K. wikenii* CBS 5671	maltose	1,40	$5,50 \times 10^5$

TABLE 17

CROSSES INVOLVING *KLUYVEROMYCES FRAGILIS* CBS 1556
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. fragilis x *K. aestuarii* CBS 4438 his	maltose	1,82	0
K. fragilis x *K. aestuarii* CBS 4438 ilv	maltose	2,63	0
K. fragilis met x *K. aestuarii* CBS 4904 his	lactose	1,35	0
K. fragilis leu x *K. africanus* CBS 2654	lactose	4,55	0
K. fragilis met x *K. africanus* CBS 2654	lactose	1,26	0
K. fragilis leu x *K. blattae* CBS 6284	lactose	1,38	0
K. fragilis met x *K. blattae* CBS 6284	lactose	1,14	0
K. fragilis leu x *K. bulgaricus* CBS 5667 lys	lactose	1,45	$1,20 \times 10^5$
K. fragilis leu x *K. bulgaricus* CBS 5667 met	lactose	0,66	$5,56 \times 10^5$
K. fragilis leu x *K. cicerisporus* CBS 4857 trp	lactose	1,59	$4,34 \times 10^3$
K. fragilis met x *K. cicerisporus* CBS 4857 ilv	lactose	1,16	$2,05 \times 10^3$
K. fragilis leu x *K. delphensis* CBS 2170	lactose	2,46	0
K. fragilis met x *K. delphensis* CBS 2170	lactose	3,07	0
K. fragilis x *K. dobzhanskii* CBS 2104 ade	maltose	2,83	$2,61 \times 10^3$
K. fragilis x *K. dobzhanskii* CBS 2104 his	maltose	2,66	$1,88 \times 10^3$
K. fragilis met x *K. dobzhanskii* CBS 2104	lactose	3,34	$9,58 \times 10^4$
K. fragilis x *K. drosophilarum* CBS 2896 trp	maltose	1,83	$5,02 \times 10^5$
K. fragilis leu x *K. drosophilarum* CBS 2896	lactose	4,83	$3,89 \times 10^5$
K. fragilis met x *K. drosophilarum* CBS 2896	lactose	1,93	$1,14 \times 10^3$
K. fragilis x *K. lactis* CBS 683 arg	maltose	2,85	$1,79 \times 10^6$
K. fragilis x *K. lactis* CBS 683 trp	maltose	2,88	$7,92 \times 10^5$
K. fragilis x *K. lactis* CBS 2359 arg ura	maltose	2,05	$2,63 \times 10^5$
K. fragilis leu x *K. lodderi* CBS 2757	lactose	1,61	0
K. fragilis met x *K. lodderi* CBS 2757	lactose	1,77	0

TABLE 17
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. fragilis met x *K. marxianus* CBS 6556 his	lactose	1,51	$4,38 \times 10^4$
K. fragilis met x *K. marxianus* CBS 6556 leu pro	lactose	0,62	$2,26 \times 10^3$
K. fragilis leu x *K. marxianus* CBS 6923 lys	lactose	0,86	$1,31 \times 10^3$
K. fragilis met x *K. marxianus* CBS 6923 lys	lactose	1,30	$6,62 \times 10^4$
K. fragilis met x *K. marxianus* CBS 6923 trp	lactose	1,43	$6,43 \times 10^4$
K. fragilis leu x *K. phaffii* CBS 4417	lactose	1,14	0
K. fragilis met x *K. phaffii* CBS 4417	lactose	1,24	0
K. fragilis leu x *K. phaseolosporus* CBS 2103	lactose	2,31	$7,10 \times 10^4$
K. fragilis met x *K. phaseolosporus* CBS 2103	lactose	2,36	$1,82 \times 10^5$
K. fragilis leu x *K. polysporus* CBS 6899	lactose	2,06	0
K. fragilis met x *K. polysporus* CBS 6899	lactose	2,23	0
K. fragilis x *K. thermotolerans* CBS 6924 thr	maltose	5,41	0
K. fragilis leu x *K. thermotolerans* CBS 6924	lactose	3,03	0
K. fragilis met x *K. thermotolerans* CBS 6924	lactose	5,42	0
K. fragilis x *K. vanudenii* CBS 4372 lys	maltose	1,75	$9,37 \times 10^5$
K. fragilis leu x *K. vanudenii* CBS 4372	lactose	4,74	$6,33 \times 10^4$
K. fragilis met x *K. vanudenii* CBS 4372	lactose	1,52	$1,32 \times 10^3$
K. fragilis leu x *K. waltii* CBS 6430	lactose	1,22	0
K. fragilis met x *K. waltii* CBS 6430	lactose	1,31	0
K. fragilis leu x *K. wickerhamii* CBS 2745	raffinose	6,14	0
K. fragilis met x *K. wickerhamii* CBS 2745	raffinose	5,87	0
K. fragilis leu x *K. wikenii* CBS 5671	lactose	1,05	$4,00 \times 10^4$
K. fragilis met x *K. wikenii* CBS 5671	lactose	1,77	$5,65 \times 10^3$

TABLE 18

CROSSES INVOLVING *KLUYVEROMYCES LACTIS* CBS 683
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lactis arg x *K. aestuarii* CBS 4438 ilv	lactose	4,35	0
K. lactis trp x *K. aestuarii* CBS 4438 ilv	lactose	3,53	0
K. lactis arg x *K. aestuarii* CBS 4904 his	lactose	3,18	0
K. lactis trp x *K. aestuarii* CBS 4904 his	lactose	3,80	0
K. lactis arg x *K. africanus* CBS 2654	lactose	4,44	0
K. lactis trp x *K. africanus* CBS 2654	lactose	4,30	0
K. lactis arg x *K. blattae* CBS 6284	lactose	3,12	0
K. lactis trp x *K. blattae* CBS 6284	lactose	2,72	0
K. lactis arg x *K. bulgaricus* CBS 5667	maltose	1,85	$1,66 \times 10^6$
K. lactis trp x *K. bulgaricus* CBS 5667	maltose	3,37	$7,98 \times 10^5$
K. lactis arg x *K. cicerisporus* CBS 4857	maltose	1,38	$8,15 \times 10^4$
K. lactis trp x *K. cicerisporus* CBS 4857	maltose	4,37	$5,46 \times 10^6$
K. lactis arg x *K. delphensis* CBS 2170	lactose	6,10	0
K. lactis trp x *K. delphensis* CBS 2170	lactose	3,82	0
K. lactis arg x *K. dobzhanskii* CBS 2140	lactose	3,35	$1,35 \times 10^5$
K. lactis trp x *K. dobzhanskii* CBS 2140	lactose	1,56	$7,37 \times 10^5$
K. lactis arg x *K. drosophilarum* CBS 2896	lactose	2,33	$1,18 \times 10^5$
K. lactis trp x *K. drosophilarum* CBS 2896	lactose	5,48	$3,59 \times 10^5$
K. lactis arg x *K. fragilis* CBS 1556	maltose	2,85	$1,79 \times 10^6$
K. lactis trp x *K. fragilis* CBS 1556	maltose	2,88	$7,92 \times 10^5$
K. lactis arg x *K. lodderi* CBS 2757	lactose	4,19	0
K. lactis trp x *K. lodderi* CBS 2757	lactose	4,05	0
K. lactis arg x *K. marxianus* CBS 6556	maltose	2,42	$2,69 \times 10^4$
K. lactis trp x *K. marxianus* CBS 6556	maltose	4,16	$5,00 \times 10^5$
K. lactis arg x *K. marxianus* CBS 6923	maltose	4,40	$5,32 \times 10^3$
K. lactis trp x *K. marxianus* CBS 6923	maltose	3,17	$1,63 \times 10^6$

TABLE 18

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lactis arg x *K. phaffii* CBS 4417	lactose	3,57	0
K. lactis trp x *K. phaffii* CBS 4417	lactose	5,72	0
K. lactis arg x *K. phaseolosporus* CBS 2103	lactose	1,27	$9,69 \times 10^5$
K. lactis trp x *K. phaseolosporus* CBS 2103	lactose	5,18	$1,31 \times 10^6$
K. lactis arg x *K. polysporus* CBS 6899	lactose	2,45	0
K. lactis trp x *K. polysporus* CBS 6899	lactose	2,46	0
K. lactis arg x *K. thermotolerans* CBS 6924	lactose	3,74	0
K. lactis trp x *K. thermotolerans* CBS 6924	lactose	6,09	0
K. lactis arg x *K. vanudenii* CBS 4372	lactose	2,45	$8,73 \times 10^5$
K. lactis trp x *K. vanudenii* CBS 4372	lactose	1,29	$7,51 \times 10^6$
K. lactis arg x *K. waltii* CBS 6430	lactose	4,11	0
K. lactis trp x *K. waltii* CBS 6430	lactose	4,99	0
K. lactis arg x *K. wickerhamii* CBS 2745	maltose	5,48	$8,94 \times 10^3$
K. lactis trp x *K. wickerhamii* CBS 2745	maltose	3,98	$1,10 \times 10^5$
K. lactis arg x *K. wikenii* CBS 5671	lactose	1,70	$2,65 \times 10^3$
K. lactis trp x *K. wikenii* CBS 5671	lactose	1,68	$5,10 \times 10^5$

TABLE 19

CROSSES INVOLVING *KLUYVEROMYCES LACTIS* CBS 2359 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lactis arg x *K. aestuarii* CBS 4438 his	lactose	1,63	0
K. lactis arg ura x *K. aestuarii* CBS 4438 ilv	lactose	2,93	0
K. lactis arg ura x *K. aestuarii* CBS 4904	maltose	2,16	0
K. lactis arg ura x *K. aestuarii* CBS 4904 ade	lactose	2,93	0
K. lactis arg ura x *K. aestuarii* CBS 4904 his	lactose	2,83	0
K. lactis arg ura x *K. aestuarii* CBS 4904 met	lactose	1,37	0
K. lactis arg ura x *K. africanus* CBS 2654	lactose	3,27	0
K. lactis arg ura x *K. blattae* CBS 6284	lactose	1,61	0
K. lactis arg ura x *K. bulgaricus* CBS 5667	maltose	4,51	$1,02 \times 10^6$
K. lactis arg ura x *K. cicerisporus* CBS 4857	maltose	2,39	$8,67 \times 10^6$
K. lactis arg ura x *K. delphensis* CBS 2170	lactose	6,85	0
K. lactis arg ura x *K. dobzhanskii* CBS 2104	lactose	1,07	$1,68 \times 10^4$
K. lactis arg ura x *K. drosophilarum* CBS 2896	lactose	3,28	$4,02 \times 10^4$
K. lactis arg ura x *K. fragilis* CBS 1556	maltose	2,05	$2,63 \times 10^5$
K. lactis arg ura x *K. lodderi* CBS 2757	lactose	1,52	0
K. lactis arg ura x *K. marxianus* CBS 6556	maltose	3,93	$1,55 \times 10^6$
K. lactis arg x *K. marxianus* CBS 6923 ade	lactose	1,43	$6,50 \times 10^5$
K. lactis arg x *K. marxianus* CBS 6923 lys	lactose	1,33	$4,57 \times 10^6$
K. lactis arg ura x *K. marxianus* CBS 6923	maltose	4,67	$9,76 \times 10^4$
K. lactis arg ura x *K. phaffii* CBS 4417	lactose	1,28	0
K. lactis arg ura x *K. phaseolosporus* CBS 2103	lactose	2,85	$1,63 \times 10^4$
K. lactis arg ura x *K. polysporus* CBS 6899	lactose	2,65	0
K. lactis arg ura x *K. thermotolerans* CBS 6924	lactose	4,32	0
K. lactis arg ura x *K. vanudenii* CBS 4372	lactose	2,99	$4,45 \times 10^4$
K. lactis arg ura x *K. waltii* CBS 6430	lactose	1,77	0
K. lactis arg ura x *K. wickerhamii* CBS 2745	raffinose	7,58	0
K. lactis arg ura x *K. wickerhamii* CBS 2745 trp	lactose	1,15	78
K. lactis arg ura x *K. wikenii* CBS 5671	lactose	1,99	$1,83 \times 10^6$

TABLE 20

CROSSES INVOLVING *KLUYVEROMYCES LACTIS* CBS 6315 AND SOME OTHER SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lactis trp x *K. aestuarii* CBS 4438 his	lactose	1,68	0
K. lactis trp x *K. aestuarii* CBS 4438 ilv	lactose	2,98	0
K. lactis trp x *K. aestuarii* CBS 4904 his	lactose	2,89	0
K. lactis ade x *K. africanus* CBS 2654	lactose	2,98	0
K. lactis ade x *K. blattae* CBS 6284	lactose	1,85	0
K. lactis ade x *K. delphensis* CBS 2170	lactose	3,61	0
K. lactis ade x *K. lodderi* CBS 2757	lactose	1,63	0
K. lactis ade x *K. phaffii* CBS 4417	lactose	1,78	0
K. lactis ade x *K. polysporus* CBS 6899	lactose	4,60	0
K. lactis ade x *K. thermotolerans* CBS 6924	lactose	5,02	0
K. lactis ade x *K. waltii* CBS 6430	lactose	1,13	0
K. lactis ade x *K. wickerhamii* CBS 2745	raffinose	4,53	0

