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TAXONOMIC IDENTIFICATION OF TWO YEASTS STRAINS ISOLATED FROM CONCENTRATED MUST AND SUPRASULFITED MUST

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Abstract:

In 2012 year two yeasts strains were isolated from yeasts populations that were active in concentrated grape must and suprasulfited must. The aim of this work was the characterization and identification of two yeasts strains and established their affiliation at osmofile or sulfites species, or proof that this property was acquired as a result of their adaptation to unfavorable environmental conditions. Research shows that these two yeasts strains marked with an 1-MC and 2-MS belong to Saccharomyces oviformis and Saccharomyces cerevisiae var. ellipsoideus species. It was found that their growth and reproduction in unfavorable environmental conditions are a result of adaptation to these conditions. This adaptation is a warning to the fact that even if the equipment of work is technologically advanced (on the construction and operation of) can not prevent biological degradation of concentrated musts and of suprasulfited musts with these yeasts strains, if the hygiene measures are not taken for the technological lines, vessels and spaces for wines storage.

Key words*: yeast strain, concentrated must, suprasulfited must, acetaldehyde*

INTRODUCTION

Loss of biological stability and degradation of wines quality are produced due to the presence of yeasts in the winemaking rooms that can be adapted to unfavorable conditions of life (Matei et al., 2012). Even in the modern and performant technological lines, the biological stability assuring of wines with sugar contents, concentrated musts and those suprasulfited is a major problem (Antoce et al., 2001). The literature in this field indicates that there are yeasts that can live in concentrated musts or protected by large amounts of SO_2, by changing musts composition (Cotea et al. 1985).

This paper aims is to identify and taxonomic classification of two yeasts strains isolated from concentrated must and suprasulfitated must (where these activities), establishing their membership to osmofile or sulfite species and prove that the property that they possess has been acquired as following a process of adaptation to unfavorable environmental conditions.

MATERIAL AND METHOD

Isolations were made, from concentrated must and suprasulfited must of SC VINEX MURFATLAR Ltd., situated in Murfatlar vineyard, in 2012. The yeast isolated from concentrated must were noted with 1-MC, and that from suprasulfited must with 2-MS. At the time of isolation concentrated must have had a sugar content of 670 g/l and the suprasulfited must have had the following composition: sugar - 200 g/l, total SO_2 - 910 mg/l and free SO_2 - 498 mg/l. Classical identification of these strains have been performed. Isolation was made from must in biological activity diluted with sterile water, and the cultivation was done on must-agar culture medium. Isolation was performed starting from a single cell using the method of successive dilutions (Domerq, 1956) and isolation in pure culture method (Barnett and Yarrow, 2001). To identify and characterize the isolated yeasts strains were used the classical methods of Lodder and Kreger van Rij (1984), Kurtzman and Fell (2011) and were followed: - cells shape and size (large and small diameters) after

cultivation for three and six days at 25°C, in liquid medium (grapes musts and Wickerham medium) and on the solid (must grapes with agarose gel); - pseudohyfae-formation, after cultivation for 12 days, in medium potato agarose gel; - sporulation on synthetic medium-Gorodkova (comments after 30 days). Physiological characteristics were determined using the following tests: - different sugars fermentation into test-tubes with Durham tubes in yeast extract medium, with 2% of each sugar tested; - the sugars and nitrate assimilation by auxanographic methods using agar medium rich in mineral salts and vitamins - using the ethyl alcohol as unique carbon source; - split arbutin. To complete the taxonomic characterization were made also the following determinations: -total number of cells increased during alcoholic fermentation (Thoma blade counting); the fermentative processes evolution by gravimetric method and sugars content metabolized; preferential metabolizing of glucose and fructose by the yeasts during fermentation, was made by paper chromatography method; total and free SO_2, alcohol, acetaldehyde, volatile acidity contents, by OIV methods. In order to check the maximum temperature supported by these yeasts, they have been thermostated at different temperatures for 48 hours, after which the viability was tested by cultivation in must-agar medium to the optimal temperature. The concentrations in sugars and SO_2 to which yeasts strains can not activate were established by their cultivation on medium with high concentrations of these compounds (500 mg/l free SO_2 and 700 g/l sugars).

RESULTS AND DISCUSSIONS

The cells shape and size of the two yeasts strains lead to the conclusion that they belong to *Saccharomyces oviformis* (1-MC) and *Saccharomyces cerevisiae* var. *ellipsoideus* (2-MS) species.

