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EXPERIMENTAL ARTICLES

Molecular Genetic Characteristics of *Saccharomyces cerevisiae* Distillers' Yeasts

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Abstract—Genomes of 36 *Saccharomyces* distillers' strains, mainly of domestic origin, were studied by PCR-RFLP analysis of rDNA 5.8S-ITS fragment, molecular karyotyping, and Southern hybridization. Molecular analysis revealed that all the strains belonged to the species *S. cerevisiae*. According to the karyotypic analysis, many of the strains were aneuploid. Accumulation of polymeric genes *SUC* and *MAL* was detected in *S. cerevisiae* distillers' strains. Accumulation of polymeric genes of sugar fermentation may be of adaptive importance and may result in increased fermentation activity of the strains. It was demonstrated that most strains fermenting maltose after one day of fermentation possessed several *MAL* genes. Thermotolerant strains with high fermentation activity were selected.

Keywords: Saccharomyces cerevisiae distillers' yeast, PCR-RFLP analysis of rDNA 5.8S-ITS fragment, molecular karyotyping, Southern hybridization, *SUC, MAL*, and *MEL* genes, thermotolerance

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Ethanol is widely used in chemical, pharmaceutical, and food industry and in recent years even finds use as a biofuel. Biotechnological production of ethanol from starch-containing raw material (rye, wheat, potato, maize) and wastes of sugar production (molasses) is based on the process of fermentation employing traditional *Saccharomyces cerevisiae* distillers' yeasts. The main disaccharide involved in starch hydrolysis is maltose, while the main component of molasses is sucrose (up to 54–63%). Moreover, molasses comprise a trisaccharide raffinose, which requires two enzymes for complete fermentation, namely, β-fructosidase and α-galactosidase. Therefore, the ability to ferment maltose, sucrose, and melibiose is an important characteristic of distillers' yeasts.

Alcohol fermentation of maltose, sucrose, and melibiose by *Saccharomyces* is controlled by the genes *MAL, SUC*, and *MEL*, which are located in the telomeric regions of various chromosomes and may accumulate in certain strains [1–7]. Fermentation of maltose by *S. cerevisiae* is determined by five loci, *MAL1–MAL4* and *MAL6*, each comprising three tightly linked genes coding for maltose permease (GENE1), maltase (GENE2), and a regulatory transcription activator (GENE3) [8]. The presence of all three genes in any of the loci is necessary for maltose fermentation. The family of the *SUC* genes is represented by nine genes *SUC1–SUC5, SUC7*, and *SUC8–SUC10* which (except for *SUC2*) are located in the telomeric regions [1, 9–12]. Melibiose fermentation is a rare feature among *S. cerevisiae* strains. Most strains do not ferment melibiose and even lack a silent sequence of the *MEL* gene. In *S. cerevisiae* strains residing in the gastrointestinal tract of mammals and olive oil production wastes, *MEL* gene accumulation was observed [3]. Eleven structure genes (*MEL1–MEL11*) were identified in *S. cerevisiae* in various combinations [13].

Modern technology of ethanol production is a multistage process comprising the processes of saccharification of dispersed biological raw material by recombinant fungal enzymes at 45–50°C followed by microbiological fermentation of sugar solution by *Saccharomyces* yeasts at temperatures optimal for their growth (28–30°C) [14–16]. Combination of the processes of saccharification and fermentation makes it possible to avoid heating/cooling of industrial vessels and promotes more efficient functioning of the hydrolyzing enzymes which, in this case, are not subjected to inhibition by the final hydrolysis products: by mono- and disaccharides, which are converted by *S. cerevisiae* directly to ethanol.

According to the modern classification, the genus *Saccharomyces* comprises *S. cerevisiae* and its six sibling species: *S. arabicola, S. bayanus, S. cariocanus, S. kudriavzevii, S. mikatae*, and *S. paradoxus* [17–20]. Phenotypically indistinguishable species of *Saccharomyces* may be differentiated by the nucleotide sequences of the internal transcribed spacers ITS1/ITS2 and by molecular karyotypes [17, 21–23].

In the present work, we performed molecular genetic study of *Saccharomyces* distillers' yeasts of dif-

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ferent origin. Strains possessing high fermentation activity and resistant to elevated temperatures were selected.

MATERIALS AND METHODS

Strains and culture media. Strains used in the work are listed in the table. Yeast cultures were grown at 28°C on a complete YPD medium (g/L): Bacto Agar (Difco, United States), 20; glucose (Merck, Germany), 20; yeast extract (Difco), 10; and Bacto Peptone (Difco), 20. Species reference strains *S. cerevisiae* VKM Y-502, *S. arboricola* CBS 10644, *S. bayanus* VKM Y-1146, *S. cariocanus* UFRJ 50816, *S. kudriavzevii* NBRC 1802, *S. mikatae* NBRC 1815, and *S. paradoxus* CBS 432 were used as the controls.

Capacity for fermentation of various sugars (maltose, sucrose, and melibiose) was determined by carbon dioxide production in a liquid YP medium in Durham fermentation tubes. YP medium composition was the same as that of YPD, but it lacked agar and contained 2% maltose, sucrose, or melibiose instead of glucose. Strains of Suc^+, Mal^+, and Mel^+ phenotype ferment the relevant sugars at the indicated concentration within one day and rarely, within two to three days. Yeasts of the phenotypes Suc^-, Mal^-, and Mel^- do not ferment corresponding sugars after 10 days.