TABLE 21

CROSSES INVOLVING *KLUYVEROMYCES LODDERI* CBS 2757 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lodderi x *K. aestuarii* CBS 4438 his	lactose	1,54	0
K. lodderi x *K. aestuarii* CBS 4438 ilv	lactose	4,09	0
K. lodderi x *K. aestuarii* CBS 4904 his	lactose	9,04	0
K. lodderi arg x *K. africanus* CBS 2654	raffinose	1,87	0
K. lodderi arg x *K. africanus* CBS 2654 ade	raffinose	1,29	0
K. lodderi asp x *K. africanus* CBS 2654	raffinose	1,37	0
K. lodderi arg x *K. blattae* CBS 6284	sucrose	1,41	0
K. lodderi asp x *K. blattae* CBS 6284	sucrose	1,84	0
K. lodderi x *K. bulgaricus* CBS 5667 lys	lactose	2,41	0
K. lodderi x *K. bulgaricus* CBS 5667 met	lactose	1,77	0
K. lodderi x *K. cicerisporus* CBS 4857 ilv	lactose	1,42	0
K. lodderi x *K. cicerisporus* CBS 4857 trp	lactose	4,21	0
K. lodderi arg x *K. delphensis* CBS 2170	raffinose	1,18	0
K. lodderi arg x *K. delphensis* CBS 2170 his	galactose	2,10	0
K. lodderi arg x *K. delphensis* CBS 2170 leu	galactose	3,73	0
K. lodderi asp x *K. delphensis* CBS 2170	raffinose	6,38	0
K. lodderi x *K. dobzhanskii* CBS 2104 ade	maltose	4,02	0
K. lodderi x *K. dobzhanskii* CBS 2104 his	maltose	2,86	0
K. lodderi x *K. drosophilarum* CBS 2896 ade	maltose	3,09	0
K. lodderi x *K. drosophilarum* CBS 2896 met thr	maltose	2,12	0
K. lodderi x *K. fragilis* CBS 1556 leu	lactose	1,61	0
K. lodderi x *K. fragilis* CBS 1556 met	lactose	1,77	0
K. lodderi x *K. lactis* CBS 683 arg	lactose	4,19	0
K. lodderi x *K. lactis* CBS 683 trp	lactose	4,05	0
K. lodderi x *K. lactis* CBS 2359 arg ura	lactose	1,52	0
K. lodderi x *K. lactis* CBS 6315 ade	lactose	1,63	0

TABLE 21

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lodderi x *K. marxianus* CBS 6556 his	lactose	2,96	0
K. lodderi x *K. marxianus* CBS 6556 leu pro	lactose	4,61	0
K. lodderi x *K. marxianus* CBS 6923 ade	lactose	1,80	0
K. lodderi x *K. marxianus* CBS 6923 arg	lactose	1,87	0
K. lodderi x *K. marxianus* CBS 6923 asp	lactose	1,40	0
K. lodderi x *K. marxianus* CBS 6923 lys	lactose	1,42	0
K. lodderi x *K. marxianus* CSIR Y293 leu	lactose	3,53	0
K. lodderi x *K. marxianus* CSIR Y293 met	lactose	5,90	0
K. lodderi arg x *K. phaffii* CBS 4417	raffinose	1,63	0
K. lodderi arg x *K. phaffii* CBS 4417 ade met	glucose	1,23	0
K. lodderi arg x *K. phaffii* CBS 4417 lys	glucose	2,20	0
K. lodderi asp x *K. phaffii* CBS 4417	raffinose	1,73	0
K. lodderi arg x *K. phaseolosporus* CBS 2103 met thr	glucose	1,45	0
K. lodderi asp x *K. phaseolosporus* CBS 2103 ade	glucose	1,09	0
K. lodderi asp x *K. phaseolosporus* CBS 2103 met thr	glucose	1,27	0
K. lodderi arg x *K. polysporus* CBS 6899 ade	glucose	1,74	0
K. lodderi arg x *K. polysporus* CBS 6899 met	glucose	1,35	0
K. lodderi arg x *K. polysporus* CBS 6899 trp ura	glucose	1,70	0
K. lodderi asp x *K. polysporus* CBS 6899 met	glucose	1,59	0
K. lodderi asp x *K. polysporus* CBS 6899 trp	glucose	1,68	0
K. lodderi x *K. thermotolerans* CBS 6924 met	maltose	1,69	0
K. lodderi x *K. thermotolerans* CBS 6924 thr	maltose	4,64	0
K. lodderi x *K. vanudenii* CBS 4372 his	maltose	1,69	0
K. lodderi x *K. vanudenii* CBS 4372 lys	maltose	3,95	0
K. lodderi arg x *K. waltii* CBS 6430	galactose	1,65	0
K. lodderi asp x *K. waltii* CBS 6430	galactose	1,38	0

TABLE 21

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lodderi x *K. wickerhamii* CBS 2745 ade	lactose	5,97	0
K. lodderi x *K. wickerhamii* CBS 2745 trp ura	lactose	2,61	0
K. lodderi arg x *K. wikenii* CBS 5671 ade	glucose	1,11	0
K. lodderi arg x *K. wikenii* CBS 5671 his	glucose	1,20	0
K. lodderi arg x *K. wikenii* CBS 5671 lys	glucose	1,21	0
K. lodderi asp x *K. wikenii* CBS 5671 ade	glucose	2,36	0
K. lodderi asp x *K. wikenii* CBS 5671 lys	glucose	1,51	0

TABLE 22

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 6556 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus x *K. aestuarii* CBS 4438 his	maltose	2,51	0
K. marxianus x *K. aestuarii* CBS 4438 ilv	maltose	3,41	0
K. marxianus his x *K. aestuarii* CBS 4438 ilv	maltose	4,36	0
K. marxianus ade x *K. aestuarii* CBS 4904 his	lactose	1,47	0
K. marxianus leu pro x *K. aestuarii* CBS 4904 his	lactose	1,29	0
K. marxianus his x *K. africanus* CBS 2654	lactose	4,88	0
K. marxianus leu pro x *K. africanus* CBS 2654	lactose	5,90	0
K. marxianus his x *K. blattae* CBS 6284	lactose	1,62	0
K. marxianus leu pro x *K. blattae* CBS 6284	lactose	1,26	0
K. marxianus his x *K. bulgaricus* CBS 5667 met	lactose	1,79	$2,84 \times 10^6$
K. marxianus leu pro x *K. bulgaricus* CBS 5667 lys	lactose	1,41	$5,59 \times 10^6$
K. marxianus his x *K. cicerisporus* CBS 4857 ilv	lactose	0,88	$3,32 \times 10^5$
K. marxianus leu pro x *K. cicerisporus* CBS 4857 trp	lactose	1,52	$5,46 \times 10^4$
K. marxianus his x *K. delphensis* CBS 2170	lactose	6,61	0
K. marxianus leu pro x *K. delphensis* CBS 2170	lactose	2,15	0
K. marxianus ade x *K. dobzhanskii* CBS 2104	lactose	3,39	$1,33 \times 10^6$
K. marxianus leu pro x *K. dobzhanskii* CBS 2104	lactose	1,23	$7,32 \times 10^4$
K. marxianus his x *K. drosophilarum* CBS 2896	lactose	1,53	$5,23 \times 10^6$
K. marxianus leu pro x *K. drosophilarum* CBS 2896	lactose	1,10	$1,26 \times 10^6$
K. marxianus his x *K. fragilis* CBS 1556 met	lactose	1,51	$4,38 \times 10^4$
K. marxianus leu pro x *K. fragilis* CBS 1556 met	lactose	0,62	$2,26 \times 10^3$
K. marxianus x *K. lactis* CBS 683 arg	maltose	2,42	$2,69 \times 10^4$
K. marxianus x *K. lactis* CBS 683 trp	maltose	4,16	$5,00 \times 10^5$
K. marxianus x *K. lactis* CBS 2359 arg ura	maltose	3,93	$1,55 \times 10^6$
K. marxianus his x *K. lodderi* CBS 2757	lactose	2,96	0
K. marxianus leu pro x *K. lodderi* CBS 2757	lactose	4,61	0

TABLE 22
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus his x *K. phaffii* CBS 4417	lactose	3,28	0
K. marxianus leu pro x *K. phaffii* CBS 4417	lactose	1,28	0
K. marxianus ade x *K. phaseolosporus* CBS 2103	lactose	2,11	$1,04 \times 10^3$
K. marxianus leu pro x *K. phaseolosporus* CBS 2103	lactose	1,46	$1,37 \times 10^4$
K. marxianus his x *K. polysporus* CBS 6899	lactose	2,51	0
K. marxianus leu pro x *K. polysporus* CBS 6899	lactose	4,12	0
K. marxianus x *K. thermotolerans* CBS 6924 met	maltose	2,78	0
K. marxianus x *K. thermotolerans* CBS 6924 thr	maltose	1,84	0
K. marxianus his x *K. thermotolerans* CBS 6924	lactose	3,07	0
K. marxianus leu pro x *K. thermotolerans* CBS 6924	lactose	4,92	0
K. marxianus his x *K. vanudenii* CBS 4372	lactose	1,94	$8,92 \times 10^5$
K. marxianus leu pro x *K. vanudenii* CBS 4372	lactose	1,20	$8,42 \times 10^5$
K. marxianus his x *K. waltii* CBS 6430	lactose	1,87	0
K. marxianus leu pro x *K. waltii* CBS 6430	lactose	2,76	0
K. marxianus his x *K. wickerhamii* CBS 2745	raffinose	6,54	0
K. marxianus leu pro x *K. wickerhamii* CBS 2745	raffinose	5,46	0
K. marxianus ade x *K. wikenii* CBS 5671	lactose	1,41	$1,32 \times 10^6$
K. marxianus leu pro x *K. wikenii* CBS 5671	lactose	1,06	$4,14 \times 10^5$

TABLE 23

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 6923
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus x *K. aestuarii* CBS 4438 his	maltose	2,92	0
K. marxianus x *K. aestuarii* CBS 4438 ilv	maltose	1,76	0
K. marxianus ade x *K. aestuarii* CBS 4438 ilv	lactose	2,63	0
K. marxianus lys x *K. aestuarii* CBS 4438	L-arabinose	2,61	0
K. marxianus lys x *K. aestuarii* CBS 4438 ilv	lactose	2,20	0
K. marxianus trp x *K. aestuarii* CBS 4438	D-ribose	2,57	0
K. marxianus ura x *K. aestuarii* CBS 4438 his	lactose	2,55	0
K. marxianus ade x *K. aestuarii* CBS 4904 his	lactose	1,87	0
K. marxianus leu x *K. aestuarii* CBS 4904	D-ribose	2,31	0
K. marxianus leu x *K. aestuarii* CBS 4904 his	lactose	3,98	0
K. marxianus lys x *K. aestuarii* CBS 4904	L-arabinose	2,61	0
K. marxianus lys x *K. aestuarii* CBS 4904 his	lactose	1,75	0
K. marxianus trp x *K. aestuarii* CBS 4904	D-ribose	2,57	0
K. marxianus ade x *K. africanus* CBS 2654	lactose	1,99	0
K. marxianus arg x *K. africanus* CBS 2654	lactose	1,60	0
K. marxianus lys x *K. africanus* CBS 2654	lactose	2,25	0
K. marxianus trp x *K. africanus* CBS 2654	lactose	1,72	0
K. marxianus leu x *K. blattae* CBS 6284	lactose	1,48	0
K. marxianus lys x *K. blattae* CBS 6284	lactose	1,12	0
K. marxianus trp x *K. blattae* CBS 6284	lactose	1,52	0
K. marxianus ura x *K. blattae* CBS 6284	lactose	2,02	0
K. marxianus ade x *K. bulgaricus* CBS 5667	cellobiose	1,15	$8,96 \times 10^4$
K. marxianus his x *K. bulgaricus* CBS 5667	cellobiose	1,58	$5,79 \times 10^4$
K. marxianus lys x *K. bulgaricus* CBS 5667 met	lactose	1,07	$5,30 \times 10^3$
K. marxianus trp x *K. bulgaricus* CBS 5667 lys	lactose	2,06	$3,20 \times 10^3$
K. marxianus ura x *K. bulgaricus* CBS 5667	cellobiose	1,49	$2,45 \times 10^4$

TABLE 23

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus lys x *K. cicerisporus* CBS 4857 trp	lactose	2,67	$2,30 \times 10^3$
K. marxianus trp x *K. cicerisporus* CBS 4857 ilv	lactose	2,61	$8,85 \times 10^2$
K. marxianus trp x *K. cicerisporus* CBS 4857 leu	lactose	2,51	$4,78 \times 10^3$
K. marxianus ade x *K. delphensis* CBS 2170	lactose	2,55	0
K. marxianus arg x *K. delphensis* CBS 2170	lactose	3,74	0
K. marxianus his x *K. delphensis* CBS 2170	lactose	1,39	0
K. marxianus trp x *K. delphensis* CBS 2170	lactose	3,95	0
K. marxianus x *K. dobzhanskii* CBS 2104 his	maltose	3,25	$4,80 \times 10^4$
K. marxianus ade x *K. dobzhanskii* CBS 2104 his	lactose	3,05	$2,58 \times 10^6$
K. marxianus asp x *K. dobzhanskii* CBS 2104	lactose	2,80	$7,57 \times 10^5$
K. marxianus his x *K. dobzhanskii* CBS 2104	lactose	1,27	$7,24 \times 10^4$
K. marxianus ade x *K. drosophilarum* CBS 2896	lactose	3,19	$1,38 \times 10^4$
K. marxianus his x *K. drosophilarum* CBS 2896	lactose	0,65	$1,15 \times 10^6$
K. marxianus lys x *K. drosophilarum* CBS 2896	lactose	0,75	$1,19 \times 10^3$
K. marxianus lys x *K. fragilis* CBS 1556 leu	lactose	0,86	$1,31 \times 10^3$
K. marxianus lys x *K. fragilis* CBS 1556 met	lactose	1,30	$6,62 \times 10^4$
K. marxianus trp x *K. fragilis* CBS 1556 met	lactose	1,43	$6,43 \times 10^4$
K. marxianus x *K. lactis* CBS 683 arg	maltose	4,40	$5,32 \times 10^3$
K. marxianus x *K. lactis* CBS 683 trp	maltose	3,17	$1,63 \times 10^6$
K. marxianus x *K. lactis* CBS 2359 arg ura	maltose	4,67	$9,76 \times 10^4$
K. marxianus ade x *K. lactis* CBS 2359 arg	lactose	1,43	$6,50 \times 10^5$
K. marxianus lys x *K. lactis* CBS 2359 arg	lactose	1,33	$4,57 \times 10^6$
K. marxianus ade x *K. lodderi* CBS 2757	lactose	1,80	0
K. marxianus arg x *K. lodderi* CBS 2757	lactose	1,87	0
K. marxianus asp x *K. lodderi* CBS 2757	lactose	1,40	0
K. marxianus lys x *K. lodderi* CBS 2757	lactose	1,42	0

