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Research Article

Study of Spontaneous Flora of Fruits and Berries with The Purpose of Breading New Strains of the Yeast for the Production of Fruit Wine

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ABSTRACT:
Experimental data on the composition of the spontaneous microflora of fruits (apple, pear, plum) and berries (blackberries, grapes) growing on the territory of the Republic of Adygeya are presented. From spontaneous microflora with the use of elective nutrient media, saccharomycete yeast was isolated, their isolates were obtained. The physiological, morphological and technological properties of saccharomycetes isolated from spontaneous microflora have been studied. On the basis of physiological, morphological and technological characteristics, strains of saccharomycetes yeast were selected. With their help, table wines are prepared by full fermentation of grape must and apple juice. It was shown that in terms of physico-chemical (volume fraction of ethyl alcohol, mass concentrations of organic acids, amino acids, aromatic components) and organoleptic indicators, experimental samples of wine materials were identical to the control, obtained using pure yeast cultures.

Keywords: microscopy; spontaneous microflora; yeast races; yeast isolate; experimental yeast strains; pure yeast cultures; selection of new strains.

INTRODUCTION.

In all regions of the world engaged in winemaking, the vital task is the selection of yeast in order to select and then use the local races best fitted and adapted to the winemaking conditions of this particular region [1,2,3]. In the last decade in the North Caucasus, including the Republic of Adygeya, the production of grape and fruit wines has significantly increased. At the same time for the fermentation of the must, imported active dry yeast are widely used that are expensive. Meanwhile, the favorable geographical position of the Republic of Adygeya, the presence of foothill and mountain zones with peculiar microfloramakes it possible to isolate and select their own yeast races, which would ensure to maximize the advantages ofthe area for the republic’s winemaking industry. Moreover, it was the local strains that were used as the basis for creating active dry yeast known throughout the wine-making world [4, 5].

Modern ideas about the local races of yeast and the technology of their application are based on studies by Italian and French scientists, according to which local races react more actively to changes in weather and climatic factors and adapt to changes in the chemical composition of fruits and berries much faster than pure cultures of yeast [4, 5,6,7,8]. In this regard, the rational method of the technology of winemaking is the selection and sectioning of such yeast races that would actively respond to changes in the chemical composition of fruits and berries under the influence of external factors [8, 9, 10].

The purpose of the work is the selection of new yeast strains isolated from the spontaneous microflora of fruits and berries of the foothill zone of the Republic of Adygeya.

Objects and methods of research.

Selection was carried out by stepped screening of yeast species Saccharomyces cerevisiae, isolated of the yeast microflora of fruits and berries in the study and in compliance with all measures of sterility. As the objects of studywere used**yeast** isolated from the spontaneous microflora of apple fruits such as the varieties Jonathan (sample No. 1) and Melba (No. 2), plums Ternovka (No. 3), pears of the Williams variety (No. 4), blackberries of the Navaho variety (No. 5),and white Chardonnay grapes (№6). Spontaneously fermented must was scattered in Petri dishes, incubated at a temperature of 20-24 ° C. The isolation was carried out according to the method modified by the authors, consisting in successive transfer to various agarized elective media with the addition of lysine (allows separating wild yeast), ethylamine (helps inhibit the growth of lactic acid and acetic acid bacteria), glycerol, etc. [11-13]. To identify individual species and genera of yeast, crops were applied to liquid and solid nutrient media with subsequent microscopy of selected cultures using polarization-interferential and luminescent microscopes. For identification of certain species and genera of yeast, crops were used for liquid solutions of various sugars [11,12]

The physiological and morphological properties of isolated yeast races were studied by direct microscopy (using dye solutions) using a complex of indicators of the shape, size, presence of vacuoles, the nature of fat inclusions [11, 12]. The morphology of the cells (shape, size of the cells, the method of micropropagation) was studied in an Olympus CX21 FS1 light microscope (Japan). Yeast sporulation was induced in two days on a standard acetate medium, g / l: baktoagar - 20; CH3COONa - 10; KCL - 5 [5,10].

Technological properties - fermentation and respiratory activity was determined by methods [9,10]. For comparison of the fermentation activity, the calculated coefficient K = QCO2 / QO2 (the ratio of fermentation intensity to respiration intensity) was used.