The rate of sugar formation was determined by the amount of carbon dioxide produced. The experiments were performed in duplicates. The tubes containing 5 mL YP medium were inoculated with yeast to the final concentration of 10^6 cells/mL and incubated for 48 h at 28°C. Then the inoculum (5 mL) was transferred to flasks with 100 mL sterile YP medium supplemented with 20% glucose. The fermentation was carried out at 28°C for 72 h. After each 24 h the flasks were weighed and the amount of released carbon dioxide (CO_2) was determined by change in the flask weight. Flasks with 100 mL YP medium without inoculum were used as the control.

PCR analysis. Amplification of the 5.8S rRNA gene and of internal transcribed spacers ITS1 and ITS2 (5.8S-ITS fragments) was carried out using the primers pITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and pITS4 (5'-CCTCCGCTTATTGATATGC-3'). To amplify the genes *MEL1, SUC2*, and *MAL62*, the following pairs of primers were used: DM1/DM2: TTCGCAGATGGGTTGGGACAA and TAAGCTTGCTGGAACAGTTGTGTT; SD1/SR: ATGCTTTTGCAAGCTTTC and GGTCATGTTCACAGATCC; and MAL62F/MAL62R: ATAACGATGGCTGGGGTGATT and AAAGCAGCAAACAGCGTCTT.

PCR was performed using a Tertsic (DNA-Technology, Russia) DNA amplifier directly on the yeast cells. A small amount of yeast biomass (at the tip of a microbiological loop) was suspended in 30 μL buffer containing 3 mM $MgCl_2$, 0.3 mM dNTP, and 50 pmol of each primer. The mixture was incubated at 95°C for 15 min to lyse the cells, and then 2.5 U of *Taq*-polymerase (Syntol, Russia) were added. Amplification products were analyzed by electrophoresis in 1% agarose gel run at 60–65 V in 0.5× TBE buffer (45 mM Tris, 10 mM EDTA, 45 mM boric acid) for 1.5 h and stained with ethidium bromide.

Restriction fragment length polymorphism (RFLP) analysis of the 5.8S-ITS fragments was performed using *Hpa*II and *Hae*III restriction endonucleases (Fermentas, Lithuania). Restriction fragments were separated in a 2.5% agarose gel run at 50–55 V in 0.5× TBE buffer for 4 h. The gel was stained with ethidium bromide for 2–3 h, then washed with distilled water, and photographed under UV light using a Vilber Lourmat (France) transilluminator.

Molecular karyotyping and Southern hybridization. Preparations of the chromosomal DNA were made according to [24]. Electrophoretic separation of the chromosomal DNA was performed using a CHEF-DR III (BioRad, United States) apparatus at 200 V under the following conditions: 15 h with field switching time of 60 s and 8 h with field switching time of 90 s. The gel was run in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, and 10 mM EDTA, pH 8.2) cooled to (14°C). Strain YNN 295 (Bio-Rad) with the known order and size of the chromosomes was used as a karyotyping standard. After electrophoresis, the gel was stained with ethidium bromide, washed in distilled water, and photographed.

Chromosomal DNA was transferred to a nitrocellulose membrane by the vacuum method in a Vacuum blotter apparatus (Bio-Rad). DNA was fixed on the membrane by annealing at 80°C during 2 h. PCR amplified fragments of the *SUC2* (strain *S. cerevisiae* X2180-1A), *MAL62* (VKM Y-1830), and *MEL1* genes (C.B.11) were used as probes. A nonradioactive digoxigenin label dig-II-dUTP was introduced according to the manufacturer's manual (Roche, Switzerland). Hybridization and visualization of the hybridization bands were carried out according to Roche's instructions.

Increased temperature resistance test. The strains' thermotolerance was assessed using a simple growth test on agarized YPD medium at temperatures of 38, 40, and 42°C. Strains growing well at 40°C were additionally tested under heat shock conditions [25]. For this purpose, one-day yeast culture biomass was resuspended in 150 μL sterile distilled water and subjected to heat shock of 46 or 48°C (the heat shock temperature was 5–6 degrees higher than the tested temperature) for 15 min. Then the tubes were cooled on ice during 5 min and the cell suspension was inoculated on petri dishes with solid YPD medium. Growth of the strains studied under specific temperatures was monitored for 48 h. Strain *Ogatae parapolymorpha* 1-IR able to grow at 48°C was used as a positive control.