TABLE 23
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus ade x *K. phaffii* CBS 4417	lactose	1,77	0
K. marxianus his x *K. phaffii* CBS 4417	lactose	1,42	0
K. marxianus lys x *K. phaffii* CBS 4417	lactose	3,15	0
K. marxianus trp x *K. phaffii* CBS 4417	lactose	1,33	0
K. marxianus ade x *K. phaseolosporus* CBS 2103	lactose	1,93	$6,68 \times 10^4$
K. marxianus lys x *K. phaseolosporus* CBS 2103	lactose	0,95	$3,16 \times 10^2$
K. marxianus trp x *K. phaseolosporus* CBS 2103	lactose	2,83	$4,06 \times 10^3$
K. marxianus ade x *K. polysporus* CBS 6899	lactose	2,97	0
K. marxianus asp x *K. polysporus* CBS 6899	lactose	3,72	0
K. marxianus his x *K. polysporus* CBS 6899	lactose	1,88	0
K. marxianus lys x *K. polysporus* CBS 6899	lactose	1,68	0
K. marxianus x *K. thermotolerans* CBS 6924 met	maltose	3,44	0
K. marxianus x *K. thermotolerans* CBS 6924 thr	maltose	4,28	0
K. marxianus ade x *K. thermotolerans* CBS 6924	lactose	1,47	40
K. marxianus his x *K. thermotolerans* CBS 6924	lactose	2,31	0
K. marxianus leu x *K. thermotolerans* CBS 6924	lactose	1,37	58
K. marxianus lys x *K. thermotolerans* CBS 6924	lactose	2,95	0
K. marxianus trp x *K. thermotolerans* CBS 6924	lactose	3,37	0
K. marxianus ura x *K. thermotolerans* CBS 6924	lactose	3,47	0
K. marxianus x *K. vanudenii* CBS 4372 his	maltose	1,16	$3,71 \times 10^6$
K. marxianus ade x *K. vanudenii* CBS 4372	lactose	2,99	$3,91 \times 10^5$
K. marxianus his x *K. vanudenii* CBS 4372	lactose	1,64	$5,85 \times 10^5$
K. marxianus ura x *K. vanudenii* CBS 4372	lactose	1,70	$2,32 \times 10^5$

TABLE 23

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus ade x *K. waltii* CBS 6430	lactose	1,37	0
K. marxianus asp x *K. waltii* CBS 6430	lactose	1,02	0
K. marxianus his x *K. waltii* CBS 6430	lactose	1,33	0
K. marxianus ura x *K. waltii* CBS 6430	lactose	2,46	0
K. marxianus ade x *K. wickerhamii* CBS 2745	raffinose	3,40	0
K. marxianus his x *K. wickerhamii* CBS 2745	raffinose	1,65	0
K. marxianus leu x *K. wickerhamii* CBS 2745	raffinose	3,08	0
K. marxianus lys x *K. wickerhamii* CBS 2745	raffinose	2,65	0
K. marxianus trp x *K. wickerhamii* CBS 2745	raffinose	3,97	0
K. marxianus ura x *K. wickerhamii* CBS 2745	raffinose	5,48	0
K. marxianus ade x *K. wickerhamii* CBS 2745 arg lys	lactose	1,26	0
K. marxianus ade x *K. wickerhamii* CBS 2745 lys	lactose	2,05	0
K. marxianus ade x *K. wickerhamii* CBS 2745 trp	lactose	1,17	0
K. marxianus asp x *K. wickerhamii* CBS 2745 trp	lactose	3,47	0
K. marxianus his x *K. wickerhamii* CBS 2745 arg lys	lactose	1,61	0
K. marxianus his x *K. wickerhamii* CBS 2745 trp	lactose	1,65	0
K. marxianus his x *K. wickerhamii* CBS 2745 ura	lactose	1,40	0
K. marxianus leu x *K. wickerhamii* CBS 2745 arg lys	lactose	1,58	0
K. marxianus lys x *K. wickerhamii* CBS 2745 trp	lactose	1,70	0
K. marxianus ura x *K. wickerhamii* CBS 2745 trp	lactose	2,09	0
K. marxianus ade x *K. wikenii* CBS 5671	lactose	1,69	$2,43 \times 10^4$
K. marxianus his x *K. wikenii* CBS 5671	lactose	1,68	$3,15 \times 10^5$
K. marxianus lys x *K. wikenii* CBS 5671	lactose	1,41	$1,03 \times 10^5$

TABLE 24

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* AND THREE ADDITIONAL STRAINS OF *K. WICKERHAMII*

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus CBS 6556 ade x *K. wickerhamii* UCD 56-40	raffinose	1,15	0
K. marxianus CBS 6556 his x *K. wickerhamii* UCD 56-40	raffinose	1,71	0
K. marxianus CBS 6556 ade x *K. wickerhamii* UCD 61-346	raffinose	6,22	0
K. marxianus CBS 6556 his x *K. wickerhamii* UCD 61-346	raffinose	1,78	0
K. marxianus CBS 6556 ade x *K. wickerhamii* UCD 68-821C	raffinose	2,57	0
K. marxianus CBS 6556 his x *K. wickerhamii* UCD 68-821C	raffinose	3,55	0
K. marxianus CBS 6923 ade x *K. wickerhamii* UCD 56-40	raffinose	1,34	0
K. marxianus CBS 6923 asp x *K. wickerhamii* UCD 56-40	raffinose	1,49	0
K. marxianus CBS 6923 lys x *K. wickerhamii* UCD 56-40	raffinose	1,41	0
K. marxianus CBS 6923 trp x *K. wickerhamii* UCD 56-40	raffinose	1,32	0
K. marxianus CBS 6923 ade x *K. wickerhamii* UCD 61-346	raffinose	4,49	0
K. marxianus CBS 6923 asp x *K. wickerhamii* UCD 61-346	raffinose	3,19	0
K. marxianus CBS 6923 lys x *K. wickerhamii* UCD 61-346	raffinose	4,27	0
K. marxianus CBS 6923 trp x *K. wickerhamii* UCD 61-346	raffinose	2,43	0
K. marxianus CBS 6923 ade x *K. wickerhamii* UCD 68-821C	raffinose	2,76	0
K. marxianus CBS 6923 asp x *K. wickerhamii* UCD 68-821C	raffinose	2,12	0
K. marxianus CBS 6923 lys x *K. wickerhamii* UCD 68-821C	raffinose	2,26	0
K. marxianus CBS 6923 trp x *K. wickerhamii* UCD 68-821C	raffinose	3,34	0
K. marxianus CSIR Y293 ade x *K. wickerhamii* UCD 56-40	raffinose	1,76	0
K. marxianus CSIR Y293 arg x *K. wickerhamii* UCD 56-40	raffinose	1,90	0
K. marxianus CSIR Y293 ade x *K. wickerhamii* UCD 61-346	raffinose	2,81	0
K. marxianus CSIR Y293 arg x *K. wickerhamii* UCD 61-346	raffinose	1,92	0
K. marxianus CSIR Y293 ade x *K. wickerhamii* UCD 68-821C	raffinose	3,55	0
K. marxianus CSIR Y293 arg x *K. wickerhamii* UCD 68-821C	raffinose	2,38	0

TABLE 25

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CSIR Y293 AND SOME OTHER SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus leu x *K. aestuarii* CBS 4438	L-arabinose	2,56	0
K. marxianus trp x *K. aestuarii* CBS 4438	D-ribose	2,55	0
K. marxianus leu x *K. aestuarii* CBS 4904	D-ribose	2,27	0
K. marxianus trp x *K. aestuarii* CBS 4904	L-arabinose	2,00	0
K. marxianus leu x *K. africanus* CBS 2654	lactose	1,45	0
K. marxianus met x *K. africanus* CBS 2654	lactose	2,73	0
K. marxianus leu x *K. blattae* CBS 6284	lactose	1,41	0
K. marxianus trp x *K. blattae* CBS 6284	lactose	2,81	0
K. marxianus met x *K. delphensis* CBS 2170	lactose	4,11	0
K. marxianus trp x *K. delphensis* CBS 2170	lactose	3,63	0
K. marxianus leu x *K. lodderi* CBS 2757	lactose	3,53	0
K. marxianus met x *K. lodderi* CBS 2757	lactose	5,90	0
K. marxianus leu x *K. phaffii* CBS 4417	lactose	2,25	0
K. marxianus met x *K. phaffii* CBS 4417	lactose	2,39	0
K. marxianus leu x *K. polysporus* CBS 6899	lactose	3,51	0
K. marxianus met x *K. polysporus* CBS 6899	lactose	2,30	0
K. marxianus x *K. thermotolerans* CBS 6924 met	maltose	3,13	0
K. marxianus x *K. thermotolerans* CBS 6924 thr	maltose	3,40	0
K. marxianus leu x *K. thermotolerans* CBS 6924	lactose	2,99	42
K. marxianus met x *K. thermotolerans* CBS 6924	lactose	3,11	0
K. marxianus trp x *K. thermotolerans* CBS 6924	lactose	3,56	0
K. marxianus met x *K. waltii* CBS 6430	lactose	1,75	0
K. marxianus trp x *K. waltii* CBS 6430	lactose	1,77	0
K. marxianus leu x *K. wickerhamii* CBS 2745	raffinose	4,31	0
K. marxianus trp x *K. wickerhamii* CBS 2745	raffinose	2,75	0

TABLE 26

CROSSES INVOLVING *KLUYVEROMYCES PHAFFII* CBS 4417
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS			Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaffii	x	*K. aestuarii* CBS 4438 his	lactose	1,59	0
K. phaffii	x	*K. aestuarii* CBS 4438 ilv	lactose	1,60	0
K. phaffii	x	*K. aestuarii* CBS 4904 his	lactose	2,78	0
K. phaffii ade met	x	*K. africanus* CBS 2654 leu	glucose	1,89	0
K. phaffii arg	x	*K. africanus* CBS 2654 met	galactose	1,78	0
K. phaffii arg his	x	*K. africanus* CBS 2654 ade	galactose	1,51	0
K. phaffii lys	x	*K. africanus* CBS 2654 met trp	galactose	1,29	0
K. phaffii ade met	x	*K. blattae* CBS 6284 his	galactose	1,08	0
K. phaffii arg	x	*K. blattae* CBS 6284 his	galactose	1,39	0
K. phaffii lys	x	*K. blattae* CBS 6284 his	galactose	1,38	0
K. phaffii lys	x	*K. blattae* CBS 6284 met	galactose	1,79	0
K. phaffii	x	*K. bulgaricus* CBS 5667 lys	lactose	1,59	0
K. phaffii	x	*K. bulgaricus* CBS 5667 met	lactose	1,22	0
K. phaffii	x	*K. cicerisporus* CBS 4857 ilv	lactose	1,05	0
K. phaffii	x	*K. cicerisporus* CBS 4857 trp	lactose	2,13	0
K. phaffii ade met	x	*K. delphensis* CBS 2170	galactose	5,03	0
K. phaffii arg	x	*K. delphensis* CBS 2170	galactose	3,74	0
K. phaffii arg	x	*K. delphensis* CBS 2170 his	galactose	1,18	0
K. phaffii lys	x	*K. delphensis* CBS 2170 his	galactose	1,21	0
K. phaffii	x	*K. dobzhanskii* CBS 2104 ade	maltose	3,33	0
K. phaffii	x	*K. dobzhanskii* CBS 2104 his	maltose	2,31	0
K. phaffii	x	*K. drosophilarum* CBS 2896 ade	maltose	5,05	0
K. phaffii	x	*K. drosophilarum* CBS 2896 trp	maltose	1,11	0
K. phaffii	x	*K. fragilis* CBS 1556 leu	lactose	1,14	0
K. phaffii	x	*K. fragilis* CBS 1556 met	lactose	1,24	0

TABLE 26
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaffii x *K. lactis* CBS 683 arg	lactose	3,57	0
K. phaffii x *K. lactis* CBS 683 trp	lactose	5,72	0
K. phaffii x *K. lactis* CBS 2359 arg ura	lactose	1,28	0
K. phaffii x *K. lactis* CBS 6315 ade	lactose	1,78	0
K. phaffii x *K. lodderi* CBS 2757 arg	raffinose	1,63	0
K. phaffii x *K. lodderi* CBS 2757 asp	raffinose	1,73	0
K. phaffii ade met x *K. lodderi* CBS 2757 arg	glucose	1,23	0
K. phaffii lys x *K. lodderi* CBS 2757 arg	glucose	2,20	0
K. phaffii x *K. marxianus* CBS 6556 his	lactose	3,28	0
K. phaffii x *K. marxianus* CBS 6556 leu pro	lactose	1,28	0
K. phaffii x *K. marxianus* CBS 6923 ade	lactose	1,77	0
K. phaffii x *K. marxianus* CBS 6923 his	lactose	1,42	0
K. phaffii x *K. marxianus* CBS 6923 lys	lactose	3,15	0
K. phaffii x *K. marxianus* CBS 6923 trp	lactose	1,33	0
K. phaffii x *K. marxianus* CSIR Y293 leu	lactose	2,25	0
K. phaffii x *K. marxianus* CSIR Y293 met	lactose	2,39	0
K. phaffii x *K. phaseolosporus* CBS 2103 ade	raffinose	2,62	0
K. phaffii x *K. phaseolosporus* CBS 2103 asp	raffinose	1,64	0
K. phaffii x *K. phaseolosporus* CBS 2103 trp	raffinose	1,80	0
K. phaffii x *K. polysporus* CBS 6899 ade	raffinose	1,71	0
K. phaffii x *K. polysporus* CBS 6899 met	raffinose	1,69	0
K. phaffii x *K. polysporus* CBS 6899 trp	raffinose	1,91	0
K. phaffii x *K. polysporus* CBS 6899 trp ura	raffinose	1,51	0
K. phaffii x *K. thermotolerans* CBS 6924 met	maltose	1,29	0
K. phaffii x *K. thermotolerans* CBS 6924 thr	maltose	2,31	0

