Study of antimicrobial activity and fermentability of the yeast Wickerhamomyces anomalus in wheat dough

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Abstract. The work is aimed at assessing the potential of using the yeast culture Wickerhamomyces anomalus CBS605T in bread baking. The in vitro antimicrobial activity of the yeast culture Wickerhamomyces anomalus (CBS605T) against the causative agent of potato disease, Bacillus subtilis, was studied. A rheoenzymometric assessment of the gas-forming and gas-retaining abilities of the studied strain during fermentation in wheat dough was carried out. The study showed that the yeast culture Wickerhamomyces anomalus (CBS605T) effectively inhibits the growth of Bacillus subtilis and has higher fermentation activity than the control organism Saccharomyces cerevisiae var. boulardii (Y3925), also known for antimicrobial activity against the pathogen of potato disease.

1 Introduction

Wickerhamomyces anomalus (formerly known as Pichia anomala, Hansenula anomala) is one of the widely used species of industrial microorganisms. This yeast is used in winemaking [1], in the production of beer [2], bioethanol [3], and in other industries. In nature they are found, in particular, on the surface of cereals and in flour; Moreover, they are also a normal part of the transient microflora of the human skin and oropharynx [4].

One of the most valuable characteristics of the yeast Wickerhamomyces anomalus is the unique flavor profile it produces, which is described as “fruity,” “floral,” and “herbal.” These features are due to the production of a wide range of flavoring components, such as 1-propanol, 2-methyl-1-propanol, methylbutanol, beta-phenylethanol, ethyl butyrate (oil ether), and others [2], as well as their special enzymatic activity, which allows metabolize associated flavoring components of raw materials [5].

In addition, this yeast is known to be highly resistant to unfavorable environmental conditions, including a wide range of pH (2.0-12.4), temperatures (3-37°C), as well as the ability to develop in conditions of high concentration of sugars in the nutrient medium (up to 160 g/l). However, within the framework of alcohol production, the problem of their low resistance to ethanol arises - this issue is also considered in the scientific community [3, 6].

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Finally, there are studies examining the antimicrobial activity of Wickerhamomyces anomalus against a number of organisms, including Acinetobacter baumannii [7], Staphylococcus aureus [8], and many others [9].

It is known that Wickerhamomyces anomalus is capable of fermenting maltose, the main sugar formed during the enzymatic breakdown of flour starch, which, combined with their native presence in flour and low sensitivity to high acidity of the environment, makes them a common component of spontaneous sourdoughs [4].

Due to the combination of these characteristics, there is interest in studying the potential of using a pure culture of the yeast Wickerhamomyces anomalus in baking. This article is devoted to the study of relevant aspects, namely, the assessment of gas-forming and gas-retaining abilities during fermentation in wheat dough, as well as the antimicrobial activity of the yeast culture Wickerhamomyces anomalus (CBS S605T).

2 Materials and methods

2.1 Materials

To conduct the study, the strain Wickerhamomyces anomalus CBS S605T was used, isolated from flour obtained from buckwheat grain that was not hydrothermally treated.

Saccharomyces cerevisiae var. was used as control samples when studying the rheological characteristics and antimicrobial properties of the resulting dough. boulardii Y3925, obtained from the bioresource center "All-Russian Collection of Industrial Microorganisms" (VKPM) of the National Research Center "Kurchatov Institute", as well as classic baker's yeast produced by Lesaffre. The antimicrobial potential of the yeast Saccharomyces cerevisiae var. has been previously reported. boulardii, which allows us to evaluate the potential of Wickerhamomyces anomalus CBS S605T as an antimicrobial agent.

Bacillus subtilis subsp. bacteria were used as a test microorganism when considering antimicrobial activity. subtilis B-9865 (correspond to strains DSM 10, ATCC 6051, CCM 2016, NCIB 3610), also obtained from the bioresource center “All-Russian Collection of Industrial Microorganisms” (VKPM) of the National Research Center “Kurchatov Institute”.

2.2 Preparation of yeast samples

Sterile barley wort with a dry matter concentration of 10 g/100 ml was used as a nutrient medium for cultivating yeast to accumulate biomass. Apparent solids concentration was assessed using a PTR46X automatic refractometer (Index Instruments).

The viability of the resulting biomass of the studied yeast was assessed by staining with methylene blue dye, followed by assessment of the number of dead cells in the culture, and microscopy of the stained preparations (Zeiss AX10 light microscope, magnification x640). For further studies, cultures containing no more than 3% dead cells were used.

The biomass for rheofermentometric studies was washed with prepared water, followed by separation of the sediment by centrifugation (Hettich Rotanta 460 centrifuge, 4500 rpm, RCF = 4573g, 20 minutes). The washing was repeated until an irreducible turbidity of the supernatant was achieved.

In the resulting biomass, using an automatic moisture meter UniBloc MOC63u, the moisture content was determined, the value of which was taken into account in further experiments.
2.3 Carrying out rheoenzyme analysis

The fermentation ability of yeast was determined using a Chopin V3 rheoenzyme meter. The volume of carbon dioxide was assessed, both retained by the dough and released into the environment.

The dough was produced according to the method specified by the manufacturer of the device for a standard measurement program. To produce it, we took 250 g of premium wheat flour, an amount of water corresponding to the moisture content of flour and yeast (121-123 ml, temperature 21 °C), 5 g of table salt, as well as yeast biomass in an amount corresponding to 1.75 g of absolutely dry substances.

