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SCREENING FOR PROMISING YEAST STRAINS FOR SHERRY WINE PRODUCTION USING GENETIC AND ENOLOGICAL MARKERS

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Abstract

Flor yeast is a special group of wine-making microflora used in the production of biologically aged wines. In the process of biological aging, flor yeast, cultivated on the surface of dry, alcohol-based wine materials, switches its metabolism from enzymatic to oxidative, which leads to deep biochemical changes in the wine with the formation of unique features of the bouquet and aroma of sherry wines. Genetic, biochemical and physiological features of flor yeast associated with their adaptation to specific conditions of sherry winemaking have been studied in sufficient detail. Earlier, the use of comparative genomic analysis methods allowed us to identify a number of genetic markers specific for flor strains, convenient for searching for strains that are promising for producing sherry-type wines. This work presents the results of using a combined approach based on primary molecular genetic screening followed by analysis of physiological, biochemical and oenological properties for the selection of strains promising for sherry winemaking in a sample of 47 strains from the Magarach Winemaking Microorganism Collection and 96 natural isolates from samples of grapes in different climatic zones in the Republic of Crimea and in the Rostov region. At the first stage, the selection of promising strains was carried out on the basis of the results of genotyping by the presence of alleles of the loci ITS, *YDR379C-A*, and *FLO11* characteristic of flor strains. According to the genotyping data, the flor allele of the ITS locus was identified in 41 strains, the flor allele of the *YDR379C-A* gene was identified in 41 strains, and a deletion of 111 nt in the promoter of the adhesin *FLO11* gene, typical of flor strains, was found in only 12 strains. A total of 59 studied strains had the flor allele of at least one of the three loci. Further study of oenological properties showed that the presence of the ITS flor locus can serve as a marker for the selection of strains with high oxidative capacity. Film formation in most strains (11 samples out of 12) with a flor variant of the *FLO11* gene promoter confirmed the main role of this adhesin gene in determining the ability of strains to surface growth on wine material. The most informative was the use of the *YDR379C-A* marker. Of the 12 strains for which the presence of only this flor locus was determined, three collection and two natural strains (I-133, I-492, I-616, No. 49, No. 78) showed the presence of all flor characteristics. The formation of a continuous film with a reproducibility of 100 % was observed in two collection strains from this group — I-133 and I-492. Nine strains (75 %) synthesized aldehydes in an amount exceeding 100 mg/l, 6 strains (50 %) formed a film with varying degrees of formation from islands on the surface to continuous growth and film reproducibility from 33 to 100 %. Aldehyde aroma and oxidation taste were determined for 58 % of the samples. Analysis of the oenological properties of six strains with three flor loci showed that all of them formed a continuous film on the surface of the fermented wort at optimal times, and five strains synthesized aldehydes during alcoholic fermentation above 100 mg/l. Tasting laboratory samples of fermented wort revealed the presence of sherry tones in aroma and taste. According to the results of genotyping and oenological sherry characteristics, these strains were close to the control strain I-329. As a result, according to the results of the screening and production check, the

collection strain I-271 can be recommended for sherrying wine materials from the Aligote grape variety, traditionally used for these purposes, as well as blended wine materials from the Rkatsiteli and Sauvignon green grape varieties, new for sherry wines, which opens up the prospect creation of new brands of this type of wine. Thus, the study showed that during the initial selection of new flor strains of S. *cerevisiae* yeast, testing them for genetic markers ITS, *YDR379C-A*, and *FLO11* allows you to quickly and reliably identify the most promising strains for the technology of sherry wine production. Of 143 strains of wine yeast, according to the results of genotyping, taking into account oenology, strain I-271 with great potential for sherrying wine materials was identified.

Keywords: *Saccharomyces cerevisiae*, flor yeast, DNA markers, flor alleles, ITS, *YDR379C-A*, *FLO11*, winemaking, oenological properties, film formation, aldehyde synthesis, adhesins

Breeding industrial flor yeast strains specific to regional raw materials and winemaking technologies is a tool to intensify wine industry and produce a certain type of wine.

Advances in genetics, chemistry and biochemistry provide in-deep study of microbiology of winemaking to develop a new methodology for selecting flor yeasts. This significantly improves genetic fund of yeast strains for winemaking and gives fundamental and practical knowledge about their properties.