TABLE 26

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaffii x *K. vanudenii* CBS 4372 his	maltose	1,55	0
K. phaffii x *K. vanudenii* CBS 4372 lys	maltose	2,91	0
K. phaffii x *K. waltii* CBS 6430 arg	raffinose	2,76	0
K. phaffii ade met x *K. waltii* CBS 6430	galactose	4,52	0
K. phaffii arg x *K. waltii* CBS 6430	galactose	2,40	0
K. phaffii lys x *K. waltii* CBS 6430	galactose	2,91	0
K. phaffii x *K. wickerhamii* CBS 2745 ade	lactose	7,86	0
K. phaffii x *K. wickerhamii* CBS 2745 trp ura	lactose	3,77	0
K. phaffii x *K. wikenii* CBS 5671 thr	raffinose	1,40	0
K. phaffii x *K. wikenii* CBS 5671 ura	raffinose	1,51	0

TABLE 27

CROSSES INVOLVING *KLUYVEROMYCES PHASEOLOSPORUS* CBS 2103 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaseolosporus x *K. aestuarii* CBS 4438 his	lactose	4,37	0
K. phaseolosporus x *K. aestuarii* CBS 4438 ilv	lactose	5,07	0
K. phaseolosporus x *K. aestuarii* CBS 4904 his	lactose	4,94	0
K. phaseolosporus arg x *K. africanus* CBS 2654	raffinose	1,45	0
K. phaseolosporus trp x *K. africanus* CBS 2654	raffinose	3,32	0
K. phaseolosporus arg x *K. blattae* CBS 6284	sucrose	2,51	0
K. phaseolosporus lys x *K. blattae* CBS 6284	sucrose	2,09	0
K. phaseolosporus x *K. bulgaricus* CBS 5667 leu	lactose	3,44	$1,37 \times 10^2$
K. phaseolosporus x *K. bulgaricus* CBS 5667 lys	lactose	1,46	0
K. phaseolosporus x *K. bulgaricus* CBS 5667 met	lactose	4,29	0
K. phaseolosporus x *K. cicerisporus* CBS 4857 ilv	lactose	4,16	$1,03 \times 10^3$
K. phaseolosporus x *K. cicerisporus* CBS 4857 trp	lactose	3,80	$2,13 \times 10^3$
K. phaseolosporus arg x *K. delphensis* CBS 2170	galactose	1,16	0
K. phaseolosporus asp x *K. delphensis* CBS 2170	galactose	2,69	0
K. phaseolosporus x *K. dobzhanskii* CBS 2104 ade	maltose	2,22	$2,29 \times 10^5$
K. phaseolosporus x *K. dobzhanskii* CBS 2104 his	maltose	3,15	$1,90 \times 10^4$
K. phaseolosporus x *K. drosophilarum* CBS 2896 ade	maltose	2,67	$5,66 \times 10^6$
K. phaseolosporus x *K. drosophilarum* CBS 2896 lys	maltose	2,90	$1,58 \times 10^6$
K. phaseolosporus x *K. drosophilarum* CBS 2896 trp	maltose	2,34	$4,83 \times 10^6$
K. phaseolosporus x *K. fragilis* CBS 1556 leu	lactose	2,31	$7,10 \times 10^4$
K. phaseolosporus x *K. fragilis* CBS 1556 met	lactose	2,36	$1,82 \times 10^5$
K. phaseolosporus x *K. lactis* CBS 683 arg	lactose	1,27	$9,69 \times 10^5$
K. phaseolosporus x *K. lactis* CBS 683 trp	lactose	5,18	$1,31 \times 10^6$
K. phaseolosporus x *K. lactis* CBS 2359 arg ura	lactose	2,85	$1,63 \times 10^4$

TABLE 27
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaseolosporus ade x *K. lodderi* CBS 2757 asp	glucose	1,09	0
K. phaseolosporus met thr x *K. lodderi* CBS 2757 arg	glucose	1,45	0
K. phaseolosporus met thr x *K. lodderi* CBS 2757 asp	glucose	1,27	0
K. phaseolosporus x *K. marxianus* CBS 6556 ade	lactose	2,11	$1,04 \times 10^3$
K. phaseolosporus x *K. marxianus* CBS 6556 leu pro	lactose	1,46	$1,37 \times 10^4$
K. phaseolosporus x *K. marxianus* CBS 6923 ade	lactose	1,93	$6,68 \times 10^4$
K. phaseolosporus x *K. marxianus* CBS 6923 lys	lactose	0,95	$3,16 \times 10^2$
K. phaseolosporus x *K. marxianus* CBS 6923 trp	lactose	2,83	$4,06 \times 10^3$
K. phaseolosporus ade x *K. phaffii* CBS 4417	raffinose	2,62	0
K. phaseolosporus asp x *K. phaffii* CBS 4417	raffinose	1,64	0
K. phaseolosporus trp x *K. phaffii* CBS 4417	raffinose	1,80	0
K. phaseolosporus ade x *K. polysporus* CBS 6899 lys	glucose	3,84	0
K. phaseolosporus arg x *K. polysporus* CBS 6899	L-sorbose	1,85	0
K. phaseolosporus arg x *K. polysporus* CBS 6899 met	glucose	2,59	0
K. phaseolosporus trp x *K. polysporus* CBS 6899	L-sorbose	2,25	0
K. phaseolosporus x *K. thermotolerans* CBS 6924 met	maltose	1,83	0
K. phaseolosporus x *K. thermotolerans* CBS 6924 thr	maltose	6,68	0
K. phaseolosporus x *K. vanudenii* CBS 4372 his	maltose	2,29	$2,09 \times 10^5$
K. phaseolosporus x *K. vanudenii* CBS 4372 lys	maltose	2,59	$1,54 \times 10^6$
K. phaseolosporus arg x *K. waltii* CBS 6430	galactose	1,63	0
K. phaseolosporus lys x *K. waltii* CBS 6430	galactose	1,93	0
K. phaseolosporus lys x *K. waltii* CBS 6430 arg	galactose	1,50	0
K. phaseolosporus trp x *K. waltii* CBS 6430	galactose	2,88	0
K. phaseolosporus x *K. wickerhamii* CBS 2745 ade	lactose	6,18	0
K. phaseolosporus x *K. wickerhamii* CBS 2745 trp ura	lactose	5,16	0
K. phaseolosporus lys x *K. wickerhamii* CBS 2745	raffinose	4,60	0

TABLE 27
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. phaseolosporus ade x *K. wikenii* CBS 5671 lys	glucose	2,75	0
K. phaseolosporus ade x *K. wikenii* CBS 5671 thr	glucose	1,66	0
K. phaseolosporus ade x *K. wikenii* CBS 5671 ura	glucose	1,98	$9,14 \times 10^3$
K. phaseolosporus arg x *K. wikenii* CBS 5671	trehalose	1,28	0
K. phaseolosporus arg x *K. wikenii* CBS 5671 lys	glucose	1,33	0
K. phaseolosporus arg x *K. wikenii* CBS 5671 thr	glucose	1,43	0
K. phaseolosporus trp x *K. wikenii* CBS 5671	trehalose	1,20	0
K. phaseolosporus trp x *K. wikenii* CBS 5671 lys	glucose	1,38	0
K. phaseolosporus trp x *K. wikenii* CBS 5671 thr	glucose	1,41	0
K. phaseolosporus trp x *K. wikenii* CBS 5671 ura	glucose	1,74	$2,42 \times 10^5$

TABLE 28

CROSSES INVOLVING *KLUYVEROMYCES POLYSPORUS* CBS 6899 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS			Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. polysporus	x	*K. aestuarii* CBS 4438 his	lactose	1,78	0
K. polysporus	x	*K. aestuarii* CBS 4438 ilv	lactose	1,65	0
K. polysporus	x	*K. aestuarii* CBS 4904 his	lactose	4,51	0
K. polysporus ade	x	*K. africanus* CBS 2654	raffinose	1,60	0
K. polysporus ade	x	*K. africanus* CBS 2654 met	raffinose	1,69	0
K. polysporus ade	x	*K. africanus* CBS 2654 met trp	raffinose	1,64	0
K. polysporus met	x	*K. africanus* CBS 2654	raffinose	1,49	0
K. polysporus trp	x	*K. africanus* CBS 2654 met	raffinose	2,32	0
K. polysporus ade	x	*K. blattae* CBS 6284	sucrose	2,41	0
K. polysporus lys	x	*K. blattae* CBS 6284	sucrose	3,15	0
K. polysporus trp	x	*K. blattae* CBS 6284	sucrose	3,23	0
K. polysporus	x	*K. bulgaricus* CBS 5667 lys	lactose	2,60	0
K. polysporus	x	*K. bulgaricus* CBS 5667 met	lactose	3,49	0
K. polysporus	x	*K. cicerisporus* CBS 4857 ilv	lactose	1,04	0
K. polysporus	x	*K. cicerisporus* CBS 4857 trp	lactose	1,28	0
K. polysporus ade	x	*K. delphensis* CBS 2170	galactose	4,11	0
K. polysporus lys	x	*K. delphensis* CBS 2170 his	galactose	4,17	0
K. polysporus trp	x	*K. delphensis* CBS 2170 his	galactose	4,20	0
K. polysporus trp ura	x	*K. delphensis* CBS 2170	galactose	4,49	0
K. polysporus	x	*K. dobzhanskii* CBS 2104 ade	maltose	3,32	0
K. polysporus	x	*K. dobzhanskii* CBS 2104 his	maltose	2,99	0
K. polysporus	x	*K. drosophilarum* CBS 2896 ade	maltose	3,56	0
K. polysporus	x	*K. drosophilarum* CBS 2896 met thr	maltose	5,71	0
K. polysporus	x	*K. fragilis* CBS 1556 leu	lactose	2,06	0
K. polysporus	x	*K. fragilis* CBS 1556 met	lactose	2,23	0

TABLE 28
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. polysporus x *K. lactis* CBS 683 arg	lactose	2,45	0
K. polysporus x *K. lactis* CBS 683 trp	lactose	2,46	0
K. polysporus x *K. lactis* CBS 2359 arg ura	lactose	2,65	0
K. polysporus x *K. lactis* CBS 6315 ade	lactose	4,60	0
K. polysporus ade x *K. lodderi* CBS 2757 arg	glucose	1,74	0
K. polysporus met x *K. lodderi* CBS 2757 arg	glucose	1,35	0
K. polysporus met x *K. lodderi* CBS 2757 asp	glucose	1,59	0
K. polysporus trp x *K. lodderi* CBS 2757 asp	glucose	1,68	0
K. polysporus trp ura x *K. lodderi* CBS 2757 arg	glucose	1,70	0
K. polysporus x *K. marxianus* CBS 6556 his	lactose	2,51	0
K. polysporus x *K. marxianus* CBS 6556 leu pro	lactose	4,12	0
K. polysporus x *K. marxianus* CBS 6923 ade	lactose	2,97	0
K. polysporus x *K. marxianus* CBS 6923 asp	lactose	3,72	0
K. polysporus x *K. marxianus* CBS 6923 his	lactose	1,88	0
K. polysporus x *K. marxianus* CBS 6923 lys	lactose	1,68	0
K. polysporus x *K. marxianus* CSIR Y293 leu	lactose	3,51	0
K. polysporus x *K. marxianus* CSIR Y293 met	lactose	2,30	0
K. polysporus ade x *K. phaffii* CBS 4417	raffinose	1,71	0
K. polysporus met x *K. phaffii* CBS 4417	raffinose	1,69	0
K. polysporus trp x *K. phaffii* CBS 4417	raffinose	1,91	0
K. polysporus trp ura x *K. phaffii* CBS 4417	raffinose	1,51	0
K. polysporus x *K. phaseolosporus* CBS 2103 arg	L-sorbose	1,85	0
K. polysporus x *K. phaseolosporus* CBS 2103 trp	L-sorbose	2,25	0
K. polysporus lys x *K. phaseolosporus* CBS 2103 ade	glucose	3,84	0
K. polysporus met x *K. phaseolosporus* CBS 2103 arg	glucose	2,59	0

TABLE 28
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. polysporus x *K. thermotolerans* CBS 6924 met	maltose	1,33	0
K. polysporus x *K. thermotolerans* CBS 6924 thr	maltose	6,30	0
K. polysporus x *K. vanudenii* CBS 4372 his	maltose	2,68	0
K. polysporus x *K. vanudenii* CBS 4372 lys	maltose	3,51	0
K. polysporus lys x *K. waltii* CBS 6430	galactose	3,47	0
K. polysporus met x *K. waltii* CBS 6430	galactose	3,52	0
K. polysporus trp x *K. waltii* CBS 6430	galactose	1,75	0
K. polysporus x *K. wickerhamii* CBS 2745 ade	lactose	4,00	0
K. polysporus x *K. wickerhamii* CBS 2745 trp ura	lactose	2,10	0
K. polysporus ade x *K. wikenii* CBS 5671 ura	glucose	1,03	0
K. polysporus met x *K. wikenii* CBS 5671 ura	glucose	1,31	0
K. polysporus trp x *K. wikenii* CBS 5671 thr	glucose	1,45	0

TABLE 29

CROSSES INVOLVING *KLUYVEROMYCES THERMOTOLERANS* CBS 6924 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. thermotolerans x *K. aestuarii* CBS 4438 his	lactose	1,56	0
K. thermotolerans x *K. aestuarii* CBS 4438 ilv	lactose	2,48	0
K. thermotolerans met x *K. aestuarii* CBS 4438 his	maltose	4,12	0
K. thermotolerans met x *K. aestuarii* CBS 4438 ilv	maltose	1,60	0
K. thermotolerans x *K. aestuarii* CBS 4904 his	lactose	3,83	0
K. thermotolerans met x *K. africanus* CBS 2654	maltose	6,70	0
K. thermotolerans thr x *K. africanus* CBS 2654	maltose	1,57	0
K. thermotolerans met x *K. blattae* CBS 6284	maltose	2,00	0
K. thermotolerans thr x *K. blattae* CBS 6284	maltose	2,34	0
K. thermotolerans x *K. bulgaricus* CBS 5667 leu	lactose	1,59	0
K. thermotolerans x *K. bulgaricus* CBS 5667 lys	lactose	1,36	0
K. thermotolerans x *K. bulgaricus* CBS 5667 met	lactose	1,54	0
K. thermotolerans met x *K. bulgaricus* CBS 5667	maltose	3,17	0
K. thermotolerans thr x *K. bulgaricus* CBS 5667	maltose	1,39	0
K. thermotolerans x *K. cicerisporus* CBS 4857 ilv	lactose	5,22	0
K. thermotolerans x *K. cicerisporus* CBS 4857 trp	lactose	2,70	0
K. thermotolerans thr x *K. cicerisporus* CBS 4857	maltose	1,85	0
K. thermotolerans met x *K. delphensis* CBS 2170	maltose	1,70	0
K. thermotolerans thr x *K. delphensis* CBS 2170	maltose	4,57	0
K. thermotolerans met x *K. dobzhanskii* CBS 2104 ade	maltose	5,48	0
K. thermotolerans thr x *K. dobzhanskii* CBS 2104 his	maltose	6,24	0
K. thermotolerans met x *K. drosophilarum* CBS 2896 lys	maltose	1,57	0
K. thermotolerans thr x *K. drosophilarum* CBS 2896 trp	maltose	1,32	0
K. thermotolerans x *K. fragilis* CBS 1556 leu	lactose	3,03	0
K. thermotolerans x *K. fragilis* CBS 1556 met	lactose	5,42	0
K. thermotolerans thr x *K. fragilis* CBS 1556	maltose	5,41	0