Characteristics of the *Saccharamyces* strains under study

Species, strain	Source and origin of isolation	Fermentation of sugars*		
		Mal	Suc	Mel
Saccharomyces arboricola				
CBS 10644	Oak bark, China	–	+	+
Saccharomyces bayanus				
VKM Y-1146	Grapes, Russia	+	+	+
Saccharomyces cariocanus				
UFRJ 50816	*Drosophila* sp., Brazil	–	+	–
Saccharomyces kudriavzevii				
NBRC 1802	Rotten leave, Japan	–	+	–
Saccharomyces mikatae				
NBRC 1815	Soil, Japan	–	+	+
Saccharomyces paradoxus				
CBS 432	Unknown	–	+	–
Saccharomyces cerevisiae				
X2180-1A	Haploid genetic line	–	+2	–
VKM Y-1830	Grapes, Michurinsk	+1	+1	–
C.B.11	Derivate of strain NCYC 74	–	–	+1
no. 1	Dry yeast, China	+1	+1	+1
no. 2	Dry yeast, China	+1	+1	+1
no. 4	Dry yeast, Austria	+2	+2	+1
no. 5	Dry yeast, China	+2	+1	+1
no. 6	Dry yeast, China	+2	+2	+1
VKPM Y-187	Soil, distiller's yeast, UkrNIISBPP	+1	+2	–
VKPM Y-408	Distiller's yeast, VNIIPBT	+1	+1	–
VKPM Y-480	Distiller's yeast, Institute of Microbiology and Virology, Latvia	+1	+2	–
VKPM Y-563	Distiller's yeast, UkrNIISP	+2	+1	+1
VKPM Y-564	Distiller's yeast, UkrNIISP	+1	+2	+2
VKPM Y-622	Distiller's yeast, Institute of Microbiology and Virology, Latvia	+1	+2	–
VKPM Y-626	Distiller's yeast	+2	+2	–
VKPM Y-1330	Distiller's yeast	+1	+1	–
VKPM Y-318	Distiller's yeast, KTIPP	+1	+2	–
VKPM Y-1334	Distiller's yeast	+2	+1	–
VKPM Y-1693	Distiller's yeast, Kotlass CBK	+1	+1	–
VKPM Y-2395	Race V	+1	+1	–
VKPM Y-2484	Race 563, VNIIPBVN	+1	+2	–
VKPM Y-795	Onega hydrolysis plant	+2	+2	–
VKPM Y-2080	Omsk race	+2	+1	–
VKM Y-380	Bread–potato grains, race II	+1	+1	–
VKM Y-381	Race XII (Krasnoyarsk 9), INMI	+2	+2	–
VKM Y-382	Race XII Ya	+1	+1	–
VKM Y-383	Race Ya	+1	+1	–
VKM Y-1169	Race XII, Berlin Institute of Fermentation	+1	+2	–
VKMY-1812	Race M	+1	+2	–

Table. (Contd.)

Species, strain	Source and origin of isolation	Fermentation of sugars*		
		Mal	Suc	Mel
VKM Y-1828	Race XV	+1	+1	–
B	Distiller's yeast, VNIIPBT	+1	+2	–
G67	Distiller's yeast, VNIIPBT	+2	+1	+2
G73	Distiller's yeast, VNIIPBT	+1	+1	+1
G660	Distiller's yeast, VNIIPBT	–	–	–
G112	Distiller's yeast, VNIIPBT	–	–	–
K81	Distiller's yeast, VNIIPBT	+1	+2	+1
XII_7	Inbred line of race XII	+1	+1	–
VS-2	Biryusinsk hydrolysis plant, Russia	–	+1	–
no. 148	Fodder yeast, Ukraine	–	+1	–

Note: * "+1", "+2", and "–" represent the ability of the strains to ferment maltose (Mal), sucrose (Suc), and melibiose (Mel) on the first and second day or the absence of the ability to ferment these sugars, respectively.
Abbreviated titles of the collections: VKM, All-Russian Collection of Microorganisms, Pushchino; VKPM, All-Russian Collection of Industrial Microorganisms, Moscow; VNIIGP, All-Russian Research Institute for Hydrolysis Industry, Moscow; VNIIPBVP, All-Russian Research Institute of Brewing, Beverages, and Wine Production, Russian Academy of Agricultural Sciences, Moscow; VNIIPBT, All-Russian Institute of Food Biotechnology, Russian Academy of Agricultural Sciences, Moscow; INMI, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow; Kotlass CBK, Kotlass Cellulose-Processing Factory, Koryazhma; KTIPP, Kiev Technology Institute of Food Production; UkrNIISP, Ukraine Research Institute of Sugar Industry, Kiev; UkrNIISBPP, Ukraine Research Institute of Alcohol and Biotechnology of Food Production, Kiev.

RESULTS

Thirty-six *Saccharomyces* distillers' strains obtained from the Institute of Food Biotechnology (Moscow), All-Russian Collection of Microorganisms (Institute of Biochemistry and Physiology of Microorganisms, Pushchino), and All-Russian Collection of Industrial Organisms (Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow), together with dry industrial yeasts (table) were the subjects of the study. According to morphology of the colonies, vegetative cells, and ascospores, and to their ability to ferment glucose, all strains under study belonged to the genus *Saccharomyces*. The analyzed strains differed by their fermentation spectra and rates of maltose, sucrose, and melibiose fermentation (table). Only 10 of 36 strains exhibited the ability to ferment melibiose, eight of them (strains nos. 1, 2, 4, 5, 6, VKPM Y-563, G73, and K81) fermented the sugar within the first day. Strains G660, G112, VS-2, and no. 148 did not ferment melibiose, and the former two did not ferment sucrose either. After one day of incubation, 22 strains fermented maltose and 19 strains fermented sucrose. Twelve strains (nos. 1, 2, G73, VKPM Y-408, VKPM Y-1330, VKPM Y-1693, VKPM Y-2395, VKM Y-380, VKM Y-383, VKM Y-1169, VKM Y-1828, and XII_7) fermented both sugars rapidly; the first three fermented melibiose as well.

RFLP analysis of rDNA 5.8S-ITS fragments. Species affiliation of the strains under study was determined by PCR-RFLP analysis of 5.8S-ITS fragments comprising the 5.8S rRNA gene and internal transcribed spacers ITS1/ITS2. Species reference strains *S. cerevisiae* VKM Y-502, *S. arboricola* CBS 10644, *S. bayanus* VKM Y-1146, *S. cariocanus* UFRJ 50816, *S. kudriavzevii* NBRC 1802, *S. mikatae* NBRC 1815, and *S. paradoxus* CBS 432 were used as the controls.