This concept is most relevant for yeast generating Jerez wines. The *Saccharomyces cerevisiae* [1-3] can form a biofilm (flor) on the wine surface and, due to oxidative metabolism, generate a characteristic sherry tone of the wine. Currently, studies of *S. cerevisiae* strains having wide practical use are actively developing [4-6]. For Jerez type wine, molecular methods ensure genetic identification of *S. cerevisiae* sherry yeasts to select biotechnologically effective strains and to ensure purity of their populations during wine production.

In early reports, the analysis of mtDNA polymorphism [7], microsatellite analysis [8], comparative genomic hybridization [9], and proportion of polymorphic loci quantified genetic diversity and phylogeny of sherry strains. Thus, the RFLP (restriction fragment length polymorphism) analysis of the ITS1 locus distinguished wine, Spanish, and French sherry yeasts [8, 10]. Most Spanish strains carry a 111 nt deletion in the *FLO11* gene promoter, leading to an increase in this adhesin expression and a denser film formation [11].

The rapid progress in genomic research has opened up new opportunities in disclosing structure, functioning, and evolution of the sherry yeast genome. In our studies and in the works of other researchers, the methods of comparative genomics revealed numerous genes specific for sherry yeast and alleles of genes that control various pathways of metabolism, transport, cell wall biogenesis, and stress resistance [12, 13], putatively associated with the adaptation of sherry strains to the specific conditions of biologically aged wines. Comparative genomics is important for understanding the origin and evolution of sherry strains [14-16] and allows us to develop convenient genetic markers for targeted selection of new sherry strains of *S. cerevisiae* yeast (17, 18). Genome-wide analysis of wine and sherry yeasts revealed a difference in the *YDR379C-A* gene sequence [17, 18].

Until recently, Russian winemakers used multiple alternations of mutagenesis and selection to create promising strains of wine yeast. This work, for the first time shows that testing of *S. cerevisiae* isolates for genetic markers ITS, *YDR379C-A* and *FLO11* allows rapid and reliable identification of yeast strains for the sherry vinification technology.

This research aimed at a multistage selection of new *Saccharomyces cerevisiae* yeast strains for the biofilm-based production of the Jerez-type wines using genetic markers in combination with oenological characteristics.

Materials and methods. A set of 143 *Saccharomyces* strains comprised 96 field isolates from grape sampled in different climatic zones (the Republic of Crimea and the Rostov region) which were genotyped and oenologically described in

part by us earlier [17] and 47 strains from the Magarach Winemaking Microorganism Collection (KMV Magarach) not recommended for the production of Jerez-type wine. The collection strain I-329 for the production of Jerez-type wine was a reference [19]. The media and cultivation modes matched the requirements and recommendations for the of Jerez wines [20, 21].

The genotyping for ITS, *FLO11*, and *YDR379C-Ac* markers was performed as described [17].

For DNA preparations, washed and lyophilized yeast cells (30-50 mg) were incubated with 500 μl of lysis buffer containing SDS (0.1 %), Triton X-100 (1%), and proteinase K (100 U/ml, Merck, Germany) for 20 min at 65 °C followed by phenol:chloroform (1:1) extraction and ethanol precipitated. The precipitate was dissolved in TE buffer (pH 8.0).

The Restriction Fragment Length Polymorphism (RFLP) analysis of PCR fragments of rDNA repeats, including two internal transcribed spacers ITS1 and ITS2 and the 5.8S rRNA gene, were used to identify and attribute the samples to wine or sherry yeast strains [8]. For PCR, primers Its1 5′-TCCGTAGGTGAACCTGCGG-3′ and Its4 5′-TCCTCCGCTTATTGATATGC-3′ were used [8]. The PCR mode was as follows: 96 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s (30 cycles). Hereinafter, a Mastercycler personal amplifier (Eppendorf, Germany) and a GoTaq® Flexi DNA polymerase were used as per the manufacturer's protocol (Promega, USA). The resulting fragments were treated with restriction endonuclease HaeIII (NEB, UK), and after analysis of electrophoregrams (1.5% agarose LE2, Lonza, Switzerland; molecular weight marker GeneRuler 1 kb DNA Ladder, Thermo Fisher Scientific, USA) the isolates were attributed to wine or sherry strains; the 311, 230, 172, and 128 bp fragments were characteristic for the *S. cerevisiae* wine strains while 311, 230, 148, and 129 bp fragments for the sherry strains.

