Residual brewing yeasts as a source of beta-glucans

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Abstract. Residual brewing yeast is one of the main solid wastes in brewing. Using residual brewing biomass as a source of biologically active substances is an important way of recycling these brewing by-products. According to the literature *S. cerevisiae* is considered as the promising source of polysaccharides, particularly beta-glucans. Beta-glucans are structural polysaccharides of the yeast cell and perform immune stimulating properties. At the same time, there is too little information about the content of these polysaccharides in brewing yeast of the genus *Brettanomyces*. The objects of this study were yeast cultures of *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis*. In this work, the cultivations of the yeasts were carried out to compare them as possible sources of beta-glucans. The yeasts were cultivated in a simple periodic culture using a laboratory fermenter (Biostat A, Sartorius). As a result, the content of beta-glucans in the yeasts *S. cerevisiae* and *B. bruxellensis* biomass was measured by enzymatic method (Megazyme, Ireland). According to the obtained data, the yeast *B. bruxellensis* contains a higher amount of beta-glucans than the yeast *S. cerevisiae*.

1 Introduction

Any brewery inevitably faces the problem of waste disposal. There is a rising manufacturers interest in recycling by-products. This is important not only from an economic but also from an environmental point of view.

Residual brewing yeast – one of the main solid wastes in brewing. Residual yeast biomass is often considered as a source of proteins, amino acids, minerals, vitamin B complex, lipids, enzymes and RNA [1, 2]. Additionally, residual brewer's yeasts contain significant amount of carbohydrates (35 to 45% dry biomass). It is known that one of the types yeast polysaccharides, beta-glucans, in the yeast cell performs the function of structural polysaccharide, is localized in the cell wall and firmly associated with the mannoprotein complex [3-7].

Polysaccharides in general, particularly beta-glucans, have a long history as immune modulators [6]. Beta-glucans mightily activate both innate and acquired (adaptive)

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immunity, providing protection to the body against invasions of foreign genetic material, as well as to fight against already penetrated infection and carcinogenesis [3, 5, 8].

As a polysaccharide with immune stimulating properties, beta-glucan can be obtained from various sources, including cereals (for example, from oats and barley grain) and certain types of lichens, fungi, including yeast. Beta-glucans obtained from different sources have different structure, for example, β -1,3 / 1,6-glucan is isolated mainly from fungi and yeast, whereas β -1,3 / 1,4-glucan is isolated from lichens and some bacteria. It has been proven that the chemical composition of this polysaccharide determines its biological activity. There are several types of beta-glucans with different levels of activity. Many of them don't have high biological activity and are used only as a source of dietary fiber. This applies to beta-glucans derived from plant materials [9, 10].

Biomedical studies have shown that the biological activity of yeast glucans is significantly higher than the activity of glucans of a different origin, which is largely due to the uniqueness of the structure of this polysaccharide [9-11]. Thus, yeast is one of the promising and easily renewable sources of beta-glucans.

Also, the study of the possible use of beta-glucans in food is of interest. In addition to a beneficial property for human health, beta-glucans also have interesting physicochemical properties. So it was shown that beta-glucans can be used as excellent thickeners, stabilizers and fat substitutes. They have the ability to form viscous solutions and emulsions that support the growth of beneficial intestinal microflora, as well as the synthesis of short-chain fatty acids. Therefore, they improve intestinal motility and contribute to the more rapid removal of harmful, potentially carcinogenic substances from the colon, which reduces the risk of developing colorectal cancer [6].

So, beta-glucans can be obtained from residual biomass, which is a secondary product and in fact waste of fermentation-process in breweries.

Yeast of the genus *Saccharomyces* is the most widely used in the brewing industry. They have many properties that explain their widespread industrial use in the production of fermented beverages and in particular in the brewing industry. These include fast growth, good ethanol production ability, resistance to high ethanol concentration and low oxygen concentration [1, 2].

Yeasts of the genus of *Brettanomyces* are also used in brewing. They are a distant relative of the classic brewing yeast *Saccharomyces cerevisiae*. Historically, these yeasts played a significant role in the fermentation of beer produced by the natural microflora. These sorts of beer are called Lambic and Gueuze [12]. In recent years interest of brewers to the genus, *Brettanomyces* is growing. The use of yeast of this genus opens up additional prospects for creating new unique sorts of beers.