1-MC yeast strain characterization:
- in liquid medium the cells are round, oval, isolated or grouped in pairs (Figure 1.a);
- budding type is polar; -cells sizes range from (4.0 to 6.0) x (9.0 to 12, 0) microns; -the strain isolated and tested does not form ring or film;
- the culture on solid medium is white to cream colored, smooth, shiny, with less lobes marked and fine ramifications at the edge of culture;
- on potato agar medium, the strain does not form pseudohyphae;
- sporulation test was performed on carrot-agar medium (Gorodkova medium);
- the yeast strain formed in asca two, rarely three spheroidal shape spores (Figure 1.b).

2-MS strain characterization:
- in liquid medium the cells are elliptical, oval, very rarely round isolated or grouped in pairs;

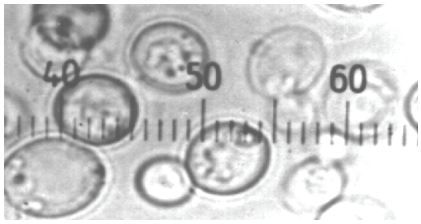

Figure 1.a. 1-MC strain – cells shape

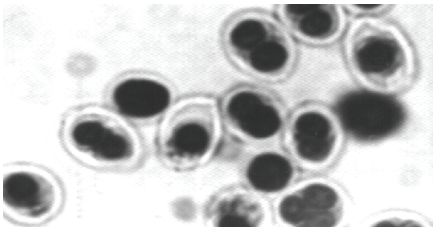

Figure 1.b. 1–MC strain – spores shape

- budding type is polar, there are cases of lateral budding (Figure 2a);
- cells sizes range from (5.0 to 8.9) x (4.0 to 8.1) microns, are similar to those in the specialized literature;
- the yeast strain isolated and tested does not form ring or film;
- culture on solid medium is white or creamy, smooth with matte or shiny aspect, straight edge;
- on potato agar medium, does not form pseudohyphae;
- in Gorodkova medium the yeast strain form 1-4 ascospores with round, spheroidal or elliptical shape (Figure 2b).

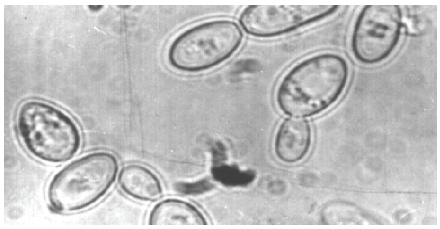

Figure 2.a. 2-MS strain-cells shape

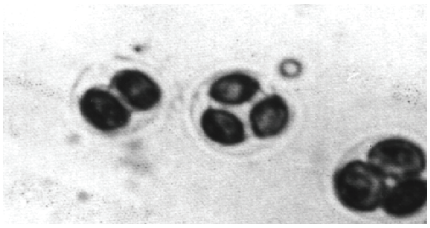

Figure 2.b. 2-MS strain: spores shape

Form cells incurred visible changes after their subjection to tests to establish maximum levels of sugar and SO_2 they support. At physiological characterization the two yeasts strains have responded as follows (Table 1, Table 2).

Table 1. Sugars fermentation at 1-MC şi 2-MS strains

Tested sugar	Fermentation	
	1-MC strain	2-MS strain
Glucose	+	+
Galactose	-	+
Zaharose	+	+
Maltose	+	+
Lactose	-	-
Rafinose	+1/3	+1/3

Table 2. Sugars asimilation at 1-MC şi 2-MS strains

Tested sugar	Asimilation	
	1-MC strain	2-MS strain
Glucose	+	+
Galactose	-	+
Zaharose	+	+
Maltose	+	+
Lactose	-	-
Rafinose	+	+

The table results shown us that the strain 1-MC, with the exception of galactose and lactose ferments and assimilates the rest of sugars, while 2- MS strain does not ferment, and do not assimilate lactose.

The tests of nitrate assimilation, using of ethyl alcohol as unique carbon source, split arbutin and the growth in the cycloheximide presence were negative for both strains and for 1-MC strain the growth in vitamin-free medium was absent.

The fermentation led in strictly aerobiosis conditions and monitored by the increase in the total cells number of yeast showed that the two yeasts strains also differ by the length of time during which the maximum cell density was achieved per unit volume. Strain 1-MC (*Saccharomyces oviformis*) recorded maximum $290x10^3$ cells/mm^3 in 5 days and 2-MS strain $245x10^3$ cells/mm^3 in 7 days (Figure 3).

