STUDIES CONCERNING MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOME YEAST STRAINS INVOLVED IN WINE FERMENTATION

CERCETĂRI PRIVIND CARACTERELE MORFOLOGICE ŞI BIOCHIMICE ALE UNOR TULPINI DE LEVURI IMPLICATE ÎN VINIFICAȚIE

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Abstract: *Study of yeasts from certain wine growing region is very important for fermentative processes and for insurance of wine stability. In this paper we characterize the yeast strains from different wine types regarding cultural, colonial, morphological and biochemical properties, and regarding the behaviour response to various physical factors. The tests for yeasts fermentative properties give the possibility to exclude the strains which have unfavourable activity in growth medium and keep those strains with useful properties*

Key words: yeast, fermentation, corona discharge.

Rezumat: *Studiul levurilor dintr-o regiune viticolă este foarte important pentru desfăşurarea proceselor fermentative şi pentru asigurarea stabilității vinurilor. În lucrarea de față tulpinile de levuri izolate din diferite vinuri au fost caracterizate din punct de vedere al caracterelor culturale, coloniale, morfologice şi biochimice, precum şi din punct de vedere al comportamentului la stresul produs de diferiți factori fizici. Testele care caracterizează levurile din punct de vedere fermentativ dau posibilitatea să fie excluse acelea care au o acțiune nefavorabilă asupra mediului pe care se dezvoltă, fiind reținute cele cu caracteristici utile pentru scopul urmărit.*

Cuvinte cheie: levuri, fermentație, descărcare corona

INTRODUCTION

Numerous studies showed important role of microorganisms in wine qualities. The species and strains of yeasts used for fermentation in wine making process contribute to developing or reduction of some properties of wine (Popa, Teodorescu, 1990).

The taxonomic study of yeasts from certain viticultural region represents the base of indispensable knowledge for rational use of these microorganisms in fermentation process and for ensuring and control of wine stability (Pomohaci et al, 2001).

MATERIAL AND METHODS

This paper contains the specifications of some particularities of certain yeast strains, isolated from two different wines, Feteasca neagra and Merlot.

We used three samples of wine at the beginning of fermentation (two samples of Feteasca neagra and one sample of Merlot). From these samples of wine we isolated seven strains of yeast with distinct cultural and colonial characteristics, by inoculating on YPG solid medium (yeast extract - peptone - glucose - agar), under the form of isolated colonies. The method for obtaining isolated colonies was the streak plate technique.

The selected yeast strains were preserved in culture tubes on the YPG solid medium slants.

The morphological study of a selected yeast strains presumed the microscopic examination of a 24 hours yeast cell suspension in YPG liquid medium. The dimensions of cells were determined with micrometer. For each sample were measured 50 cells for five repetitions.

The capacity of selected yeast for nitrate assimilation was studied, using a proper liquid medium (with glucose, potassium acid phosphate, magnesium sulphate, calcium chloride, sodium chloride, growth factors and potassium nitrate). The inoculated samples were maintain 15 days at 25°C and the level of cultures development was observed.

The capacity of yeast to ferment sugars was studied, using a liquid medium with rigorous amount of sugars (glucose, saccharose, galactose, maltose, raffinose, lactose) and Durham tubes for distinguish gas production. The capacity of yeast to ferment certain sugars depends of presence in cells of some specific enzymes. It is a firm character, which may helps to identify yeasts (Popa, Teodorescu, 1990).

In the last stage of our experiment, the behaviour of selected strains of yeast in electrical field (negative corona discharge) was observed. The exposure in electrical field is an alternative method to sterilisation of fresh grape juice in wine making process (Aubrecht et al, 1999).

We used a device with one anode and one cathode, like two wire with small radius of curvature; the exposure dose represents the product between electric intensity (17 μA) and exposure time. The exposure time varied between 5 minutes to 15 minutes. We used a witness lot (without exposure) to compare the results. The experiences were repeated for three times.

After exposure, the number of yeast cells were estimated with Thoma chamber. It was observed the modification of viable cells number. The cells count was repeated for five times for each sample and then was calculated their arithmetic average.

RESULTS AND DISCUSSIONS

The yeast cultures were relatively uniform, with exuberant growth in liquid medium. On the YPG solid medium, the yeast colonies from wine samples had regular form and appearance, with complete bounds, white or yellow colour, gentle convex. We selected seven strains (three white colonies and four yellow colonies.

The morphological study of all seven selected yeast strains showed the spherical - ovoidal cells, with various dimension: for $1b_1$ strain the values of diameters were 4,05 x (2,7 - 4,05)μm; for $1b_2$ strain the values of diameters were 2,07 - 4,05μm; $2b_1$ strain presented cells with relatively constant dimension, (4,05 - 5,4) x (2,7 - 4,05)μm; for $2b_2$ strain the values of diameters were (2,7 - 5,4) x

(2,7 - 4,05)µm; $2a_2$ strain presented cells similar with $1b_1$ strain, and $2a_3$ strain with $2b_2$ strain; for $3b_2$ strain the values of diameters were (4,05 - 5,4) x (2,7 - 5,4)µm.

The yeast strains isolated from Feteasca neagra wine and Merlot wine were not capable to assimilate nitrate; the witness tube and test tubes had the same turbidity of the medium. These results suggest that the selected yeasts were not from *Hansenula*, *Candida* and *Pichia genus*.

În sugar fermentation experiments, the glucose, saccharose, galactose, maltose, raffinose were fermented by all selected yeast strains; lactose wasn't fermented by any of them.

These result suggest the isolated strains belong to another species than: *Saccharomyces bayanus, S. oviformis* and *Schizosaccharomyces pombe* (because of galactose); *S. italicus* şi *S. steineri* (because of maltose); *Torulopsis stellata* (which don't have the ability to ferment galactose and maltose); *Rhodotorula mucilaginosa*, which don't have the ability to ferment any of these sugars; *Kloeckera apiculata*, which have the ability to ferment only glucose and had distinctive form of cells (Popa, Teodorescu, 1990).

For the exposure in electrical field (negative corona) we pick out three yeast strains (from seven); we had three experimental variants, depending of exposure time: 5 minutes, 10 minutes and 15 minutes.

It was remarks that the number of cells in suspensions varies inversely proportional with exposure time (decreases while the exposure time increases), comparative with cells from witness lot (Table 1). For 15 minute exposure in negative electrical discharge, the number of cells decreases with 40 % comparative with initial number of cells in suspension.

Table 1.

The effect of corona discharge for number of yeast cells

Exposed strain (cells/ml) / Time (minutes)	**$1b_1$**	**$2b_1$**	**$3b_2$**
0 minutes (unexposed witness)	1284×10^4	324×10^4	894×10^4
5 minutes	1038×10^4	216×10^4	642×10^4
10 minutes	990×10^4	174×10^4	564×10^4
15 minutes	954×10^4	126×10^4	354×10^4

CONCLUSIONS

1. All seven isolated yeast strains may be included in *Saccharomyces* genus, regarding for cultural and morphological properties.

2. None of investigated strains don't have the ability to assimilate nitrate and to ferment lactose; all these yeast strains fermented glucose, saccharose, galactose, maltose, raffinose, and produced various amounts of gas, at 28°C.

3. Regarding to effect of electrical discharge negative corona on yeast cells, the number of cells in suspension decreased while exposure time increased. All three yeast strains exposed in electrical field had similar behaviour.

4. In Feteasca neagra wine and Merlot wine used in these experiments were yeast which belong to *Saccharomyces* genus; their properties recommend these strains for selection and utilisation at industrial level.

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