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REVIEW

Taxonomy, Ecology, and Genetics of the Yeast *Saccharomyces bayanus*: A New Object for Science and Practice

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Abstract—The review considers various aspects of the biology of the yeast *Saccharomyces bayanus*, which is distantly related to the cultured yeast *S. cerevisiae*. The cryotolerant *S. bayanus* strains found in wine-making became the second most important yeast for basic and applied studies. Introduction of natural and experimental hybrids of *S. cerevisiae* × *S. bayanus* in a range of fermentation processes indicates the high breeding importance of *S. bayanus*. The biological species *S. bayanus* acts as a new gene pool for the scientific and breeding projects.

Keywords: Saccharomyces bayanus, Saccharomyces uvarum, cryotolerant yeasts, interspecies hybrids, molecular polymorphism, wine-making, viral dsRNA.

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The scientific and practical importance of the cultured yeast *Saccharomyces cerevisiae* Meyen ex Hansen is well known [1]. For genetic, physiological, and molecular biological research, this yeast species is a good model of higher eukaryotes. It has been used for millennia in baking and the production of alcoholic beverages. *S. cerevisiae* is the first eukaryotic organism for which the complete genome nucleotide sequence was determined [2]. Genetic and molecular analysis revealed six sibling species of *S. cerevisiae: S. arboricola* Wang et Bai, *S. cariocanus* G. Naumov et al., *S. bayanus* Saccardo, *S. kudriavzevii* G. Naumov et al., *S. mikitae* G. Naumov et al., and *S. paradoxus* Batchinskaja [3–6].

The subject of the present review is the biological species *S. bayanus*. Its remote relation to *S. cerevisiae* and other *Saccharomyces* species [7], as well as their biochemical and biotechnological characteristics, make *S. bayanus* a promising organism for the evolutionary and breeding programs. A previous review on *S. bayanus*, dealing with its other aspects was published in 2002 [8].

TAXONOMY

The melibiose-fermenting (Mel$^+$) yeasts *S. bayanus* have been previously known (mostly in wine-making) as *S. uvarum* Beijerinck [9]. Since Mel$^+$ strains occur, albeit very seldom, among *S. cerevisiae*, physiological tests are not absolutely reliable for differentiation between the wine strains of these species. Physiological identification of environmental *Saccharomyces* isolates is still more difficult. All the known *S. mikitae* strains of Japanese origin, as well as the rare North American *S. paradoxus* strains also possess the Mel$^+$ phenotype [11–13]. For example, using genetic analysis we identified four Mel$^+$ strains of *"S. uvarum"* from the UCD collection (http://phaffcollection.org), which have been isolated from *Drosophila* in California, as *S. bayanus* (51-206, 61-137), *S. cerevisiae* (61-190), and *S. paradoxus* (61-248) [12].

The metabolic characteristics of *S. bayanus*, including glycerol accumulation in wine, were originally determined by Arabidze [14]. The cryophilic nature of *S. bayanus* was originally shown for Moldavian wine strains [15, 16]. While *S. cerevisiae* predominated at 25°C, *S. bayanus* prevailed at 8–10°C. These features of *S. bayanus* were subsequently confirmed by other works, e.g. [17–22]. Experiments on DNA–DNA reassociation [23–25] and genetic analysis [26–30] unequivocally confirmed the status of *S. bayanus* as an independent species. Rosini et al. [24] determined the conspecificity of the type cultures of *S. bayanus* and *S. uvarum* (95% DNA homology). Until recently, the name *S. uvarum* has been the synonym for *S. bayanus* according to the priority of description of the latter species [31]. Sequence analysis of the D1/D2 domain of 26S rRNA and of the 5.8S-ITS site,

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Table 1. Occurrence of *S bayanus* var. *uvarum* in the fermenting botrytised grape must from the Bordeaux region and in the fermenting sweet must from Jurançon [51]

