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EXPERIMENTAL ARTICLES

The Molecular Genetic Differentiation of Cultured *Saccharomyces* Strains

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Abstract—A comparative molecular genetic study of cultured *Saccharomyces* strains isolated from the surface of berries and various fermentation processes showed that baker's yeast and black-currant isolates contain not only *Saccharomyces cerevisiae* but also *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids. The molecular karyotyping of baker's, brewer's, and wine yeasts showed their polyploidy. The restriction enzyme analysis of noncoding rDNA regions (5.8S-ITS and IGS2) makes it possible to differentiate species of the genus *Saccharomyces* and to identify interspecies hybrids. The microsatellite primer $(GTG)_5$ can be used to study the populations of cultured *S. cerevisiae* strains.

Key words: *Saccharomyces, S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids, RFLP analysis, rDNA, molecular karyotyping.

The role of *Saccharomyces* yeasts in fermentation-based industries is difficult to overestimate. The genus *Saccharomyces* sensu Kurtzman 2003 currently includes six species (*S. cerevisiae, S. bayanus, S. cariocanus, S. kudriavzevii, S. mikatae*, and *S. paradoxus*) [1–3]. European projects on the sequencing and functional analysis of the genome of *S. cerevisiae* genetic lines [4] have formed the basis for investigating the genetic pool of various *Saccharomyces* species. However, little is known about the comparative organization of cultured and wild *Saccharomyces* strains. *Saccharomyces* are primarily represented by *S. cerevisiae* and *S. bayanus* species, which rarely produce wild populations in nature [2, 5]. The specific ecological niche of *S. bayanus* is various low-temperature fermentation processes. This species is represented by two partly genetically isolated varieties, *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum*, which differ in the nucleotide sequences of the internal transcribed rDNA spacers, ITS1 and ITS2 [3, 6]. *S. bayanus* var. *bayanus* includes the type strain CBS 380 and some other strains contaminating brewing. *S. bayanus* var. *uvarum* is a melibiose-fermenting cryophilic wine yeast variety, which is associated with the production of some sweet wines, champagne, and cider [7, 8]. *S. cerevisiae* and *S. bayanus* var. *uvarum* can occur in mixed populations in wine making. The allotetraploid strain isolated earlier was found to contain the whole genomes of *S. cerevisiae* and *S. bayanus* var. *uvarum* [9]. The brewer's yeast *S. pastorianus* (synonym *S. carlsbergensis*) is likely to be an allotetraploid of *S. cerevisiae* and *S. bayanus* [10, 11].

This work aimed at a comparative molecular genetic analysis of various cultured *Saccharomyces* strains and their natural hybrids.

MATERIALS AND METHODS

Strains and cultivation media. Experiments were carried out with 32 strains of *Saccharomyces* yeasts, of which 13 were isolated from the surface of berries gathered in western Belarus and the others obtained from the collection of yeasts at the Magarach Institute of Grape and Wine in Yalta, Crimea. The latter strains were isolated from various materials used in fermentation-based industries, such as beer brewing; bread baking; and the manufacture of alcohol, primary wine, and champagne. The origin of all these strains is shown in the table. The yeasts were identified by standard procedures [11] using the type strains *S. cerevisiae* CBS 1171, *S. paradoxus* CBS 432, and *S. bayanus* CBS 380; the hybrid strain *S. pastorianus* CBS 1538; and the variety *S. bayanus* var. *uvarum* VKM Y-1146 as the reference strains. The yeasts were cultivated at 28°C on complete YPD medium containing (g/l) yeast extract, 5; peptone, 10; glucose, 20; and agar, 20.

PCR assay and the analysis of products. DNA was isolated as described earlier [12]. The 5.8S rDNA and internal transcribed ITS1 and ITS2 spacers (5.8S–ITS fragments) were amplified with the primers pITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and pITS4

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The origin of the *Saccharomyces* strains under study

