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# ASSESSMENT CRITERIA FOR *SACCHAROMYCES CEREVISIAE* YEAST CULTURE IN SELECTION FOR BIOTECHNOLOGICAL USE

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*Saccharomyces cerevisiae* (Meyen ex E. C. Hansen) yeast cultures, isolated from local zone grape cultivars, which are especially valuable for the biotechnology of winemaking during fermentation of carbohydrates in grape must, were investigated. The main criteria for yeast selection are: quantity of produced alcohol, and quality of wine including: flavour, taste, after-taste, ethanol concentration. Other important and valuable parameters include: the concentration of titratable acidity, volatile acids, and total phenolics in the wine obtained from carbohydrate fermentation in grape musts are very important and valuable parameters. During wine production, yeast cells are affected by several conditions that are adverse to growth (oxidative, temperature, osmotic, sulfitation and ethanol stress). Yeast cells should detect and respond to these adverse conditions. If they fail to do this, alcoholic fermentation can be negatively affected and even stop.

*Key words*: selection, assessment criterion, wine yeast culture, *Saccharomyces cerevisiae*, biotechnology.

## INTRODUCTION

*Saccharomyces cerevisiae* (Meyen ex E. C. Hansen, 1883) yeast cultures have an extensive history of use in food processing [6]. Also known as wine yeast, this organism has been used for centuries as leavening for bread and as a fermenter of alcoholic beverages [1]. With a prolonged history of industrial applications, this yeast has been either the subject of, or model for, various studies in the principles of microbiology. Currently, *S. cerevisiae* is the subject of a major international effort to characterise a eukaryotic genome [5]. Yeast can exist either as a single-celled organism or as pseudomycelia. The cells reproduce by multilateral budding. Yeast produces from one to four ellipsoidal, smooth-walled ascospores, and can be differentiated from other yeasts based on growth characteristics and physiological traits: principally, the ability to ferment individual sugars.

*Saccharomyces cerevisiae*, in addition to its use in food processing, is widely used for the production of macromolecular cellular components such as lipids, proteins (including enzymes), and vitamins [4]. *Saccharomyces cerevisiae* is commonly recovered from a variety of fresh fruit (generally, those fruit with high levels of fermentable sugars) and vegetables. It is not, however, listed as the causative agent of food spoilage for fruit and vegetables [11]. The only adverse effect to the environment noted in the literature is the presence of «killer toxins», which are active against other strains of Saccharomyces.

*Saccharomyces cerevisiae* is a normal inhabitant of soils and is widespread in nature. It would be expected to survive well in soil. Releases could result in human and environmental exposure. It is currently estimated that over one million tons of naturally-occurring yeast are produced annually during wine, brewing and wine distilling practices *S. cerevisiae* is able to take up a wide variety of sugars and amino acids. These traits enhance the organism's ability for long term survival. *Saccharomyces cerevisiae* can be

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isolated from fruit, grain and other materials with a high concentration of carbohydrates [2, 8].

*Saccharomyces*, as a genus, represents a low risk to human health and/or the environment. Criteria used to differentiate between species are based on their ability to utilise specific carbohydrates, without relevance to pathogenicity. Nonetheless, this risk assessment applies to those organisms that fall under the classical definition of *S. cerevisiae* as described by van der Walt [7, 15].

Yeasts used for wine production produce organic acids and ethanol, which create an extremely harsh environment where few microorganisms can grow.

Fermentations are often monitored using simple indicators of microbial growth such as decreases in pH or sugar concentrations or increases in cell numbers (turbidity, direct microscopic examination), acidity or alcohol concentrations. Fermentation alone can lead to inhibition of microbial growth and, in some cases, to microbial death although this will be dependent on both the type and concentration of metabolic products produced by the fermenting organisms.

Developing a laboratory-based approach to assessing resilience of wine yeasts is, however, not a straightforward exercise; particularly when one is interested in their robustness in a commercial winemaking setting. First, one has to identify 'indicators' of robustness, and for this we have decided to use ethanol-tolerance. We have chosen this criterion because it is widely believed in the industry and in yeast research that yeast ethanol stress is a major cause of suboptimal fermentations. But what makes ethanol toxic to cells and how might it induce suboptimal fermentation performance in wine yeast [10, 13].

