

# Isolation and Technological Characterisation of *Brettanomyces Anomalus* in Wine

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**ABSTRACT:** Natural fermentation of grape must is carried out by a sequence of different yeast species. The non - *Saccharomyces* yeasts dominate in early stages of fermentation and are replaced by *Saccharomyces* yeast that finish the fermentation process. Since the non - *Saccharomyces* yeast showed a great interests in industrial fermentation, in this paper we present the study of 4 *Brettanomyces anomalus* strains for fermentation abilities. These strains were isolated in the first stage of fermentation. The results showed that three strains of *Brettanomyces anomalus* had not fermentative activity. KB2' strain of the species *Brettanomyces anomalus* presented with significant fermentation activity was studied for technological characteristics (fermentation power in controlled and low temperature, fermentation power in the presence and in the absence of SO<sub>2</sub> and fermentation energy). The results showed that the strain KB2' ends with high alcoholic degree in controlled temperature fermentation, compared with low temperature fermentation. About the presence of SO<sub>2</sub> in fermentation, KB2' strain result very sensitive, having as a result the absence of fermentative activity.

**KEYWORDS:** Identification, Fermentation, non – *Saccharomyces*, *Brettanomyces anomalus*.

## I. INTRODUCTION

Biochemical reactions that occur during the fermentation depends on the nature of microorganisms, which are on the surface of the grapes [3], [5]. Natural fermentation of grape juice carried out by a sequence of different yeast genera and species. The early stages of fermentation are dominated by non - *Saccharomyces* yeast, which are characterized by a low fermentation power, since fermenters with high metabolic activity inhibit their development [10]. After 3-4 days non - *Saccharomyces* yeast are replaced by the highly genera *Saccharomyces* yeasts that continue and finish the fermentation process [6]. Recently have been many studies on non - *Saccharomyces* yeasts that contribute to the fermentation, competing with *Saccharomyces* yeast for the nutrients and may produce secondary compounds, affecting the bouquet of the final wine [11]. In red wine, *Brettanomyces anomalus* is considered a spoilage yeast [4], [9], [7]. These yeasts can be found in fermenting must and in wine. Their populations are usually minor compared with numerous other yeasts with high fermentation power. Their increase in numbers only occurs during more nutritionally favourable conditions that suit their characteristics. These conditions are created once alcoholic fermentation is completed and traces of residual sugars allow them to proliferate more easily. Wines affected by this yeast are characterized by unpleasant odors, as a result from the production of phenol volatile, e.g.: 4-ethylphenol and 4-ethylguaiacol during fermentation [8]. The control of *Brettanomyces anomalus* in wine is usually achieved through the use of SO<sub>2</sub> which serves as inhibitor.

The main aim of this study was to identify and define the technological characteristics of the species *Brettanomyces anomalus* in wine fermentation. The influence of two physical – chemical factors such as temperature and SO<sub>2</sub> on these yeasts during the fermentation process.

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## II. MATERIALS AND METHODS

### Isolation and identification of *Brettanomyces anomalus*.

Samples were taken in grape musts before fermentation. They were serially diluted and then were plated in Petri dishes, with three repetitions for each sample and incubated at 25°C for 72 hours. The mediums used for cultivation of these samples were PDA (Potato - Dextrose - Agar) (42 g/L) and YMA (Must - Agar) (47 g/L). Identification of yeasts was determined according to the requirements of identifying keys [1], [2], [13].

### Determination of fermentative activity.

In a test tubes with 10 mL must was placed upside down Durham tubes. Once the test tubes were closed with corks and sterilized, were inoculated cells of *Brettanomyces anomalus* yeast. They were incubated in thermostat at 25°C and after 2 days the examination of cultures became [14].

### Study of technological characteristics.

For the study of technological characteristics of *Brettanomyces anomalus*, was used densimetric method [12]. According to this method, the microfermentations were carried out in 250 mL Erlenmeyer flasks equipped with corks traversed by Pasteur pipette, containing 100 mL must of 26.5% sugar content. After sterilization at 90°C for 15 minutes, were inoculated with 10<sup>6</sup> cells/mL. culture 48 hour in PD broth (potato-dextrose). This practice has the function of recording the weight loss by the liberation of dioxide carbon produced during fermentation. Determination of the weight loss is used for monitoring the fermentation process. Weights were taken every day, when the weight was stable, fermentation was considered to have been finished.

### Analytical determinations.

The indicators devised during the experiments were:

- *Fermentation power* which was defined as the alcohol produced during the fermentations, under a controlled and low temperature conditions [12].
- *Resistance to SO<sub>2</sub>*, which indicates the ability of fermentation of the strains, in the presence of SO<sub>2</sub> [12].
- *Fermentation energy*, which shows the speed that a strain begins the fermentation and ends it. The fermentation energy was determined for 7 and 11 days of fermentation [12].