Original name	Collection number	Origin	Location
VV-1	KBP-3792	Grape	Volkovysk region, Belarus
VV-2	KBP-3793	Grape	Volkovysk region, Belarus
GV-1	KBP-3790	Grape	Grodno, Belarus
GV-4	KBP-3791	Grape	Grodno, Belarus
GSCh-1	KBP-3781	Black currant	Grodno, Belarus
GSCh-5	KBP-3782	Black currant	Grodno, Belarus
GSCh-8	KBP-3783	Black currant	Grodno, Belarus
GSCh-11	KBP-3784	Black currant	Grodno, Belarus
GM-8	KBP-3786	Wild raspberry	Grodno, Belarus
GM-15	KBP-3787	Wild raspberry	Grodno, Belarus
GM-17	KBP-3788	Wild raspberry	Grodno, Belarus
DChK-1	KBP-3785	Blackberry	Dyatlov, Belarus
VChR-3	KBP-3789	Black chokeberry	Volkovysk region, Belarus
Apple-7	M-132	Apple juice	Kursk, Russia
Cherry-18	M-17	Cherry juice	Kursk, Russia
Cider-101	M-56	Apple juice	Kursk, Russia
Kharkov-39	M-212	Champagne acratophores	Kharkov, Ukraine
Champagne-11/12	M-239	Champagne	Unknown
Kornet		Champagne acratophores	Moscow, Russia
Steinberg-92	M-93	Champagne	Unknown
47-K	M-527	Champagne	Unknown
Kokur-3	M-279	Grape must	Crimea, Ukraine
Feodosiya-1-19	M-271	Grape must	Crimea, Ukraine
L-80-4	–	Baker's yeast	Moscow yeast-producing plant
301	–	Baker's yeast	Moscow yeast-producing plant
261	–	Baker's yeast	Moscow yeast-producing plant
125	–	Baker's yeast	Moscow yeast-producing plant
Spanish-XII	–	Beer	Unknown
P-148	–	Beer	Unknown
Ya	–	Molasses wort	Vinnitsya, Ukraine
985-T	–	Ethanol	VNIISP, Moscow
U-717	–	Ethanol	VNIISP, Moscow
–	CBS 1171	Top yeasts	Netherlands
–	CBS 380	Beer	Unknown
–	CBS 432	Unknown	Unknown
–	CBS 1538	Beer	Denmark
–	VKM Y-1146	Grape berries	Michurinsk, Russia

Note: The abbreviations used: VNIISP, All-Russia Research Institute of the Alcohol Industry; KBP, culture collection at the Department of Soil Biology, Moscow State University; VKM, All-Russia Collection of Microorganisms; VKPM, All-Russia Collection of Industrial Microorganisms; M, collection at the Magarach Institute of Grape and Wine, Yalta; CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. The correspondence of some culture numbers: KBP-3791 = VKPM Y-3098; KBP-3781 = VKPM Y-3100; KBP-3786 = VKPM Y-3097; KBP-3785 = VKPM Y-3096; KBP-3789 = VKPM Y-3099.

(5'-CCTCCGCTTATTGATATGC-3') [12]. The intergeneric spacer 2 (IGS2) was amplified with the primers NTS2 (5'-AACGGTGSTTTSTGGTAG-3') and ETS1 (5'-TGTCTTCAACTGCTTT-3') [13]. The reaction mixture (30 μl) contained PCR buffer with 20 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.25 mM each dNTP, 1 μl each primer, 0.5 U *Taq* polymerase (Sintol, Russia), and 20 ng of genomic DNA. The IGS2 fragment was amplified with the aid of a Tertsik thermocycler manufactured by the DNA technology company (Russia) with the initial DNA denaturation step at 94°C for 5 min, followed by 35 cycles of DNA denaturation at 94°C for 45 s, primer annealing at 52°C for 30 s, and DNA synthesis at 72°C for 2 min, with the final extension step at 72°C for 10 min. The procedures for PCR analysis with the microsatellite primer $(GTG)_5$, the amplification of the *MEL* genes, and the electrophoretic analysis of amplified products were performed as described earlier [14, 15]. The restriction fragment length polymorphism (RFLP) analysis of the 5.8S-ITS and IGS2 regions of rDNA was carried out with *Hpa*II and *Hae*III (in the case of the 5.8S-ITS region) and *Alu*I and *Ban*I (in the case of the IGS2 region) restriction endonucleases. The amplified *MEL* genes were analyzed with the *Hin*cII and *Hin*dIII restriction endonucleases. All these restriction enzymes were purchased from Fermentas (Lithuania). The restriction digests were analyzed by electrophoresis in 1.6% agarose gel at 60 V in 0.5× TBE buffer for 3 h. The gel was stained with ethidium bromide and photographed under UV light using a Vilber Lourmat transilluminator (France).

Pulsed-field electrophoresis. Chromosomal DNA was isolated as described elsewhere [15] and separated by using a CHEF-DRTMIII apparatus (Bio-Rad, United States). Pulsed-field electrophoresis was carried out in 0.5× TBE (45 mM Tris, 45 mM boric acid, and 10 mM EDTA; pH 8.2; temperature 14°C) at 200 V for the total 24 h (15 h at a switching time of 60 s and 9 h at a switching time of 90 s). After electrophoresis, the gel stained with ethidium bromide was washed in distilled water and photographed.

Southern hybridization. Chromosomal DNA was transferred to a nitrocellulose membrane by Southern blotting and fixed by baking at 80°C for 2 h. The probe was a *Hin*dIII fragment of the *ARF4* gene with a length of about 3000 bp, which was isolated from plasmid pINAI [9]. A nonradioactive marker was incorporated according to the manufacturer's instruction (Roche Applied Science, Germany) with the use of the DIG-II-dUTP digoxygenin. Hybridization and the analysis of hybridization products were performed according to the same instruction.