The aim of this investigation was to specify criteria for the assessment of *S. cerevisiae,* in wine yeast culture and biotechnological evaluation.

This work should provide winemakers with an increased range of options when it comes to choosing wine yeasts. We aim to develop strains that not only have the capacity to provide sought-after flavour and aroma profiles, but will perform quickly and reliably in the winery.

## MATERIAL AND METHODS

Anaerobic alcoholic fermentation was carried out at a temperature of between 17 and 24°C. During the fermentation process daily homogenisation of fermenting grape must was carried out in order to mix it carefully.

We studied yeast cultures isolated from grape must and raw materials directly from the vineyards. Maun was spontaneously fermented and, after fermentation for 10–12 days, we proceeded to the isolation of yeast cultures. Fermented grape musts were plated on Inhibitory Mold Agar (IMA) containing 300 μg/ml Chloramphenicol to suppress bacterial growth. After incubation under aerobic conditions at a temperature of 26–32°C, yeast culture was isolated and biochemical and morphological properties were evaluated. Evaluation criteria were: the growth of yeast cultures incubated at high temperature 42°C and at lower 4°C, cryoresistance (lysis and autolysis of individual cells during storage in the frozen state were determined), thermo resistance, growth at low pH 2.7–3.0 (acidity resistance), growth in the presence of sulphur dioxide (sulphite resistance), and the ability

to grow the yeast culture at concentrations of alcohol of 5–10–15% (ethanol resistance) [12, 16].

Biotechnological characteristics of yeast include: lifting power, the enzyme activity of yeast, and sugar content. On the basis of yeast selection, we carried out screening of active wine yeast cultures by their fermentation activity, aromatics, flavour, and other technologically important physiological and biochemical characteristics [3, 9].

We isolated and investigated 84 regional blighty yeast cultures obtained from Koblevo, Nikolaev region and Tairov Research Institute, and the Odessa Region of Ukraine. From these, we selected from this amount 18 stable wine yeast cultures, which were suitable for use in wine biotechnology.

**Typical signs of yeast cultures:**

**Morphological properties:** yeast cells are oval in shape, with a cell size of 7–8 μm. Cultural features include: growth on solid media, colonies are of a rounded shape, and a thick consistency, pale-yellow colour of yeast colonies.

**Physiological characteristics:** yeast strains of *S. cerevisiae* are able to:

- Fermenting carbohydrates such as: xylose, maltose, arabinose, glucose, and sucrose;
- Assimilate fermentable carbohydrates;
- Urea hydrolysis;
- Form ethers;
- Grow well in a medium containing 50% glucose;
- Growth observed in saline solutions (Halotolerant 4.5% NaCl);
- Grow at temperatures of 28–34°C and 37–39°C.

**Biotechnological properties:** The main stage in alcoholic drink technology is based on wine grape must, which is fermenting glucose, fructose, and sucrose, in addition to the formation of ethyl. Some of the sugar is fermented by yeast culture to form ethanol in the expected product.

Using yeast cultures allows the attainment of a harmonious combination of valuable components of grape must and products of yeast fermentation (wine and ethanol).

The ability of yeast cultures to form ethanol from grape must is an integral part of the investigation in grapes obtained from different grape varieties. The yield of ethanol reached secondary to this process lies between approximately 7.2% and 14.6%.

**Technological properties:** Yeast culture cultivation was carried out at optimal temperature for yeast growth – 28 to 30°C for 6 hours, with the total sugar content in the grape musts of 15 to 22% (pH 4.5 to 5.0).

Long-term storage of yeast culture was carried out at a temperature of 4 to 10°C on Wort Agar (Malt Extract Agar) or Sabouraud agar.

## RESULTS AND DISCUSSION

The most important yeast culture criteria evaluated were:

**Fermentation activity** – fermentation kinetics are represented by the speed of fermentation, in terms of time, or of $CO_2$ released.

**The kinetics of glucose and fructose consumption** – In order to evaluate and differentiate the capacity of yeasts their fructose uptake, the glucose and fructose contents were measured throughout fermentation to evaluate the kinetics of sugar consumption.

