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Genetic identification of biological species in the *Saccharomyces* sensu stricto complex[a]

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Studies on taxonomic and evolutionary genetics of the *Saccharomyces* sensu stricto complex are considered in light of the biological species concept. Genetic variability of some physiological properties traditionally used in yeast taxonomy is discussed. Genetic hybridization analysis and molecular karyotyping revealed six biological species in the *Saccharomyces* sensu stricto complex. DNA–DNA reassociation data are concordant with the data obtained by genetic analysis. A new system for naming the cultivated *Saccharomyces* yeast (groups of cultivars) is proposed.

Keywords: *Saccharomyces* sensu stricto; sibling species; genetic taxonomy; yeast cultivars

Introduction: polymorphism of yeast physiological properties

For almost 30 years we have been studying taxogenetics of the *Saccharomyces* sensu stricto complex. Genetic principles and methods were used to analyze the natural polymorphism of different physiological properties of yeast taxonomy: ability to utilize different sugars (maltose, α-methylglucoside, sucrose, galactose, melibiose), peculiarities of the life cycle (homo-heterothallism), and antagonistic relationships (formation of killer toxins, sensitivity and resistance to toxins). In 1970 we summarized our experimental and literature data on the mutational and combinative variability of *Saccharomyces* yeasts to ferment different sugars, concluding that genes for sugar utilization were suitable as convenient markers for strain identification but not for species delimitation [66]. The DNA–DNA reassociation data obtained in Phaff's laboratory in 1978 showed that strains with different sugar capacities often had high homology of their genomes, while strains utilizing the same sugars could have quite different genomes [76]. The more one examines strains, the higher one can expect variability for any sugar utilization property. The fermentation of sucrose, maltose, α-methylglucoside, melibiose and starch is controlled in the yeast *Saccharomyces* by the gene families: (*SUC*)n, (*MAL*)n, (*MGL*)n, (*MEL*)n, and (*STA*)n [30,65,87]. If, for example, two sucrose-fermenting strains containing different polymeric *SUC* genes are crossed, it is possible to find among meiotic progeny non-fermenting sucrose recombinants without *SUC* genes. Polymeric genes of sugar fermentations are located on telomeric regions of chromosomes and can migrate from one chromosome to another. The hypothesis has been proposed that the families of polymeric sugar genes have arisen by recombinational rearrangements of the telomere-associated sequences [6,7]. In some populations of *Saccharomyces* yeasts an accumulation of polymeric genes *MAL*, *MEL*, *SUC* and *STA* takes place [30,48,52,56–58,65]. Strains incapable of fermenting different sugars are natural mutants. When natural maltose non-fermenting *Saccharomyces* strains were crossed, we obtained complementation as the hybrids were able to ferment maltose [26,28]. Genetic analysis revealed interaction of genes both within the same locus and between different loci. Each *MAL* locus is a complex of three genes: maltose permease Gene 1, α-glucosidase Gene 2 and regulatory Gene 3. All three gene functions are required for a strain to ferment maltose. Natural *S. cerevisiae* strains unable to ferment maltose carry one or two defective genes (Gene 1 and/or Gene 3) but active α-glucosidase Gene 2 [52]. The results obtained suggest that *MAL*1 is the progenitor locus from which the other loci were derived.

While studying natural *Saccharomyces* strains unable to ferment galactose we found that they were natural mutants with one, two or three mutant alleles [37,38]. Genetic analysis of *Saccharomyces* strains of different origin allowed us to reconstruct a process of regressive microevolution of the Gal^- property.

Homo-heterothallism is not suitable for differentiation of yeast species either. Mutations in three genes, HMR, HML and HO, could convert homothallic strains into heterothallic ones [18,64,72]. Many authors propose to use a sensitivity of yeasts to some killer toxins for species differentiation. However, genetic analysis of *Saccharomyces* strains revealed that such tests were not suitable for taxonomic purposes [27,70].