Phylogenetic analysis. The DNA relatedness of the yeast strain was evaluated by comparing the PCR products amplified with the microsatellite primer $(GTG)_5$. The dendrogram was constructed by the neighbor-joining method [17] with the aid of the TREECON software package and using the strain *S. bayanus* var. *uvarum* VKM Y-1146 as the outgroup. The bootstrap values, indicated on the tree, were derived by using 100 replications.

RESULTS AND DISCUSSION

The yeasts were preliminarily identified through the analysis of the morphology of colonies, vegetative cells, and ascospores with allowance made for the ability of the yeasts to ferment glucose. All the strains examined proved to belong to the genus *Saccharomyces.* Three strains (VV-2, Kharkov-39, and Champagne-11/12) were found to be able to ferment melibiose, which is a typical characteristic of the species *S. bayanus* and *S. pastorianus.*

The RFLP analysis of the amplified 5.8S-ITS fragments of rDNA. The yeasts were assigned to particular species using three molecular genetic methods: RFLP analysis of the noncoding regions of rDNA, molecular karyotyping, and PCR with the microsatellite primer $(GTG)_5$. The species of the genus *Saccharomyces* differ in the sequences of the inner transcribed spacers ITS1 and ITS2 [3] and can be differentiated based on RFLP analysis of these rDNA regions [12, 18]. The species *S. cerevisiae* can be distinguished from the species *S. bayanus* and *S. pastorianus* with the aid of *Hae*III restriction endonuclease. At the same time, the *Hpa*II restriction profiles of *S. cerevisiae, S. bayanus* and the hybrid species *S. pastorianus* are identical, and the PCR products of *S. paradoxus* do not contain the typical restriction site of *Hpa*II. The size of the amplified 5.8S-ITS fragments of all studied and reference strains was equal to about 850 bp, which is typical of members of the genus *Saccharomyces.* The analysis of PCR products with the *Hpa*II and *Hae*III endonucleases showed that all the strains had identical *Hpa*II profiles with two fragments, 730 and 120 bp in size. This suggested that none of the strains under study belonged to the species *S. paradoxus.* At the same time, according to their *Hae*III restriction profiles, these strains could be divided into three groups (Figure 1 shows the RFLP patterns of some strains). The first group comprised the type culture *S. cerevisiae* CBS 1171, 10 strains from Belarus (VV-1, VV-2, GV-1, GV-4, GSCh-1, DChK-1, VChR-3, GM-8, GM-15, and GM-17), and 17 strains from the Magarach collection. The members of this group were distinguished by the presence of four *Hae*III restriction fragments about 320, 230, 170, and 130 bp in size (Fig. 1, lanes *3, 10–18*). The second group included two type cultures, *S. pastorianus* CBS 1538 and *S. bayanus* var. *bayanus* CBS 380, and the reference strain *S. bayanus* var. *uvarum* VKM Y-1146, the restriction patterns of which had three *Hae*III fragments, approximately 490, 230, and 130 bp in size (Fig. 1, lanes *1, 2*, and *4*, respectively). The third group consisted of three strains from Belarus (GSCh-5, GSCh-8, and GSCh-11) and two strains from the Magarach collection (Champagne-11/12 and L-80-4), which exhibited unique *Hae*III

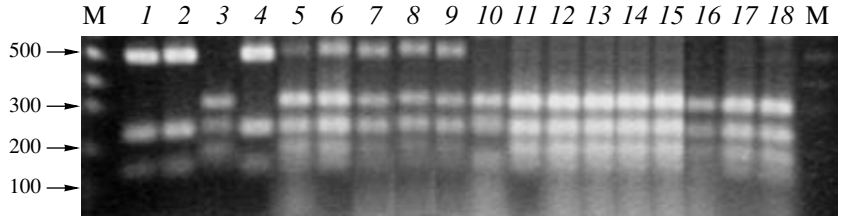

Fig. 1. *Hae*III restriction enzyme analysis of the amplified 5.8S-ITS fragments of the rDNA of *Saccharomyces* strains. Lanes: (*1*) *S. pastorianus* CBS 1538; (*2*) *S. bayanus* var. *bayanus* CBS 380; (*3*) *S. cerevisiae* CBS 1171; (*4*) *S. bayanus* var. *uvarum* VKM Y-1146: *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids: (*5*) GSCh-5; (*6*) GSCh-8; (*7*) GSCh-11; (*8*) L-80-4; (9) Champagne-11/12; *S. cerevisiae* strains: (*10*) Kornet; (*11*) Steinberg-92; (*12*) Kharkov-39; (*13*) 301; (*14*) 261; (*15*) 125; (*16*) GV-1; (*17*) GV-4; (*18*) GSCh-1. M is the molecular weight (bp) marker "100 bp DNA Ladder" (Fermentas, Lithuania).

