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# Metabolic Potential of Alcoholic Fermentation Yeasts

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ABSTRACT. **On the strength of reported evidence and our own findings, the metabolic potential of *Saccharomyces* yeasts utilized in bread, beer and wine production was revealed.**

**Using $^{14}$C-compounds, transformation products of ethanol, carbon dioxide and the urine main organic acids and aminoacids were studied during secondary alcoholic fermentation (sparkling wine production). The ways of using separate atoms of carbon skeleton of glycerine, ethanol, glycine, acetic and succinic acids by yeast cells in the process of the yeast endogenic and exogenic exchange have been established.**

**The results obtained are essential for further perfection of sparkling wine-making biotechnological processes through goal-directed synthesis of major compounds with *Saccharomyces cerevisiae* yeasts.** *© 2009 Bull. Georg. Natl. Acad. Sci.*

***Key words***: *yeasts, secondary alcoholic fermentation, organic acids, aminoacids.*

Yeasts represent facultative organisms which belong to the lowest eucaryotes-metacaryotes. They are united in the kingdom fungi (*Mycota*) where division of fungi (*Eumycota*) is separately singled out. In accordance with the current classification, the majority of investigators, according to sexual process and type, unite three subdivisions into fungi domain*: Ascomycetes*, *Basidiomycetes* and incomplete fungi in which sexual propagation process has been detected. The yeasts involved in fermentation process are representatives of *Ascomycetes*, which are included in the *Hemiascomycetes* class, belong to the order of *Endomycetales*, to the family of *Saccharomycetaceae*, to the subfamily of *Saccharomycetoideae* wherein *Saccharomyces* constitute one of the genera [1, 2].

The yeast taxonomy and systematics are made according to different signs and undergo permanent perfection. For this reason, in up-to-date research, the methods for assessment of phenotypic, genetic, biochemical and molecular structure are extensively employed. As a result, eventually genus and species are established. Nowadays over 60 genera and more than 500 species of yeasts have been described. Of alcoholic fermentation yeasts *Saccharomyces cerevisiae* appears to be a commonly used culture. The yeasts *Saccharomyces cerevisiae*, utilized in the wine, beer and bread production represent physiological races. They are sporogenic yeasts. Diploid and polyploidy races of *Saccharomyces* genus are used in industry. *Candida, Torulopsis, Pullularia, Rhodotorula* and representatives of other genera belong to asporogenic yeasts.

Yeasts and yeast-like fungi are widely spread in nature. They are characterized by adsorption type of nutrition. Many of them are saprophytic and occur in the plant pilosphere and rhizosphere, in the soil, seas and fresh waters, in sugar-containing substrates - fruit, vegetables, grain. Yeasts are found in the organisms of insects, honey, flowers, etc. Some species of yeasts have a broad area of spread. Definite forms of them exist in specific, only restricted, definite conditions of life.

Some representatives of yeasts are used as industrial producers of B group vitamins, enzymes, organic acids and aminoacids. In addition to the well-known beneficial properties, yeasts are characterized by some

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deleterious action too. They pollute the casks where beer is brewed or undergoes marination. Some species are known as parasites, pathogenic forms for humans, animals and plants.

People have used yeasts for some thousand years in wine, beer and bread production. Wine-making history originated 6000 years ago. Many investigators think that wine production, at least its generation started in the South Caucasus [3]. Now this territory involves the North-western part of Turkey, Northern Iraq, Azerbaijan and Georgia. Characteristically, the yeasts used in biotechnological processes, isolated from different soil-climatic zones of Georgia, are distinguished for a great variety. They are characterized by quite specific physiological properties and by genetically determined high metabolic potential. Detection of these metabolic processes, transformation ability of different class organic compounds occurs at different levels of research. It is closely related with the composition of nutrition media, functioning of enzyme systems under definite conditions of biotechnological processes, with cytological, biochemical and genetic studies of endogenic and exogenic metabolisms.

Yeast cells possess a fairly large surface area. In 1 g pressed beer yeast with 25% dry mass this value reaches $3x9\ m^2$. As a result of this, nutritive substances rapidly penetrate the yeast cells. 1 g beer yeast is able in about 1 sec to break down $10^7$ molecules of maltose by forming ethanol and carbon dioxide. Accordingly, glucose assimilation velocity is $2x10^7$ molecule per second [4].