As the control variants, the well-known races of the yeast family Saccharomycetaceae, the genus Saccharomyces cerevisiae, the race Champagne 7-10C and the active dry yeast Cabernet-Merlot (France) were chosen. Standard GOST techniques were usedto determine the physico-chemical indicators of must, juices and wines (mass concentration of sugars,titratable and volatile acids). Mass concentrations of organic acids, glycerol, and amino acids were determined by the chromatography method ofhigh performance using an “Agilent Technologies” instrument.

RESULTS AND DISCUSSION.

Analysis of research results (table 1) indicates a significant difference in the physiological and morphological properties of the studied microorganisms.

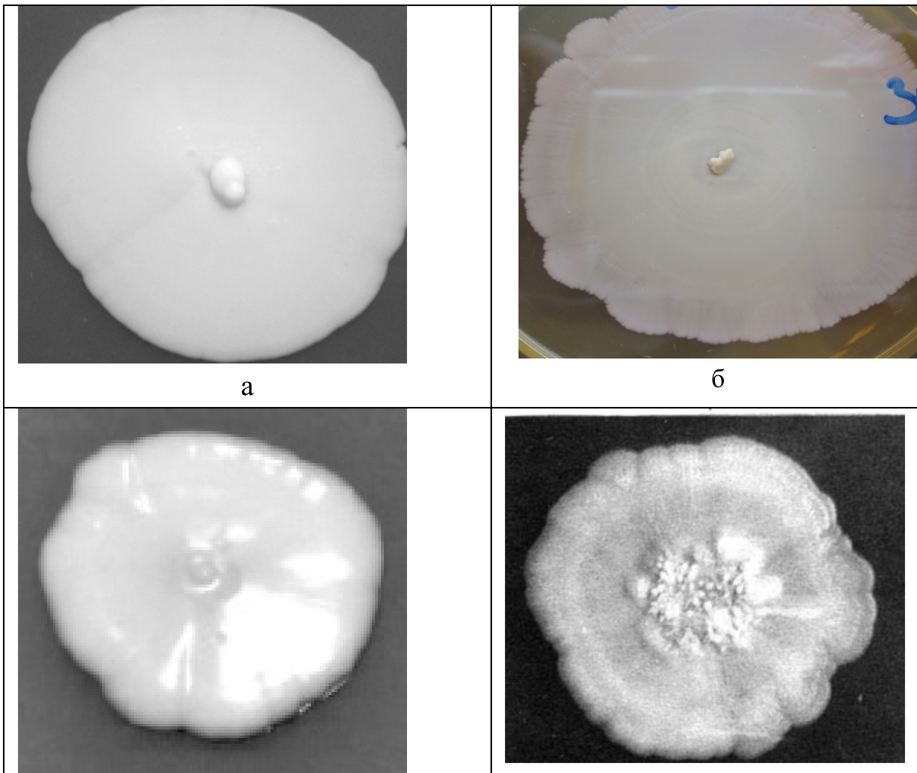
Table 1 - Physiological and morphological characteristics

Sample number	Evaluation of the microbiological state on the environment		Morphological features of yeast cells
	liquid	solid	
1	abundant emission of gas, fermenting liquid slightly turbid, a creamy wrinkled film is formed on the surface, a wall ring on the walls of the tube	grayish-white moist, smooth colonies with deformed edges	round (3-4 microns), oval (3x5 microns), elongated 2-3 h5-6 microns; there are pointed and oval-elongated cells; sporulation proceeded easily with the formation of asci mainly with 2, less often with 4 oval spores.
2	abundant emission of gas with the formation of a stable foam	shiny colonies pinkish-cream, grainy, with little	elliptical (mostly 3 x4 microns); elongated (up to 6 microns) arranged

	with a large number of large bubbles	branched edges	separately or in pairs; hemispheric spores; in a month, asci were observed with 2-4 spores, round or slightly oval; fusion before the formation of asci was rarely observed.
3	the fermenting medium is turbid covered with gray-white thin foam	white moist, smooth colonies with low cut edges	elliptical, elongated (up to 5-9 microns) are found, grouped in pairs and in chains; no sporulation detected
4	the fermenting medium was covered with a dry matte film of black and gray color	white, matt, thincolonies	elongated, oval form chains, branched; hemispheric spores; asci with 2-4 spores
5	abundant emission of gas with formation of a stable foam, turbid medium, folded wrinkled film	gelatinouswhitecolonies	elongated (up to 5-8 microns) singles and pairs with pointed edges are found in chains; hemispheric spores; asci with 2-4 spores
6	abundant emission of gas, wrinkled film, wall ring on the walls of the tube	white moist, smooth colonies with low cut and feathery edges	elliptical (predominantly 3 x4 microns); oval (3x5 microns), ovate-elongated (up to 6 microns); sporogenesis inactive