To analyze the *FLO11* gene promoter polymorphism, strains with full-length and truncated (having a 111-nt deletion) *FLO11* gene promoter were identified by PCR technique using primers Flo11D.REV (5′-TTGGGCGACATTTTTCTTGT-3′) and Flo11D.FOR (5′-CCACGGGTGAGATTTGTTCT-3′) [11]. The PCR mode was 96 °C for 40 s, 51 °C for 40 s, 72 °C for 40 s (30 cycles). The fragments of approx. 400 nt or 300 nt in size characterized the wild and Jerez alleles of the *FLO11* gene, respectively [11].

To detect strains with polymorphic *YDR379C-A* gene, RFLP analysis with PCR primers F_sdh6 (5′-TCGCGTCAACTTGTTTTTGAG-3′) and R_sdh6 (5′-ATTCGTCAGTTCAG-3′) was applied. The PCR mode was 96 °C for 40 s, 52 °C for 40 s, 72 °C for 40 s (30 cycles). Fragments approx. 800 bp in length were treated with restriction enzyme AflIII (NEB, Great Britain) as per the manufacturer's protocol [18]. Restricts of 450 and 350 bp were characteristic of the sherry alleles of the *YDR379C-A* gene.

In lab tests, strains were cultured on one batch of must from Aligote grapes harvested in 2018 (the mass concentration of sugars 210 g/l, of titratable acids 7.0 g/l, pH 3.2). The wort was prepared according to the usual procedure in winemaking [5]. The strains from the collection were cultured at 26±0.5 °C in tubes with pasteurized grape must under cotton-gauze plugs. The obtained yeast inoculum was ready for use when the cell number was not less than 80 million/cm^3, budding cells not less than 30%, and the dead cells not more than 2% as assessed by light microscopy.

To obtain yeast biofilms in lab conditions, the pasteurized grape must was added with 2% yeast wort and fermented at 18±0.5 °C. By the end of fermentation when the mass concentration of sugars was not more than 4 g/l the wine material was

alcoholized to 15.3 vol.% ethyl alcohol and allowed at 18±0.5 °C until a folded light beige biofilm was formed. The physiological state of the biofilm was assessed using microscopy by the proportion of living cells not less than 50%.

The ability to form a film was determined by the rate of its growth on the surface of the fermented wort and on wine material alcoholized to 15.3 vol.%. In lab conditions, pasteurized wine-grape wort was fermented at 18±0.5 °C in 200 ml flasks with cotton gauze plugs; in micro-winemaking conditions, freshly squeezed wort was fermented at 18±0.5 °C in 3-liter glass bottles under cotton gauze plugs. Yeast wort was added to grape must (2% by volume). After the end of alcoholic fermentation, yeast film growth was monitored daily.

In lab tests of the yeast alcohol resistance, a film grown in 3-liter bottles was applied to the surface of the alcoholized wine material. Film quality and growth rate were assessed visually and by microscopy [19].

Yeasts were adapted to the 15.3 vol.% alcohol by successive frequent passages using fresh wine materials with a gradually increasing alcohol concentration as per Sayenko's method.

The selected yeast strain was tested in a winery (the Chernyavsky's farm, Evpatoria, Republic of Crimea). Oak 5003-liter wine barrels (Nos. 1, 2, and 3) were filled $^2/_3$ with different wine materials, the Aligote 50% + Sauvignon green 25% + Rkatsiteli 25% (blend 1, barrel No. 1), Sauvignon green 50% + Rkatsiteli 50% (blend 2, barrel No. 2) and Aligote wine material (barrel No. 3). Equal amounts of well-developed young yeast films grown on Aligote wine materi-al alcoholized to 15.3 vol.% in lab conditions, after checking the physiological state of the yeast, were transferred to 30% surface of the wine material in each barrel. The upper openings of the barrels were closed with cotton-gauze plugs. The barrels were allowed at 18±1 °C. During wine aging, the film quality (its appearance, the living cells at least 50%, the presence of foreign microflora) was assessed microbiologically, as well as the accumulation of aldehydes was measured. After 3-month exposure under the formed biofilm, the wine samples were taken from the barrels for biochemical analysis.

The mass concentration of volatile acids in the samples was determined as per the state standard GOST 32001-2012 ("Alcoholic products and raw materials for its production". Moscow, 2014), the mass concentration of aldehydes as per GOST 12280-75 ("Wines, wine materials, cognac and fruit spirits". Moscow, 2003), the mass concentration of residual sugars as per GOST 13192-73 ("Wines, wine materials and cognacs". Moscow, 2005), and the volume concentration of ethyl alcohol as per GOST 32029-2013 ("Alcoholic products and raw materials for its production". Moscow, 2014).