Metabolic potential of yeasts manifests itself largely via two metabolic pathways; in unaerobic conditions almost 90% conversion occurs through the Embden-Meyerhop pathway. In aerobic conditions the hexose monophosphate pathway is used by 30-50% with *C. utilis* and by 6-30% with *S. cerevisiae*.

Yeasts are able to unaerobically ferment hexoses: D-glucose, D-fructose and D-manose. Most yeasts cause fermentation of Saccharose. Bread and beer yeasts ferment maltose. This process is activated by hexoses. In intact bread yeasts breakdown of maltose is hindered by trehalose whose transformation rate is rather low. Pentoses, namely xylose, have an inhibitory action on fermentation in beer yeasts.

In *Saccharomyces cerevisiae* glycine, manite, ethyl and other alcohols, organic acids (lactic, acetic, malic, citric) can be used as carbon sources. Assimilation of different carbon sources with their simultaneous presence in the nutrition medium occurs by the polyauxia principle. During periodic cultivation glucose and fructose are assimilated in the first place. Acetic acid contributes to the assimilation of lactic acid, malic acid - to the assimilation of glycolic acid. Acetic acid and glucose are assimilated by yeasts simultaneously. As a rule, from the mixture of carbon sources assimilated first are compounds which make for high intensity of yeast growth [5]. Different species of yeasts diversely assimilate one and the same carbon source. For example, fermentation of galactose in *S. cerevisiae* starts only on the second day of cultivation. Organic acids are essential for carbon metabolism, energy exchange in microorganisms, for the processes of synthesis and dissimilation. The use of fatty acids as carbon source is determined by the yeast species, strain, concentration of the acid used, the length of its carbon chain and degree of electrolytic dissociation. From this point of view good substrates are $C_2$-$C_4$ organic acids. As to the fatty acids of higher order, only yeasts of certain genus and species assimilate them. Any intermediate product of the Krebs cycle (pyruvic acid, citric acid, succinic acid, fumaric acid, malic acid) can be utilized by the yeasts as a single carbon source.

Alongside carbon sources yeasts use also nitric ones. With the application of chromatographic and microbiological methods a definite order of assimilation of aminoacids has been established. First occurs assimilation of aliphatic aminoacids (methionine, lysine, leucine, aspartic acid, isoleucine), then come cyclic aminoacids (phenylalanine, thyrosine, triptophane, histidine), proline, which is not assimilated by all strains of yeasts forms an exception. At the same time, by the results obtained with beer yeasts, aminoacids are arranged according to their assimilation rate by yeasts. Most rapidly assimilated are: glutaminic acid, aspartic acid, glutamine serine, threonine, lysine, arginine. Then - valine, methionine, leucine, isoleucine, histidine. Then - glycine, thyrosine, triptophane, alanine, ammonia and, last - proline. Apart from aminoacids, yeasts assimilate peptides whose structure affects their assimilation rate. Yeast may also assimilate polypeptides from the fermentation medium. Of other nitric sources, yeasts may use urine, different bases of purine and pyridine. In general, yeasts are characterized by the ability of rather active transformation of organic compounds, owing to which detoxication of chemicals used in viticulture and horticulture is in principle feasible. Proper selection of yeast races may provide for a substantial reduction during fermentation, or complete conversion of various toxicants into yeast-specific metabolites that largely account for the production of ecologically pure products [6].

Synthesis of extracellular compounds should also be considered one of the manifestations of yeast meta-

bolic potential. Bread, beer and wine yeasts in the process of growth and fermentation release nitrogen containing substances into the medium. In addition to aminoacids, oligopeptides are released into the medium. There is evidence that yeast cells release nucleotides too. Exogenic enzymes synthesized by yeasts (namely: amylase, chitinase, phosphatase, urease, protease, cellulose, lipase, polygalacturonase) are identified in the medium. Some yeast species are revealed which exhibit high activity of these enzymes, which is the basis for their industrial production.

Metabolic potential of yeasts somewhat rises in the presence of natural growth factors (biotin, pantothenic acid, inosite, thiamine, nicotinic acid, pyridoxine). Reverse carbon-containing compounds (glycogen, trehalose, lipids) are also of major importance for normal metabolism in yeasts.

It is clear that numerous, diverse and complex factors determine metabolic processes ongoing in the yeast cells, manifestation of their metabolic potential. These are enzyme synthesis and activity, cellular control mechanisms, transfer of anabolic and catabolic information from genes onto ribosomes, composition of nutrition medium, physiological status of membrane systems, etc.