Thus, many phenotypic characteristics are not suitable for classification and identification of yeasts within the *Saccharomyces* sensu stricto complex. What can genetics propose instead of the physiological tests? Genetics relate to the concept of biological species and provide objective methods of species identification. Although the concepts of biological species in higher eukaryotes are central to modern biology, the studies concerning the biological species concept in lower eukaryotes are very limited. Only during recent decades have mycologists noticed that within both ascomycetous and basidiomycetous fungi there are reproductively isolated populations representing different biological species [74,75]. However zymologists, traditionally

[a]This paper is dedicated to Danish scientists Ö Winge and V Jensen in recognition of their contributions to zymology.

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using the bacteriological methodology for their studies, were more conservative.

The Danish geneticist Winge, having great experience in plant and animal genetics, was the first to characterize a reproductive genetic isolation of some yeasts. Having observed in 1939 that in some crossings combinations with strains of different origin produced hybrids with reduced ascospore viability, Winge and Lausten came to the conclusion that mating capability and viability of sexual progeny could be used as a criterion for species differentiation within the genus *Saccharomyces* [85]. The authors proposed to examine spore viability of parental strains as controls. However, studies conducted in other laboratories showed that natural and, especially, industrial strains often had low ascospore viability due to numerous genetic factors, in particular, polyploidy, aneuploidy, recessive lethals, deletions, translocations and inversions present in heterozygotic stages. Spores of such strains are genetically heterogeneous and their total viability provides anomalous results when used in hybridization. Objective estimation of strain relatedness is possible using specially constructed inbred lines having high ascospore viability instead of initial natural or industrial strains. It is necessary to compare fertility of hybrids with fertility of their inbred parents. In order to obtain parents with high ascospore viability it is usually enough to clone homothallic strains from one spore. For heterothallic yeasts intratetrad crosses may be needed. Monosporic cloning of strains leads to an elimination of lethal factors [25] and, therefore, to increased ascospore viability.

Cultured yeast *Saccharomyces cerevisiae*

The genetics of a large collection of *Saccharomyces* sensu stricto strains isolated from different sources (fermentation processes, soil, animal intestines, etc) in various geographic areas (Europe, Middle Asia, Siberia, Russian Far East, Japan, Africa, North and South America) was studied in our laboratory. Strains having different sporulation ability have been used. First, all strains were cloned to obtain monosporic fertile cultures. For some strains it was necessary to inbreed over several generations to achieve this genetic property. Among monosporic cultures, clones with good sporulation and high ascospore viability were selected. Strains with reduced ascospore viability showed, for the most part, segregation for colony size. Similar observations have been made by others [25]. Colonies of standard size were used further for crossing experiments. Hybrids of homothallic yeasts were obtained by a 'spore-to-spore' mating method using a micromanipulator [39]. Hybrids of heterothallic yeasts were obtained by mass mating of cells and by following isolation of zygotes with a micromanipulator.

The hybrid nature of yeasts and haploidy of spores were investigated by analyzing the segregation of control of natural markers (maltose, sucrose, raffinose, melibiose and galactose fermentations, mating types and homoheterthallism) or induced auxotrophic mutations. We analyzed over 100 hybrids in all. Part of this genetic analysis is presented in Table 1. Viability data on hybrid ascospores convincingly demonstrated that all the cultivated yeasts originally designated as *S. cerevisiae*, *S. aceti*, *S. capensis*, *S. gaditensis*, *S. hienipiensis*, *S. lindneri*, *S. mangini*, *S. norbensis*, *S. oleaceus*, *S. oleaginosus*, *S. oviformis*, *S. oxidans* and *S. hispanica* belonged to a single biological species *S. cerevisiae* [40]. Ascospore viability varied from 44 to 100%. Most hybrids (84%) showed 70–100% ascospore viability. Unfortunately, some of the type cultures of the taxonomic species were asporogenic and, therefore, could not be studied by genetic hybridization analysis in our laboratory.

Other authors showed that yeasts belonging to the taxonomic species *S. diastaticus*, *S. italicus*, *S. chevalieri* and *S. cordubensis* formed fertile hybrids with *S. cerevisiae* [8–10,79,86]. The DNA–DNA reassociation data (86–100% DNA homology) confirmed that the taxonomic species mentioned above and some others (*S. beticus*, *S. coreanus*, *S. cheresiensis*, *S. hispalensis*, *S. ellipsoideus*, *S. lindneri*, *S. logos*, *S. odessa*, *S. steineri* and *Candida robusta*) belong to the biological species *S. cerevisiae* [77,81,82,88].