The organoleptic quality was determined by a commonly used sensory evaluation method [17].

The data were processed statistically using the Microsoft Excel program. All experiments were arranged in three biological replicates with two technical replicates of each analytical measurements. Means (M) and standard errors of means (±SEM) are calculated at a confidence level of $P = 0.95$.

Results. Figure 1 shoes the scheme we developed to find *S. cerevisiae* strains producing Jerez wines by biofilm formation on the wine material surface.

Genotyping and oenological characterization of *Saccharomyces* yeast strains. Of 143 strains involved in molecular genotyping, the ITS sequence was characteristic of the "wine" type in 102 strains (71%), and of the "sherry" type in 41 strains (29%). The *YDR379C-A* gene allele specific for sherry yeast was detected in 41 strains, and a 111 nt deletion in the adhesin gene *FLO11*

promoter typical for Sherry strains — only in 12 strains. Fifty-nine strains had a "sherry" allele in at least one of the three loci tested.

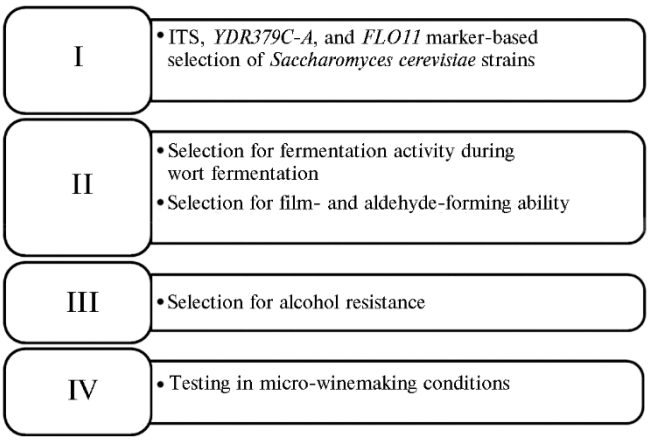

Fig. 1. The use of genetic and oenological markers to select yeast *Saccharomyces cerevisiae* candidate strains for the production of Jerez wines.

The selection of candidate strains was based on both genotyping data and the assessment of specific Jerez oenological characteristics in laboratory samples of fermented grape must (Table 1), namely, film-forming and aldehyde-forming ability and generation of characteristic properties of Jerez-type wines. Physiological, biochemical and technological studies showed that sherry yeast strains from the the Magarach Winemaking Microorganism Collection can produce 132 to 352 mg/l aldehydes during grape must fermentation phase, and it takes from 1 week to 1 month for a biofilm to appear on the fermented wort [19]. For high-quality table wine materials, the amount of aldehydes is 20-100 mg/l and often at a sensory threshold level of 40-100 mg/l [18]. This is largely due to the yeast strains with low oxidative activity. The candidate sherry strains should synthesize more than 100 mg/l aldehydes.

1. Genetic and oenological markers of *Saccharomyces cerevisiae* strains for Jerez-type wine production (n = 143, the Magarach Winemaking Microorganism Collection, Republic of Crimea)

Strain	Organoleptic quality	MCA, mg/l (M±SEM)	Film formation	
			F	R
		ITS locus		
I-31	Oxidized, salty taste	107.4±6.5	Film	66%
I-43	–	103.4±6.3	–	
I-53	Sherry tone, oxidized, salty taste	132.0±8.0	Film (ring)	66%
I-214	–	114.4±6.9	–	
I-307	Sherry tone, oxidized taste	160.6±9.7	–	
I-440	Sherry tone, oxidized, salty taste	148.7±9.0	–	
I-471	–	178.2±10.8	–	
I-527	–	123.2±7.5	–	
I-630	–	92.4±5.6	–	
I-651	–	171.6±10.4	–	
I-653	–	123.2±7.5	–	
No. 19	–	71.3±4.3	–	
No. 28	–	73.0±4.4	–	
No. 74	–	37.8±2.3	–	
No. 75	–	128.5±7.8	–	
No. 76	–	124.9±7.6	–	
		YDR379C-A locus		
I-133	Sherry tone, oxidized, salty taste	171.6±10.4	Film	100%
I-308	–	114.4±6.9	–	
I-492	Sherry tone, oxidized, salty taste	180.4±10.9	Film	100%
I-616	Sherry tone, oxidized taste	184.8±11.2	Film	66%
No. 45	–	84.5±5.1	–	
No. 49	Slight sherry tone, oxidized taste	129.2±7.8	Film	66%