During the period of intensive fermentation of the must, the number of Saccharomycetes yeast gradually increased and by the end of fermentation it became predominant. This allows us to conclude that on the fermentation stage, the Saccharomycete yeast is able to compete with the selection cultures of yeast [8,9]. Meanwhile, studies have shown the presence of a large number of so-called wild yeast from which,Pichia Hansen (up to 2.5%), HansenulaSydow and Brettanomyces (from 0.5 to 1.8%), Torulopsis (0.1-0.5%), Candida (0.08– 0.15%) prevailed.

It was established that according to cultural and morphological characteristics (table 1), the appearance of colonies on solid nutrient medium (Figure 1), the saccharomycete yeast of spontaneous microflora differed significantly, their sizes significantly varied. The largest cells (up to 9 microns) are isolated from the surface of plum fruits and blackberries.



а б в г

Figure 1 - Isolates of yeast isolated from the surface:

а and б are grapes;

в - blackberries;

г - apple fruit

It is known [11,12] that the most characteristic feature used in the classification of yeast is the fermentation of solutions of various sugars (Kudryavtsev key). This ability of cells depends on the presence in them of various enzymes, peculiar only to this particular type of cells. Most types of wine yeast ferment glucose, fructose, maltose, sucrose, galactose, raffinose partially, and lactose, melibiose, pectose, dextrins, starch are not fermented at all due to the lack of appropriate enzymes.

For the experiment, a model environment with a sugar content of 8%was used. The experiments and the processing of the data obtained (Table 2) showed that the isolated yeast strains belong to the glucosophilic yeast of the genus Saccharomyces, fermented a significant proportion of sugars from the model medium [13,14]. It is these yeasts that end fermentation, many of them participate in the formation of the flavour of young wines. Sugars such as lactose, arabinose and raffinose are practically not fermented and remained in the medium in almost the original concentration.

Table 2 - Fermentation of various sugars by cell cultures, selected fruits of apple varieties Jonathan (1) and Melba (2), plums Ternovka (3), pears, variety Williams (4), blackberries, variety Navaho (5) and white Chardonnay (6).

Sugar	Sugarconcentration,%, duringfermentation											
	on the third day of fermentation						on the fifth day of fermentation					
	variant											
	1	2	3	4	5	6	1	2	3	4	5	6
glucose	4,6	4,8	4,2	4,8	4,4	3,8	0,2	0,2	0,1	0,3	zero	zero
sucrose	4,8	5,4	5,6	5,3	4,1	3,9	0,4	0,2	0,1	0,4	0,1	0,1
maltose	5,2	5,4	5,3	5,5	4,7	4,4	0,6	0,3	0,3	0,3	0,2	0,2
lactose	7,8	8,0	7,9	7,9	8,0	7,6	7,6	7,9	7,9	7,8	7,8	7,8
galactose	6,4	6,7	5,7	6,1	6,3	6,4	0,8	0,7	0,4	0,5	0,3	0,3
arabinose	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0
raffinose	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0	8,0

Studies have shown that yeast cells isolated from single colonies of isolates from the surface of blackberries and grapes were the most actively fermenting sugars of the model medium.

As a result of the studies (table 3), it was found that the respiration activity (total and specific) of yeast isolated from the surface of fruits and berries is not significantly different from the control races. A greater difference was found in fermentation activity: the greatest fermentation activity, close to the control races, was shown by strains isolated from the surface of blackberries and grapes. These strains were used for further studies to assess the effect on the chemical composition and organoleptic properties of wines.

Table 3 - The respiratory activity of the studied cultures of saccharomycetes

Variant number	respiratory activity		К (Q_{CO2} / Q_{O2})
	total, mm^3/g	specific, mm^3/g	
1	4,87	36	5,8
2	3,64	28	4,7
3	5,06	34	7,4
4	4,42	34	6,9
5	5,20	36	8,7
6	4,32	32	9,3
Control variants			
Champagne7-10C	4,12	28	10,4
CabernetMerlot	3,86	32	9,8

For research, must of grape varieties Chardonnay (white) and Cabernet-Sauvignon (red) was fermented with sugar cubes isolated from the surface of Chardonnay grapes, and yeast extracted from the surface of blackberry was used to ferment apple juice. The condition of the original must (juice): Chardonnay - mass concentration of sugars 22.5 g / 100 cm3, titrated acidity - 8.6 g / dm3; Cabernet Sauvignon - 25.4 and 6.7, respectively; apple juice is 15.2 and 5.4.