Table 1 Genetic study of the synonyms of the biological species *S. cerevisiae*

Origin of hybrids	No. of ascospores isolated	No. of viable ascospores of hybrids (%)
S. cerevisiae ATCC 48498 × *S. cerevisiae* CBS 5287	172	79
S. cerevisiae ATCC 48498 × *S. aceti* CBS 4054	176	74
S. cerevisiae L2-43 × *S. capensis* M3-33	164	90
S. cerevisiae ATCC 48498 × *S. gaditensis* CBS 6006	96	93
S. cerevisiae ATCC 48498 × *S. hienipiensis* CBS 4903	100	84
S. cerevisiae ATCC 48498 × *S. lindneri* CBS 403	84	90
S. cerevisiae ATCC 48498 × *S. mangini* CBS 405	88	83
S. cerevisiae ATCC 48498 × *S. norbensis* CBS 5378	96	56
S. cerevisiae CBS 5287 × *S. oleaceus* CBS 3093	32	50
S. cerevisiae CBS 5287 × *S. oleaginosus* CBS 3081	76	66
S. cerevisiae ATCC 48498 × *S. oviformis* M 180	10	86
S. cerevisiae ATCC 48498 × *S. oxidans* CBS 4093	100	99
S. cerevisiae ATCC 48498 × *S. hispanica* CBS 5835	56	64

CBS 403 = VKM Y-407; CBS 405 = VKM Y-481; CBS 5287 = VKM Y-502; CBS 5378 = VKM Y-1232; ATCC 48498 = M 437; CBS 4093 = SBY 2592. ATCC = American Type Culture Collection, Rockville, USA; VKM = All-Russian Collection of Microorganisms, Moscow, Russia; CBS = Centraalbureau voor Schimmelcultures, Delft, Holland; M 437 is from Magarach Scientific Research Institute of Viticulture and Wine Making, Yalta, Crimea, Ukraine. SBY = Seccion de Bioquimica, Instituto Nacional de Investigaciones Agrarias, Madrid, Spain. For all taxonomic species the type cultures were studied, except *S. cerevisiae*, *S. capensis* and *S. oviformis*.

Thus, the biological species *S. cerevisiae* includes strains with a common gene pool and this is confirmed by recombination of numerous biochemical and physiological markers in so-called 'interspecific' crosses.

So far, two Asian populations of wild *S. cerevisiae* yeasts have been described: one in Central Siberia and another in Japan [42,59]. *S. cerevisiae* strains were isolated from exudates of broad-leaved trees and soils. The hypothesis of an East-Asian origin for the domesticated species of *S. cerevisiae* was put forward [61]. Recently some *Saccharomyces* strains isolated by A Capriotti from soils in Holland, Finland and Spain have been genetically reidentified as *S. cerevisiae* [47,53]. Electrophoretic karyotyping showed a very close karyotypic similarity between wild *S. cerevisiae* yeasts despite the variable geographic origin of the strains [47,49]. At the same time cultivated strains of *S. cerevisiae* are characterized by molecular chromosomal polymorphism [14,49,83]. Probably European semi-wild soil strains of *S. cerevisiae* are rare synantropic populations originating directly or indirectly from human activity.

Saccharomyces paradoxus and other wild species

The distribution of *S. cerevisiae* is linked to production of alcoholic beverages and baking. Despite the practical significance and century-long intensive study of the yeast, its closest wild relatives remained unknown until recently. There were several reports in the literature of isolation of *Saccharomyces*-like yeasts from different natural sources, such as exudates of broad-leaved trees, uncultivated soils and insects (see references in [31,47]).

Genetic study of seven *S. cervisiae* var *terrestris* strains isolated from Danish forest soil by Jensen [13] showed that they formed fertile hybrids with one another (70% ascospore viability) and sterile hybrids with the *S. cerevisiae* reference strain [29,33]. Thus, genetic analysis revealed a new sibling species of *S. cerevisiae*.