TABLE 29
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count x10^8 per ml	Number of recombinants/10^8 mated cell population
K. thermotolerans x *K. lactis* CBS 683 arg	lactose	3,74	0
K. thermotolerans x *K. lactis* CBS 683 trp	lactose	6,09	0
K. thermotolerans x *K. lactis* CBS 2359 arg ura	lactose	4,32	0
K. thermotolerans x *K. lactis* CBS 6315 ade	lactose	5,02	0
K. thermotolerans met x *K. lodderi* CBS 2757	maltose	1,69	0
K. thermotolerans thr x *K. lodderi* CBS 2757	maltose	4,64	0
K. thermotolerans x *K. marxianus* CBS 6556 his	lactose	3,07	0
K. thermotolerans x *K. marxianus* CBS 6556 leu pro	lactose	4,92	0
K. thermotolerans met x *K. marxianus* CBS 6556	maltose	2,78	0
K. thermotolerans thr x *K. marxianus* CBS 6556	maltose	1,84	0
K. thermotolerans x *K. marxianus* CBS 6923 ade	lactose	1,47	40
K. thermotolerans x *K. marxianus* CBS 6923 his	lactose	2,31	0
K. thermotolerans x *K. marxianus* CBS 6923 leu	lactose	1,37	58
K. thermotolerans x *K. marxianus* CBS 6923 lys	lactose	2,95	0
K. thermotolerans x *K. marxianus* CBS 6923 trp	lactose	3,37	0
K. thermotolerans x *K. marxianus* CBS 6923 ura	lactose	3,47	0
K. thermotolerans met x *K. marxianus* CBS 6923	maltose	3,44	0
K. thermotolerans thr x *K. marxianus* CBS 6923	maltose	4,28	0
K. thermotolerans x *K. marxianus* CSIR Y293 leu	lactose	2,99	42
K. thermotolerans x *K. marxianus* CSIR Y293 met	lactose	3,11	0
K. thermotolerans x *K. marxianus* CSIR Y293 trp	lactose	3,56	0
K. thermotolerans met x *K. marxianus* CSIR Y293	maltose	3,13	0
K. thermotolerans thr x *K. marxianus* CSIR Y293	maltose	3,40	0
K. thermotolerans met x *K. phaffii* CBS 4417	maltose	1,29	0
K. thermotolerans thr x *K. phaffii* CBS 4417	maltose	2,31	0

TABLE 29
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. thermotolerans met x *K. phaseolosporus* CBS 2103	maltose	1,83	0
K. thermotolerans thr x *K. phaseolosporus* CBS 2103	maltose	6,68	0
K. thermotolerans met x *K. polysporus* CBS 6899	maltose	1,33	0
K. thermotolerans thr x *K. polysporus* CBS 6899	maltose	6,30	0
K. thermotolerans x *K. vanudenii* CBS 4372 his	cellobiose	1,35	0
K. thermotolerans x *K. vanudenii* CBS 4372 leu	cellobiose	1,57	0
K. thermotolerans met x *K. vanudenii* CBS 4372 his	maltose	1,54	0
K. thermotolerans thr x *K. vanudenii* CBS 4372 ura	maltose	1,02	0
K. thermotolerans met x *K. waltii* CBS 6430	maltose	1,65	0
K. thermotolerans thr x *K. waltii* CBS 6430	maltose	3,14	0
K. thermotolerans x *K. wickerhamii* CBS 2745 ade	lactose	2,12	0
K. thermotolerans x *K. wickerhamii* CBS 2745 trp ura	lactose	3,88	0
K. thermotolerans met x *K. wickerhamii* CBS 2745	raffinose	6,84	0
K. thermotolerans thr x *K. wickerhamii* CBS 2745	maltose	5,58	0
K. thermotolerans met x *K. wikenii* CBS 5671	maltose	1,34	0
K. thermotolerans thr x *K. wikenii* CBS 5671	maltose	3,20	0

TABLE 30

CROSSES INVOLVING *KLUYVEROMYCES VANUDENII* CBS 4372
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. vanudenii x *K. aestuarii* CBS 4438 his	lactose	2,08	0
K. vanudenii x *K. aestuarii* CBS 4438 ilv	lactose	1,44	0
K. vanudenii x *K. aestuarii* CBS 4904 his	lactose	1,98	0
K. vanudenii his x *K. africanus* CBS 2654	maltose	1,73	0
K. vanudenii lys x *K. africanus* CBS 2654	maltose	2,48	0
K. vanudenii his x *K. blattae* CBS 6284	maltose	2,91	0
K. vanudenii lys x *K. blattae* CBS 6284	maltose	1,79	0
K. vanudenii x *K. bulgaricus* CBS 5667 met	lactose	1,00	$2,20 \times 10^5$
K. vanudenii his x *K. bulgaricus* CBS 5667	maltose	1,92	$3,65 \times 10^6$
K. vanudenii lys x *K. bulgaricus* CBS 5667	maltose	1,52	$1,19 \times 10^6$
K. vanudenii x *K. cicerisporus* CBS 4857 ilv	lactose	2,98	$1,28 \times 10^3$
K. vanudenii x *K. cicerisporus* CBS 4857 trp	lactose	1,58	$1,01 \times 10^3$
K. vanudenii his x *K. cicerisporus* CBS 4857	maltose	1,64	$1,31 \times 10^5$
K. vanudenii his x *K. delphensis* CBS 2170	maltose	1,90	0
K. vanudenii lys x *K. delphensis* CBS 2170	maltose	2,99	0
K. vanudenii his x *K. dobzhanskii* CBS 2104 ade	maltose	4,74	$1,81 \times 10^5$
K. vanudenii ilv x *K. dobzhanskii* CBS 2104 his	maltose	1,35	$2,99 \times 10^4$
K. vanudenii lys x *K. dobzhanskii* CBS 2104 ade	maltose	3,71	$3,49 \times 10^5$
K. vanudenii his x *K. drosophilarum* CBS 2896 met thr	maltose	0,87	$4,04 \times 10^6$
K. vanudenii his x *K. drosophilarum* CBS 2896 trp	maltose	2,34	$7,61 \times 10^6$
K. vanudenii ura x *K. drosophilarum* CBS 2896 ade	maltose	2,36	$7,03 \times 10^5$
K. vanudenii x *K. fragilis* CBS 1556 leu	lactose	4,74	$6,33 \times 10^4$
K. vanudenii x *K. fragilis* CBS 1556 met	lactose	1,52	$1,32 \times 10^3$
K. vanudenii lys x *K. fragilis* CBS 1556	maltose	1,75	$9,37 \times 10^5$
K. vanudenii x *K. lactis* CBS 683 arg	lactose	2,45	$8,73 \times 10^5$
K. vanudenii x *K. lactis* CBS 683 trp	lactose	1,29	$7,51 \times 10^6$
K. vanudenii x *K. lactis* CBS 2359 arg ura	lactose	2,99	$4,45 \times 10^4$

TABLE 30
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. vanudenii his x *K. lodderi* CBS 2757	maltose	1,69	0
K. vanudenii lys x *K. lodderi* CBS 2757	maltose	3,95	0
K. vanudenii x *K. marxianus* CBS 6556 his	lactose	1,94	$8,92 \times 10^5$
K. vanudenii x *K. marxianus* CBS 6556 leu pro	lactose	1,20	$8,42 \times 10^5$
K. vanudenii x *K. marxianus* CBS 6923 ade	lactose	2,99	$3,91 \times 10^5$
K. vanudenii x *K. marxianus* CBS 6923 his	lactose	1,64	$5,85 \times 10^5$
K. vanudenii x *K. marxianus* CBS 6923 ura	lactose	1,70	$2,32 \times 10^5$
K. vanudenii his x *K. marxianus* CBS 6923	maltose	1,16	$3,71 \times 10^6$
K. vanudenii his x *K. phaffii* CBS 4417	maltose	1,55	0
K. vanudenii lys x *K. phaffii* CBS 4417	maltose	2,91	0
K. vanudenii his x *K. phaseolosporus* CBS 2103	maltose	2,29	$2,09 \times 10^5$
K. vanudenii lys x *K. phaseolosporus* CBS 2103	maltose	2,59	$1,54 \times 10^6$
K. vanudenii his x *K. polysporus* CBS 6899	maltose	2,68	0
K. vanudenii lys x *K. polysporus* CBS 6899	maltose	3,51	0
K. vanudenii his x *K. thermotolerans* CBS 6924	cellobiose	1,35	0
K. vanudenii leu x *K. thermotolerans* CBS 6924	cellobiose	1,57	0
K. vanudenii his x *K. thermotolerans* CBS 6924 met	maltose	1,54	0
K. vanudenii ura x *K. thermotolerans* CBS 6924 thr	maltose	1,02	0
K. vanudenii his x *K. waltii* CBS 6430	maltose	1,52	0
K. vanudenii lys x *K. waltii* CBS 6430	maltose	1,56	0
K. vanudenii x *K. wickerhamii* CBS 2745 ade	lactose	0,71	$2,52 \times 10^4$
K. vanudenii his x *K. wickerhamii* CBS 2745 trp	maltose	3,81	$2,68 \times 10^3$
K. vanudenii lys x *K. wickerhamii* CBS 2745	maltose	7,50	$6,09 \times 10^5$
K. vanudenii his x *K. wikenii* CBS 5671	maltose	2,63	$5,62 \times 10^6$
K. vanudenii lys x *K. wikenii* CBS 5671	maltose	2,89	$1,94 \times 10^6$

TABLE 31

CROSSES INVOLVING *KLUYVEROMYCES WALTII* CBS 6430
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS			Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. waltii	x	*K. aestuarii* CBS 4438 his	lactose	2,53	0
K. waltii	x	*K. aestuarii* CBS 4438 ilv	lactose	2,43	0
K. waltii	x	*K. aestuarii* CBS 4904 his	lactose	2,54	0
K. waltii	x	*K. africanus* CBS 2654 ade	galactose	3,85	0
K. waltii	x	*K. africanus* CBS 2654 met	galactose	3,28	0
K. waltii arg	x	*K. africanus* CBS 2654 ade	galactose	4,12	0
K. waltii	x	*K. blattae* CBS 6284 his	galactose	1,67	0
K. waltii	x	*K. blattae* CBS 6284 met	galactose	2,27	0
K. waltii arg	x	*K. blattae* CBS 6284	sucrose	1,72	0
K. waltii his met	x	*K. blattae* CBS 6284	sucrose	1,47	0
K. waltii	x	*K. bulgaricus* CBS 5667 lys	lactose	1,67	0
K. waltii	x	*K. bulgaricus* CBS 5667 met	lactose	2,54	0
K. waltii	x	*K. cicerisporus* CBS 4857 ilv	lactose	2,17	0
K. waltii	x	*K. cicerisporus* CBS 4857 trp	lactose	3,06	0
K. waltii arg	x	*K. delphensis* CBS 2170	sucrose	4,59	0
K. waltii his met	x	*K. delphensis* CBS 2170	sucrose	3,65	0
K. waltii	x	*K. dobzhanskii* CBS 2104 ade	maltose	4,51	0
K. waltii	x	*K. dobzhanskii* CBS 2104 his	maltose	5,48	0
K. waltii	x	*K. drosophilarum* CBS 2896 ade	maltose	1,78	0
K. waltii	x	*K. drosophilarum* CBS 2896 lys	maltose	2,21	0
K. waltii	x	*K. drosophilarum* CBS 2896 met thr	maltose	1,62	$2,11 \times 10^6$
K. waltii	x	*K. drosophilarum* CBS 2896 trp	maltose	1,36	$1,47 \times 10^5$
K. waltii	x	*K. fragilis* CBS 1556 leu	lactose	1,22	0
K. waltii	x	*K. fragilis* CBS 1556 met	lactose	1,31	0

TABLE 31
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. waltii x *K. lactis* CBS 683 arg	lactose	4,11	0
K. waltii x *K. lactis* CBS 683 trp	lactose	4,99	0
K. waltii x *K. lactis* CBS 2359 arg ura	lactose	1,77	0
K. waltii x *K. lactis* CBS 6315 ade	lactose	1,13	0
K. waltii x *K. lodderi* CBS 2757 arg	galactose	1,65	0
K. waltii x *K. lodderi* CBS 2757 asp	galactose	1,38	0
K. waltii x *K. marxianus* CBS 6556 his	lactose	1,87	0
K. waltii x *K. marxianus* CBS 6556 leu pro	lactose	2,76	0
K. waltii x *K. marxianus* CBS 6923 ade	lactose	1,37	0
K. waltii x *K. marxianus* CBS 6923 asp	lactose	1,02	0
K. waltii x *K. marxianus* CBS 6923 his	lactose	1,33	0
K. waltii x *K. marxianus* CBS 6923 ura	lactose	2,46	0
K. waltii x *K. marxianus* CSIR Y293 met	lactose	1,75	0
K. waltii x *K. marxianus* CSIR Y293 trp	lactose	1,77	0
K. waltii x *K. phaffii* CBS 4417 ade met	galactose	4,52	0
K. waltii x *K. phaffii* CBS 4417 arg	galactose	2,40	0
K. waltii x *K. phaffii* CBS 4417 lys	galactose	2,91	0
K. waltii arg x *K. phaffii* CBS 4417	raffinose	2,76	0
K. waltii x *K. phaseolosporus* CBS 2103 arg	galactose	1,63	0
K. waltii x *K. phaseolosporus* CBS 2103 lys	galactose	1,93	0
K. waltii x *K. phaseolosporus* CBS 2103 trp	galactose	2,88	0
K. waltii arg x *K. phaseolosporus* CBS 2103 lys	galactose	1,50	0
K. waltii x *K. polysporus* CBS 6899 lys	galactose	3,47	0
K. waltii x *K. polysporus* CBS 6899 met	galactose	3,52	0
K. waltii x *K. polysporus* CBS 6899 trp	galactose	1,75	0

