Evaluation of the effectiveness of killer toxins produced by some strains of yeasts against other strains killer

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Abstract. To make the wine, you do not need killer strains used for the purpose of dominance. It is only necessary that the yeasts are neutral. The aim of this study was to evaluate the effectiveness of killer toxins produced by some strains to produce a lethal effect on other strains killer. The experiments were conducted using the medium must Lorena M80: 20 with methylene blue. The killer test strains used were: 7M, 12M, 24M, 25M and 30M isolated from grape berries derived from the cultivar Merlot municipality of Pinto Bandeira (RS, Brazil). These strains were tested against other strains isolated killer Pinto Bandeira, they being the 3CF lineage derived from the cultivar Cabernet Franc, and the 24A strain isolated from cultivar Ancelota,; and also against strains isolated from the city of Colombo (PR, Brazil), called 4C, 6C, 7C, 10C, 11C, 12C, 13C, 14C, 15C, 17C, 19C, 20C, 22C and 24C. Were also used in the reference 1B, 91B and K1 strains. The inoculated plates were placed in an oven at 18 ° C 48 to 72 hours. The results revealed that none of the isolates showed sensitivity of these regions with the killer of five strains isolated killer test Merlot. However, the pattern and 91B K1 strains are sensitive to the killer factor produced by strains 30M and 12M. The 7M line introduced himself as killer only with respect to strain 91B. The taxonomic identification was first performed by amplifying the ITS region. Thus, three of the five strains analyzed, 7M, 24M and 25M, showed an amplicon at 610 base pairs.

1 Introduction

The killer factor in yeast was discovered in 1963 by Markower and Bevan, observed that some strains of *Saccharomyces cerevisiae* produced a substance capable of killing other yeast strains. This agent produced by certain cells cause the death of susceptible yeast, but is harmless to the neutral lines, so named because they do not produce the toxin and are not affected by it. The killer toxin production, glycoprotein, is caused by segments of double-stranded RNA, located in the cell cytoplasm, these segments is M-dsRNA and L dsRNA-, the former being responsible for the killer toxin production[4]. There is a broad description of killer toxin producing microorganisms in the literature. Currently there are numerous techniques for identification of yeasts of oenological interest. The biggest challenge lies in identifying yeasts on the need to differentiate genera and species that are taxonomically very close, but have different properties with respect to their fermentative and organoleptic characteristics. Molecular biology has provided new techniques for taxonomic determination of wine yeast. The ease, speed, versatility and sensitivity of PCR to make powerful molecular genetic studies involving large numbers of individuals of any living organism [1].

Against this backdrop, this paper carried out near Pinto Bandeira- RS demonstrates the importance of the selection of strains of *Saccharomyces cerevisiae*, determining their oenological characteristics to assist the demarcation of the typicality of the products. Improving the quality of wine produced in this region would add value to the product and reputation, increasing its market insertion and contributing to the socioeconomic development of the municipality.

2 Material and methods

The experimental part of the project was held at the National Research Center Embrapa Grape and Wine (CNPUV) in Microbiology and Fermentation Lab, located in Bento Gonçalves, Rio Grande do Sul-Brazil.

Three strains killer the species *Saccharomyces cerevisiae*, designated EMBRAPA 1B ($K + R^w$), where in killer suicidal and 91B characterized by killer resistant were isolated by Silva (1996), and also the commercial line K1 ($K^+ R^+$) obtained were used company Lallemand, Canada. Was suspended using a 10^7cél / ml using the scale Kreger-van Rij (1984).

To perform the test, the culture medium used was described by M 80:20 with the addition of ELNC [9]. The experiment aims to detect killer strains that are sensitive to other killer strains. The test was conducted as follows: 100mL seeded suspension of 10^7cel / ml of the test strain and killer spread throughout its length with the aid of the handle Drigalski. After applied point masses of other strains in test that also had killer behavior. The strains tested were: 7M, 12M, 24M, 25M and 30M against other strains derived from Pinto Bandeira-RS 3CF (Cabernet Franc) and 24A (Ancelota), 4C, 6C, 7C, 10C, 11C, 12C, 13C, 14C, 15C, 17C, 19C, 20C, 22C, 24C from the region of Colombo-PR and finally against the reference strains 1B, 91B and K1. The plates were placed in an oven at 18 ° C 48 to 72 hours.

For extraction of DNA of the strains was made from the freezing and thawing of the suspensions [9].

The PCR wa used ITS ITS 1 and 4 primers to amplify a region of rDNA named ITS1 and ITS2 with the 5.8s rDNA gene [2]. Afterwards the PCR product was applied to a 2% agarose gel and stained with ethidium bromide and photographed, the 100bp marker was used to visualize the band size.

3 Results and discussion

A strain "killer" may have become resistant to other lineage killer, neutral or may be sensitive to it. This test aimed to detect strains which are sensitive to other yeast killer then compared with one another to observe the formation of the halo or not the death or resistance to it.

None of the strains tested showed sensitivity to the other lineage killer. However the standard K1 and 91B strains were killed by strains 30M and 12M, 7M already presented only halo of death in strain 91B. As we can see in Figure 1.

Figure 1. Test killer/killer 91B and k1

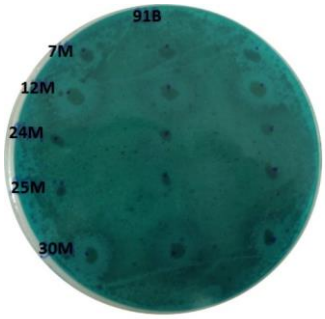

Table 1. killer strains of cultivar Merlot that kill other killer

Pattern strains			
Strain tested	**K1**	**1B**	**91B**
7 M	-	-	+
12 M	-	+	+
24 M	-	-	-
25 M	-	-	-
30 M	-	+	+

PCR were performed for all five killer strains to identify its kind. The identification and characterization of yeast strains and species have been based on their morphology and physiological capabilities [3]. During the initial phase of the fermentation of grape must yeasts are usually the dominant species *Kloeckera apiculata* also called *Hanseniaspora uvarum*. To a lesser extent species of *Candida, Rhodotorula, Pichia, Kluyveromyces, Hansenula* can also be detected [6,5,7]

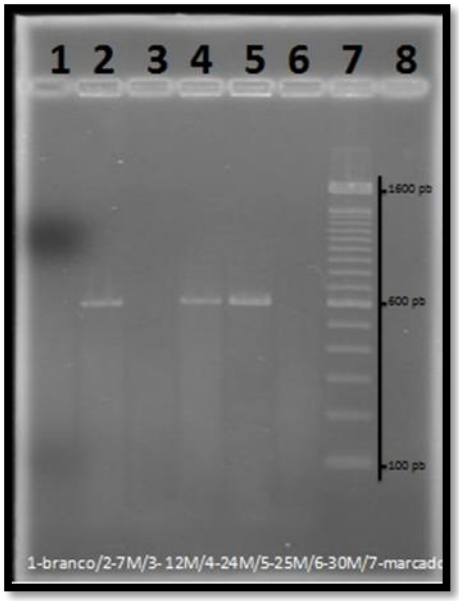

Figure 2. photo agarose gel 2% killer strains of cultivar Merlot

Just 7M; 24M and 25M show amplification of 610 base pairs. The 610 base pairs of Candida species can be *sarbosa* or *Hansemula mrakii* species [2]. The 12M, 30M showed no amplification, as visualized in Figure 2.

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