Morphological properties of yeast cultures are shows in fig. 1–2.

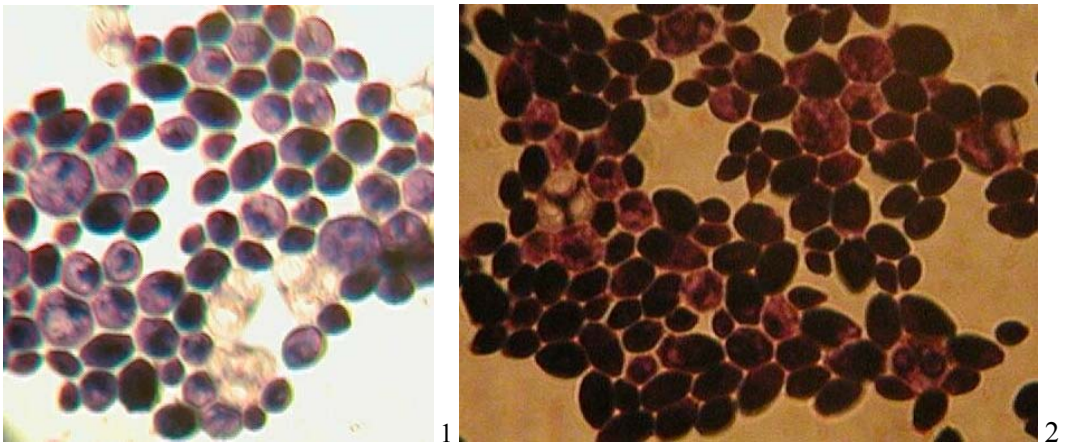

Fig. 1–2. Morphology of *Saccharomyces cerevisiae* yeast cultures isolated from grape cultivars «Traminer» MAFF Y-230141; NRRL Y-63652 (1) and «Bastardo» MAFF Y-230125; NRRL Y-63641 (2)

Magnification – ×900. Yeast cells that stained blue (in fig. 1) or brown (in fig. 2) in colour represent live and active cells. The cells are large, and are round / or a rounded oval shape in fig. 1 and large and oval / or a round oval shape in fig. 2.

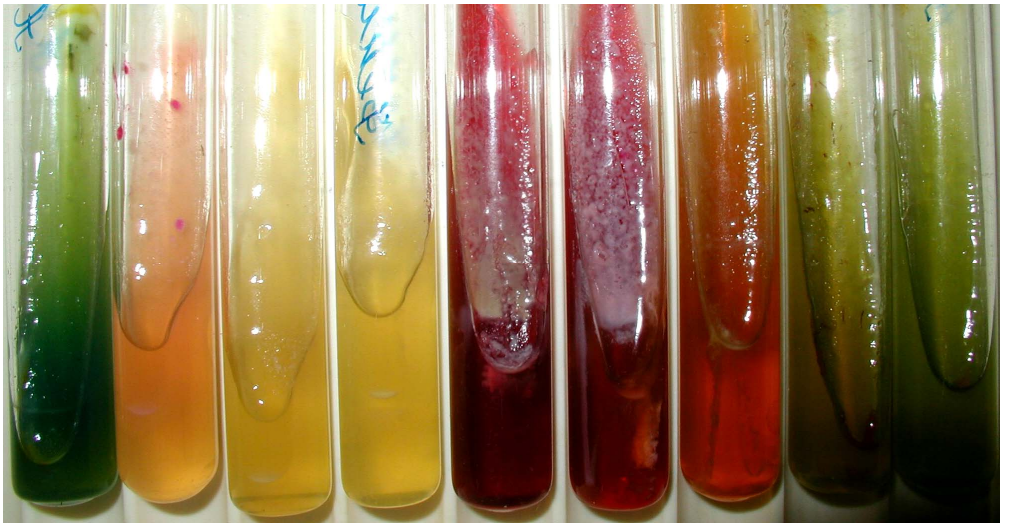

Fig. 3. Carbohydrate assimilation in tested *Saccharomyces cerevisiae* yeast cultures

Fig. 1 shows *S. cerevisiae* isolated from grape cultivar «Traminer», OAO «Koblevo», Nikolaev region of Ukraine, deposited in the GeneBank of Japan (MAFF culture collection), also deposited in the National Research Regional Laboratory of USA (NRRL

culture collection), and in the British National Culture Yeats Collection (NCYC), MAFF Y-230141; NRRL Y-63652. Fig. 2 shows yeast culture *S. cerevisiae* isolated from grape cultivar «Bastardo» OAO «Koblevo», Nikolaev region of Ukraine, also deposited in the international culture collections: MAFF Y-230125; NRRL Y-63641.