The fermentation speed expressed by the release of CO_2 and daily sugars consumption has characteristic values for each strain.

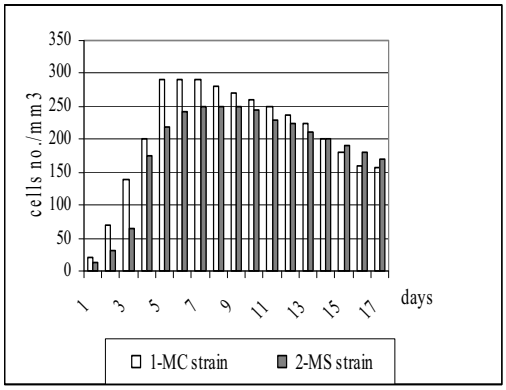

Figure 3. The total cells number increase under strict aerobiosis conditions

Regarding the selective sugars fermentation it was found that both species ferment glucose preferentially.

For both strains, in the time when 80% of the glucose has been fermented the proportion of fructose has not exceeded 50%, while the 1-MC strain fermented sugars at a higher speed and in a shorter time.

Alcoholic degree produced by 1-MC strain was higher with almost 2 vol% than 2-MS yeast strain and the capacity to produce volatile acids was higher for the 2-MS yeast strain (Table 3).

Table 3. The production of alcohol and volatile acids during alcoholic fermentation process

Yeasts strain	Alcohol vol. %	Volatile acids g/l CH_3COOH	Acetaldehyde mg/l
1-MC	12,0	0,90	113,1
2-MS	10,1	1,12	120,0

In the evolution of acetaldehyde content some differences were observed , in the sense that 1-MC yeast strain produced a larger amount by alcoholic fermentation than 2-MS yeast strain (Table 2 and Figure 4).

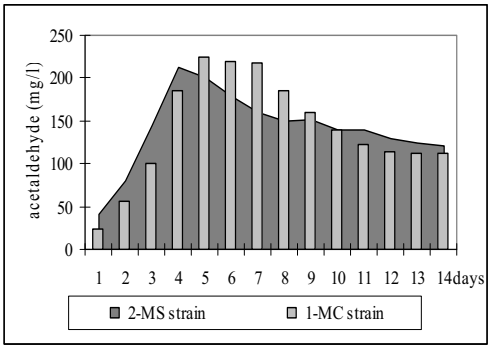

Figure 4. The evolution of the amount of acetaldehyde during the alcoholic fermentation

Keeping their viability at different temperatures was performed by thermostat for 2 days. It was observed that the lethal ranged temperature for both yeasts strains was around 47^0C. The MC-1 yeast strain was tested to establish the maximum quantity of sugars that it can support and the researches showed that at values about 650-700 g/l it activity is inhibited, while at the sugar level around 450 - 500 g/l, this yeast metabolizes sugars to 5-6 vol.% alcohol in about 52 days. The 2-MS yeast strain isolated from a suprasulfited must (498 mg/l SO_2), after isolation and purification was tested for the maximum level of SO_2 that can activate; it was found that it does not support large concentrations than 390-400 g/l free SO_2.

CONCLUSIONS

As a result of morphological and physiological measurements determined in laboratory conditions, the two strains belong to the species *Saccharomyces oviformis* (1-MC) and *Saccharomyces cerevisiae* var. *ellipsoideus* (2-MS).

The two yeasts strains were differentiated between them by cells shape and size, the aspect of giant colonies, by the sugars fermentation and assimilation tests and oenological characteristics (production of alcohol, volatile acids and acetaldehyde).

The property of 1-MC yeast strain (*Saccharomyces oviformis*) to activate in concentrated musts medium and to produce partial sugars fermentation in an inversely proportional quantity to the sugars concentration leads to the idea that the alcohol- their own product of metabolism- is an inhibitor of the yeasts activity that can be used in the preservation of concentrated grape must. In this case, the must can not be considered sterile because it contains yeasts which can become active.

The property of 2-MS yeast strain (*Saccharomyces cerevisiae* var. *ellipoideus*) to be active in suprasulfitated musts represents an adaptation that could be maintained if the yeast continues to be present in this medium. When the must is concentrated the microorganisms are concentrated too and thus the source of infection increased.

To ensure biological stability it is recommended to apply preventive must treatments and to ensure aseptic conditions as much as possible throughout the technological flow.

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