Research of many years' standing along these lines started with a direct study of unaerobic breakdown of carbohydrates - study of alcoholic fermentation chemism. In keeping with current views, alcoholic fermentation can be considered as an unaerobic multistep process of sugar breakdown through the action of enzymes available in the cell, or secreted within it resulting in obtaining secondary products together with the main products of fermentation. In this multistep process the reaction sequence and velocity are regulated by enzymes - on the level of phosphor-fructokinase and pyruvatekinase before formation of pyruvate from glucose-6-phosphate.

It is calculated that each yeast cell can give rise to fermentation of such an amount of sugar as to 30-fold exceed its own weight.

During a normal course of alcoholic fermentation there is a certain quantitative balance between the fermentation products and deviation from it indicates that for some reason, the biotechnological process has not gone normally.

The existing quantitative ratio between the fermentation products is expressed in the equilibrium equations [7]. These equations are of definite technological importance in viniculture for evaluation of the normal course of fermentation. However, it does not envisage further conversions of the compounds existing in the process or new generations to other substances which had not been reflected in the equations. It also excludes the possibility of using in the secondary products' biosynthesis of those compounds which do not represent the products of glucose conversion.

Use of radioactive compounds has revealed in quite a new way the metabolic potential of yeast in the process of alcoholic fermentation. Experimental findings have made it clear that it is impossible to place the processes ongoing in a natural fermentation medium by yeast within strict frames of any equation. The main and secondary products of sugar breakdown may again be implicated in the primary process of fermentation, undergo endogenic and exogenic conversions and take part in the synthesis of compounds of different classes.

From this point of view, secondary alcoholic fermentation that is a biotechnological basis for sparkling wine production has not been studied at all for a long time and it essentially differs by its conditions from natural alcoholic fermentation; carbon dioxide high pressure, alcoholic environment, availability of volatile acids and alcohols of high order in the wine material, low temperature of fermentation and other factors affect the intensity and direction of the yeasts' endogenic and exogenic metabolism. Thus far, viability of yeasts under secondary alcoholic fermentation conditions goes beyond the classical fermentation ranges and may be fancied as a biotechnological process occurring in extreme conditions.

Manifestation of the yeasts' metabolic potentials, study of the transformation pathways of low-molecular compounds of wine during secondary alcoholic fermentation allows to ascertain the molecular mechanisms of yeasts' adaptation under extreme fermentation conditions which is essential for the management of biotechnological processes of secondary alcoholic fermentation [8, 9].

As a result of many years' research we have obtained statistically significant data dealing with the study of main, secondary and side products. As a fermentation agent for qualitative and quantitative analysis pure cultures of *Saccharomyces cerevisiae*, *va*r. *vini* were used, which are traditionally employed in Georgia: Kakhuri-42, Manavi-86, Chinuri-79, Rkatsiteli-61. In the experiments with $_{14}$C-compounds industrial strain of *Saccharomyces cerevisiae, var. vini-39* was introduced into the fermentation medium. In the experiments we have used isotopes having high specific activity, due to which pH in the fermentation medium did not virtually alter and the amount of $^{14}$C-compound introduced into the medium did not exceed its normal content in the wine.

The results of analysis have shown that the yeast cells during acute fermentation period before the onset

of noticeable lysis, take an active part in the metabolism of the compounds explored. In the process of secondary fermentation the industrial strain of *Saccharomyces cerevisiae var. vini-39* assimilates the carbon skeleton of ethanol, carbon dioxide, acetladehyde, acetic acid, succinic acid, glycerine and glycine. In metabolic processes participate $1^{14}$C-lactic acid, $1^{14}$C-malic acid, $1^{14}$C-citric acid, $1^{14}$C-alanine, $3^{14}$C-serine, $1^{14}$C-lysine, $4^{14}$C-aspartic acid and $5^{14}$C-glutaminic acid. The results of yeast and wine analysis indicate (Table 1) that, secondary and side major products of fermentation are characterized by different levels of transformation. In the conditions of our experiments yeasts maximally assimilate and convert glucose (98.9%), malic acid (92.4%), citric acid (91.7%), glycine (90.3%) and serine (90.1%). Ethyl alcohol (0.7%) undergoes insignificant assimilation and conversion. From the same evidence it is seen that during fermentation going on with the classical method of champagnization, although yeast contact with wine components is short-lasting (exposure for 12-20 days), most of the endogenic metabolism products of the compounds explored was transferred into wine. The qualitative and quantitative composition of the fermented wine was essentially altered. The carbon skeleton of the studied compounds did thereat partially oxidize to carbon dioxide (Table 2).