In this case, residual biomass can be used as a raw material for the production of beta-glucans - natural immune modulators. In addition to the immune- modulating properties yeasts beta-glucans can be used in food production as a prebiotic, thickener, emulsifier and stabilizer [6, 13].

Unfortunately, at present there is insufficient data on the quantitative content of beta-glucans in the yeast of genus *Brettanomyces*. This opens up new opportunities in the search for the additional sources of beta-glucans.

2 Materials and methods

The objects of this study were the yeasts *Saccharomyces cerevisiae* strain W68 (Hefebank Weihenstephan, GmbH) and *Brettanomyces bruxellensis* strain WY5112 (Wyeast Laboratories, USA).

Yeast strains of the genus *Saccharomyces* and *Brettanomyces* were selected based on the fact that these strains have the same range of optimal growth temperatures, so that this particularity does not affect the physiology of the yeasts during the experiment.

Yeast was grown on sterile malt wort with a dry mass fraction of 12%. Preparation of the wort: 200 g of ground barley malt with an extract content of 80-81% (produced by Nosters, Russia) was added to 1 l of heated water (50 ± 1) °C. The mixture was thoroughly mixed for 30 minutes. It was heated in a water bath at a rate of 1°C per minute to (55 ± 1) °C, paused for 15 minutes; then the temperature was raised to 63-65°C with the same speed and kept at this level for 1 hour. Then the temperature was increased at a rate of 1°C per minute to (72 ± 1) °C and the wort was kept at this temperature until complete saccharification. Lugol's solution was used to check the degree of saccharification of the wort.

The obtained wort was filtered through the filter cloth, topped up to 1 liter and sterilized for 30 minutes at 107-110 °C. Then the wort was decanted. The filtered wort was diluted with water to a dry matter concentration of $11.5 \pm 0.5\%$. Ammonium sulfate (99.9% purity, produced by Basf, Belgium) was added to the medium as an additional source of nitrogen at the concentration of 2.4 g / l of wort. As an additional source of phosphorus was used diammonium phosphate (>98% purity, obtained from Lenreactiv, Russia) - 0.8 g / l of wort. The medium was dispensed to sterile flasks and sterilized for 15 minutes at (116 ± 1)°C.

To prepare the inoculum, the prepared wort was inoculated with pure yeast culture. Cultivation is carried out in a thermostat at the temperature 30°C for five days (for the yeast of the genus *Brettanomyces*) and for three days (for yeast of the genus *Saccharomyces*). The difference in cultivation time is due to the different metabolic rates of these yeasts.

Cultivation was carried out in a simple periodic culture in a laboratory bioreactor BIOSTAT A (Sartorius, Germany) with a working volume of 2 l in the absence of forced aeration at the temperature 30°C. The initial concentrations of the yeasts were 12 million cells in 1 ml of medium. The pH was kept up at a constant level of 4.5 ± 0.2 by automatically adding 10% solutions of phosphoric acid and ammonium hydroxide (Lenreactiv, Russia).

The accumulation of biomass (absolutely dry yeast biomass) was determined gravimetrically after drying the suspension of the washed yeast to constant mass at 105°C. The dry matter content in the nutrient medium was determined using an Alcozyer Beer Analyzing System equipped with density meter Anton Paar DMA 4500 (Anton Paar GmbH, Austria).

The economic coefficient (biomass yield) was calculated as the ratio of the yield of absolutely dry yeast biomass to the amount of substrate consumed and estimated in %.

The content of beta-glucans in *S. cerevisiae* and *B. bruxellensis* yeast biomass was determined using the β -glucan Assay Kit (Yeast & Mushroom) (Megazyme, Ireland).

3 Results and discussion

The cultivation processes were controlled by indicators of biomass accumulation, and a decrease in the concentration of dry substances in the medium. The morphological state of the culture was estimated using a Zeiss light microscope (Germany) equipped with a camera. The results are shown in Figs 1, 2.

The culture *S. cerevisiae* (W68) was grown for 22.5 hours. The growth curve of *S. cerevisiae* in the BIOSTAT A bioreactor (Sartorius, Germany) is shown in the Fig. 1. The culture *B. bruxellensis* (WY5112) was grown under the same conditions for 135 hours (Fig. 2).