Sample	Stage of fermentation*	Origin	Year of isolation	Colonies analyzed	Frequency of occurrence, %**
TBII	b, c, d	Sauternes	1992	83	7
TBIV	b, c, d	Sauternes	1992	89	5
YI	c, d	Sauternes	1992	57	0
YII	c, d	Sauternes	1992	58	0
YII	c, d	Sauternes	1993	46	4
TBI	c, d	Sauternes	1995	57	0
TBIV	c, d	Sauternes	1995	58	8
GI	c, d	Sauternes	1995	55	0
DDI	c	Barsac	1995	27	15
DDII	c	Barsac	1995	13	31
PM	c	Jurançon	1998	8	100

Notes: * b, c, and d indicate the initial, intermediate, and final stages of fermentation.
** The remaining colonies were identified as *S. cerevisiae*.

including the ITS1/ITS2 internal transcribed spacers and the 5.8S rRNA gene, demonstrated the heterogeneity of *S. bayanus*, containing two groups, "bayanus" and "uvarum" [32–35]. Genetic analysis demonstrated production of semisterile hybrids by the strains from different groups. This was the basis for description of two varieties, *S. bayanus* Saccardo var. *bayanus* and *S. bayanus* var. *uvarum* G. Naumov [36]. The *S. bayanus* varieties may be also differentiated based on their molecular karyotypes, especially the number of the smallest chromosomes [34, 36, 37]. Due to the presence of several *S. cerevisiae* telomere genes in the genome of the *S. bayanus* var. *bayanus* type culture [38, 39], some researchers [40–42] suggest its hybrid origin and propose the use of only the "correct" name *S. uvarum*. However, the presence of several allogenic telomere genes does not change the species affiliation of the type strain of *S. bayanus*. Similar to other authors [22, 43–49], we are using the species name *S. bayanus*.

ECOLOGY AND DISTRIBUTION

Karyotypic and genetic analyses were used to confirm that the melibiose-fermenting *Saccharomyces* strains isolated from wine-making in Moldova, Slovakia, France, Italy, and Russia belonged, with very few exceptions, to *S. bayanus* var. *uvarum* [10, 29, 37, 50]. Investigation of genetic diversity of the *Saccharomyces* yeasts from certain wines in Bordeaux (Sauternes), Val de Loire, and Jurançon revealed association of *S. bayanus* var. *uvarum* with some types of wines [51]. The relevant data are presented in Tables 1 and 2. The composition of the yeast flora in the three regions studied differed significantly and varied from year to year at the same site. The frequency of occurrence of *S. bayanus* var. *uvarum* was analyzed in the samples of botrytised and sweet must from Bordeaux and Jurançon (Table 1) and white must from Val de Loire and Jurançon (Table 2). Apart from *S. bayanus* var. *uvarum*, S. *cerevisiae* was also detected. The sample PJS.94 containing 15% of *Hanseniaspora uvarum* was exceptional. Different concentrations of *S. bayanus* var. *uvarum* were found in 16 samples out of 24. One sample of sweet must from the chateau (winery) PM in Jurançon (Table 1) and two samples from chateaus PJP an LC in the Val de Loire (Table 2) contained only *S. bayanus* var. *uvarum*. Thirteen samples contained both *S. cerevisiae* and *S. bayanus* var. *uvarum*. The remaining eight samples contained only *S. cerevisiae*. In spontaneously fermented botrytised must, the frequency of occurrence of *S. bayanus* var. *uvarum* was low (4–31%) (Table 2). Importantly, the sweet must from Jurançon was not prepared from botrytised grapes. *S. bayanus* var. *uvarum* could dominate in spontaneously fermented white must from the Val de Loire and Jurançon (Table 2). The composition of the yeast flora varied from year to year. For example, in the PJP chateau of the Val de Loire, the frequency of occurrence for *S. bayanus* var. *uvarum* was 100% in 1994 and 89% in 1996, but in 1995–1996 it decreased to 22–26% (Table 2). The samples of red must from Bordeaux for many years did not contain *S. bayanus* var. *uvarum*. Plating of the yeasts from the must of aseptically collected Val de Loire grapes revealed that 26 colonies out of 28 belonged to *S. bayanus* var. *uvarum*. Thus, their presence on the grapes in the vineyard was shown.

