METHODOLOGICAL APPROACHES FOR ISOLATION OF YEASTS BELONGING TO THE BRETTANOMYCES / DEKKERA GENERA FROM THE WINE

ABORDĂRI METODOLOGICE PENTRU IZOLAREA LEVURILOR DIN GENUL BRETTANOMYCES/ DEKKERA DIN VIN

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Abstract: *Among the various causes of spoilage of wine and wine materials, some genera and types of wild yeast are especially common. One of the most harmful microorganisms is the yeast of the genus Brettanomyces / Dekkera. Timely detection and quantification of these fungi is essential to prevent wine spoilage. The analysis of referenses describing various methods of detecting yeast in wines and raw wine materials, from classical microbiological methods to modern molecular genetic methods, was carried out. The potential of wine microbiological monitoring was also assessed as a result of research on wines produced in the Microvinification of the Department of Oenology and Chemistry of Faculty of Food Technology TUM, when the advantages and disadvantages of the "gold standard" of microbiology, a culture method for isolating yeast of the genus Brettanomyces / Dekkera, were assessed.*
Key words: *Brettanomyces, wine, spoilage, cultivation, culture media, RT- PCR.*

Rezumat: *Printre diferitele cauze ale alterării vinului și a materialelor vinicole, unele genuri și tipuri de drojdie sălbatică sunt deosebit de periculoase. Unul dintre cele mai dăunătoare microorganisme este drojdia din grupa Brettanomyces / Dekkera. Detectarea și cuantificarea în timp util a acestor ciuperci este esențială pentru a preveni deteriorarea vinului. A fost efectuată analiza referințelor care descriu diferite metode de detectare a drojdiei în vinuri și materii prime de vin, de la metodele microbiologice clasice la metodele genetice moleculare moderne. Potențialul monitorizării microbiologice a vinului a fost, de asemenea, evaluat ca rezultat al cercetării vinurilor produse în Microvinificarea Departamentului de Enologie și Chimie al Facultății de Tehnologie Alimentară UTM, când avantajele și dezavantajele „standardului de aur" al microbiologiei, o cultură a fost evaluată metoda de izolare a drojdiei din genul Brettanomyces / Dekkera.*
Cuvinte cheie: *Brettanomyces, vin, alterare, cultivare, cultura media, RT-PCR*

Introduction

The wine industry is traditionally considered the main and even strategic industry in the Republic of Moldova. It is an important source of direct and indirect income for a significant part of the country's population. In a difficult economic situation in the country, wineries are making significant efforts to produce wines that are competitive in foreign markets [1].

Wines are susceptible to chemical and microbiological spoilage, and the yeast of the genus Brettanomyces / Dekkera is often the cause of these problems, especially in red wines, producing ethyl phenols and other unpleasant odor compounds that mask the desired fruity character of the wine. Contaminated wines have a tainted organoleptic perception, reduced fruitiness and, therefore, are rejected by consumers [1], which entails tangible economic problems.

Timely detection of these organisms in wine is critical to maintaining the quality of the wine. Therefore, the study and use of modern, fast and effective methods of analysis for testing the quality of wines becomes highly relevant. For this, molecular methods of rapid testing, especially real-time PCR, are becoming increasingly important [3].

Research Methodology

In the analyzed literature samples of red wine obtained from producers from different geographic regions and at different stages of the winemaking process or storage were used as the test material for the determination of Brettanomyces [2].

Methods for the determination and identification of yeasts of the genus Brettanomyces are include microbiological and molecular genetic.

Microbiological methods for detecting yeast are based on the cultivation of microorganisms on elective and differential diagnostic media with subsequent determination of physiological and biochemical parameters and morphological data. In our own experiments - cultivation on the nutrient media for the isolation of Brettanomyces, dry red wines produced in the Microvinification of the Department of Oenology and Chemistry TUM from two grape varieties: Feteasca Neagra and Rara Neagra were taken as the test material. And as culture media have been chosen: Sabouraud 4% Glucose Agar, Malt Extract Agar Base and FastOrangeTM Yeast Agar.[1]

Molecular genetic methods. Molecular PCR - tools include:
species specific PCR, PCR-RFLP PCR-DGGE,
multicomplex PCR, LAMP-PCR,
quantitative PCR.

Traditional PCR methods involve amplification of the target DNA in a thermal cycler and separation of the product by gel electrophoresis followed by visual detection. This is a laborious and manual process, but PCR products can also be detected with fluorescent probes in real-time quantitative PCR. The labeled probe anneals the target DNA during the reaction, and as the DNA expands, it emits a fluorescent signal that can be detected by a light cycle. The concentration of the released fluorescence is proportional to the concentration of the PCR product generated in each cycle. Such detection of a real-time PCR product involves the use of a specific fluorescent probe (eg Taqman) in the CFX96 TouchTM BIORAD real-time PCR detection systems as described according to the manufacturer's protocol. A description of such study, conditions and results are described in detail in the publication of moldovan authors "The methodological aspects of using Real-Time Polymerase Chain Reaction (RT-PCR) in Brettanomyces / Dekkera detection" [4]. In this work the potential of the real-time PCR method in microbiological monitoring of wines was assessed and the analysis process was optimized in accordance with the PIKA WeihenstephanTM SO Detection HH Brettanomyces / Dekkera protocol.

Conclusions

1. Wild yeast Brettanomyces / Dekkera is the cause of wine spoilage. They grow well on nutrient media, but Sabouraud 4% glucose agar is the most suitable medium for their cultivation

2. The detection of Brettanomyces / Dekkera by bacterial culture, together with the advantages associated with the availability and clarity of these methods, also has disadvantages: the long duration of the tests (from 7 days to 3 weeks), which does not allow for timely and effective reactivity if necessary.

3. Molecular genetic testing, such as the RT-PCR method for the determination of Brettanomyces / Dekkera in wines, is fast, accurate, sensitive and very promising as it allows a quick response if detected.

4. Using the most optimal protective measures in a timely manner and taking into account the physiological capabilities of the Brettanomyces / Dekkera yeast, it is possible to control the situation and prevent wine spoilage.

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