Then we focused our attention on two taxonomic species described as natural *Saccharomyces* sensu stricto isolates: *S. paradoxus* Batschinskaya and *S. cerevisiae* var *tetrasporus* (Bejerink ex Dekker) Phaff *et al. Saccharomyces paradoxus* was isolated for the first time by Batschinskaya from oak and birch sap in St Petersburg and the Poltava region [4]. According to the data of Kudrjawzew [19], these yeasts were widely spread in oak exudates in the European part of Russia, Ukraine (Crimea) and Russian Far East.

S. paradoxus strains isolated by Batshinskaya and Kudrjawzew from exudates of trees have been lost. In 1980–1993 we isolated 45 pure cultures of a *Saccharomyces* species from mixed populations of yeasts, fungi and bacteria, occurring in exudates of *Quercus robur* (in Europe and Siberia) and *Q. mongolicus* (in Russian Far East) [31,33,36,42,43,47,53,62]. Inbred lines of wild *Saccharomyces* were obtained and crossed with genetic testers of the biological species *S. cerevisiae* (CBS 5287) and *S. paradoxus* (CBS 5829). Reference strains were marked by UV-induced adenine (*ade*) auxotrophies (red colonies). Effective hybridization of the strains with the reference testers indicated that they belonged to the genus *Saccharomyces* sensu stricto. The genomes of monosporic cultures of wild strains were compared with genomes of the test strains by tetrad analysis of hybrids. The high viability of hybrid ascospores and the monogenic segregation of the control marker *ade* in random spore analysis gave evidence of a high homology of the genomes compared and indicated that the parent strains belonged to the same biological species. Conversely, inviability of hybrid ascospores allowed us to assign the corresponding parent strains to different species. Results of genetic studies of some natural strains are presented in Table 2. Forty out of 45 strains gave fertile hybrids with *S. paradoxus*, but sterile hybrids with *S. cerevisiae*. Five *Saccharomyces*-like strains isolated in Caucasus (one strain), Siberia (three strains) and Russian Far East (one strain) were assigned to *S. cerevisiae*, as their crosses with *S. cerevisiae* showed high ascospore viability.

Taking into account the priority of Batschinskaya's description of the *S. paradoxus* species, we reinstated this taxon but not *S. cerevisiae* var *terrestris* [31,33]. Genetic analysis of *S. cerevisiae* var *tetrasporus* and *S. cerevisiae* var *terrestris* yeasts showed that they belonged to the biological species *S. paradoxus* (Table 3). The sterility of hybrids between *S. cerevisiae* and *S. cerevisiae* var *tetrasporus* was first demonstrated by Gilliland in 1949 [8]. Genetic reidentification of the yeast *S. douglasii* (CBS 7400) which is widely used in molecular and genetic studies showed that the species name *S. douglasii* nom nud is a synonym for *S. paradoxus* [41]. Extensive examination of *Saccharomyces* strains from different culture collections gave further evidence of the wide distribution of the *S. paradoxus* yeast throughout the world [31,33,43,47,50,53–55]. Probably, for the first time *S. paradoxus* strains isolated from oak exudates were studied by Lindner [21] and Ludwig [23], however it was done without a taxonomic description.

The data on DNA–DNA reassociation and PCR-analysis

Table 2 Genetic identification of *S. paradoxus* strains isolated from oak exudates

Origin of hybrids	No. of tetrads isolated	No. of viable ascospores of hybrids (%)	Segregation of control marker *ade* : *ADE*
	S. paradoxus × *S. paradoxus*		
N7 × CBS 5829	22	75	31 : 24
N8 × CBS 5829	30	63	35 : 45
N9 × CBS 5829	25	76	35 : 40
N10 × CBS 5829	16	88	23 : 28
N11 × CBS 5829	18	78	27 : 29
N12 × CBS 5829	16	78	23 : 27
N13 × CBS 5289	15	68	20 : 21
	S. paradoxus × *S. cerevisiae*		
N7 × CBS 5287	16	0	–
N8 × CBS 5287	29	0	–
N9 × CBS 5287	17	0	–
N10 × CBS 5287	10	0	–
N11 × CBS 5287	14	0	–
N12 × CBS 5287	25	0	–
N13 × CBS 5287	25	0	–

The strains originate from geographic regions: N7 from St Petersburg, Nos 8 and 13 from Moscow region, N9 from Uzbekistan, Nos 10 and 11 from Novgorod region, N12 from the Caucasus and CBS 5829 from Denmark.