This identification of yeasts by molecular and genetic techniques is supplemented by the data of earlier studies on the yeasts from Bordeaux and the Val de Loire. For example, Peynaud and Domercq [52] iso-

Table 2. Occurrence of *S bayanus* var *uvarum* in the fermenting white must of different samples from the Val de Loire and Jurançon [51]

Sample*	Origin	Year of isolation	Colonies analyzed	Frequency of occurrence, %**
VS	Sancerre	1994	20	20
PJS	Sancerre	1994	26	50
PJP	Pouilly Fumé	1994	13	100
VS	Sancerre	1995	16	0
PJS	Sancerre	1995	29	90
PJP	Pouilly Fumé	1995	23	22
LC	Sancerre	1995	30	100
VS	Sancerre	1996	6	0
PJP	Pouilly Fumé	1996	9	89
SV	Sancerreð	1996	18	0
GM	Jurançon	1998	17	53
PJP	Pouilly Fumé	1999	23	26
PJS	Sancerre	1999	17	0

Notes: * All samples were collected at mid-fermentation.
** The remaining colonies were identified as *S. cerevisiae*. Sample PJS.94 contained 15% of the yeast *Hanseniaspora uvarum*.

lated 9 strains of *S. bayanus*/*S.varum* from nine samples of red must and one sample of white must near Bordeaux (Médoc and Graves). Henceforth, the double name is restricted to the strains identified as *S. uvarum* according to the standard physiological tests. Other authors [55] also found these yeasts in the Val de Loire vineyards. Two strains isolated in Bordeaux and 19 strains isolated in the Val de Loire were reidentified as *S. bayanus* var. *uvarum* [10]. Moreover, four strains from in Val de Loire region and one strain each from Alsace and Savoy were identified as *S. bayanus* var. *uvarum* [33]. Importantly, *S. bayanus* var. *uvarum* predominates in the Val de Loire region, i.e., in the zone of northern French wine-making, where this cryophilic species probably gains a selective advantage over *S. cerevisiae* [51]. Grapes for all the Sauternes and sweet Jurançon wines is indeed collected in November, when the temperature falls to 15–20°C. After three years of investigation of the yeast flora of Alsatian wine-making, the authors [54] made a similar conclusion. Association of *S. bayanus* var. *uvarum* and spontaneous fermentation of white wines was also found in the Basque Country in northern Spain [55]. Our preliminary conclusion [26, 29] that viniculture and wine-making at low temperatures are the specific ecological niche for *S. bayanus* var. *uvarum* was therefore confirmed.

Technology of the Sauternes white wines is somewhat similar to the technology of the Tokay wines. Minárik [56] demonstrated frequent occurrence of the Mel^+ *Saccharomyces* yeasts in the Tokay wines of Slovakia. According to genetic and karyotype analyses, these strains, together with the Hungarian Tokay strains, belong to *S. bayanus* var. *uvarum* [37, 57, 58]. The *S. bayanus*/*S. uvarum* yeasts were found also in the wine-making of other countries. According to the literature data [59–61], these yeasts have been regularly observed in central and southern Italy with the frequency of 5%. Six strains isolated from grapes by Castelli were reidentified as *S. bayanus* var. *uvarum* [10]. One of the populations of wine yeasts in Israel was found to contain 10% of *S. bayanus*/*S. uvarum* [61]. Usseglio-Tomasset et al [62] reported high content of these yeasts in the Amarone wine from Valpolicella (Italy) when the fermentation was carried out at 5–15°C but not at higher temperatures (up to 25°C). The subsequent RAPD-PCR and restriction mtDNA analysis indeed demonstrated that, among the 20 yeast strains from the Amarone wine, 13 belonged to *S. cerevisiae* and 7, to *S. bayanus* var. *uvarum* [63]. The technologies of the Amarone and Tokay wines are similar: in both cases, botrytised grapes are used and the must is fermented at low temperature. Some data indicate that *S. bayanus*/*S. uvarum* yeasts are important for production of the Portuguese Alentejo white wine (Aperitivo type) [64]. The role of *S. bayanus*/*S. uvarum* in production of champagne wines and cider [65–67] should also be mentioned. Information on the natural occurrence of *S. bayanus* var. *uvarum* is scarce. Several strains from various locations are known (Table 3).