Saccharomyces species differed by the sequence of rDNA internal transcribed spacers ITS1 and ITS2 and may be differentiated on the basis of the restriction fragment length analysis of the region. Using restriction endonucleases *Hae*III and *Hpa*II, *S. cerevisiae* may be distinguished from the species groups *S. paradoxus*/*S. cariocanus*, *S. bayanus*/*S. kudriavzevii* and *S. arboricola*/*S. mikatae* [23]. Inside each group, the species may be differentiated according to their molecular karyotypes: the patterns of *S. bayanus*, *S. cariocanus*, and *S. mikatae* are species-specific [17, 19, 26, 17].

We carried out amplification of the 5.8S-ITS fragments in the 36 strains under analysis and 7 species reference strains. The size of the amplified fragments was the same in all the studied and control strains and was approximately 850 bp, which is typical of *Saccharomyces*. PCR products were analyzed by enzymatic cleavage with endonucleases *Hpa*II and *Hae*III (Fig. 1). All analyzed strains possessed identical *Hpa*II profiles comprising two fragments, approximately 730 and 120 bp long, typical of *S. cerevisiae* (Fig. 1a, lanes *1*, *8–13*). Upon *Hae*III restriction, the strains had the patterns of four fragments, approximately 320, 230, 170, and 130 bp long (Fig. 1b, lanes *1*, *8–13*). Therefore, according to *Hpa*II and *Hae*III profiles, all

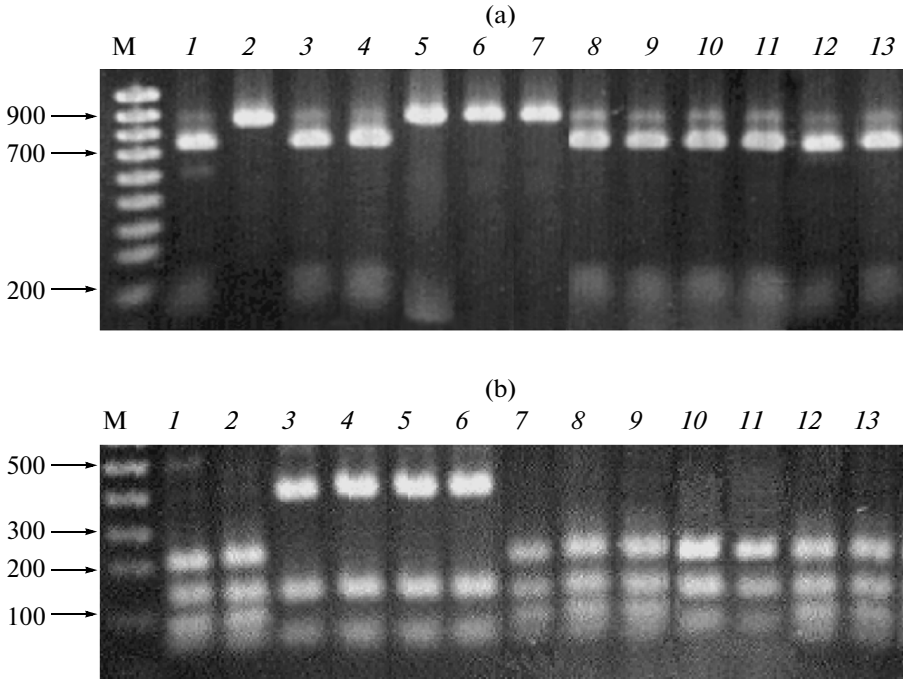

Fig. 1. Restriction analysis of amplified rDNA 5.8S-ITS fragments of *Saccharomyces* strains with restriction endonucleases *Hpa*II (a) and *Hae*III (b). Lanes: *1*, *S. cerevisiae* VKM Y-502; *2*, *S. paradoxus* CBS 423; *3*, *S. bayanus* VKM Y-1146; *4*, *S. kudryavzevii* NBRC 1802; *5, S. mikatae* NBRC 1815; *6*, *S. arboricola* CBS 10644; *7*, *S. cariocanus* UFRJ 50816; *S. cerevisiae*: *8*, VKM Y-380, *9*, VKM Y-381, *10*, VKMP Y-187; *11*, VKPM Y-795; *12*, G67; and *13*, K81. M, a 100 bp DNA Ladder (Fermentas, Lithuania) molecular weight marker (bp).

36 strains under study belonged to the species *S. cerevisiae*.

Molecular karyotyping. We compared molecular karyotypes of 36 distillers' strains (Figs. 2 and 3). Identification of the individual chromosomal bands was carried out by comparison with the *S. cerevisiae* YNN 295 reference strain karyotype (Figs. 2 and 3, lane *1*).

Karyotype analysis confirmed the affiliation of all strains under study to *S. cerevisiae*. At the same time, comparative analysis of the patterns revealed profound polymorphism of the chromosome size and number of bands. Five strains (nos. 1, 2, and 4–6) of dry yeast were exceptional, with identical karyotypes (Fig. 2a, lanes *3–7*). Chromosomal DNA of most strains separated into 13–20 electrophoresis bands. According to the fluorescence intensity of the stained bands, some of them contained several chromosomes. In practically all strains under study chromosomes XIII and XVI migrated in a doublet. Six strains of the VKM collection (381, 382, 383, 1169, 1812, and 1828), four strains of the Institute of Food Biotechnology collection (V, G67, G73, and K81), and eight strains of the VKPM collection (187, 563, 1330, 318, 1334, 2395, 795, and 2080) had more than 16 chromosomal bands in their karyotypes. The patterns of these strains were characterized by the presence of four chromosomal bands of 245–370 kb and additional chromosomes, 580–945 kb long (Fig. 2, lanes *9–17* and *20* and Fig. 3, lanes *3, 6, 10–12, 14, 16*, and *17*).