TABLE 31

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. waltii x *K. thermotolerans* CBS 6924 met	maltose	1,65	0
K. waltii x *K. thermotolerans* CBS 6924 thr	maltose	3,14	0
K. waltii x *K. wanudenii* CBS 4372 his	maltose	1,52	0
K. waltii x *K. wanudenii* CBS 4372 lys	maltose	1,56	0
K. waltii x *K. wickerhamii* CBS 2745 ade	lactose	1,76	0
K. waltii x *K. wickerhamii* CBS 2745 trp ura	lactose	2,21	0
K. waltii x *K. wikenii* CBS 5671 lys	galactose	1,05	0
K. waltii x *K. wikenii* CBS 5671 thr	galactose	1,59	0
K. waltii x *K. wikenii* CBS 5671 ura	galactose	2,14	0

TABLE 32

CROSSES INVOLVING *KLUYVEROMYCES WICKERHAMII* CBS 2745 AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wickerhamii x *K. aestuarii* CBS 4438 his	raffinose	6,62	0
K. wickerhamii x *K. aestuarii* CBS 4438 ilv	maltose	4,01	0
K. wickerhamii trp x *K. aestuarii* CBS 4438 ilv	lactose	5,81	0
K. wickerhamii ura x *K. aestuarii* CBS 4438 his	lactose	4,51	0
K. wickerhamii x *K. aestuarii* CBS 4904 his	raffinose	4,27	0
K. wickerhamii ade x *K. africanus* CBS 2654	lactose	7,94	0
K. wickerhamii trp ura x *K. africanus* CBS 2654	lactose	3,31	0
K. wickerhamii leu x *K. blattae* CBS 6284	lactose	4,44	0
K. wickerhamii lys x *K. blattae* CBS 6284	lactose	1,25	0
K. wickerhamii x *K. bulgaricus* CBS 5667 lys	raffinose	4,03	0
K. wickerhamii x *K. bulgaricus* CBS 5667 met	raffinose	4,10	0
K. wickerhamii trp x *K. bulgaricus* CBS 5667	cellobiose	1,70	0
K. wickerhamii x *K. cicerisporus* CBS 4857 ilv	raffinose	4,32	0
K. wickerhamii x *K. cicerisporus* CBS 4857 trp	raffinose	4,89	0
K. wickerhamii lys x *K. delphensis* CBS 2170	lactose	2,34	0
K. wickerhamii trp ura x *K. delphensis* CBS 2170	lactose	2,28	0
K. wickerhamii x *K. dobzhanskii* CBS 2104 ade	maltose	4,62	$7,88 \times 10^5$
K. wickerhamii x *K. dobzhanskii* CBS 2104 his	maltose	4,05	$2,19 \times 10^3$
K. wickerhamii trp x *K. dobzhanskii* CBS 2104	lactose	2,69	$4,16 \times 10^4$
K. wickerhamii x *K. drosophilarum* CBS 2896 ade	maltose	4,90	$6,65 \times 10^5$
K. wickerhamii x *K. drosophilarum* CBS 2896 trp	maltose	4,62	$2,06 \times 10^5$
K. wickerhamii trp x *K. drosophilarum* CBS 2896	lactose	0,93	$2,83 \times 10^3$
K. wickerhamii x *K. fragilis* CBS 1556 leu	raffinose	6,14	0
K. wickerhamii x *K. fragilis* CBS 1556 met	raffinose	5,87	0

TABLE 32
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wickerhamii x *K. lactis* CBS 683 arg	maltose	5,48	$8,94 \times 10^3$
K. wickerhamii x *K. lactis* CBS 683 trp	maltose	3,98	$1,10 \times 10^5$
K. wickerhamii x *K. lactis* CBS 2359 arg ura	raffinose	7,58	0
K. wickerhamii trp x *K. lactis* CBS 2359 arg ura	lactose	1,15	78
K. wickerhamii x *K. lactis* CBS 6315 ade	raffinose	4,53	0
K. wickerhamii ade x *K. lodderi* CBS 2757	lactose	5,97	0
K. wickerhamii trp ura x *K. lodderi* CBS 2757	lactose	2,61	0
K. wickerhamii x *K. marxianus* CBS 6556 his	raffinose	6,54	0
K. wickerhamii x *K. marxianus* CBS 6556 leu pro	raffinose	5,46	0
K. wickerhamii x *K. marxianus* CBS 6923 ade	raffinose	3,40	0
K. wickerhamii x *K. marxianus* CBS 6923 his	raffinose	1,65	0
K. wickerhamii x *K. marxianus* CBS 6923 leu	raffinose	3,08	0
K. wickerhamii x *K. marxianus* CBS 6923 lys	raffinose	2,65	0
K. wickerhamii x *K. marxianus* CBS 6923 trp	raffinose	3,97	0
K. wickerhamii x *K. marxianus* CBS 6923 ura	raffinose	5,48	0
K. wickerhamii arg lys x *K. marxianus* CBS 6923 ade	lactose	1,26	0
K. wickerhamii arg lys x *K. marxianus* CBS 6923 his	lactose	1,61	0
K. wickerhamii arg lys x *K. marxianus* CBS 6923 leu	lactose	1,58	0
K. wickerhamii lys x *K. marxianus* CBS 6923 ade	lactose	2,05	0
K. wickerhamii trp x *K. marxianus* CBS 6923 ade	lactose	1,17	0
K. wickerhamii trp x *K. marxianus* CBS 6923 asp	lactose	3,47	0
K. wickerhamii trp x *K. marxianus* CBS 6923 his	lactose	1,65	0
K. wickerhamii trp x *K. marxianus* CBS 6923 lys	lactose	1,70	0
K. wickerhamii trp x *K. marxianus* CBS 6923 ura	lactose	2,09	0
K. wickerhamii ura x *K. marxianus* CBS 6923 his	lactose	1,40	0
K. wickerhamii x *K. marxianus* CSIR Y293 leu	raffinose	4,31	0
K. wickerhamii x *K. marxianus* CSIR Y293 trp	raffinose	2,75	0

TABLE 32

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wickerhamii ade x *K. phaffii* CBS 4417	lactose	7,86	0
K. wickerhamii trp ura x *K. phaffii* CBS 4417	lactose	3,77	0
K. wickerhamii x *K. phaseolosporus* CBS 2103 lys	raffinose	4,60	0
K. wickerhamii ade x *K. phaseolosporus* CBS 2103	lactose	6,18	0
K. wickerhamii trp ura x *K. phaseolosporus* CBS 2103	lactose	5,16	0
K. wickerhamii ade x *K. polysporus* CBS 6899	lactose	4,00	0
K. wickerhamii trp ura x *K. polysporus* CBS 6899	lactose	2,10	0
K. wickerhamii x *K. thermotolerans* CBS 6924 met	raffinose	6,84	0
K. wickerhamii x *K. thermotolerans* CBS 6924 thr	maltose	5,58	0
K. wickerhamii ade x *K. thermotolerans* CBS 6924	lactose	2,12	0
K. wickerhamii trp ura x *K. thermotolerans* CBS 6924	lactose	3,88	0
K. wickerhamii x *K. vanudenii* CBS 4372 lys	maltose	7,50	$6,09 \times 10^5$
K. wickerhamii ade x *K. vanudenii* CBS 4372	lactose	0,71	$2,52 \times 10^4$
K. wickerhamii trp x *K. vanudenii* CBS 4372 his	maltose	3,81	$2,68 \times 10^3$
K. wickerhamii ade x *K. waltii* CBS 6430	lactose	1,76	0
K. wickerhamii trp ura x *K. waltii* CBS 6430	lactose	2,21	0
K. wickerhamii ade x *K. wikenii* CBS 5671	lactose	3,34	0
K. wickerhamii arg lys x *K. wikenii* CBS 5671	lactose	3,05	0
K. wickerhamii trp x *K. wikenii* CBS 5671	lactose	3,54	0
K. wickerhamii trp ura x *K. wikenii* CBS 5671	lactose	3,09	0
K. wickerhamii ura x *K. wikenii* CBS 5671	lactose	2,05	0

TABLE 33

CROSSES INVOLVING *KLUYVEROMYCES WIKENII* CBS 5671
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wikenii x *K. aestuarii* CBS 4438 his	lactose	2,88	0
K. wikenii x *K. aestuarii* CBS 4438 ilv	lactose	1,80	0
K. wikenii x *K. aestuarii* CBS 4904 his	lactose	2,43	0
K. wikenii his x *K. africanus* CBS 2654	raffinose	3,07	0
K. wikenii lys x *K. africanus* CBS 2654	raffinose	4,06	0
K. wikenii thr x *K. africanus* CBS 2654	raffinose	3,18	0
K. wikenii ura x *K. africanus* CBS 2654	raffinose	2,14	0
K. wikenii ade x *K. blattae* CBS 6284	sucrose	1,47	0
K. wikenii his x *K. blattae* CBS 6284	sucrose	1,12	0
K. wikenii x *K. bulgaricus* CBS 5667 lys	lactose	2,53	$5,93 \times 10^3$
K. wikenii x *K. bulgaricus* CBS 5667 met	lactose	1,90	$7,89 \times 10^2$
K. wikenii x *K. cicerisporus* CBS 4857 ilv	lactose	5,33	$5,23 \times 10^3$
K. wikenii x *K. cicerisporus* CBS 4857 trp	lactose	1,49	$8,05 \times 10^3$
K. wikenii thr x *K. delphensis* CBS 2170	raffinose	1,80	0
K. wikenii ura x *K. delphensis* CBS 2170	raffinose	4,18	0
K. wikenii x *K. dobzhanskii* CBS 2104 ade	maltose	3,11	$1,47 \times 10^6$
K. wikenii x *K. dobzhanskii* CBS 2104 his	maltose	3,45	$5,41 \times 10^3$
K. wikenii x *K. drosophilarum* CBS 2896 ade	maltose	1,27	$5,59 \times 10^6$
K. wikenii x *K. drosophilarum* CBS 2896 met thr	maltose	1,45	$4,48 \times 10^6$
K. wikenii x *K. drosophilarum* CBS 2896 trp	maltose	1,40	$5,50 \times 10^5$
K. wikenii ura x *K. drosophilarum* CBS 2896 ade	maltose	0,96	$2,00 \times 10^5$
K. wikenii x *K. fragilis* CBS 1556 leu	lactose	1,05	$4,00 \times 10^4$
K. wikenii x *K. fragilis* CBS 1556 met	lactose	1,77	$5,65 \times 10^3$
K. wikenii x *K. lactis* CBS 683 arg	lactose	1,70	$2,65 \times 10^3$
K. wikenii x *K. lactis* CBS 683 trp	lactose	1,68	$5,10 \times 10^5$
K. wikenii x *K. lactis* CBS 2359 arg ura	lactose	1,99	$1,83 \times 10^6$

TABLE 33
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wikenii ade x *K. lodderi* CBS 2757 arg	glucose	1,11	0
K. wikenii ade x *K. lodderi* CBS 2757 asp	glucose	2,36	0
K. wikenii his x *K. lodderi* CBS 2757 arg	glucose	1,20	0
K. wikenii lys x *K. lodderi* CBS 2757 arg	glucose	1,21	0
K. wikenii lys x *K. lodderi* CBS 2757 asp	glucose	1,51	0
K. wikenii x *K. marxianus* CBS 6556 ade	lactose	1,41	$1,32 \times 10^6$
K. wikenii x *K. marxianus* CBS 6556 leu pro	lactose	1,06	$4,14 \times 10^5$
K. wikenii x *K. marxianus* CBS 6923 ade	lactose	1,69	$2,43 \times 10^4$
K. wikenii x *K. marxianus* CBS 6923 his	lactose	1,68	$3,15 \times 10^5$
K. wikenii x *K. marxianus* CBS 6923 lys	lactose	1,41	$1,03 \times 10^5$
K. wikenii thr x *K. phaffii* CBS 4417	raffinose	1,40	0
K. wikenii ura x *K. phaffii* CBS 4417	raffinose	1,51	0
K. wikenii x *K. phaseolosporus* CBS 2103 arg	trehalose	1,28	0
K. wikenii x *K. phaseolosporus* CBS 2103 trp	trehalose	1,20	0
K. wikenii lys x *K. phaseolosporus* CBS 2103 ade	glucose	2,75	0
K. wikenii lys x *K. phaseolosporus* CBS 2103 arg	glucose	1,33	0
K. wikenii lys x *K. phaseolosporus* CBS 2103 trp	glucose	1,38	0
K. wikenii thr x *K. phaseolosporus* CBS 2103 ade	glucose	1,66	0
K. wikenii thr x *K. phaseolosporus* CBS 2103 arg	glucose	1,43	0
K. wikenii thr x *K. phaseolosporus* CBS 2103 trp	glucose	1,41	0
K. wikenii ura x *K. phaseolosporus* CBS 2103 ade	glucose	1,98	$9,14 \times 10^3$
K. wikenii ura x *K. phaseolosporus* CBS 2103 trp	glucose	1,74	$2,42 \times 10^5$
K. wikenii thr x *K. polysporus* CBS 6899 trp	glucose	1,45	0
K. wikenii ura x *K. polysporus* CBS 6899 ade	glucose	1,03	0
K. wikenii ura x *K. polysporus* CBS 6899 met	glucose	1,31	0