More than a half of radioactivity of $1^{14}$C-cirtric acid, $1^{14}$C-malic acid assimilated by yeasts, as well as $1^{14}$C-alanine, is released in the form of $^{14}CO_2$ [10]. Complex mechanisms of carbon skeleton oxidative conversions are indicated by $^{14}CO_2$ radioactivity indices during conversions of $1^{14}$C- and $2^{14}$CF-glycine, $1^{14}$C- and $2^{14}$C-acetic acid, $1^{14}$C- and $2^{14}$C-ethyl alcohol.

The results of chromatographic and autoradiographic assessment show that out of transformation products of the compounds explored, both in yeast cells and wine components, the essential ones are Krebs and glyoxalate acids and those aminoacids whose genesis is associated with the conversions of relevant ketoacids [11, 12].

In the process of secondary alcoholic fermentation, in spite of the limiting factors (high $CO_2$ pressure, high ethanol concentration, presence of high order alcohols,

Table 1

Relative assimilation and conversion of wine components by *S. cerevisiae var. vini-39* during secondary alcoholic fermentation

| $^{14}$C-compounds introduced into the medium | Assimilated and converted $^{14}$C-radioactivity (%) from initial activity of fermentation medium | % distribution of radioactivity | |
|---|---|---|---|
| | | in yeast | in wine components |
| 1-$6^{14}$C-glucose | 98.9 | 2.5 | 97.5 |
| $1^{14}$C-malic acid | 92.4 | 0.2 | 99.8 |
| $1^{14}$C-citric acid | 91.7 | 0.01 | 99.9 |
| $1^{14}$C-glycine | 90.3 | 16.3 | 83.7 |
| $3^{14}$C-serine | 90.1 | 1.7 | 98.3 |
| $1^{14}$C-alanine | 76.3 | 2.7 | 97.3 |
| $2^{14}$C-glycine | 76.1 | 11.1 | 88.9 |
| $5^{14}$C-glutaminic acid | 75.5 | 3.9 | 96.1 |
| $1^{14}$C-lysine | 60.1 | 24.8 | 75.2 |
| $2^{14}$C-acetic acid | 52.2 | 3.4 | 96.6 |
| $1^{14}$C-acetic acid | 40.7 | 3.3 | 96.7 |
| $1^{14}$C-lactic acid | 36.1 | 2.4 | 97.6 |
| $4^{14}$C-aspartic acid | 35.5 | 9.2 | 90.8 |
| 2,$3^{14}$C-succinic acid | 35.1 | 2.6 | 97.4 |
| 1,$4^{14}$C- succinic acid | 26.8 | 3.5 | 96.5 |
| 1,$2^{14}$C-acetaldehyde | 23.3 | 5.2 | 94.8 |
| $1^{14}$C-glycerine | 22.7 | 0.3 | 99.7 |
| $2^{14}$C-glycerine | 19.5 | 0.9 | 99.1 |
| $^{14}$C-carbon dioxide | 6.3 | 3.2 | 96.8 |
| $2^{14}$C-ethyl alcohol | 0.7 | 0.5 | 99.5 |
| $1^{14}$C-ethyl alcohol | 0.6 | 0.4 | 99.6 |

restricted oxygen amount, etc.), yeast metabolic potential, interrelation of transformation processes in the wine main components are strongly manifested.

Autoradiographic analysis has shown that the main source for succinic acid formation during fermentation is carbon dioxide, 2$^{14}$C-ethyl alcohol, 1$^{14}$C- and 2$^{14}$C-acetic acid, 1$^{14}$C-malic acid, 1,2$^{14}$C-acetaldehyde, 1$^{14}$C-glycine and 5$^{14}$C-glutaminic acid. Succinic acid carbon skeleton and 4$^{14}$C-aspartic acid are mainly involved in the synthesis of malic acid. 1,4$^{14}$C- and 2,3$^{14}$C-succinic acid and 2$^{14}$C-ethyl alcohol constitute the principal source for citric acid formation. 1$^{14}$C-alanine and carbon dioxide are main participants in lactic acid synthesis. Glyoxal acid is preferentially yielded by 1$^{14}$C-glycerine, 1$^{14}$C-acetic acid, 1$^{14}$C- and 2$^{14}$C-glycine, 1$^{14}$C-lysine.