The research results (Table 4) indicate similar physical and chemical indicators of wine materials produced using experimental yeast strains and pure yeast cultures: the use of all strains provided complete attenuation of sugars, rather high accumulation of natural fermentation of alcohol. It should be noted that when using experimental strains of yeast in wine materials more glycerin was accumulated - a secondary product of alcoholic fermentation, giving the wine materials fullness and softness of taste. The cycle of tricarboxylic acids in the experimental and control variants was identical, as evidenced by the close values of the concentrations of all the studied organic acids.

The concentration of amino acids in experimental and experimental variants also has similar values, or vary in a small range.

A slight difference was found in the accumulation of aromatherapy compounds in wine materials. Thus, in the production of white and red wine materials using experimental strains, a greater accumulation of esters and higher alcohols was noted.

The results of the organoleptic analysis indicate the high quality of wine materials, especially apple, produced using yeast isolated from spontaneous microflora. The presence of a bright flavour with a predominance of varietal shades is especially noted.

Table 4 - Physical and chemical indicators of wine materials withsaccharomycetes isolated from spontaneous microflora

Thenameofindicators	Fermentation medium					
	Must Chardonnay		must Cabernet Sauvignon		Apple juice	
	yeast					
	Champagne 7-10C	test	CabernetMerlot	test	Champagne 7-10C	test
volume fraction of ethyl alcohol,%	13,5	13,6	13,8	13,5	10,4	10,4
Massconcentration						
sugars, g / dm3	2,2	1,8	2,8	3,0	2,1	2,2
titratableacids, g / dm3	8,3	8,0	6,5	6,4	5,3	5,3
volatileacids, g / dm3	0,36	0,42	0,34	0,44	0,40	0,46
glycerin, g / dm3	5,4	6,1	4,2	4,4	2,8	2,6
acetaldehyde, mg / dm3	117	97	87	103	78	84
amounts of esters, mg / dm3	187	212	164	188	112	93
higheralcohols, mg / dm3	388	412	328	356	273	257
Mass concentration of organic acids, g / dm3						
8. tartaric	3,1	3,0	2,7	2,8	0,2	zero
9. apple	2,3	2,6	2,2	2,0	3,4	3,4
10. citric	0,3	0,3	0,2	0,3	zero	zero
11. amber	1,1	0,8	0,5	0,8	0,2	0,2
12. lactic	0,3	zero	0,3	0,2	0,4	0,4
Mass concentration of amino acids, mg / dm3						
Arginine	14,1	17,2	22,6	21,4	18,6	15,4
Lysine	нет	0,83	1,12	1,25	0,80	0,63
ά-aminobutyric	0,34	1,08	1,04	1,06	0,34	0,21
Tyrosine	9,2	12,4	10,2	9,7	6,3	6,0
β – phenylalanine	14,3	11,0	15,5	16,7	3,7	2,2
Histidine	6,8	7,7	5,5	5,2	0,6	0,8
Glutamicacid	136	144	138	146	62	53
Leucine	4,8	4,7	10,1	12,1	6,6	5,2
Methionine	5,5	7,3	10,6	11,4	3,4	5,2
Valin	3,5	2,7	3,3	3,8	2,7	2,4
Proline	195	145	153	163	117	114
Threonine	12,5	11,4	11,2	12,8	4,1	6,7
Tryptophan	2,8	5,9	8,1	6,4	2,7	3,2
Serine	6,4	6,2	4,6	4,3	3,7	4,2
α–alanine	13,4	17,6	14,1	17,5	13,3	14,2
Glycine	6,7	6,7	5,4	8,5	6,8	7,3
Cystine	0,12	0,12	0,16	0,08	zero	zero
Cysteine	0,10	0,09	0,15	0,15	zero	zero
Cystathionine	0,07	0,09	0,10	0,07	zero	zero
tastingassessment, mark	83	81	84	84	78	80

The presented experimental data allows to make the following main conclusions:

- on the surface of all stages of the investigated fruits and berries different groups of microorganisms (representatives of wild microflora) are revealed;
- with the same qualitative composition, the frequency of their occurrence varies depending on the type of studied fruits and berries;
- the presence of wild yeast-Sakharomycetes confirms the need for selection of new races of yeast with high fermentation activity;
- new strains of Saccharomycetes yeast with high fermentation activity allowing to produce high-quality wine products were isolated and investigated.