Continued Table 1

No. 71	–	75.7±4.6	Film (floating islets)	33%
No. 78	Slight sherry tone, oxidized taste	154.9±9.4	Film (floating islets)	33%
No. 79	–	91.5±5.5	–	
No. 97	Oxidized taste	202.4±12.3	–	
No. 108	Oxidized taste	183.0±11.1	–	
No. 112	–	176.0±10.7	–	
	FLO11 locus			
No. 52	Sherry tone, oxidized taste	140.3±8.5	Complete flor covering	
No. 53	–	34.3±2.1	Film (floating islets)	100%
No. 90	–	74.5±4.5	–	66%
	ITS + *YDR379C-A* loci			
I-14	Aldehyde tone in the aroma, oxidized, salty taste	198.0±12.0	Film (ring)	66%
I-118	Oxidized, salty taste	162.8±9.9	Film (ring)	66%
I-137	Aldehyde tone in the aroma, oxidized taste	103.4±6.3	–	
I-280	–	149.6±9.1	–	
I-310	Aldehyde tone in the aroma, salty taste	316.8±19.2	Film (floating islets)	100%
I-340	Oxidized, slight salty taste	101.2±6.1	Film (ring)	100%
I-374	–	123.2±7.5	Film	100%
I-380	Slight bread tones in the aroma	167.2±10.1	Film	100%
I-448	Oxidized taste	123.2±7.5	Film (ring)	33%
I-515	Oxidized, salty taste	198.0±12.0	Film (floating islets)	100%
I-516	–	149.6±9.1	Film (ring)	100%
I-523	Oxidized, salty taste	85.4±5.2	Film (floating islets)	33%
I-525	Sherry tone in the aroma, oxidized, salty taste	149.6±9.1	–	
I-654	Oxidized, salty taste	132.0±8.0	Film (floating islets)	33%
No. 4	Oxidized, salty taste	67.8±4.1	Complete flor covering	66%
No. 18	–	134.0±8.1	Film (floating islets)	33%
No. 27	Oxidized taste	98.7±6.0	Film (floating islets)	33%
No. 77	–	184.8±11.2	Film (ring)	66%
	ITS + *FLO11* loci			
	No strains found			
	YDR379C-A + *FLO11* loci			
No. 46	–	109.7±6.7	Film (ring)	33%
No. 54	Sherry tone in the aroma, oxidized, salty taste	148.7±9.0	Complete flor covering	100%
No. 110	Oxidized taste	253.4±15.4	Film (ring)	100%
	ITS + *YDR379C-A* + *FLO11* loci			
I-271	Sherry tone in the aroma and in the taste	343.2±20.8	Complete flor covering	66%
No. 3	Sherry tone in the aroma and in the taste	65.9±4.0	Complete flor covering	100%
No. 23	Sherry tone in the aroma and in the taste	105.6±6.4	Complete flor covering	100%
No. 109	Slight sherry tone in the aroma and in the taste	283.4±17.2	Complete flor covering	100%
No. 111	Sherry tone in the aroma and in the taste	279.8±17.0	Complete flor covering	100%
No. 113	Sherry tone in the aroma and in the taste	176.0±10.7	Complete flor covering	100%
	Control			
I-329	Sherry tone, oxidized, salty taste	350.6±21.3	Complete flor covering	100%

Note. MCA — mass concentration of aldehydes, F — yeast biofilm on the surface, R — reproducibility. Dashes indicate the absence of the trait.

Sixteen strains carrying only the ITS sherry marker presented a high frequency of samples (75%) with active aldehyde formation and a low frequency of those (13%) capable of surface growth (from the rings on the bottle walls formed by the collection strain I-53 and up to complete biofilm formation by collection strain I-31) with 66% reproducibility. Organoleptic evaluation of lab cultured wine materials revealed aldehyde tone in aroma and oxidation in taste in four collection strains (I-31, I-53, I-307, I-440) which produced 107.4-160.6 mg/l aldehydes.

Testing for the sherry allele of only the *FLO11* locus revealed three field isolates (Nos. 52, 53, and 90) of which two (Nos. 52, 53) developed a film. For one strain (No. 52), all oenological sherry characteristics appeared (complete film formation with a 100% reproducibility, production of 140.3 g/l aldehydes, an aldehyde tone in the aroma and oxidation in the taste).