GENETICS AND BREEDING

We investigated almost 100 strains of *S. bayanus* var. *uvarum* [72, 73] of different natural and cultural origin, with a homothallic life cycle and usually with high viability of meiotic ascospores. In their natural

Table 3. Origin of the *S. bayanus* var. *uvarum* strains isolated from environmental sources

Strain no.	Substrate and region of isolation	Reference
CBS 7001=MCYC 623	Caddis fly *Mesophylax adopersus*, Spain	[26]
CCY 21-31-12=CBS 2698	Fungus *Amanita citrina*, Slovakia	[68]
NCAIM Y.00789	Hornbeam *Carpinus betulus* exudate, Hungary	[36]
UCD 51-206=CBS 8697	*Drosophila persimilis*, California, United States	[11]
UCD 61-137=CBS 8696	*D. pseudoobscura*, California, United States	[11]
136.01	Elm *Ulmus pumula* exudate, Blagoveshchensk, Amur oblast, Russia	[69]
148.01	Elm *U. pumula* exudate, Blagoveshchensk, Amur oblast	[69]
UWO 99-807.1.1	Beech *Nothofagus* sp. exudate, Patagonia, Argentine	[70]
UWO 99-808.3	Beech *Nothofagus* sp. exudate, Patagonia, Argentine	[70]
№№ 1–8	Oak *Quercus garryana* bark, Hornby Island, Canada	[71]
№ 9	Bark of *Arbutus* sp., Hornby Island, Canada	[71]
№ 10	Bark of *Prunus* sp., Hornby Island, Canada	[71]

environment, these yeasts probably undergo a life cycle, ascospore formation being an important survival factor. Unlike *S. cerevisiae* from different fermentation processes, the *S. bayanus* var. *uvarum* strains isolated from wine-making are probably not cultured yeasts in the strict meaning of the term. Hybridization of *S. bayanus* with *S. cerevisiae* and with other species suggests a universal system of mating types in the genus *Saccharomyces*. This notion is supported by experimental production of the heterothallic strains of *S. bayanus* var. *uvarum* and *S. paradoxus* by disruption of the *HO* endonuclease gene, which is responsible for conversion of the mating types in the homothallic strains [45, 74]. Genetic work with the species *S. cerevisiae* and *S. bayanus* differs only in small details [26, 29, 45]. It should be remembered, however, that for the cryophilic species *S. bayanus* the temperature for cultivation, hybridization, and sporulation should not exceed 26°C.

Recently, numerous investigations dealt with the intraspecific diversity of *S. cerevisiae* [75–78]. Similar works on *S. bayanus* are still scarce. Restriction analysis (RPLP) of the mitochondrial DNA using four endonucleases (*Alu*I, *Dde*I, *Hinf*I, and *Rsa*I) was recently used to reveal the genetic relations between 41 *S. bayanus* var. *uvarum* strains from different wine-making regions of Europe and 4 environmental isolates [72]. No strict correlation was found between the origin, source of isolation, and the mtDNA restriction profiles. The mtDNA of *S. bayanus* var. *uvarum* were found to be less polymorphic than those of *S. cerevisiae*. This is in agreement with the results of molecular karyotyping. Unlike the *S. cerevisiae* wine yeasts, *S. bayanus* var. *uvarum* exhibits low polymorphism in the size of chromosomes [38, 79].