REFERENCES

1. Mercado, C., A. Dalcero, R. Masuelli, M. Combina. Diversity of Saccharomyces strains on grapes and winery surfaces: Analysis of their contribution to fermentative flora of Malbec wine from Mendoza (Argentina) during two consecutive years // Food Microbiology. – 2007, №24. – P.403.
2. The yeasts. A taxonomic study. Eds. C.P.Kurtzman, J.W.Fell. Fourth revised and enlarged edition. Amsterdam: Elsevier Science B.V., 1998, 1055 p.
3. J. Ribero-Gaillon, E. Peyno, P. Ribero-Gaillon, P. Sydro. Theory and practice of winemaking. Characteristic of wines. Ripening grapes. Yeast and bacteria / M .: Food. prom-st, 1979. - T.2. - 348 s.

4.B.S.Valles, R.P. Bedrinana, N.F. Tascon, A. Q. Simon, R.R. Madrera. Yeast species associated with the spontaneous fermentation of sider //Food Microbiology. – 2007. – №24. – P.25-31.

5. E. di Maro, D. Ercolini, S.Coppola. Yeast dynamics during spontaneous wine fermentation of the Catalanesce grape// International Journal of Food Microbiology.// International Journal of Wine Research. – 2007. – № 117. – P.201-210.

6. Barnett J.A., Payne R.W., Yarrow D. A guide to identifying and classifying yeasts. Cambridge: Cambridge Univ. Press, 1991.

5. Kurtzman C.P. Methods for Isolation, Phenotypic Characterization and Maintenance of Yeasts / Kurtzman C. P., Fell J. W., Boekhout T., Robert V. // The Yeast, a Taxonomic Study / Eds. Kurtzman C.P., Fell J.W., Boekhout T.- Amsterdam: Elsevier, 2011.– P. 88–110.

6. Ageeva N.M., Nasonov A.I., Dust A.V., Suprun I.I., Sosyura E.A. Investigation of the composition of the microflora of grapes in order to identify natural populations of Saccharomyces cerevisiae // Bulletin of the APK of Stavropol, №1 (25), 2017, p.115-119.

7. Abdullabekova D. A., Magomedova E. S. Magomedov G. G. Study of the physiological and biochemical properties of the Saccharomycetes yeast depending on its location on the plant substrate / Journal of the Samara Scientific Center of the Russian Academy of Sciences. Issue number 1-5.t. 13. - 2011. - p. 36-44.

8. Eldarov M.A., Kishkovskaya S.A., Tanashchuk T.N., Mardanov A.V. Genomics and biochemistry of wine strains of Saccharomyces cerevisiae yeast // Advances in Biological Chemistry, Vol. 56, 2016, p. 155–196.

9. PalágyiZs., Ferenczy L., Vágvölgyi Cs. Carbon-source assimilation pattern of the astaxanthin-producing yeast Phaffiarhodozyma// World Journal of Microbiology and Biotechnology. — 2001. — Vol. 17, no. 1. — P. 95—97.

10. Fleet G. H. Yeast interactions and wine flavour // Int. J. Food Microbiol. — 2003. — Vol. 86, no. 1—2. — P. 11—22.

11.Babieva I.P., ChernovI.Yu. Yeast biology. M .: MGU Publishing House, 2004, ... p.240

12. Buryan N.I. Microbiology of winemaking // Simferopol: Tavria. - 2002. - 403 s.

13. Buryan N.I., Kishkovskaya S.A., Zagoruiko V.A., Skorikova T.K., Ivanova E.V., Chernikova T.V. Reidentification of some yeast strains - saccharomycetes from the national collection of microorganisms for winemaking using modern methods of analysis // Magarach. Viticulture and winemaking. - №1. -2011. - pp. 56-59.

14.Kotenko S.Ts., Khalilova E.A., Islammagomedova E.A., Aliverdiyeva D.A. Physiological and biochemical features of the Saccharomyces cerevisiae Y-3980 wine strain // Fundamental research. - 2015. - № 7-2. - p. 255-264.