The most informative was the use of the *YDR379C-A* marker. Of the 12 strains for which we determined only this sherry locus, three collection strains (I-133, I-492, I-616) and two isolates (Nos. 49, No. 78) showed all sherry characteristics. Of these, two collection strains (I-133 and I-492) developed flor covering the entire surface of the wine material with a 100% reproducibility. Nine

strains (75%) synthesized aldehydes (more than 100 mg/l), six strains (50%) formed a biofilm on the surface (from islets to entire covering) with 33 to 100% reproducibility. Aldehyde aroma and oxidation in taste were characteristic to 58% of the samples tested.

For combination of two sherry loci, the ITS + *YDR379C-A* (19 strains) or *YDR379C-A* + *FLO11* (3 strains), the number of strains with oenological sherry characteristics increased. Of these, two strains (collection strain I-380 and isolate No. 54) formed a complete biofilm with a reproducibility of 100% and synthesized aldehydes (167.2-148.7 g/l) during alcohol fermentation phase resulting in an aldehyde tone in the aroma and oxidation taste.

Oenological examination of six strains (I-271, Nos. 3, 23, 109, 111, and 113) carrying combination of three sherry loci showed that all of them developed biofilm on the entire surface of the fermented wort during optimal periods of time, and five strains produced aldehydes (more than 100 mg/l) in the course of alcohol fermentation. Tasting laboratory samples of fermented wort revealed sherry tones in aroma and taste. According to the results of genotyping and oenological sherry characteristics, these strains are close to the collection strain I-329 (control).

Our findings indicate that the sherry ITS locus of *S. cerevisiae* can serve as a marker to select strains for high oxidative capacity. Flor developed by 11 out of 12 strains with the *FLO11* gene directly responsible for biofilm formation confirms the main role of this gene in the selection of strains capable of surface growth on wine material. Locus *YDR379C-A* can be a selection marker for several characteristics of sherry, for example, high oxidative activity and the ability to grow on the surface of wine material. The *YDR379C-A* marker also makes it possible to reveal sherry strains that, according to the ITS typing, are classified as wine strains.

Therefore, ITS, *YDR379C-A*, and *FLO11* sherry alleles are indicative of promising strains for the production of Sherry wines. Based on genotyping, six samples, the I-271 (industrial strain), Nos. 3, 23, 109, 111 and 113 (field isolates), were involved in technological tests.

Oenological characterization of strains during preparation of wine materials in micro-winery conditions. In the test, we used the I-271 strain and two isolates, No. 3 and No. 23 selected in our previous work [17]. Grape must fermentation in micro-winery conditions showed that the prepared wine materials met all the requirements for sherry-type wine materials. The strains differed slightly in fermentation activity and production of volatile acids. They fermented sugars during the optimal time and produced 12.5-13.1 vol.% alcohol, while the residual sugars (1.2-2.5 g/l) did not exceed the permissible level. We considered the latter indicator as the main one at this stage of selection, since the low fermentation activity associated with incomplete fermentation of grape must sugars could negatively affect the biofilm formation [28]. The mass concentration of volatile acids was 0.10-0.57 g/l, of aldehydes — 65.9-105.6 mg/l, with no extraneous tones in the taste and aroma.

In breeding sherry yeas, the most important features are the ability to rapidly develop a surface biofilm on the wine containing 15-16 vol.% alcohol and to synthesize aldehydes amounting at least 350 mg/l final concentration in the sherry wine material under the biofilm [21]. Acetaldehyde being 90% of all wine aldehydes is an important component of Jerez wines, mainly affecting their aromatic characteristics [22-24]. The rate of biofilm formation on the wine material and the accumulation of aldehydes during biological aging largely depend on the strain used. Basically, the formation of a sherry biofilm occurs within a period of 3 to 30 days, depending on the strain and alcohol content [25], and the acetaldehyde concentration in the wine material can reach 1000 mg/l [23, 26-28].

The yeast strains tested (No. 3, No. 23, I-271, and I-329) were similar in the rate of surface growth. It began in 8-10 days from the end of fermentation phase, and the biofilm covered the entire surface of the wine material in 13-16 days. In terms of the amount of aldehydes and organoleptic characteristics, wine materials aged under the biofilm met the requirements for biologically aged wine materials (Table 2).