As the result of conversion of 1$^{14}$C- and 2$^{14}$C-acetic acid, 1,4$^{14}$C-succinic acid, 1$^{14}$C-malic acid, 1$^{14}$C-citric acid mainly identified in wine aminoacids are: valine, leucine, glutaminic acid. In the synthesis of glycine, serine, and triptophan 2$^{14}$C-acetic acid and 1,4$^{14}$C-succinic acid participate preferentially. Synthesis of glutaminic acid is linked mainly with 1$^{14}$C-acetic and 1$^{14}$C-citric acid conversion. Intensive formation of valine is noticeable during biotransformation of virtually all the compounds explored.

The principal metabolic pathways of different class compounds are established; carbon atoms of ethanol and

Table 2

Formation of carbon dioxide by *S. cerevisiae var. vini-39* from different wine components

| $^{14}$C-compounds introduced into the medium | Amount of initial substances ($C^{12}$+$C^{14}$) mg/400 ml | % Radioactivity of released $^{14}CO_2$ from the assimilated and converted by yeasts compound |
|---|---|---|
| 1$^{14}$C-citric acid | 168.8 | 65.7 |
| 1$^{14}$C-malic acid | 661.0 | 53.6 |
| 1$^{14}$C-alanine | 49.1 | 50.0 |
| 1$^{14}$C-glycine | 13.1 | 38.0 |
| 1-6$^{14}$C-glucose | 8892.5 | 25.7 |
| 4$^{14}$C-aspartic acid | 179.5 | 18.6 |
| 2$^{14}$C-glycine | 41.4 | 10.2 |
| 1$^{14}$C-lactic acid | 396.7 | 8.5 |
| 1$^{14}$C-lysine | 19.8 | 4.7 |
| 1$^{14}$C-ethyl alcohol | 42014.6 | 4.3 |
| 1,4$^{14}$C- succinic acid | 519.4 | 2.5 |
| 1$^{14}$C-acetic acid | 425.5 | 2.0 |
| 5$^{14}$C-glutaminic acid | 118.2 | 0.5 |
| 3$^{14}$C-serine | 134.0 | 0.4 |
| 1,2$^{14}$C-acetaldehyde | 143.0 | 0.3 |
| 2$^{14}$C-acetic acid | 345.0 | 0.2 |
| 2,3$^{14}$C-succinic acid | 540.4 | 0.1 |
| 1$^{14}$C-glycerine | 1623.1 | 0.1 |
| 2$^{14}$C-ethyl alcohol | 32834.3 | 0.1 |
| 2$^{14}$C-glycerine | 1709.1 | 0 |

carbon dioxide participate preferentially in the synthesis of wine organic acids and yeast protein aminoacids. Transformation of a large part of organic acids is accomplished by Krebs and glyoxalate cycle. Carbon atoms of aminoacids take part mainly in the intermediate exchange. At the same time, assessment of the carbon skeleton transformation products of the compounds explored shows that a large part of them represent the products of synthetic processes. At every separate moment of fermentation dynamic changes occur in the fermentation medium qualitative and quantitative composition, in oxidation-reduction potential, temperature, enzyme activity, their adsorption and release, formation of different class compounds. Accumulation of metabolic products in the medium according to Le Chateler's principle may disturb the equilibrium with enhancement of re-synthesis (synthesis of reserve carbohydrates, lipids) that may result in a temporary inhibition of a sugar breakdown process.

Extreme fermentation conditions alongside other factors causes profound changes of the principal metabolic pathways which does not rule out the functioning of as yet unknown separate pathways.

The results obtained enable to study every question of fermentation yeasts'metabolism, to reveal comprehensively their metabolic potential. Thus far, rather important task is to establish optimal composition of wines with the use of selectively chosen yeast strains, to reveal genetic peculiarities of functioning of the enzyme systems having specific biochemical characteristics that are responsible for biosynthesis of technologically important compounds. This is essential for further perfection of sparkling wine production biotechnological processes.