The results obtained indicate profound chromosome length polymorphism of the distillers' yeast *S. cerevisiae*. According to molecular karyotypes, many strains contained additional chromosomes and probably were aneuploid.

Using Southern hybridization we studied the chromosomal polymorphism of the genes *SUC, MAL*, and *MEL* in the 36 distillers' strains.

Southern hybridization. Chromosomal DNA of the strains presented in Figs. 2 and 3 was transferred via Southern blot onto a nitrocellulose membrane and hybridized with the *MAL62* and *SUC2* probes. The results of hybridization with the *MAL62* probe are presented in Figs. 2b and 3b. The VKM Y-1830 strain containing all five known *MAL* genes in the telomeric regions of different chromosomes (*MAL1*, on chromosome VII, *MAL2*, III, *MAL3*, II, *MAL4*, XI, and *MAL6*, VIII) was used as the control. Most strains contained several *MAL* loci. The *MAL1* locus was present in all strains under study; 29 strains also contained the locus *MAL3*. No new *MAL* loci were revealed in the strains. Strains G660, G112, BC-2, and no. 148, which were unable to ferment maltose, did not hybridize with the *MAL63* probe (data not shown).

As we have already pointed out, nine polymeric *SUC* genes are known; they are located on different chromosomes: *SUC1* (on chromosome VII), *SUC2* (IX), *SUC3* (II), *SUC4* (XIII), *SUC7* (VIII), *SUC8* (X),

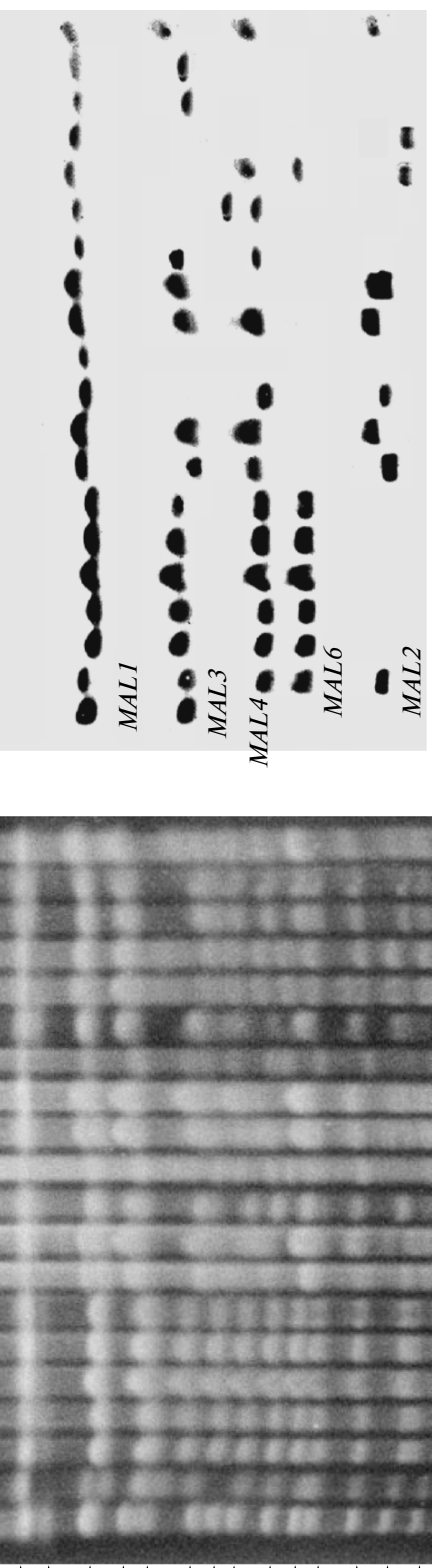

Fig. 2. Pulsed-field electrophoresis of native chromosomal DNA of *S. cerevisiae* strains obtained from the VKM and VNIIPBT collections (a) and Southern hybridization with the *MAL62* (b) and *SUC2* (c) probes. Lanes: *1*, YNN 295 (chromosomal standard), *2*, VKM Y-1830 (b)/X2180-1A (c), *3*, no. 1, *4*, no. 2, *5*, no. 4, *6*, no. 5, *7*, no. 6, *8*, VKM Y-380, *9*, VKM Y-381, *10*, VKM Y-382, *11*, VKM Y-383, *12*, VKM Y-1169, *13*, VKM Y-1812, *14*, VKM Y-1828, *15*, V, *16*, G67, *17*, G73, *18*, G660, *19*, G112, and *20*, K81. Chromosome order is reported according to that of YNN 295 strain.