2. Characterization of promising *Saccharomyces cerevisiae* strains with combination of three sherry loci (ITS + *YDR379C-A* + *FLO11*) **by flor formation and aldehyde production in a micro-winery test** (the Magarach Winemaking Microorganism Collection, Republic of Crimea)

Strain	Biofilm growth, days		Biofilm appearance in 1 month	Aldehydes, mg/l (M±SEM)	Tasting characteristic
	islets	entire surface covered			
I-271	9	15	Well-formed, thin, elastic	426,8±5,7	Sherry tone in the aroma and in the taste
No. 3	9	16	Thin, heterogeneous in density, with gaps, light beige	492,9±7,7	Slight sherry tone in the aroma and in the taste
No. 23	10	16	Well-formed, uniform, light beige	768,0±5,8	Sherry tone in the aroma and in the taste
I-329	8	15	Well-formed, folded, dark beige	506,0±8,8	Strong sherry tone in the aroma and in the taste

Alcohol tolerance. In biofilms of the yeast strains Nos. 3, 23 and I-271 developed on the wort with 12.5-13.1% ethanol and transferred to the wine material containing 16% ethanol, growth sharply slowed down followed by the yeast cell death. We applied the Sayenko's method to gradually adapt sherry yeasts to increased alcohol concentrations [26]. The collection strain I-271 adapted well and quickly formed the biofilm over the entire surface of the wine material (Fig. 2). Strains No. 3 and No. 23 at an alcohol concentration of 15.3% reduced growth activity which resulted in 70-80% surface covered. In these strains, we noted a good physiological state of the film (up to 70% of living cells), despite a decrease in growth rate [27].

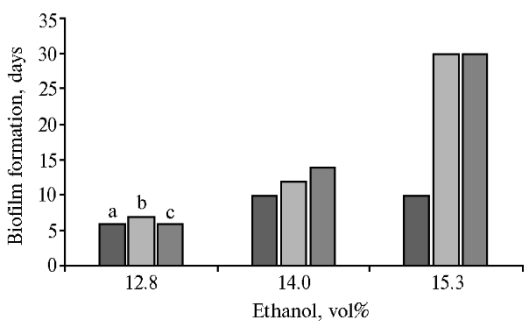

Fig. 2. Biofilm formation in promising *Saccharomyces cerevisiae* strains combining three sherry loci (ITS + *YDR379C-A* + *FLO11*) as depends on the ethanol concentration in the wine material: a — collection strain I-271, b — field isolate No. 3, c — field isolate No. 23 (a micro-winery test; the Magarach Winemaking Microorganism Collection, Republic of Crimea).

The collection strain I-271 was close to the control sherry strain I-329 in terms of alcohol resistance, biofilm formation, organoleptic parameters, accumulation of aldehydes, and the physiological state of the film cells.

Testing strains in a winery conditions. Experimental samples of Jerez-type wines were produced by biological aging under biofilm [28]. As per the chemical analyzes, biofilm growth rate, mass concentration of aldehydes, and tasting characteristics, all the blends corresponded to the sherry type wine material biologically aged for 3 months. The complete overgrowth of the biofilm on the surface of the wine materials occurred almost simultaneously: in barrels No. 1 and No. 2 — on day 12 after biofilm inoculation, in barrel No. 3 on day 10. The aldehyde concentrations after a 3-month exposure under the biofilm was 618.4 mg/l in barrel No. 1, 506.0 mg/l in barrel No. 2, and 537.7 mg/l in barrel No. 3 (5% measurement error), which corresponds to the technological instructions [21]. All wine materials were transparent, straw-colored, with a clean light aroma and sherry tones in aroma

and taste.

Based on the screening data and production tests, the collection strain I-271 can be recommended to produce sherry-type wines from both the traditionally used Aligote white grape variety and the blends of Rkatsiteli and Sauvignon green grape varieties, new for sherry wines, which opens up prospects to generate new Jerez wine brands.

Thus, our findings show that the pre-selection of *Saccharomyces cerevisiae* strains by the genetic markers ITS, *YDR379C-A*, and *FLO11* allows rapid and reliable identification of sherry strains most promising for wine production. Genotyping of 143 wine yeast strains identified strain No. I-271 with a high potential for sherry-type wine production due to alcohol resistance, formation of a complete, thin, elastic biofilm and sherry tone in aroma and taste of the wine materials.

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