Morphological, cultural and physiological characteristics of the *S. cerevisiae* yeast presented in tab. 1.

*Table 1*

Basic assessment criteria: morphological, cultural and physiological characteristics of the *Saccharomyces cerevisiae* yeast strains

| **Properties** | **Signification** |
|---|---|
| Description of stroke | Milky colour, dense consistence |
| Shape of yeast cells | Round, oval |
| Cell size | from (1.5–3.5) × (3–6.5) to (3–5.5) × (5.5–7.5) µm |
| Sexless and filamentous structures | Budding of globalistic, multilateral, pseudomycelia forming |
| Growth in vitamin-free medium | No growth (for growth requirement pyridoxine, thiamine and biotin) |
| Halotolerant | 6–10% sodium chloride (NaCl) |
| Growth at temperature 25°C | Yes |
| Growth at temperature 28°C | Yes |
| Growth at temperature 34°C | Yes |
| Growth at temperature 37°C | Yes |
| Growth at temperature 40°C | Yes |
| Growth at temperature 42°C | Yes |
| Ethanol forming | Yes |
| Hydrolysis of urea | No |
| Amylogenesis (Starch formation) | No |

For identification of yeast cultures, their physiological properties, were studied, along with careful study of their morphology. Important properties in the identification of yeast cultures are their cultural properties and their growth on solid and liquid media. On their growth on solid nutrient media, the following yeast culture properties are examined: size, shape, colour, and general characteristics of yeast colonies.

Most yeast species require the optimal temperature for growth of between 20 and 28°C; however, there are yeast cultures which grow optimally at lower or higher temperatures. An important differentiating feature of yeast cultures is the temperature for a particular yeast culture to achieve maximal growth. Depending on the yeast genera, a usually important measurable parameter is in the differences in the ability of the yeast culture to grow at temperature ranges of: 20–25°C, 28–34°C, 37–39°C, and 40–45°C.

We found that at temperatures of 10–15°C, all tested yeast strains were characterized by weak growth, whereas at temperatures of 28–39°C, all tested yeast strains were

observed to display abundant growth. At temperatures of 40–45°C, six strains showed poor growth, whereas other strains did not grow in the same temperature range.

Thus, it was found that the growth of the selected, deposited yeast strains, when cultured on nutrient media, depends strongly on the cultivation temperature. The optimal growth temperature lay between 28°C and 39°C. Optimal growth was observed at 35°C, with the presence of thiamine (vitamin $B_1$) and pyridoxine (vitamin $B_6$) in the growth media.

Identification of the studied strains by the major physiological characteristics involves fermentation using various sugars, including: glucose, galactose, maltose, melibiose, sucrose, raffinose, and trehalose; in addition to assimilation (growth in the presence of the following sugars): glucose, sucrose, maltose, xylose, lactose, salicin, inulin, starch, sorbose, trehalose, raffinose, ribose, lysine, mannitol, and ethanol. The assimilation of carbohydrates is shown in fig. 3. Basic criteria for the assessment isolated *S. cerevisiae* yeast and industrial yeast cultures presented in tab. 2.

It was established that all 84 tested yeast strains ferment such carbohydrates as: glucose, galactose, sucrose, raffinose, maltose (with the exception of 4 strains); and assimilate trehalose, raffinose, and ethanol. Practically, no one of the tested strains did not assimilate: starch, salicin, lactose, lysine, xylose, and sorbose. Lactose and trehalose were not fermented by the tested yeast strains. The majority of yeast strains studied do not ferment melibiose. On testing the cultural properties of the isolated different grape cultivar yeast strains, yeast culture growth on solid media was determined. Yeast formed different colonies: of round, oval or elongated shapes; the texture of biomass stroke was thick, viscous, pasty or mucosal. Colonies were formed with smooth edges and a convex shape, with a matte, rough, smooth or glossy pale cream, off white, light yellow or white colouration. Yeast cells under the microscope were observed to have an oval, round or elongated form. The diameter of yeast cells ranged from between 2 and 8 μm in length, to between 3 and 12 μm.