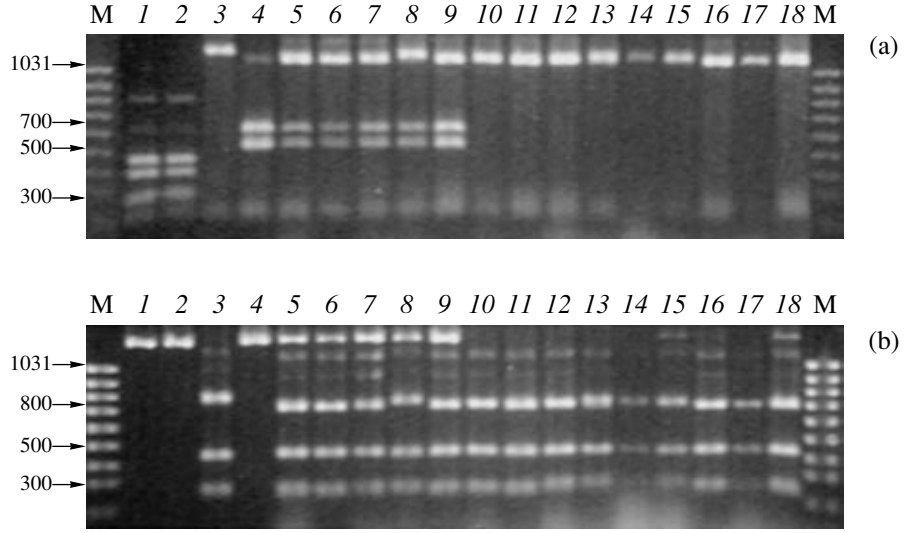

Fig. 2. *Alu*I (a) and *Ban*I (b) restriction enzyme analysis of the amplified IGS2 fragments of the rDNA of *Saccharomyces* strains. Lanes: (*1*) *S. pastorianus* CBS 1538; (*2*) *S. bayanus* var. *bayanus* CBS 380; (*3*) *S. cerevisiae* CBS 1171; (*4*) *S. bayanus* var. *uvarum* VKM Y-1146; *S. bayanus* var. *uvarum* hybrids: (*5*) GSCh-5; (*6*) GSCh-8; (*7*) GSCh-11; (*8*) L-80-4; (*9*) Champagne-11/12; *S. cerevisiae* strains: (*10*) Kornet; (*11*) Steinberg-92; (*12*) Kharkov-39; (*13*) 301; (*14*) 261; (*15*) 125; (*16*) GV-1; (*17*) GV-4; (*18*) GSCh-1. M is the molecular weight (bp) marker "100 bp DNA Ladder" (Fermentas, Lithuania).

restriction patterns of the 5.8S-ITS region of rDNA (Fig. 1, lanes *5–9*, respectively). The complex restriction patterns of the members of the latter group included three fragments (approximately 490, 230, and 130 bp in size) typical of the species *S. bayanus* and four fragments (approximately 320, 230, 170, and 130 bp in size) typical of *S. cerevisiae.*

The RFLP analysis of the amplified IGS2 fragments of rDNA. The varieties *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum*, which have identical restriction maps of the 5.8S-ITS region of rDNA, could be differentiated based on RFLP analysis of the IGS2 region of rDNA with the aid of the *Alu*I restriction enzyme [13]. The yeasts *S. bayanus* var. *bayanus* and *S. pastorianus* have identical *Alu*I restriction patterns, whereas *Ban*I sites are only specific for the IGS2 fragment of *S. cerevisiae.* The amplified IGS2 fragments of all the strains under study had the same size of ca. 1300 bp. The amplified fragments were analyzed by using the *Alu*I and *Ban*I restriction endonucleases. The restriction patterns of some yeast strains are shown in Fig. 2. The type cultures *S. pastorianus* CBS 1538 and *S. bayanus* var. *bayanus* CBS 380 displayed the absence of the *Ban*I-specific site, whereas the *Alu*I restriction patterns of these yeasts were identical and had four fragments, about 480, 380, 270, and 170 bp in size (Fig. 2, lanes *1* and *2*). The reference strain *S. bayanus* var. *uvarum* VKM Y-1146 exhibited no *Ban*I-specific site. The *Alu*I restriction pattern of this strain was characterized by the presence of three fragments, about 610, 520, and 170 bp in size (Fig. 2, lane *4*). Faint bands, the sizes of which were approximately 850 and 650 bp in the type strains CBS 1538 and CBS 380 and approximately 1300 bp in the strain VKM Y-1146, were likely due to the partial restriction of PCR products [13].

Actually, the 32 strains under study displayed two different restriction patterns. The pattern of the 10 strains from Belarus and 17 strains from the Magar-

ach collection was identical to that of the type culture *S. cerevisiae* CBS 1171 and had two *Alu*I restriction fragments (approximately 1130 and 170 bp in size) and three *Ban*I fragments with sizes of approximately 720, 400, and 180 bp (Figs. 2 and 2b, lanes *3, 10–18*). Five strains (GSCh-5, GSCh-8, GSCh-11, Champagne-11/12, and L-80-4) exhibited unique restriction patterns (Fig. 2, lanes *5–9*) with five *Alu*I fragments, three of which were specific to the reference strain *S. bayanus* var. *uvarum* VKM Y-1146 and three were specific to the type culture *S. cerevisiae* CBS 1171. The *Ban*I restriction patterns of these strains had four fragments, three exhibiting the same size as in the case of the type culture *S. cerevisiae* and one having the size of the initial PCR product, that is 1300 bp.