Fig. 1. Growth and substrate consumption in a batch culture of *Saccharomyces cerevisiae* (W68).

Fig. 2. Growth and substrate consumption in a batch culture of *Brettanomyces bruxellensis* (WY5112).

From the figures it is clear, that the *Saccharomyces cerevisiae* yeast (W68) demonstrates a higher rate of biomass accumulation compared to *Brettanomyces bruxellensis* yeast (WY5112).

In general, both growth curves have the expected sigmoidal shape. Substrate consumption curves correlate well with growth curves for both *S. cerevisiae* and *B. bruxellensis*. The final substrate concentration for the *B. bruxellensis* strain was 4.8 g / 100 ml, which is slightly higher than the final substrate concentration for the *S. cerevisiae* strain (2.5 g / 100 ml) as shown in Fig. 2. This may be due to the inhibitory effect of some secondary metabolites of yeast of the genus *Brettanomyces* [12, 14].

The strain of the yeast *Saccharomyces cerevisiae* reached the stationary growth phase in 22.5 hours, while the strain *Brettanomyces bruxellensis* reached the stationary phase after

105 hours of cultivation. This data is in accordance with the well-known data on the slow metabolism of yeast of the genus *Brettanomyces* [12, 14].

The economic factors were calculated: for the culture *S. cerevisiae* - 8.9%, for *B. bruxellensis* - 7.3%.

Samples for micrographs were taken in the logarithmic growth phase (*S. cerevisiae* - 16.5 hours of cultivation, *B. bruxellensis* - 69 hours). Micrographs cultures are shown in Figs 3-4.

Cells of *S. cerevisiae* have an oval shape, characteristic of this species of yeast. Yeast *B. bruxellensis* has smaller vegetative forms, but at the same time show a tendency to the formation of pseudomycelium. The formation of pseudomycelium is one of the possible ways of adapting yeast and has not been studied enough in yeast of this genus up to now [14].

Fig. 3. The micrograph of yeast culture *Saccharomyces cerevisiae* (W68) (x640).

Fig. 4. The micrograph of yeast culture *Brettanomyces bruxellensis* (WY5112) (x640).

The formation of pseudomycelium occurs through cell elongation due to the mechanism that disturbs their division. The reason for this is a delay in cell development during the G2 / M phase, which leads to long-lasting directed polarized growth without separation after cytokinesis [14]. As a result, pseudomycelium is formed. Compare to true hyphae, pseudomycelium are filaments that do not possess multiple nucleuses [15, 16].

Changes in cell morphology are directly related to changes in the composition of the yeast cell walls polysaccharides. Usually, elongated forms contain more beta-glucans [17, 18]. At the next stage of the study the content of total glucans, alfa-glucans (glycogen) and beta-glucans in *S. cerevisiae* and *B. bruxellensis* yeast was evaluated using the β -glucan assay kit (Yeast & Mushroom) (Megazyme, Ireland). The results of measurement are in Table 1.

Table 1. The content of different types of glucans in yeasts

Yeast species	Content of glucans, % of ADB*		
	total glucans	α -glucans (glycogen)	β -glucans
Saccharomyces Cerevisiae	13.5 $\pm$ 0.3	5.6 $\pm$ 0.2	7.9 $\pm$ 0.3
Brettanomyces bruxellensis	11.6 $\pm$ 0.2	0.8 $\pm$ 0.2	10.8 $\pm$ 0.2
* ADB - absolutely dry biomass			

Studies have shown that yeasts of the genus *Brettanomyces* contain more beta-glucans and synthesize much less α -glucans compared to *Saccharomyces* yeasts.

It was noted that the metabolism of the *Brettanomyces* yeast is slower than the metabolism of *Saccharomyces*; therefore, at this stage of research, the yeast of the genus *Brettanomyces* cannot be recommended as a producer of beta-glucans. Despite this, the biomass of the yeast which is a waste product of the brewing industry can probably be used in industrial processing in order to obtain these natural immune modulators.

4 Conclusion

This paper presents comparison of yeasts from genus of *Brettanomyces* and *Saccharomyces* according to the following parameters: the morphological characteristics, the metabolic characteristics, and the qualitative and quantitative composition of glucans, which are natural immune modulators.

The obtained data indicate the possibility of using the residual yeast biomass of *B. bruxellensis* as a source of beta-glucans.

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