One of the most important properties used to identify yeast is the ability of the yeast to use and assimilate nitrogen substance as a sole nitrogen source [14]. There are yeast strains that can grow at a low content of nitrogen in the nutrient medium. This test is an important physiological sign used in the identification of yeast cultures.

Yeast strains with the ability to assimilate nitrogen from nitrogen-containing substances such as: $(NH_4)_2SO_4$ – ammonium sulphate, $(NH_2)_2SO$ – (urea), $KNO_3$ – potassium nitrate, and $(NH_4)_2CO_3$ – (ammonium carbonate) assimilates all strains, were investigated.

During yeast species identification, an additional test is cultivation of yeast strains without vitamins in growth medium, as different yeast species are capable of synthesizing certain vitamins. A very important test in the yeast species identification is the ability to grow in a nutrient medium that does not contain vitamins: e.g. thiamine and pyridoxine.

An additional important test in the identification of yeast cultures is determination of the yeast cultures' osmo-tolerance. An important identifying indicator is the ability of the yeast strain to grow on nutrient media containing 50–60% of glucose. We found that all the tested yeast strains give intense growth in media with 50% glucose.

Medium with sodium chloride concentrations of between 1% and 25% were used to determine halo-tolerant yeast. Most yeast strains were determined to be of higher and

*Table 2*

Basic criteria for the assessment of isolated (author's) *Saccharomyces cerevisiae* yeast cultures and industrial *S. cerevisiae* wine yeast strains

| **Evaluated criteria** | **Laboratory (author's) yeast strains** | **Industrial yeast strains** |
|---|---|---|
| Level of hydrogen sulphide production ($H_2S$) | Shouldn't produce $H_2S$ gas | Low $H_2S$ gas producing |
| Level of sulphur dioxide production ($SO_2$) | Low | Moderate |
| Level of foaming | Low and moderate amount | Low and moderate |
| Level of titratable acidity | Moderate and high | Moderate and high |
| Level of volatile acids | Moderate and low | Moderate and low |
| Level of triglyceride production | Moderate | Moderate |
| Level of residual sugar | Low | Low |
| Acid resistance to low pH values | Resistant | Resistant |
| Resistance to sulphites | Resistant | Resistant |
| Resistance to ethanol: 5, 10, 15% concentration | Resistant | Resistant |
| Resistance to low temperatures (cold resistance) (+4°C) | Resistant | Resistant |
| Resistant to high temperatures (thermal stability) (+42°C) | Resistant | Resistant |
| Resistance to antifungal antibiotics and remedies | Sensitive to antifungal remedies | Sensitive to antifungal remedies |
| Consumption of sulphur dioxide during fermentation | Consumes | Consumes |
| The phenotype of culture, the type of killer toxin ($K_2$) produced | Killer phenotype | Killer phenotype |
| Assimilation of carbohydrate sources | Assimilates | Assimilates |
| Auxotrophy of yeast strains to amino acids, purine and pyrimidine bases | Auxotrophy rarely observed | Auxotrophy rarely observed |
| The quantitative content of organic acids: tartaric, malic, lactic, citric, acetic after fermentation in wine | High level | High level |
| The quantitative content of phenolic compounds in wine | Moderate level | Moderate level |
| Degradability of starch (split of starch) | Some yeast strains degrade starch | No |
| Duration of the lag phase | Short lag phase | Short lag phase |
| Rate of fermentation | High | High |
| The necessity in assimilative nitrogen | Requires | Requires |
| Ability to recover 2,3,5-triphenyltetrazolium chloride (TTC) | Some yeast strains recover | Do not recover |

lower halo-tolerance, and grow when salt concentration was 1.0, 4.0%, and 4.5% sodium chloride. It was found that the studied yeast cultures' halo-tolerance differed greatly from

each other. In the process of the growth and evolution of yeast cultures, they may acidify their environment to varying degrees, as a result of emphasising organic acids (acetic, lactic, etc.). When this occurs, it is necessary to slightly alkalise the growth medium with a 4% sodium hydrogen carbonate solution.