In general, the RFLP analysis of the two noncoding regions of the rDNA of 32 yeast strains showed that 27 strains belong to the species *S. cerevisiae* and five strains (GSCh-5, GSCh-8, GSCh-11, Champagne 11/12, and L-80-4) are likely to be *S. cerevisie* × *S. bayanus* var. *uvarum* hybrids.

Molecular karyotyping. It is known that six species of the genus *Saccharomyces* possess the same basic karyotypic characteristics: the identical set of 16 haploid chromosomes and the identical minimal and maximal sizes of chromosome bands (245 and 2200 kb) [3]. At the same time, the size and the pattern of chromosome bands in the species *S. cerevisiae* and *S. bayanus* are different [16, 19]. First, the karyotype of *S. bayanus* is characterized by the presence of a chromosome band with a size of approximately 1300 bp. Second, unlike the variety *S. bayanus* var. *bayanus*, which has three or more chromosomal bands in the region 245–370 kb, the variety *S. bayanus* var. *uvarum* has two chromosomal bands in this region.

The molecular karyotypes of the 32 yeast species under study are presented in Fig. 3a. These karyotypes were compared to that of the chromosome standard strain *S. cerevisiae* YNN 295 with known sizes of chromosome bands (Fig. 3, lane *1*). Karyotypic analysis confirmed that most of the strains under study belong to the species *S. cerevisiae.* The chromosomal DNA patterns of these strains contained 11–14 bands. As is evident from a comparison of the intensity of these bands, some strains contain more than one chromosome. The karyotypes of four baker's yeast strains (L-80-4, 301, 261, and 125), two brewer's yeast strains (Spanish-XII and P-148), and two distiller's strains (985-T and U-717) contained more than 16 chromosomal bands, of which four bands from 245 to 370 kb in size and several bands within the range 580–945 kb (Fig. 3, lanes *9–12*).

The karyotypes of the strains GSCh-5, GSCh-8, and GSCh-11 with unique RFLP patterns did not differ from that of the strain GSCh-1, which was also isolated from black currant berries and, according to its RFLP pattern, belongs to the species *S. cerevisiae* (Fig. 3, lanes *5–8*). Only the strain Champagne-11/12 displayed a chromosomal band approximately 1300 kb in size,