## III. RESULTS AND DISCUSSION

### Isolation and identification of *Brettanomyces anomalus*.

For isolating and identifying *Brettanomyces anomalus*, is used traditional method based on the scheme below Figure 1.

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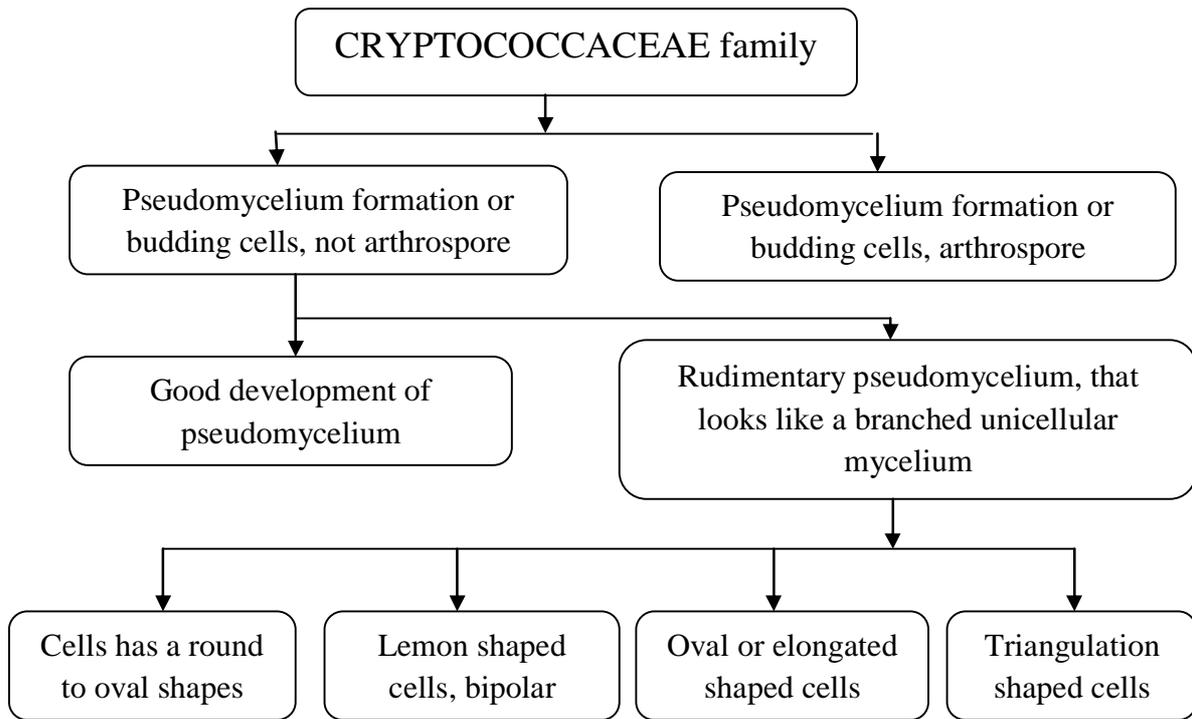


Figure 1. Identification scheme of *Brettanomyces anomalus* up to genus of Cryptococcaceae family.

Isolation of yeasts were made in grape musts before fermentations. Identification of them was given by the morphological and macro-morphological characteristics. These strains form colonies by irregular margins, raised mass ruffled on the top like “floral”, creamy white color, not shiny, smooth consistency. Their cells are oval or elongated shape and reproduce by budding. The selected strains were named KS7, MP2, KL1’ and KB2’, and they belonged to *Brettanomyces anomalus* species.

### Determination of fermentative activity of *Brettanomyces anomalus* strains.

Determination of fermentative activity of *Brettanomyces anomalus* strains, was conducted in must, this ability is observed through the release of CO<sub>2</sub> (product of fermentation). Table 1 show the results obtained from the experiments.

Table 1. Determination of fermentative activity of selected *Brettanomyces anomalus* strains.

Nr.	Strains	Results
1.	MP2	-
2.	KS7	-
3.	KL1’	-
4.	KB2’	+++

- No fermentation; +++ Good fermentation

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As seen in Table 1. three strains had no fermentative activity, while the strain KB2' presents fermentative activity, therefore this strain was selected for study the technological characteristics.