In carrying out the tests to identify yeast species, it is important to investigate their metabolic activity. For more complete characterisation of the yeast; urease acitivity; the ability to hydrolyse glycerol; the formation of organic acids, esters and starch-like compounds; and liquefaction of gelatine were tested. It was established that all tested yeast strains liquefy gelatine, and have urease activity, i.e. the ability to degrade urea. There are a few strains, however, which are exceptions to this general rule.

**CONCLUSIONS**

*Saccharomyces cerevisiae* yeast culture is generally recognised as a safe micro-organism for food industry use. The only adverse effect to the environment observed is the presence of the killer toxins phenotype, which is active against other strains of *Saccharomyces*. This effect is widely used in the wine industry. *Saccharomyces cerevisiae* is a organism which has an extensive history of safe use in the food industry. Developing a laboratory-based approach to assessing the resilience of wine yeasts, however, is not a simple task; particularly when interested in their robustness in a commercial winemaking setting. First, it is necessary to identify their ethanol-tolerance. Second it is necessary to identify a cross-section of commercially available wine yeasts that perform quite differently in wine ferments; i.e. imparting different sensory attributes, and reportedly differing in their reliability or fermentation efficiency. With an assay for signs of stability and robustness in place, it would then be possible to design a yeast-selective breeding program aimed at improving the robustness of poor performers that impart desirable sensory attributes to wine.

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**Байрактар В. М. Критерії оцінки дріжджів *Saccharomyces cerevisiae* при їх селекції для біотехнологічних цілей** // Екосистеми, їх оптимізація та охорона. Сімферополь: ТНУ, 2013. Вип. 9. С. 197–205.

Були досліджені дріжджові культури *Saccharomyces cerevisiae* (Meyen ex E. C. Hansen) виділені з місцевих, районованих сортів винограду, які особливо цінні в селекції для біотехнології виноробства в процесі бродіння вуглеводів у виноградному суслі. Основними критеріями для селекції дріжджів є кількість утвореного спирту і якість вина в тому числі: аромат, смак, присмак, концентрація етанолу. Також важливі такі показники, як: титруемая кислотність, летючі кислоти, загальні фенольні сполуки у виноматеріалах отриманих в результаті бродіння вуглеводів у виноградному суслі. Під час виробництва вина, дріжджові клітини залежить від декількох умов, несприятливих для зростання (окислювання, температури, осмосу, сульфітації і етанольного стресу), і вони повинні виявляти і реагувати на ці умови, в іншому випадку може негативно вплинути на спиртове бродіння та зупинити його.

*Ключові слова*: селекція, критерії оцінки, культури винних дріжджів, *Saccharomyces cerevisiae*, біотехнологія.

**Байрактар В. Н. Критерии оценки дрожжей *Saccharomyces cerevisiae* при их селекции для биотехнологических целей** // Экосистемы, их оптимизация и охрана. Симферополь: ТНУ, 2013. Вып. 9. С. 197–205.

Были исследованы дрожжевые культуры *Saccharomyces cerevisiae* (Meyen ex E. C. Hansen) выделенные из местных, районированных сортов винограда, которые особенно ценны в селекции для биотехнологии виноделия в процессе брожения углеводов в виноградном сусле. Основными критериями для селекции дрожжей являются количество образуемого спирта и качество вина в том числе: аромат, вкус, послевкусие, концентрация этанола. Также важны такие показатели, как: титруемая кислотность, летучие кислоты, общие фенольные соединения в виноматериалах полученных в результате брожения углеводов в виноградном сусле. При производстве вина, дрожжевые клетки зависят от нескольких условий, неблагоприятных для роста (окисление, температуры, осмоса, сульфитации и этанольного стресса), и они должны выявлять и реагировать на эти условия, в противном случае может негативно повлиять на спиртовое брожение и остановить его.

*Ключевые слова*: селекция, критерии оценки, винные культуры дрожжей, *Saccharomyces cerevisiae*, биотехнология.

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