Table 3 Genetic study of the synonyms of the biological species *S. paradoxus*

Origin of hybrids	No. of ascospores isolated	No. of viable ascospores (%)
S. cervisiae var *terrestris* CBS 5829 × *S. cerevisiae* var *terrestris* N1	96	70
S. cerevisiae var *terrestris* CBS 5829 × *S. paradoxus* CBS 432	96	67
S. cerevisiae var *terrestris* CBS 5829 × *S. cerevisiae* var *tetrasporus* CBS 406	108	55
S. cerevisiae var *terrestris* CBS 5829 × *S. cerevisiae* var *tetrasporus* CBS 2980	108	40
S. cerevisiae var *terrestris* CBS 5289 × *S. douglasii* CBS 7400	112	69

obtained in different laboratories confirmed the existence of the biological species *S. paradoxus* [24,80,88]. The DNA–DNA homology between *S. cerevisiae* and *S. paradoxus* is 50%. Electrophoretic karyotypes of these two sibling species are nearly identical, supporting their genomic similarities [49]. A truly wild yeast, *S. paradoxus* is of interest for comparative and evolutionary genetics studies [12,22,32,59,68].

Recently three new biological species were identified as genetically isolated populations of the *Saccharomyces* sensu stricto yeasts [44,50]. Two of them were found in Japan and one in Brazil. The strains yielded sterile hybrids with all three species testers, indicating that they could not be assigned to any of the known species in the *Saccharomyces* sensu stricto complex. Two Japanese biological species were shown by DNA–DNA reassociation to have low DNA similarity to the type cultures of *S. cerevisiae*, *S. paradoxus* and *S. bayanus* [15,88] while the Brazilian species is closely related to *S. paradoxus* according to some preliminary data [20].

The cultured yeast *Saccharomyces bayanus*

The existence of one more biological species was shown in 1970 on the basis of ecological and molecular data in three different laboratories [3,5,73]. Cryophilic wine strains isolated in Moldavia were classified as *S. uvarum* [3]. Mixed cultivation of *S. cerevisiae* and *S. uvarum* yeasts in grape must at different temperatures showed that *S. cerevisiae* predominated at 25°C and *S. uvarum* predominated at 8–10°C. The DNA–DNA homology between *S. cerevisiae* and *S. uvarum* (NRRL Y-969) was only 40% [5]. Rossini *et al* [78] showed that the type cultures of *S. bayanus* and *S. uvarum* had 98% homology of their total DNAs and the latter species was synonymous with *S. bayanus*. The following list of synonyms of *S. bayanus* has been compiled on the basis of DNA–DNA reassociation data: *S. abuliensis*, *S. globosus*, *S. heterogenicus*, *S. intermedius* var *validensis*, *S. tubiformis*, *S. inusitatus* and *S. willianus* [81,82]. The DNA–DNA homology between *S. cerevisiae and S. bayanus* was 20% and between *S. paradoxus* and *S. bayanus* 30%. The cryophilic character and DNA peculiarities of *S. bayanus* were confirmed by Japanese authors [2,15,17,88].

The biological species *S. bayanus* was genetically identified in our laboratory using strains CBS 7001 (= MCYC 623 = NRRL Y-11845), NRRL Y-969 and VKM Y-1146 [33,60]. All three strains formed fertile hybrids with one another, but sterile hybrids with both *S. cerevisiae* and *S. paradoxus*.