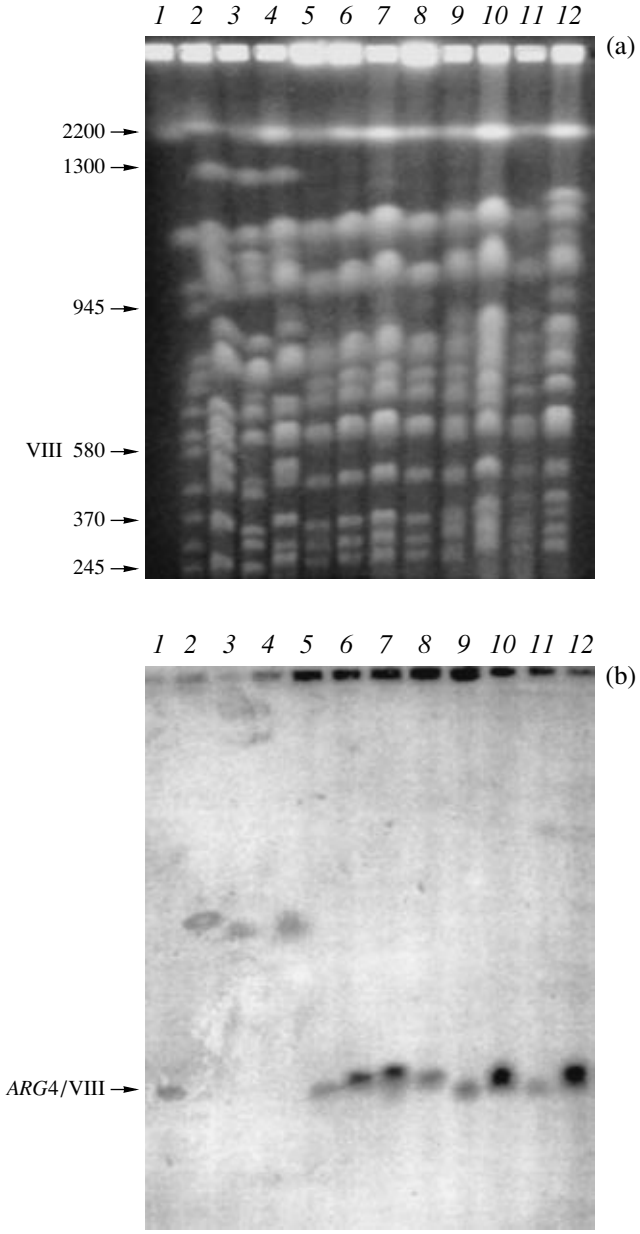

Fig. 3. Pulsed-field electrophoresis of (a) chromosomal DNA from *Saccharomyces* yeasts and (b) Southern hybridization with the *ARG4* probe. Lanes: (*1*) *S. cerevisiae* YNN 295 (chromosomal standard); (*2*) *S. bayanus* var. *uvarum* VKM Y-1146; (*3*) *S. pastorianus* CBS 1538; *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids: (*4*) Champagne-11/12; (*5*) GSCh-5; (*6*) GSCh-8; (*7*) GSCh-11; (*9*) L-80-4; *S. cerevisiae* strains: (*8*) GSCh-1; (*10*) 301; (*11*) 985-T; (*12*) U-717.

which is specific to the species *S. bayanus* and *S. pastorianus* (Fig. 3, lanes *2–4*). In the ability to ferment melibiose and according to its karyotype, the strain Champagne-11/12 resembles the hybrid yeast species *S. pastorianus.* At the same time, the two other melibiose-fermenting strains, VV-2 and Kharkov-39, have karyotypes that are specific to the species *S. cerevisiae.*

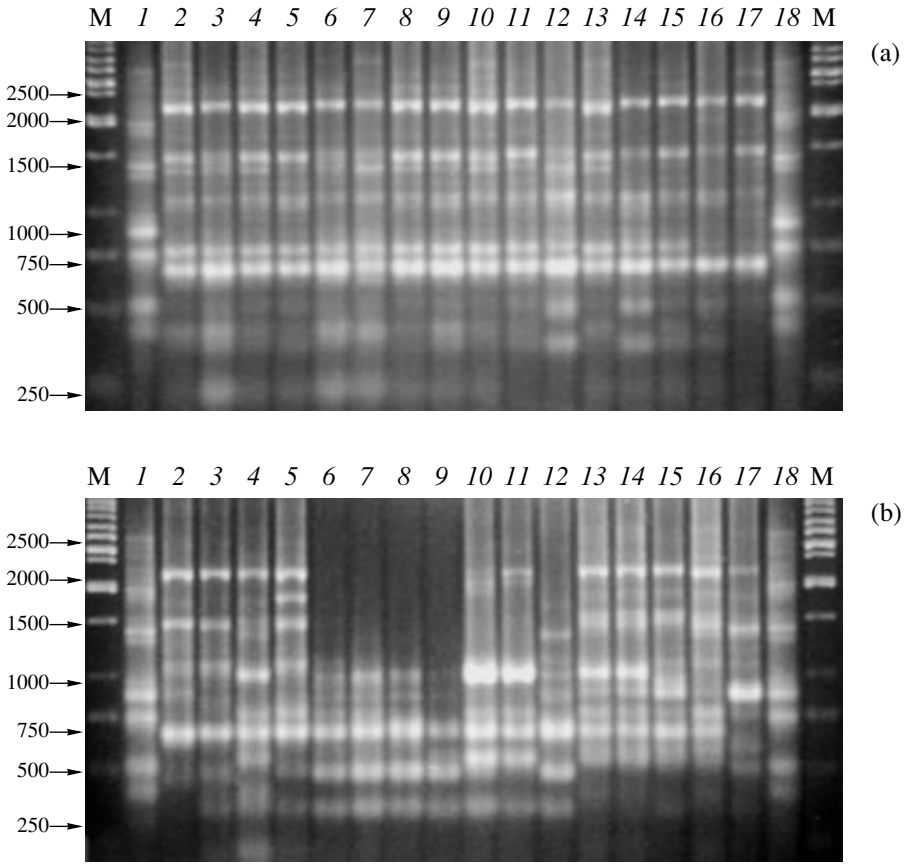

Fig. 4. PCR analysis of *Saccharomyces* yeasts with the microsatellite primer $(GTG)_5$. Lanes in panel a: (*1, 18*) *S. bayanus* var. *uvarum* VKM Y-1146; *S. cerevisiae* strains: (*2*) VV-1; (*3*) GM-8; (*4*) GM-15; (*5*) GM-17; (*6*) DChK-1; (*7*) VChR-3; (*8*) Apple-7; (*9*) Spanish-XII; (*10*) P-148; (*11*) Ya; (*12*) 985-T; (*13*) Steinberg-92; (*14*) Cherry-18; (*15*) 47-K; (*16*) 301, (*17*) VV-2. Lanes in panel b: (*1, 18*) *S. bayanus* var. *uvarum* VKM Y-1146; *S. cerevisiae* strains: (*2*) Kharkov-39; (*3*) Kornet; (*4*) GSCh-1; (*5*) Cider-101; (*6*) GV-1; (*7*) GV-3; (*8*) Feodosiya-1-19; (*9*) Kokur-3; (*10*) 261; (*11*) 125; (*12*) U-717; *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids: (*13*) GSCh-5; (*14*) GSCh-11; (*15*) L-80-4; (*16*) GSCh-8; (*17*) Champagne-11/12. M is the molecular weight (bp) marker "100 bp DNA Ladder" (Fermentas, Lithuania).