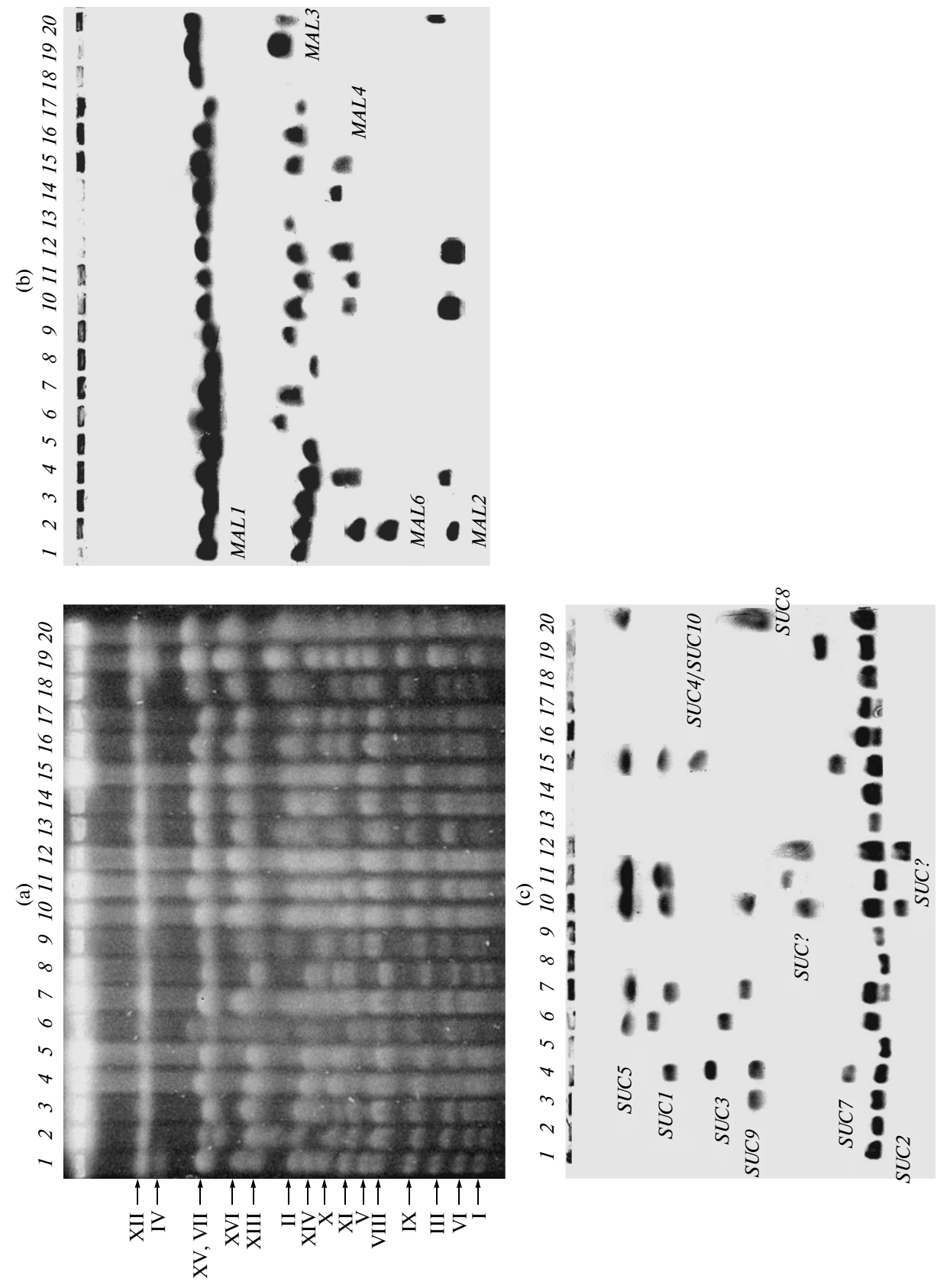

Fig. 3. Pulsed-field electrophoresis of native chromosomal DNA of *S. cerevisiae* strains obtained from the VKPM collection (a) and Southern hybridization with the *MAL62* (b) and *SUC2* (c) probes. Lanes: *1*, YNN 295 (chromosomal standard), *2*, VKM Y-1830 (b)/X2180-1A (c), *3*, VKPM Y-187, *4*, VKPM Y-408, *5*, VKPM Y-480, *6*, VKPM Y-563, *7*, VKPM Y-564, *8*, VKPM Y-622, *9*, VKPM Y-626, *10*, VKPM Y-1330, *11*, VKPM Y-318, *12*, VKPM Y-1334, *13*, VKPM Y-1639, *14*, VKPM Y-2395, *15*, VKPM Y-2484, *16*, VKPM Y-795, *17*, VKPM Y-2080, *18*, VS-2, *19*, no. 148, and *20*, XII_7. Chromosome order is reported according to that of YNN 295 strain.