TABLE 33
Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. wikenii x *K. thermotolerans* CBS 6924 met	maltose	1,34	0
K. wikenii x *K. thermotolerans* CBS 6924 thr	maltose	3,20	0
K. wikenii x *K. vanudenii* CBS 4372 his	maltose	2,63	$5,62 \times 10^6$
K. wikenii x *K. vanudenii* CBS 4372 lys	maltose	2,89	$1,94 \times 10^6$
K. wikenii lys x *K. waltii* CBS 6430	galactose	1,05	0
K. wikenii thr x *K. waltii* CBS 6430	galactose	1,59	0
K. wikenii ura x *K. waltii* CBS 6430	galactose	2,14	0
K. wikenii x *K. wickerhamii* CBS 2745 ade	lactose	3,34	0
K. wikenii x *K. wickerhamii* CBS 2745 arg lys	lactose	3,05	0
K. wikenii x *K. wickerhamii* CBS 2745 trp	lactose	3,54	0
K. wikenii x *K. wickerhamii* CBS 2745 trp ura	lactose	3,09	0
K. wikenii x *K. wickerhamii* CBS 2745 ura	lactose	2,05	0

TABLE 34

CROSSES INVOLVING *KLUYVEROMYCES* STRAIN DSM 70885
AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
DSM 70885 x *K. aestuarii* CBS 4438 ilv	lactose	3,38	0
DSM 70885 x *K. aestuarii* CBS 4904 his	lactose	1,55	0
DSM 70885 ade x *K. africanus* CBS 2654	D-xylose	4,25	0
DSM 70885 trp x *K. africanus* CBS 2654	D-xylose	2,92	0
DSM 70885 ade x *K. blattae* CBS 6284	sucrose	2,00	0
DSM 70885 trp x *K. blattae* CBS 6284	sucrose	1,55	0
DSM 70885 x *K. bulgaricus* CBS 5667 lys	lactose	0,95	$4,21 \times 10^6$
DSM 70885 x *K. bulgaricus* CBS 5667 met	lactose	3,17	$9,46 \times 10^3$
DSM 70885 x *K. cicerisporus* CBS 4857 ilv	lactose	1,07	$1,08 \times 10^3$
DSM 70885 x *K. cicerisporus* CBS 4857 trp	lactose	2,36	$2,12 \times 10^3$
DSM 70885 ade x *K. delphensis* CBS 2170	galactose	4,51	0
DSM 70885 trp x *K. delphensis* CBS 2170	galactose	2,70	0
DSM 70885 x *K. dobzhanskii* CBS 2104 ade	maltose	3,08	$9,38 \times 10^5$
DSM 70885 x *K. dobzhanskii* CBS 2104 his	maltose	2,13	$1,18 \times 10^4$
DSM 70885 x *K. drosophilarum* CBS 2896 met thr	maltose	1,09	$7,34 \times 10^2$
DSM 70885 x *K. drosophilarum* CBS 2896 trp	maltose	3,16	$9,17 \times 10^5$
DSM 70885 x *K. fragilis* CBS 1556 leu	lactose	3,88	$7,89 \times 10^3$
DSM 70885 x *K. fragilis* CBS 1556 met	lactose	1,37	$8,03 \times 10^2$
DSM 70885 x *K. lactis* CBS 683 trp	lactose	3,06	$1,63 \times 10^2$
DSM 70885 x *K. lactis* CBS 2359 arg ura	lactose	2,38	$2,02 \times 10^6$
DSM 70885 ade x *K. lodderi* CBS 2757	L-arabinose	4,53	0
DSM 70885 trp x *K. lodderi* CBS 2757	L-arabinose	1,73	0
DSM 70885 x *K. marxianus* CBS 6556 his	lactose	4,85	$8,10 \times 10^5$
DSM 70885 x *K. marxianus* CBS 6556 leu pro	lactose	6,63	$1,22 \times 10^5$
DSM 70885 x *K. marxianus* CBS 6923 lys	lactose	4,73	$2,39 \times 10^3$
DSM 70885 x *K. marxianus* CBS 6923 trp	lactose	4,55	$1,01 \times 10^3$

TABLE 34

Continued

PARENT STRAINS	Carbon source in recovery medium	Viable count $\times 10^8$ per ml	Number of recombinants/10^8 mated cell population
DSM 70885 ade x *K. phaffii* CBS 4417	raffinose	2,77	0
DSM 70885 trp x *K. phaffii* CBS 4417	raffinose	1,75	0
DSM 70885 ade x *K. phaseolosporus* CBS 2103	L-arabinose	3,87	0
DSM 70885 trp x *K. phaseolosporus* CBS 2103	L-arabinose	3,39	0
DSM 70885 ade x *K. phaseolosporus* CBS 2103 arg	glucose	1,59	0
DSM 70885 ade x *K. phaseolosporus* CBS 2103 trp	glucose	3,83	0
DSM 70885 trp x *K. phaseolosporus* CBS 2103 ade	glucose	3,13	0
DSM 70885 trp x *K. phaseolosporus* CBS 2103 arg	glucose	2,66	0
DSM 70885 ade x *K. polysporus* CBS 6899	L-arabinose	1,55	0
DSM 70885 trp x *K. polysporus* CBS 6899	L-arabinose	1,92	0
DSM 70885 x *K. thermotolerans* CBS 6924 met	maltose	4,03	0
DSM 70885 x *K. thermotolerans* CBS 6924 thr	maltose	1,96	0
DSM 70885 x *K. vanudenii* CBS 4372 his	maltose	2,97	$6,56 \times 10^5$
DSM 70885 x *K. vanudenii* CBS 4372 lys	maltose	2,64	$2,48 \times 10^6$
DSM 70885 ade x *K. waltii* CBS 6430	galactose	1,80	0
DSM 70885 trp x *K. waltii* CBS 6430	galactose	1,37	0
DSM 70885 x *K. wickerhamii* CBS 2745 leu	lactose	3,29	0
DSM 70885 x *K. wickerhamii* CBS 2745 lys	lactose	3,34	0
DSM 70885 ade x *K. wikenii* CBS 5671	trehalose	1,43	0
DSM 70885 trp x *K. wikenii* CBS 5671	trehalose	1,46	0

TABLE 35

CROSSES INVOLVING *KLUYVEROMYCES LACTIS* AND THE PRESUMPTIVE IMPERFECT FORMS OF SOME *KLUYVEROMYCES* SPECIES

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. lactis CBS 683 arg x *C. kefyr* CBS 834	maltose	1,04	0
K. lactis CBS 683 lys x *C. kefyr* CBS 834	maltose	2,77	0
K. lactis CBS 683 trp x *C. kefyr* CBS 834	maltose	2,41	$4,02 \times 10^5$
K. lactis CBS 2359 arg ura x *C. kefyr* CBS 834	maltose	1,62	0
K. lactis CBS 683 lys x *C. macedoniensis* CBS 600	maltose	2,60	$1,08 \times 10^5$
K. lactis CBS 683 trp x *C. macedoniensis* CBS 600	maltose	2,70	$3,63 \times 10^4$
K. lactis CBS 683 ura x *C. macedoniensis* CBS 600	maltose	1,76	$2,61 \times 10^4$
K. lactis CBS 2359 arg ura x *C. macedoniensis* CBS 600	maltose	4,43	$5,60 \times 10^5$
K. lactis CBS 683 arg x *C. pseudotropicalis* CBS 607	maltose	1,72	0
K. lactis CBS 683 lys x *C. pseudotropicalis* CBS 607	maltose	3,28	0
K. lactis CBS 683 trp x *C. pseudotropicalis* CBS 607	maltose	2,79	0
K. lactis CBS 2359 arg ura x *C. pseudotropicalis* CBS 607	maltose	2,54	0
K. lactis CBS 683 lys x *T. sphaerica* CBS 141 ade	lactose	1,95	$3,59 \times 10^5$
K. lactis CBS 683 trp x *T. sphaerica* CBS 141 ilv	lactose	3,04	$3,23 \times 10^7$
K. lactis CBS 2359 arg ura x *T. sphaerica* CBS 141 ade	lactose	1,15	$2,00 \times 10^7$
K. lactis CBS 2359 arg ura x *T. sphaerica* CBS 141 ilv	lactose	2,60	$8,07 \times 10^6$
K. lactis CBS 6315 ade x *T. sphaerica* CBS 141 ilv	lactose	2,43	$4,58 \times 10^6$
K. lactis CBS 6315 trp x *T. sphaerica* CBS 141 ade	lactose	1,41	$2,80 \times 10^7$

TABLE 36

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 6556 AND REPRESENTATIVES OF OTHER ASCOGENOUS SPECIES

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus his x *P. etchellsii* CBS 2011	lactose	4,99	0
K. marxianus leu pro x *P. etchellsii* CBS 2011	lactose	3,52	0
K. marxianus his x *Sacch. cerevisiae* CBS 432	lactose	5,18	0
K. marxianus leu pro x *Sacch. cerevisiae* CBS 432	lactose	3,37	0
K. marxianus his x *Sacch. cerevisiae* CSIR Y48	lactose	1,87	0
K. marxianus leu pro x *Sacch. cerevisiae* CSIR Y108	lactose	1,57	0
K. marxianus his x *Sacch. chevalieri* CBS 400	lactose	3,46	0
K. marxianus leu pro x *Sacch. chevalieri* CBS 400	lactose	5,11	0
K. marxianus his x *Sacch. kloeckerianus* CBS 5503	lactose	3,85	0
K. marxianus leu pro x *Sacch. kloeckerianus* CBS 5503	lactose	4,67	0
K. marxianus his x *Sacch. montanus* CBS 4506	lactose	7,37	0
K. marxianus leu pro x *Sacch. montanus* CBS 4506	lactose	5,89	0
K. marxianus his x *Torulaspora rosei* CSIR Y857	lactose	5,67	0
K. marxianus leu pro x *Torulaspora rosei* CSIR Y857	lactose	4,70	0
K. marxianus his x *Z. rouxii* CBS 4838	lactose	3,41	0
K. marxianus leu pro x *Z. rouxii* CBS 4838	lactose	2,61	0
K. marxianus his x *Z. rouxii* CSIR Y849	lactose	1,59	0
K. marxianus leu pro x *Z. rouxii* CSIR Y849	lactose	2,31	0

TABLE 37

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 6923 AND REPRESENTATIVES OF OTHER ASCOGENOUS SPECIES

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus lys x *P. etchellsii* CBS 2011	lactose	5,12	0
K. marxianus trp x *P. etchellsii* CBS 2011	lactose	3,15	0
K. marxianus ura x *P. etchellsii* CBS 2011	lactose	4,18	0
K. marxianus ade x *Sacch. cerevisiae* CBS 432	lactose	1,65	0
K. marxianus lys x *Sacch. cerevisiae* CBS 432	lactose	1,28	0
K. marxianus lys x *Sacch. cerevisiae* CSIR Y2	lactose	2,78	0
K. marxianus ade x *Sacch. cerevisiae* CSIR Y48	lactose	1,38	0
K. marxianus ade x *Sacch. cerevisiae* CSIR Y108	lactose	1,74	0
K. marxianus lys x *Sacch. chevalieri* CBS 400	lactose	3,06	0
K. marxianus trp x *Sacch. chevalieri* CBS 400	lactose	2,77	0
K. marxianus lys x *Sacch. kloeckerianus* CBS 5503	lactose	4,19	0
K. marxianus trp x *Sacch. kloeckerianus* CBS 5503	lactose	2,56	0
K. marxianus lys x *Sacch. montanus* CBS 4506	lactose	3,36	0
K. marxianus trp x *Sacch. montanus* CBS 4506	lactose	3,02	0
K. marxianus ura x *Sacch. montanus* CBS 4506	lactose	3,38	0
K. marxianus lys x *Torulaspora rosei* CSIR Y857	lactose	4,67	0
K. marxianus trp x *Torulaspora rosei* CSIR Y857	lactose	1,62	0
K. marxianus his x *Z. rouxii* CBS 4838	lactose	1,27	0
K. marxianus lys x *Z. rouxii* CBS 4838	lactose	1,49	0
K. marxianus trp x *Z. rouxii* CBS 4838	lactose	1,40	0
K. marxianus ura x *Z. rouxii* CBS 4838	lactose	1,47	0
K. marxianus asp x *Z. rouxii* CBS 5499	lactose	2,84	0
K. marxianus ade x *Z. rouxii* CBS 5499	lactose	2,02	0
K. marxianus trp x *Z. rouxii* CSIR Y849	lactose	3,03	0
K. marxianus ura x *Z. rouxii* CSIR Y849	lactose	4,56	0
K. marxianus lys x *Z. rouxii* CSIR Y850	lactose	2,72	0
K. marxianus trp x *Z. rouxii* CSIR Y850	lactose	3,32	0

TABLE 38

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CSIR Y293 AND REPRESENTATIVES OF OTHER ASCOGENOUS SPECIES

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. marxianus leu x *P. etchellsii* CBS 2011	lactose	7,07	0
K. marxianus met x *P. etchellsii* CBS 2011	lactose	8,91	0
K. marxianus trp x *P. etchellsii* CBS 2011	lactose	3,15	0
K. marxianus leu x *Sacch. cerevisiae* CBS 432	lactose	2,57	0
K. marxianus met x *Sacch. cerevisiae* CBS 432	lactose	1,69	0
K. marxianus met x *Sacch. cerevisiae* CSIR Y2	lactose	2,77	0
K. marxianus leu x *Sacch. cerevisiae* CSIR Y48	lactose	2,34	0
K. marxianus met x *Sacch. cerevisiae* CSIR Y108	lactose	2,89	0
K. marxianus met x *Sacch. chevalieri* CBS 400	lactose	4,85	0
K. marxianus trp x *Sacch. chevalieri* CBS 400	lactose	3,26	0
K. marxianus leu x *Sacch. kloeckerianus* CBS 5503	lactose	1,63	0
K. marxianus met x *Sacch. kloeckerianus* CBS 5503	lactose	3,87	0
K. marxianus leu x *Sacch. montanus* CBS 4506	lactose	5,67	0
K. marxianus met x *Sacch. montanus* CBS 4506	lactose	6,89	0
K. marxianus leu x *Torulaspora rosei* CSIR Y857	lactose	5,52	0
K. marxianus met x *Torulaspora rosei* CSIR Y857	lactose	3,22	0
K. marxianus leu x *Z. rouxii* CBS 4838	lactose	3,16	0
K. marxianus met x *Z. rouxii* CBS 4838	lactose	1,80	0
K. marxianus leu x *Z. rouxii* CSIR Y850	lactose	3,70	0
K. marxianus trp x *Z. rouxii* CSIR Y850	lactose	1,86	0