It was of interest to compare restriction maps the *MEL* genes of the three aforementioned strains. These genes can easily be distinguished with the aid of the *Hin*cII and *Hin*dIII restriction enzymes, since the *MEL* gene of *S. cerevisiae* contains no *Hin*dIII restriction sites and the *MEL* genes of *S. bayanus* and *S. pastorianus* contain no *Hin*cII restriction sites [15]. The PCR-amplified fragments of the strains Champagne-11/12, VV-2, and Kharkov-39 had the same size (1300 kb), although the *MEL* genes of these strains had different restriction maps. Namely, the strain Champagne-11/12 had the *MEL* gene typical of *S. bayanus* and *S. pastorianus*, whereas the strains VV-2 and Kharkov-39 had the *MEL* genes typical of *S. cerevisiae*. The attempts to perform the amplification of the *MEL* genes of the remaining 29 strains under study, which are unable to ferment melibiose, showed that only four of them (GSCh-5, GSCh-8, GSCh-11, and L-80-4) gave rise to the 1300-bp amplified fragments. The restriction maps of these fragments from the four strains were identical to those of the *MEL* genes of the yeasts *S. bayanus* and *S. pastorianus.*

The chromosomal DNA of the strains listed in Fig. 3a were transferred to nitrocellulose membranes by Southern blotting and hybridized with the *ARG4* probe (Fig. 3b). It is known that this molecular marker is present in chromosome VIII of *S. cerevisiae*, which differs from the respective chromosome of *S. bayanus* in size [9, 19]. Southern hybridization analysis showed that all the strains studied displayed one hybridization band. In the chromosome pattern of the strain Champagne-11/12, the molecular marker *ARG4* was located in the position specific to the species *S. bayanus* and *S. pastorianus* (Fig. 3b, lanes 2–4). In the case of the other strains studied, including the hybrid strains GSCh-5, GSCh-8, GSCh-11, and L-80-4, this marker was in the position specific to *S. cerevisiae* (Fig. 3b, lanes *1, 5–12*).

PCR analysis with the microsatellite primer $(GTG)_5$ makes it possible to differentiate *S. cerevisiae* and *S. bayanus*, as well as to distinguish the particular strains of these species [14, 20]. Figure 4 shows the results of the PCR analysis of the 32 strains under study

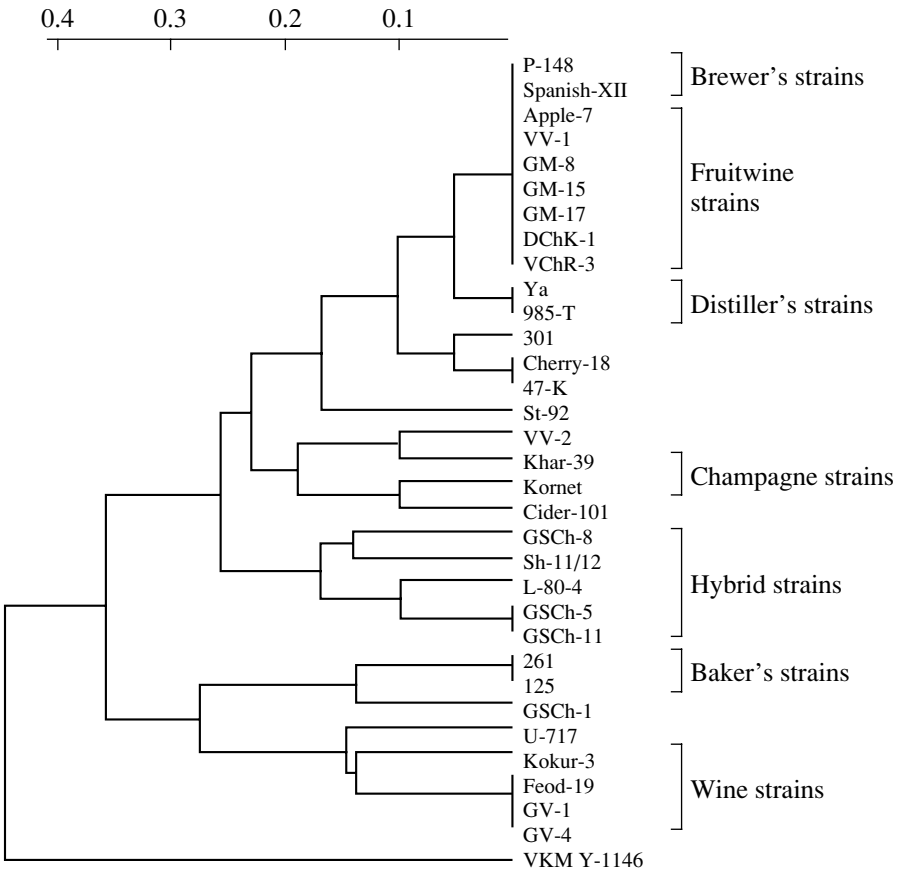

Fig. 5. A dendrogram showing the relatedness of *Saccharomyces* strains based on their PCR profiles with the microsatellite primer $(GTG)_5$. The dendrogram is constructed with the strain *S. bayanus* var. *uvarum* VKM Y-1146 as the outgroup. The data are processed by the neighbor-joining method with the aid of the TREECON software package [17].