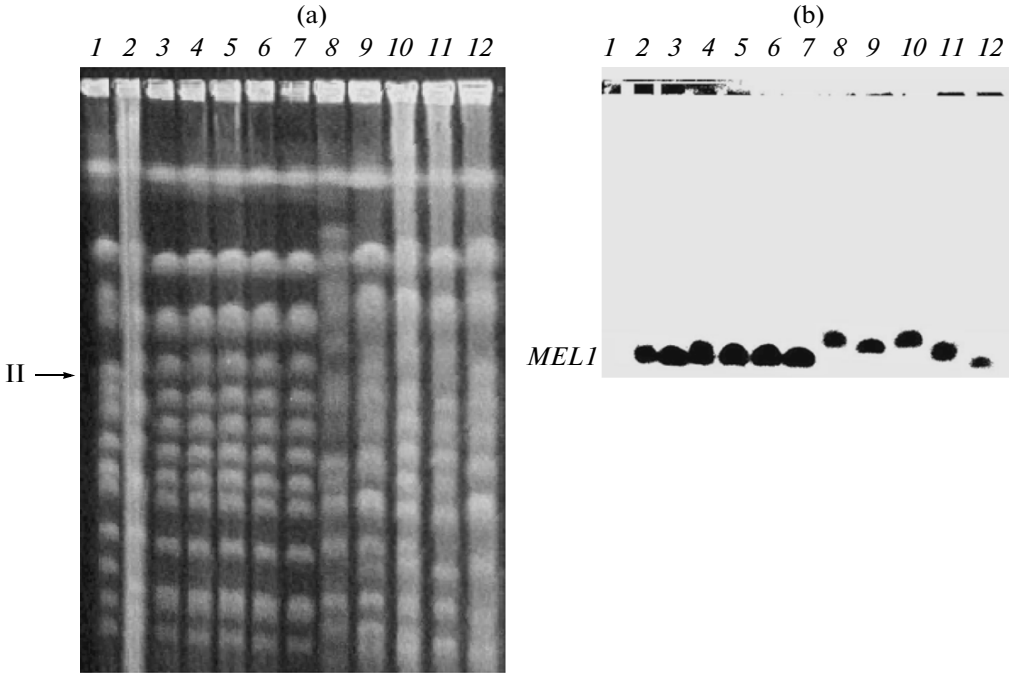

Fig. 4. Pulsed-field electrophoresis of native chromosomal DNA of *S. cerevisiae* melibiose-fermenting yeast strains (a) and Southern hybridization with the *MEL1* (b) probes. Lanes: *1*, YNN 295 (chromosomal standard), *2*, C.B.11 (control), *3*, no. 1, *4*, no. 2, *5*, no. 4, *6*, no. 5, *7*, no. 6, *8*, VKPM Y-563, *9*, VKPM Y-564, *10*, G67, *11*, G73, and *12*, K81.

SUC9 (XIV), and *SUC10* (XVI) [1, 10–12]. Southern hybridization with the *SUC2* probe revealed considerable polymorphism of hybridization profiles among the strains under study (Figs. 2c and 3c). From one to seven hybridization signals were detected in different strains. All 36 strains possessed the *SUC2* gene localized in chromosome IX. Strains VKM Y-381, VKM Y-383, G67, G112, K81, VKPM Y-564, VKPM Y-1330, VKPM Y-1334, VKPM Y-2395, VKPM Y-795, and VKPM Y-2080 had two hybridization signals, each in the region of chromosome IX of the YNN 295 reference strain (Fig. 2c, lanes *9, 13, 16, 19*, and *20* and Fig. 3c, lanes *7, 10, 12, 14, 16*, and *17*). In 19 strains the *SUC2* probe hybridized with chromosome VII where the *SUC1* gene is located (Fig. 2c, lanes *8–20* and Fig. 3c, lanes *6, 7, 10, 11, 15*, and *20*). Moreover, the VKM Y-380 strain had an additional chromosome VII (Fig. 2c, lane *8*). In strains VKM Y-380, VKM Y-381, VKM Y-1169, G67, VKPM Y-1330, VKPM Y-318, and VKPM Y-1334, the *SUC2* probe hybridized with a chromosomal band matching by size with chromosome V of the reference strain, YNN 295 (Fig. 2c, lanes *8, 9, 12, 16*, and *20*, and Fig. 3c, lanes *10–12*). Apparently, the eight strains indicated possessed a previously unknown *SUC* gene. Another new *SUC* gene located on chromosome VI was found in strains VKM Y-381, VKM Y-382, VKM Y-1169, VKM Y-1812, G73, K81, VKPM Y-1330, and VKPM Y-1334 (Fig. 2c, lanes *9, 10, 12, 13,17*, and *20* and Fig. 3c, lanes *10* and *12*). Therefore, the results indicate accumulation of the polymeric *SUC* genes in *S. cerevisiae* distillers' yeast.

To determine the number of the *MEL* genes, encoding α-galactosidase, and their chromosomal localization, 10 melibiose-fermenting strains were subjected to Southern analysis. *S. cerevisiae* strain C.B.11 containing the *MEL1* gene (Fig. 4, lane *2*) was used as a control. According to the results, all ten distillers' strains possessed a single *MEL* gene. In six strains (no. 1, 2, 4–6, and K81), the *MEL1* probe hybridized with the chromosomal band matching by size chromosome II of the control strain *S. cerevisiae* YNN 295, while in strains VKPM Y-563, VKPM Y-564, G67, and G3 the hybridization signal was located somewhat higher (Fig. 4, lanes *8–11*). Apparently, in the latter strains chromosome II was larger than in the reference strain. It is known that meiotic crossing-over may result in the chromosome size changes.

Thermotolerance. All 36 strains grew well at the temperature of 37–39°C. Twenty strains grew at 40°C as well, although good growth was noted only for ten of them: nos. 1, 2, 4–6, G67, VKPM Y-187, VKPM Y-564, VKM Y-380, and VKM Y-1812. The indicated strains were tested for their ability to grow at 40°C after heat shock of 46°C, as well as at temperatures of 42 and 44°C after heat shock of 48 and 50°C, respectively. All ten strains grew well at 40°C after heat shock, but none of them grew at temperatures above 40°C.