The microsatellite technique is promising for the intraspecific and interspecific differentiation between the *Saccharomyces* yeasts. Microsatellites or single-sequence repeats (SSR) consist of the tandem repeats of short DNA motifs (usually less than 10 base pairs). In eukaryotic organisms, they are distributed throughout the genome, are extremely variable in length, and may differentiate the members of the same species. For the characterization of the *S. bayanus* var. *uvarum* isolates from different regions of France, four microsatellite loci were used: $(GT)_n$, $(TA)_n$, $(ATT)_n$, and $(CTG)_n$ [48]. Analysis of these microsatellites made it possible to differentiate four populations—Sancerre, Loire, South West, and Alsace. Intraspecific polymorphism of *S. bayanus* var. *uvarum* was also studied by PCR with the $(GTG)_5$ microsatellite primer [73]. Analysis of 69 strains of different origins (France, Slovakia, Hungary, Spain, Italy, and Moldova) revealed the correlation between the PCR profiles and the sources of isolation (wine type and wine-making region). Southern hybridization was used to reveal the introgression between *S. cerevisiae* and *S. bayanus* var. *uvarum*. Two strains isolated from Hungarian alcoholic beverages and identified by genetic analysis as *S. bayanus* var. *uvarum* possessed some subtelomeric *S. cerevisiae* sequences: *Y', SUC, RTM*, and *MAL* [73].

Investigation of the viral dsRNA (fractions L and M) of *S. bayanus* var. *uvarum* deserves special attention. This type of viruses (fraction M) is known to control production of the killer toxins in *S. cerevisiae* [80]. The recent analysis [81, 82] of 71 *S. bayanus* var. *uvarum* strains of different origin revealed that most of the strains did not possess dsRNA, 7 strains had only the L fraction, while 11 strains contained both the L and M fractions. Similar to the cultured yeast *S. cerevisiae*, the size of the L fraction (4.5 kbp) was not variable.

The size of the M fraction varied from 1.2 to 1.8 kbp. Seven types of M-dsRNA (M_1–M_3 and M_8–M_{11}) were found, primarily among the French strains. Phenotypic analysis revealed that the M-dsRNA found were cryptic and probably were the mutant forms of the killer plasmids [81]. We may therefore suggest that the French strains are the most archaic wine forms of *S. bayanus* var. *uvarum*, while the wine strains from other regions had completely lost the nonfunctional viral dsRNA. An alternative point of view is possible. The presence of nonfunctional dsRNA may indicate the recent acquisition of the killer plasmids by the French population of *S. bayanus* var. *uvarum*, which is still unable to express the gene(s) of toxin production due to the absence of the special genes (mutations?) Several dozen nuclear genes are known to be involved in the expression of the KIL1 toxin in *S. cerevisiae* [80]. Each of these hypotheses requires experimental confirmation.

The demonstration [25, 83, 84] of the hybrid origin of the beer yeast *S. pastorianus* E.C. Hansen (*S . bayanus* × *S. cerevisiae*), which is better known to applied microbiologists as *S. carlsbergensis* E.C. Hansen, was an additional confirmation of the value of the *S. bayanus* genome. Both the allotetraploid and allodiploid hybrid strains of *S. bayanus* var. *uvarum* × *S. cerevisiae* were also found among wine yeasts [49, 85, 86]. One of the strains of cider production turned out to be an even more complex hybrid: *S. bayanus* var. *uvarum* × *S. cerevisiae* × *S. kudriavzevii* [39, 87, 88].

Genetic identification of the wine strains of *S. bayanus* var. *uvarum* made it possible to obtain the promising experimental hybrids of *S. bayanus* var. *uvarum* × *S. cerevisiae* in the laboratories of Russia and Ukraine [89], Japan [18], Italy [21], and France and Russia [90]. It is presently beyond doubt that a number of *S. cerevisiae* sibling species are a valuable gene pool for the breeding of *Saccharomyces* yeasts for various purposes [91]. In conclusion, it should be noticed that the taxa *S. bayanus* and *S. bayanus* var. *uvarum* have been accepted by the latest taxonomic guide on yeasts [92].

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