The specific ecological niche of *S. bayanus* is found in viticulture and wine making at low temperatures. It was shown that melibiose-fermenting *Saccharomyces* strains isolated from wine making belonged, for the most part, to the biological species *S. bayanus*. Among 45 wine Mel^+ strains of *Saccharomyces* sensu stricto isolated in Russia, Moldavia, Slovakia, Switzerland, France, Italy and Spain only three strains were genetically identified as *S. cerevisiae*, while the others were assigned to *S. bayanus* [33,34,45,53,63,67]. Our results showed, however, that the Mel^+ phenotype cannot be used as a discriminative character for differentiation of *S. cerevisiae* and *S. bayanus*, since *bona fide S. cerevisiae* can be found in wine. Besides, three so-called '*S. uvarum*' strains isolated in due time from *Drosophila* in California have been recently assigned by genetic analysis to three biological species: *S. cerevisiae*, *S. bayanus* and *S. paradoxus* [54]. The distribution of *S. bayanus* species in nature has not been studied practically. Rare wild strains of *S. bayanus* were isolated from *Mesophylax adoperus*, fruit bodies of mushrooms and *Drosophila* [33,47,54]. Electrophoretic karyotyping revealed that yeast *S. bayanus* possessed a species-specific karyotype readily distinguishable from those of *S. cerevisiae* and *S. paradoxus* [17,45–47,49,67]. It should be noted that the Mel^+ yeast of bottom fermentation *S. pastorianus* (syn *S. carlsbergensis*) is probably an interspecific hybrid of *S. cerevisiae* and *S. bayanus* [71,81,82].

Identification of the biological species *S. bayanus* among wine yeasts opens the possibility of using its genetic pool in breeding programs. In particular, it has been shown already that interspecific hybridization *S. cerevisiae* × *S. bayanus* led to the creation of highly productive Champagne yeasts with improved fermentation ability at low temperatures [16,69,89]. *S. bayanus* strains can improve wine composition [1,11].

Karyosystematics of the *Saccharomyces* sensu stricto yeasts

Chromosomal DNAs of monosporic cultures of the six biological species were compared by contour-clamped homogeneous electric field (CHEF) gel electrophoresis and Southern blot hybridization with 22 cloned *S. cerevisiae* genes assigned to fifteen different chromosomes (except chromosome VI) [44,46,49, unpublished data]. For electrophoretic karyotyping we made a monosporic cloning of the

strains because this led to the homozygosity of chromosomal sets and an elimination of supernumerary chromosomes. Despite the big divergence of their genomes as determined by total DNA–DNA reassociation, six biological species display similar basic karyotypic characters, ie the same haploid number of chromosomes ($n = 16$) and the same range of chromosomal bands (from 250 to 2200 kb) (Figure 1). However, individual sizes of each chromosome in different strains can vary because of chromosome length polymorphisms. Karyotype patterns of *S. cerevisiae* and *S. paradoxus* are nearly identical, while *S. bayanus* and a Brazilian *Saccharomyces* sp have species-specific karyotypes [17,44,45,47,49,67]. Two Japanese species displayed karyotypes similar to those of *S. cerevisiae* and *S. paradoxus* [50]. Southern blot analysis clearly demonstrated the identity of karyotypes of *S. cerevisiae* and *S. paradoxus*. The order and the sizes of the homeologous chromosomes in the two sibling species are the same. The intensity of DNA–DNA hybridization in *S. paradoxus* was variable with different cloned genes of *S. cerevisiae*. As a rule, *S. paradoxus* strains showed weaker hybridization than *S. cerevisiae* strains. The hybridization data showed that genomic DNAs of the remaining four sibling species are more divergent from *S. cerevisiae* genomic DNA than *S. paradoxus* from *S. cerevisiae*. It is likely that only four chromosomes are similar in size in all six biological species [46, unpublished data].

First separated by van der Walt [84] on the basis of peculiar morphological and physiological properties, the *Saccharomyces* sensu stricto complex was then identified as a system of crossed species [33] having chromosomal similarity [44,45,47,49,50]. Thus, the gene pool of the *Saccharomyces* sensu stricto yeasts is discrete as it is represented by reproductively isolated, biological species. The biological yeast species is not only a theoretical, but also an operational concept.