*ბიოფიზიკა*

# სპირტული დუღილის საფუვრების მეტაბოლური პოტენციალი

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**ნაშრომში წარმოდგენილია ალკოჰოლურ დუღილში გამოყენებული საფუვრების კლასიფიკაცია ტაქსონომიისა და თანამედროვე სისტემატიკის პრინციპების გათვალისწინებით.**

**ლიტერატურის მონაცემებისა და საკუთარი გამოკვლევების საფუძველზე გამოვლენილია პურის, ლუდისა და ღვინის წარმოებაში გამოყენებული საფუვრების მეტაბოლური პოტენციალი. გაანალიზებულია სპირტული დუღილის ქიმიზმის ძირითადი ასპექტები.**

***Saccharomyces*-ის გვარის საფუვრებში $^{14}C$ ნაერთების გამოყენებით შესწავლილია დუღილის ძირითადი პროდუქტების (ეთანოლი, ნახშირორჟანგი), ორგანული მჟავების (ძმარმჟავას, რძემჟავას, ქარვამჟავას, ვაშლმჟავას, ლიმონმჟავას), ამინომჟავების (ალანინის, გლიცინის, სერინის, ლიზინის, ასპარაგინმჟავას და გლუტამინმჟავას) ტრანსფორმაციის პროდუქტები მეორეული სპირტული დუღილის პროცესში (ცქრიალა ღვინოების წარმოებისას), როგორც საფუარში, ისე დადუღებულ არეში.**

**დადგენილია საფუვრების უჯრედების მიერ გლიცერინის, ეთანოლის, გლიცინის, ძმარმჟავასა და ქარვამჟავას ნახშირბადოვანი ჩონჩხის ცალკეული ნახშირბადატომების გამოყენების გზები მეორეული სპირტული დუღილის ექსტრემალურ პირობებში. ეთანოლისა და ნახშირორჟანგის ნახშირბადატომები უპირატესად ღვინის ორგანული მჟავებისა და საფუვრის ცილების ამონომჟავათა სინთეზში მონაწილეობს.**

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**მიღებულ შედეგებს არსებითი მნიშვნელობა აქვს შესწავლილი საფუვრების (*Saccharomyces cerevisiae var. vini*) ენერგეტიკული და კონსტრუქციული ცვლის იმ თავისებურებათა გამოყენებისთვის, რომელნიც განაპირობებს მზა პროდუქციის ხარისხისათვის მნიშვნელოვანი ნაერთების სინთეზს, ცქრიალა ღვინოების წარმოების ბიოტექნოლოგიური პროცესების შემდგომი სრულყოფისთვის.**

## REFERENCES


1. *E.I. Kvasnikov, I.F. Shchelokova* (1991), Drozhzhi. Biokhimiya, puti issledovaniya, Kiev, 94-119 (in Russian).
2. *C. Navarre, F. Langland* (2004), Enology, London-Paris-New-York, 27-93.
3. *R.S. Jackson* (2000), Wine science: Principles, Practice, Perceptin. Acad. Press: 5-18.
4. The yeasts. Ed. by *Rose A.H. and Harrison I.S.* (1971). Acad. Press, v. 2, 430.
5. *E.G. Kirtadze, T.M. Kurdovanidze* (1992), Biokhimicheskie osobennosti vtorichnogo spirtovogo brozheniya, Tbilisi, 6-28 (in Georgian).
6. *E. Kirtadze, N. Gagelidze, M. Kiknadze, Kh. Tusishvili, L. Amiranashvili* (2007). Proc. Georg. Acad. Sci. Biol. Ser. B, **5**, 2: 66-70.
7. *Zh. Ribero-Gaion, E. Peino, P. Ribero-Gaion, P. Surdo* (1979),Teoriya i praktika vinodeliya. t. **2**: 327-328, M. (in Russian).
8. *E. Cebollero, R. Gonzalez* (2006). Appl. Envir. Microbiol., **72**: 4121-4127.
9. *M. Belovic, A. Pives, I. Kosmerl, M. Wonda, S. Celan* (2007), Journal of Bioscience and Bioengineering, **193**, 2: 135-139.
10. *N. Nutsubidze, E. Kirtadze, N. Saginadze, V. Aplakov* (2008), Bull. Georg. Natl. Acad. Sci., **2**, 1: 83-87.
11. *V. Aplakov, E. Kirtadze, N. Abuladze, R. Aplakov* (2006), Bull. Georg. Acad. Sci., **173**, 3: 575-577.
12. *V. Aplakov, E. Kirtadze, R. Aplakov* (2005), Bull. Georg. Acad. Sci., **172**, 3: 537-539.


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