## Fermentation dynamics.

The performance of dynamics in different fermentation conditions of strain KB2', was conducted by Baranyi model and presented graphically in Figure 2:

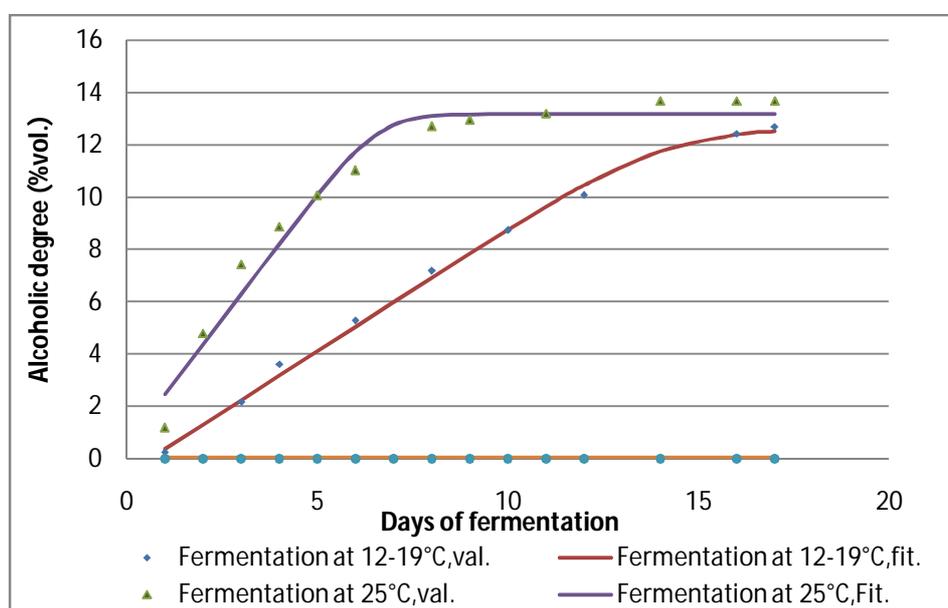


Figure 2. The performance of dynamics in different fermentation conditions of strain KB2'.

As seen from the graph in Figure 2, KB2' strain developed a good fermentation in controlled temperature (25°C) and completed it for a period of 10 days, giving 13.69% vol. alcoholic degree. This strain at low temperatures, developed the fermentation for a period of 17 days and gave an alcoholic degree lower than the controlled temperature fermentation. In the presence of SO<sub>2</sub> this strain showed high sensitivity and therefore no fermentation of must.

Table 2. The performance of dynamics in different conditions of fermentation of the strain KB2'.

Strain KB2'	yDatMin	yDatMax	rate	lag	y0	yEnd	se(fit)	R <sup>2</sup> _stat
Fermentation at 25°C	1,20	13,69	1,92	-*	2,46	13,19	0,72	0,96
Fermentation at 12-19°C	0,24	12,69	0,93	-	0.35	12.66	0,35	0,99
Fermentation with SO <sub>2</sub>	-	-	-	-	-	-	-	-

\* - No data

From the statistical data presented in Table 2, it is observed that the strain KB2' in controlled temperature of fermentation showed that variability of the alcoholic degree is from 1.20 - 13.69% vol. with R<sup>2</sup> = 0.96, while in low temperature this variability presents R<sup>2</sup> = 0.99.

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## Fermentation energy.

The fermentation energy is an important indicator in determining the technological characteristics of the wine. In this study, were determined the energy fermentation for 7 and 11 days of fermentation. Fermentation were conducted in controlled temperature (25°C) and low temperature (12-19 °C).

Table 3. Fermentation energy.

Strain KB2'	Fermentation energy (% vol per 7 days)	Fermentation energy (% vol per 11 days)
Fermentation at 25°C	1.81* ± 0.02	1.22 ± 0.02
Fermentation at 12-19°C	1.03 ± 0.02	0.92 ± 0.04

\* Mean ±SD

As seen from Table 3 in the fermentation with controlled temperature, the fermentation speed value is higher during 7 days and 11 days of fermentation compared to fermentation at low temperature. This high value of fermentation energy is associated with a high alcoholic degree (13.69% vol.).

*Brettanomyces anomalus* is considered a yeast with negative impact in wine, because of the production of not desirable aromatic components [4], [9], [7] but in this study it was observed that this yeast showed fermentative activity with a high alcoholic degree.

## IV. CONCLUSIONS

4 strains of *Brettanomyces anomalus* were isolated, identified and named KS7, MP2, KL1' and KB2'.

Strains KS7, MP2 and KL1' resulted with no fermentative activity.

KB2' strain showed significant fermentative activity in wine fermentation at controlled temperature (25°C) and ended it with high alcoholic degree, for a 10-day period. This strain at low temperatures, develops fermentation for a period of 17 days and gives a lower alcoholic gradation compared with controlled temperature fermentation.

In the presence of sulfur dioxide, KB2' strain showed high sensitivity and did not ferment the must.

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