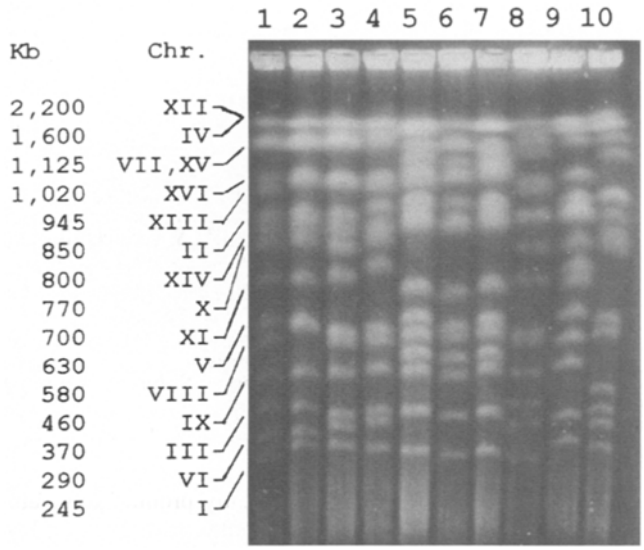

Figure 1 CHEF gel electrophoresis analysis of chromosomal DNAs from *Saccharomyces* sensu stricto yeasts. Lanes 1–3, *S. cerevisiae*, YNN 295, X2180-1A and CBS 5287, respectively; lane 4, *S. paradoxus*, CBS 432; lanes 5–7, *S. bayanus*, VKM Y-1146, CECT 1363 and CECT 1884, respectively; lane 8, *Saccharomyces* sp, IFO 1803; lane 9, *Saccharomyces* sp, IFO 1815; lane 10, Brazilian *Saccharomyces* sp, UFRJ 50791. All cultures are monosporic isolates. The linkage group numbering and chromosome sizes refer to the chromosomes of the strain YNN 295. Preparation of chromosomal DNAs has been described elsewhere [47]. A CHEF-DRII apparatus (Bio-Rad, Richmond, CA, USA) was used to separate chromosomal DNAs. The electrophoresis buffer was 0.5 × TBE. The buffer was circulated around the gel and cooled to 14°C. Electrophoresis was carried out at 200 V for 15 h with a switching time of 60 s and then for 8 h with a switching time of 90 s. A standard set of *S. cerevisiae* YNN 295 chromosomes was obtained commercially (Bio-Rad).

Cultivars of *Saccharomyces* sensu stricto

The data presented show that the gene pool of the cultivated *Saccharomyces* yeasts consists of two biological species: *S. cerevisiae* and *S. bayanus*, and one hybrid taxon *S. pastorianus*.

This classification has a good scientific basis, however it does not take into account the interests of applied microbiologists. *Saccharomyces* strains from different industrial processes were traditionally considered as separate species. Wild yeasts contaminating fermentation productions had their own species names as well. This was suitable for applied microbiologists. Biological species of *S. cerevisiae* and *S. bayanus* contain both cultivated and wild strains. Applied microbiologists are also confused by repeated revisions of the genus *Saccharomyces*. Undoubtedly, strains from different industrial processes and their wild relatives have some genetic differences which, however, should not be taken into consideration in scientific taxonomic classification.

Unlike bacteria, yeast strains are classified in accordance with the International Code of Botanical Nomenclature. That is why we propose to use the International Code of Nomenclature for Cultivated Plants [35] for naming the cultivated yeasts. It is reasonable to use the name for a group of cultivars but not for a single cultivar. This type of naming is allowed by the Code. In complete accordance with the Code we have chosen the initial Latin names of the revised taxa which are important for the define industries or for selection. We divided cultivated strains of the biological species *S. cerevisiae* into six groups of cultivars: 'Cerevisiae', 'Ellipsoideus', 'Oviformis', 'Diastaticus', 'Cheresanus' and 'Logos', whereas the biological species *S. bayanus* and the hybrid taxon *S. pastorianus* would include the Uvarum and Carlsbergensis groups of cultivars, respectively. The main peculiarities of the groups of cultivars are as follows.