TABLE 39

CROSSES INVOLVING *KLUYVEROMYCES THERMOTOLERANS* CBS 6924 AND *SACCHAROMYCES BAYANUS*

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
K. thermotolerans met x *Sacch. bayanus* CSIR Y119	galactose	2,90	0
K. thermotolerans met x *Sacch. bayanus* CSIR Y120	galactose	3,30	0
K. thermotolerans thr x *Sacch. bayanus* CSIR Y119	galactose	2,17	0
K. thermotolerans thr x *Sacch. bayanus* CSIR Y120	galactose	1,33	0

TABLE 40

VIABILITY OF HYBRID ASCOSPORES

HYBRID STRAINS	Total count[1] of ascospores/ml	Viable count[2] of ascospores/ml	% viability
K. marxianus CBS 6923 trp x *K. bulgaricus* CBS 5667 lys	$1,71 \times 10^7$	$2,81 \times 10^6$	16,4
K. marxianus CBS 6923 lys x *K. cicerisporus* CBS 4857 trp	$7,65 \times 10^6$	$8,55 \times 10^5$	11,2
K. marxianus CBS 6556 leu pro x *K. dobzhanskii* CBS 2104	$2,50 \times 10^4$	$5,05 \times 10^3$	20,2
K. marxianus CBS 6923 his x *K. drosophilarum* CBS 2896	$6,58 \times 10^6$	$2,48 \times 10^6$	37,7
K. marxianus CBS 6923 trp x *K. fragilis* CBS 1556 met	$6,65 \times 10^6$	$2,67 \times 10^5$	4,0
K. marxianus CBS 6923 x *K. lactis* CBS 2359 arg ura	$1,19 \times 10^7$	$3,67 \times 10^6$	30,8
K. marxianus CBS 6923 ade x *K. phaseolosporus* CBS 2103	$2,66 \times 10^7$	$1,31 \times 10^7$	49,2
K. marxianus CBS 6923 ade x *K. thermotolerans* CBS 6924	$4,80 \times 10^6$	$8,90 \times 10^5$	18,5
K. marxianus CBS 6923 ura x *K. vanudenii* CBS 4372	$2,82 \times 10^6$	$1,24 \times 10^6$	43,9
K. marxianus CBS 6556 ade x *K. wikenii* CBS 5671	$7,25 \times 10^6$	$1,94 \times 10^6$	26,8
K. dobzhanskii CBS 2104 ade x *K. fragilis* CBS 1556	$1,92 \times 10^7$	$3,40 \times 10^6$	17,7
K. drosophilarum CBS 2896 trp x *K. waltii* CBS 6430	$2,80 \times 10^6$	$8,35 \times 10^5$	29,8
K. vanudenii CBS 4372 lys x *K. wickerhamii* CBS 2745	$5,80 \times 10^6$	$4,06 \times 10^5$	7,1

[1] Results obtained from a direct count in a haemacytometer of the ascospore suspensions in paraffin.

[2] Results obtained from YM agar plates on which the ascospore suspensions in paraffin were plated.

TABLE 41

CROSSES INVOLVING *KLUYVEROMYCES MARXIANUS* CBS 6923 ade (MUTANT M160) AND THE REMAINING SPECIES OF THE GENUS

PARENT STRAINS	Carbon source in recovery medium	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population
Mutant M160 x *K. aestuarii* CBS 4438 ilv	lactose	1,51	0
Mutant M160 x *K. aestuarii* CBS 4904	D-ribose	2,22	0
Mutant M160 x *K. africanus* CBS 2654	lactose	1,72	0
Mutant M160 x *K. blattae* CBS 6284	lactose	1,39	0
Mutant M160 x *K. bulgaricus* CBS 5667	cellobiose	2,69	0
Mutant M160 x *K. dacerisporus* CBS 4857 trp	lactose	1,63	0
Mutant M160 x *K. delphensis* CBS 2170	lactose	2,19	0
Mutant M160 x *K. dobzhanskii* CBS 2104	lactose	2,74	0
Mutant M160 x *K. drosophilarum* CBS 2896	lactose	2,09	0
Mutant M160 x *K. fragilis* CBS 1556 met	lactose	1,93	0
Mutant M160 x *K. lactis* CBS 683 trp	maltose	1,69	0
Mutant M160 x *K. lactis* CBS 2359 arg	maltose	1,03	0
Mutant M160 x *K. lodderi* CBS 2757	lactose	3,21	0
Mutant M160 x *K. phaffii* CBS 4417	lactose	1,78	0
Mutant M160 x *K. phaseolosporus* CBS 2103	lactose	3,13	0
Mutant M160 x *K. polysporus* CBS 6899	lactose	1,57	0
Mutant M160 x *K. thermotolerans* CBS 6924	lactose	1,78	0
Mutant M160 x *K. vanudenii* CBS 4372	lactose	3,42	0
Mutant M160 x *K. waltii* CBS 6430	lactose	1,77	0
Mutant M160 x *K. wickerhamii* CBS 2745	raffinose	1,40	0
Mutant M160 x *K. wikenii* CBS 5671	lactose	1,45	0

TABLE 42

INTRAGENERIC AND INTERGENERIC CROSSES

	Crosses within the genus *Kluyveromyces*		Crosses between representatives of the genus *Kluyveromyces* and representatives of other genera		TOTAL
	Intraspecific crosses	Interspecific crosses	Crosses between *K. lactis* and presumed imperfect states of *Kluyveromyces*	Crosses between *K. marxianus* and representatives of other sporogenous genera	
Number of crosses	16	758	18	69	861
Crosses in which recombinants were detected	16	203	11	0	230
Crosses in which recombinants were not detected	0	555	7	69	631

TABLE 43

RECOMBINANT FORMATION INVOLVING AUXOTROPHIC MUTANTS OF HYBRID STRAINS

PARENT STRAINS	Viable count $x10^8$ per ml	Number of recombinants/10^8 mated cell population recovered on basal medium containing	
		lactose	maltose
K. lactis CBS 2359 arg ura x Hybrid A ade	2,31	$3,03 \times 10^3$	-
K. lactis CBS 2359 arg ura x Hybrid A his	1,53	-	$2,01 \times 10^3$
Hybrid A ade x Hybrid A his	1,71	$3,39 \times 10^5$	$2,91 \times 10^5$
Hybrid B ura x Hybrid C trp	1,23	$2,18 \times 10^3$	$2,25 \times 10^3$
Hybrid B ade x Hybrid C trp	1,52	$2,56 \times 10^3$	$2,64 \times 10^3$
Hybrid D trp x Hybrid E his	1,14	$3,07 \times 10^2$	$3,68 \times 10^2$

Hybrid A = *K. marxianus* CBS 6923 ade x *K. thermotolerans* CBS 6924

Hybrid B = *K. marxianus* CBS 6923 x *K. lactis* CBS 2359 arg ura

Hybrid C = *K. marxianus* CBS 6556 x *K. lactis* CBS 2359 arg ura

Hybrid D = *K. marxianus* CBS 6923 trp x *K. bulgaricus* CBS 5667 lys

Hybrid E = *K. drosophilarum* CBS 2896 trp x *K. vanudenii* CBS 4372 his

TABLE 44

MEAN MOLAR PERCENTAGE OF GUANINE PLUS CYTOSINE (MOL % G+C)
VALUES AS REPORTED BY VARIOUS AUTHORS

SPECIES	Nakase & Komagata (1971)	Martini, Phaff & Douglass (1972)	Poncet & Fiol (1972)	Phaff (personal communication, 1978)
K. aestuarii	-	39,5	35,4 - 35,6	39,8 - 39,9
K. africanus	-	38,0	35,6 - 35,8	38,5
K. blattae	-	-	-	33,1
K. bulgaricus	-	42,4	33,9	41,3 - 41,5
K. cicerisporus	-	43,4	39,2 - 39,5	41,2 - 41,4
K. delphensis	-	39,8	36,3	40,2
K. dobzhanskii	-	43,3	41,2 - 41,9	42,5 - 42,6
K. drosophilarum	40,0	40,7	38,5 - 39,0	39,9 - 40,9
K. fragilis	40,0 - 40,7	41,3	35,6 - 37,0	41,1 - 41,3
K. lactis	39,3 - 40,0	41,4	38,8 - 39,0	40,3
K. lodderi	-	37,7	34,4 - 34,6	35,4 - 35,7
K. marxianus	41,0	40,0	38,8 - 39,3	41,2 - 41,3
K. phaffii	-	37,4	33,6	35,3
K. phaseolosporus	-	40,8	38,5	40,5
K. polysporus	33,4	35,3	34,4 - 34,6	35,3
K. thermotolerans	44,4 - 45,9	47,4	44,0 - 44,5	46,2
K. vanudenii	-	41,7	38,0 - 38,3	40,1 - 40,3
K. waltii	45,6*	-	-	-
K. wickerhamii	-	41,5	40,2 - 40,9	41,9 - 42,4
K. wikenii	-	43,1	34,6 - 35,6	41,1 - 41,2

* Value from Kodama (1974)

TABLE 45

CROSSES BETWEEN STRAINS SHOWING DEGREES OF DNA-DNA HOMOLOGY ≥ 70%

PARENT STRAINS			Carbon source in recovery medium	Number of recombinants/10^8 mated cell population	DNA-DNA homology [1]
K. cicerisporus CBS 4857 ilv	x	*K. bulgaricus* CBS 5667 met	lactose	$4{,}90 \times 10^6$	117%
K. marxianus CBS 6923 lys	x	*K. fragilis* CBS 1556 met	lactose	$6{,}62 \times 10^4$	97%
K. phaseolosporus CBS 2103	x	*K. drosophilarum* CBS 2896 trp	maltose	$4{,}83 \times 10^6$	70%
K. vanudenii CBS 4372	x	*K. lactis* CBS 683 arg	lactose	$8{,}73 \times 10^5$	97%
K. wikenii CBS 5671	x	*K. bulgaricus* CBS 5667 lys	lactose	$5{,}93 \times 10^3$	120%
K. wikenii CBS 5671	x	*K. cicerisporus* CBS 4857 ilv	lactose	$5{,}23 \times 10^3$	103%

[1] Data from Martini (1973)

TABLE 46

CROSSES BETWEEN STRAINS SHOWING DEGREES OF DNA-DNA HOMOLOGY ≤ 36% IN WHICH RECOMBINATION WAS NOT OBSERVED

PARENT STRAINS	Carbon source in recovery medium	Recombinants/10^8 mated cell population	DNA-DNA homology [1]
K. aestuarii CBS 4438 ilv x *K. lactis* CBS 683 trp	lactose	not detected	32%
K. aestuarii CBS 4438 his x *K. vanudenii* CBS 4372	lactose	not detected	32%
K. africanus CBS 2654 x *K. vanudenii* CBS 4372 his	maltose	not detected	36%
K. bulgaricus CBS 5667 x *K. thermotolerans* CBS 6924 met	maltose	not detected	21%
K. cicerisporus CBS 4857 x *K. aestuarii* CBS 4438 his	maltose	not detected	32%
K. delphensis CBS 2170 x *K. phaffii* CBS 4417 arg	galactose	not detected	30%
K. lodderi CBS 2757 asp x *K. phaffii* CBS 4417	raffinose	not detected	20%
K. polysporus CBS 6899 ade x *K. africanus* CBS 2654	raffinose	not detected	12%
K. polysporus CBS 6899 met x *K. lodderi* CBS 2757 arg	glucose	not detected	4%
K. polysporus CBS 6899 trp x *K. phaffii* CBS 4417	raffinose	not detected	5%
K. thermotolerans CBS 6924 x *K. lactis* CBS 683 arg	lactose	not detected	32%
K. wickerhamii CBS 2745 x *K. marxianus* CBS 6923 ade	raffinose	not detected	30%

[1] Data from Martini (1973)

TABLE 47

CROSSES BETWEEN STRAINS SHOWING DEGREES OF DNA-DNA HOMOLOGY < 36% IN WHICH RECOMBINANT FORMATION WAS DETECTED

PARENT STRAINS			Carbon source in recovery medium	Number of recombinants/10^8 mated cell population	DNA-DNA homology [1]
K. bulgaricus CBS 5667	x	*K. lactis* CBS 683 trp	maltose	$7{,}98 \times 10^5$	12%
K. cicerisporus CBS 4857	x	*K. lactis* CBS 683 trp	maltose	$5{,}46 \times 10^6$	12%
K. dobzhanskii CBS 2104	x	*K. lactis* CBS 683 trp	lactose	$7{,}37 \times 10^5$	4%
K. drosophilarum CBS 2896 lys	x	*K. dobzhanskii* CBS 2104 his	maltose	$2{,}80 \times 10^5$	13%
K. fragilis CBS 1556	x	*K. dobzhanskii* CBS 2104 ade	maltose	$2{,}61 \times 10^3$	14%
K. fragilis CBS 1556	x	*K. lactis* CBS 683 trp	maltose	$7{,}92 \times 10^5$	16%
K. marxianus CBS 6556 leu pro	x	*K. drosophilarum* CBS 2896	lactose	$1{,}26 \times 10^6$	27%
K. marxianus CBS 6923	x	*K. lactis* CBS 2359 arg ura	maltose	$9{,}76 \times 10^4$	12%
K. phaseolosporus CBS 2103	x	*K. dobzhanskii* CBS 2104 ade	maltose	$2{,}29 \times 10^5$	35%
K. wickerhamii CBS 2745	x	*K. lactis* CBS 683 trp	maltose	$1{,}10 \times 10^5$	6%
K. wikenii CBS 5671	x	*K. lactis* CBS 683 trp	lactose	$5{,}10 \times 10^5$	16%
K. wikenii CBS 5671	x	*K. vanudenii* CBS 4372 lys	maltose	$1{,}94 \times 10^6$	3%

[1] Data from Martini (1973)