and the reference strain *S. bayanus* var. *uvarum.* The amplified fragments of the DNA of these strains varied from 250 to 2100 bp in size. Twenty-eight strains had similar RFLP patterns with four major DNA fragments (approximately 2100, 1500, 750, and 670 bp). The slight polymorphism of the members of this group of strains manifested itself in the presence or absence of some minor fragments. The remaining four strains (GV-1, GV-4, Feodosiya-1-19, and Kokur-3) differed from the aforementioned 28 strains in the presence of three major fragments about 670, 500, and 375 bp in size (Fig. 3b, lanes *6–9*). These strains, which are used in wine making, were isolated from grapes cultivated in different geographical zones (Belarus, Ukraine, and Georgia). In their PCR profiles, the hybrid strains are similar to the other strains studied rather than to the reference strain *S. bayanus* var. *uvarum* (Fig. 4b, lanes *13–18*), except that the strain Champagne-11/12 lacks the major fragment approximately 670 bp in size and some hybrid strains exhibit the same fragments as do the species *S. bayanus* var. *uvarum*.

Figure 5 shows the dendrogram based on the PCR profiles that were obtained with the microsatellite primer $(GTG)_5$. All the 32 strains under study formed a separate cluster with respect to the reference strain *S. bayanus* var. *uvarum* VKM Y-1146, which was isolated from grapes. The cluster can be divided into two groups of slightly dissimilar strains.

The first group, which comprises most fruit wine, champagne, and distiller's strains, is subdivided into three subgroups. The first subgroup includes five hybrids. The second subgroup includes the champagne strains Kornet and Kharkov-39 and the fruit wine strains VV-2 and Cider-101. The third subgroup contains seven fruit wine strains and two brewer's yeast strains, with two adjoining distiller's strains, Ya and 985-T.

The second group can be divided into two subgroups. The first subgroup comprises the baker's strains 261 and 125 and the GSCh-1 strain isolated from black currant. The second subgroup contains the strains GV-1, GV-4, Feodosiya-1-19, and Kokur-3, which were isolated from grapes, and the alcohol-producing strain U-717.

Molecular genetic analysis showed that the strains isolated from the surface of red-berry fruits, as a rule, belong to the species *S. cerevisiae*, except that three

strains (GSCh-5, GSCh-8, and GSCh-11), which were isolated from black currant, are *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids. It should be noted that hybrid strains were earlier isolated only from grapes [9], whereas their isolation from red-berry fruits has not previously been described in the literature. It is known that interspecies hybrids possess a high fermenting ability at low temperatures and can improve wine flavor. Of interest is the fact that the type culture *S. uvarum* CBS 395 was isolated from fermenting black currant juice.

We failed to detect the variety *S. bayanus* var. *uvarum* among the strains studied. Two melibiose-fermenting strains (VV-1 and Kharkov-39) were identified as *S. cerevisiae*, whereas the third melibiose-fermenting strain (Champagne-11/12) was a *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrid. Unlike the three hybrid strains isolated from the surface of black currant, the strain Champagne-11/12 has a complex karyotype resembling that of the hybrid species *S. pastorianus.* Three other champagne strains (Kornet, Steinberg-92, and 47-K), as well as two brewer's strains (Spanish-XII and P-148), also belong to *S. cerevisiae.* It should be noted that bottom brewer's yeasts are usually referred to the hybrid species *S. pastorianus* and the top brewer's yeasts to the species *S. cerevisiae.* Unlike the other *S. cerevisiae* strains under study, the strains Spanish-XII and P-148 are likely to be polyploids, since they have a complex karyotype with more than 16 chromosome bands. Complex karyotypes were also found in two distiller's strains (985-T and U-717) and four baker's yeast strains (L-80-4, 301, 261, and 125). According to the RFLP analysis of two noncoding rDNA regions, the strain L-80-4 is a *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrid.

Thus, the RFLP analysis of the 5.8S-ITS and IGS2 regions of rDNA and molecular karyotyping make it possible to differentiate the yeast species *S. cerevisiae* and *S. bayanus* and to detect interspecies hybrids. The microsatellite primer $(GTG)_5$ is a promising tool in studying the populations of cultured *S. cerevisiae* strains. It is expedient to continue this study by using a greater number of yeast strains.

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