Fermentation activity. According to the results of fermentation tests, Southern analysis, and thermotol-

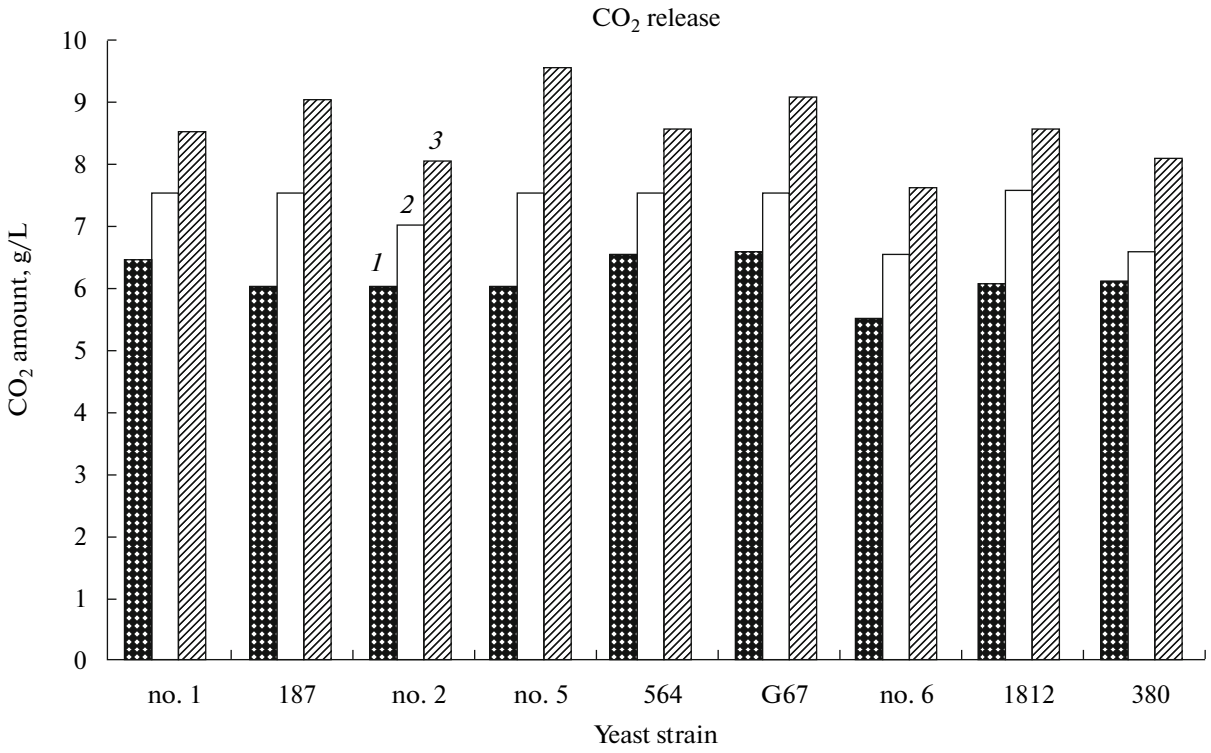

Fig. 5. Fermentation intensity of the thermotolerant *S. cerevisiae* strains. Fermentation rate of a 20 g/L glucose solution was determined by the amount of released carbon dioxide after 24 (*1*), 48 (*2*), and 72 h (*3*).

erance tests, we selected nine strains for which the fermentation activity was assessed in a liquid YP medium supplemented with 20% glucose (Fig. 5). The rate of glucose fermentation was determined by the amount of carbon dioxide released during 72 h. On the first day the most intense fermentation was noted in strains no. 1, VKPM Y-564, and G67. After 48 h another three strains started to ferment intensely: no. 5, VKPM Y-187, and VKM Y-1812. The six indicated strains demonstrated the best results after 72 h as well. Strain G67, characterized by high fermentation activity after 24, 48, and 72 h, stands out. This strain has polymeric genes *MAL* and *SUC*, together with the *MEL1* gene (Figs. 2–4).

DISCUSSION

By means of PCR–RFLP analysis of the rDNA 5.8S-ITS fragments, molecular karyotyping, and Southern hybridization, genomes of 36 distillers' strains, mostly of domestic origin (table), were studied. Molecular analysis demonstrated that all strains belonged to the species *S. cerevisiae*. Karyotype analysis revealed profound polymorphism of chromosomal DNA of the *S. cerevisiae* distillers' yeasts; practically each strain under study possessed an individual karyotype. The karyotypes of most strains were characterized by the presence of additional chromosomal bands.

It is known that many industrial strains of *S. cerevisiae* are aneuploid [28]. Aneuplody is considered a mechanism of yeast adaptation to industrial fermentation by increasing the number of copies of the relevant genes [29]. This agrees well with the presence of additional chromosomes carrying the *MAL* and *SUC* genes in many of the distillers' strains under study. Strains with multiple *MAL* and *SUC* genes are often found among distillers', bakers', and brewers' yeasts [5–7, 30]. Accumulation of polymeric genes of sugar fermentation with cumulative effect in a single strain leads to intensification of the process [31]. Indeed, most strains under study which fermented maltose on the first day had several *MAL* genes. Subtelomeric repeats of sugar fermentation genes could appear in the genome of *S. cerevisiae* under the effect of selection in the course of their domestication. It should be noted that accumulation of the polymeric genes *MAL, SUC*, and *MEL* is found only among the cultured *S. cerevisiae* and has not been detected in other six species of the *Saccharomyces* genus [2, 32, 33].

High-temperature alcohol fermentation is one of the ways to cheapen and intensify ethanol production. In this connection, selection of thermotolerant *S. cerevisiae* strains of good fermentation capacity is of current concern. Among the 36 studied distillers' strains, we selected six which were able to grow at 40°C and exhibited high fermentation activity: no. 1, no. 5, VKPM Y-564, G67, VKPM Y-187, and VKM Y-1812. It

should be noted that the four former strains are able to ferment sucrose, maltose, and melibiose. The indicated strains are of interest for further molecular genetic studies and breeding.

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