The kneading was done on a Kenwood Chef planetary mixer with a hook attachment for 1 minute at speed 1 and 6 minutes at speed 2, according to the instructions for the measurement program. The resulting dough at a temperature of 28.5°C was placed in a rheoenzyme meter glass. The measurement was carried out for 3 hours for each sample.

2.4 Testing for antimicrobial activity

Antimicrobial activity was assessed in vitro on agar medium.

Agar and liquid YEPD media (1% yeast extract, 2% peptone, 2% glucose, if necessary 2% microbiological agar, 93-95% distilled water) were used for evaluation. Bacillus subtilis bacteria were uniformly inoculated onto the surface of the YEPD agar medium in an amount of 106 CFU per 1 cup using a Drigalski spatula. After this, holes with a diameter of 5 mm were cut out in each of the cups using a sterile instrument. Cultures of Wickerhamomyces anomalus or the studied strains of Saccharomyces cerevisiae were added to these wells. The dishes were incubated in a thermostat for 24 hours at a temperature of 37°C, after which the radius of the zone of inhibition of the growth of Bacillus subtilis bacteria was measured. The intensity of antimicrobial activity was judged by the size of this radius.

3 Results

3.1 Results of rheoenzyme analysis

At the end of cultivation, the cultures showed high viability: the content of living cells in all samples was at least 99%.

A comparison of the dynamics of carbon dioxide emissions by the studied organisms is presented in Figure 1.

The yeast Wickerhamomyces anomalus CBS605T is significantly inferior to industrial samples of Saccharomyces cerevisiae in its ability to ferment dough, but in this parameter it is superior to Saccharomyces cerevisiae var. boulardii Y3925, known for their antimicrobial activity.
3.2 Results of antimicrobial activity assay

Evaluation of antimicrobial activity in vitro showed that Wickerhamomyces anomalous CBS605T has pronounced antagonism to Bacillus subtilis, like the yeast Saccharomyces cerevisiae var. boulardii Y3925, in contrast to the classic strain of baker's yeast (Lesaffre).

Thus, after 24 hours, the growth inhibition zone of Bacillus subtilis around the wells with Saccharomyces cerevisiae var. boulardii Y3925 was 9-10mm; the radius of the growth inhibition zone around the wells with Wickerhamomyces anomalous CBS605T was 10-11mm. A visual comparison is shown in Figure 2.

Fig. 1. Dynamics of carbon dioxide release by the yeasts Wickerhamomyces anomalous CBS605T and Saccharomyces cerevisiae var. boulardii Y3925 in comparison with the industrial sample Saccharomyces cerevisiae.

Fig. 2. Zones of Bacillus subtilis growth inhibition around wells with different yeast samples (from left to right: Saccharomyces cerevisiae var. boulardii Y3925, Wickerhamomyces anomalous CBS605T and classic baker's Saccharomyces cerevisiae).

4 Discussion

The antimicrobial activity of the yeast Wickerhamomyces anomalous is widely covered in the scientific literature [7-9]; Moreover, the mechanisms of formation of killer proteins in yeast of this species have been studied and the genetic sequences responsible for this have been identified [11].
However, knowledge about the antimicrobial activity of Wickerhamomyces anomalus against a number of industrially important spoilage microorganisms is currently significantly limited or non-existent. One of these organisms is Bacillus subtilis, which causes potato bread disease. Taking into account the fact that Wickerhamomyces anomalus can be part of the natural microflora of flour and can multiply in wheat sourdoughs [4], the study of the antagonism of Wickerhamomyces anomalus in relation to Bacillus subtilis is an important part of the study of the general antimicrobial effect of sourdoughs; In addition, a high level of antagonism in combination with remarkable organoleptic characteristics may become a prerequisite for the use of Wickerhamomyces anomalus as a pure yeast culture in baking.

The key difficulty in using Wickerhamomyces anomalus in this area seems to be its lower fermentation activity compared to industrial strains of Saccharomyces cerevisiae. Despite the ability to assimilate maltose, the studied strain of Wickerhamomyces anomalus CBS605T is significantly inferior in fermentation intensity to industrial strains of Saccharomyces cerevisiae, which may lead to the need for a longer technological process. Potential solutions may include the search for new strains of Wickerhamomyces anomalus with higher fermentation activity, as well as the study of mixed yeast cultures of Wickerhamomyces anomalus and Saccharomyces cerevisiae.

5 Conclusion

Thus, the Wickerhamomyces anomalus strain CBS605T is superior in the combination of antimicrobial activity and rheological characteristics to the Saccharomyces cerevisiae var. boulardii Y3925, previously studied and recommended as a microorganism that prevents the development of potato disease in bread.

Moreover, despite the ability of Wickerhamomyces anomalus CBS605T to ferment maltose, the rate of this process is significantly lower than that of the industrially used yeast Saccharomyces cerevisiae (Lesaffre).

At the next stage, a study of the antimicrobial effect of the yeast Wickerhamomyces anomalus CBS605T directly in wheat bread will be carried out. In addition, microbial starters and yeast compositions that are promising for the industrial production of bread using this yeast strain will be selected.

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References

2. P. Satora, A. Pater, Applied Sciences, 13, 2872 (2023)