The *S. cerevisiae* Cerevisiae group includes top brewer's, baker's and distiller's strains. Basionym: *S. cerevisiae* Hansen 1883. Fermentation of sugars: Gal^+, Mal^+, Suc^+, Mel^-, Sta^-. The natural mutants Mal^-, Suc^- rarely occur. This group is characterized by intensive maltose fermentation. Genetic and Southern blot analyses revealed an accumulation of polymeric *MAL* genes [30,52].

The *S. cerevisiae* Ellipsoideus group contains yeasts from primary wine fermentations. Basionym: *S. ellipsoideus* Hansen 1883. The fermentation pattern is the same as in the Cerevisiae group. Synonyms: *S. cerevisiae* var *ellipsoideus* (Hansen) Dekker, *S. vini* Meyen ex Kudriavzev.

The *S. cerevisiae* Cheresanus group consists of yeasts from secondary wine-making industry (sherry-like wines)

which are able to form a sherry film on the surface of wine during ethanol oxidation. Basionym: *S. cheresanus* Chowrenko et Babenko 1925 (we proposed strain CBS 1250 as the neotype). The fermentation profile is similar to those of the Cerevisiae and Ellipsoideus groups, but Gal$^-$ strains very often occur in the Cheresanus group. Synonyms: *S. aceti* Santa Maria, *S. beticus* Marcilla ex Santa Maria, *S. cheresiensis* Prostoserdov et Afrikian, *S. cordubensis* Santa Maria, *S. gaditensis* Santa Maria, *S. hispanica* Santa Maria, *S. oviformis* var *cheresiensis* (Prostoserdov et Afrikian) Kudriavzev, *S. oxidans* Santa Maria.

The *S. cerevisiae* Oviformis group includes wine yeasts which do not ferment galactose and which are resistant to high concentrations of ethanol and sulphites. These yeasts accumulate at the end of grape juice fermentation, during wine storage or champagne-making. Basionym: *S. oviformis* Osterwalder 1924. Fermentation of sugars: Gal$^-$, Mal$^+$, Suc$^+$, Mel$^-$, Sta$^-$. There is an accumulation of multiple Gal$^-$ mutants in the Oviformis populations [37,38].

The *S. cerevisiae* Diastaticus group is characterized by the ability to ferment soluble starch (dextrins). Basionym: *S. diastaticus* Andrews et Gilliland ex v.d. Walt 1965. Fermentation of sugars: Gal$^+$, Mal$^+$, Suc$^+$, Mel$^-$, Sta$^+$. Starch fermentation is controlled by polymeric genes accumulated in the genome [30]. There were many attempts to use *STA* genes in breeding programs.

The *S. cerevisiae* Logos group includes yeasts which ferment melibiose. Basionym: *S. logos* van Laer et Denamur ex Jorgensen 1909. Strains of this group may differ in galactose, maltose and sucrose fermentations. Synonyms: *S. coreanus* Saito, *S. hienipiensis* Santa Maria, *S. italicus* Castelli var *melibiosi* van Uden et Assis-Lopes, *S. norbensis* Santa Maria, *S. oleaceus* Santa Maria, *S. oleaginosus* Santa Maria. An accumulation of polymeric *MEL* genes is found in the genome of these yeasts [48,51,57,58,65]. Melibiose genes are used for construction of industrial Mel$^+$ strains which can more completely utilize molasses.

The *S. bayanus* Uvarum group contains cryophilic wine strains fermenting melibiose. Fermentation of sugars: Gal$^+$, Mal$^+$, Suc$^+$, Mel$^+$, Sta$^-$. Basionym: *S. uvarum* Beijerink 1896.

The *S. pastorianus* Carlsbergenesis group includes brewer's yeasts of bottom fermentations with the phenotype Gal$^+$, Mal$^+$, Suc$^+$, Mel$^+$, Sta$^-$. Basionym: *S. carlsbergensis* Hansen 1908. The cryophilic property of this hybrid group seems to have originated from the Uvarum group.

The proposed system is intended to solve three main problems: 1) determination of the taxonomic status by applying the biological species concept for the cultivated *Saccharomyces* sensu stricto yeasts; 2) description of distinct genetic sub-populations to offer guidance to applied microbiologists; 3) conservation of traditional names.

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