Evaluation of breadmaking potential of the yeasts *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3)

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Abstract. In a study, Wickerhamomyces anomalus (CBS S605T) and Torulaspora delbrueckii (YIT3) yeasts were analyzed in comparison with a breadmaking strain of Saccharomyces cerevisiae ("Luxe", SAF-Neva LLC). The study has shown that respective yeast species all have moderate or high levels of acid and osmotic tolerance and are able to ferment fructose, glucose, maltose and sucrose. According to the results of gas chromatrographic analysis, the yeast Wickerhamomyces anomalus (CBS S605T) accumulates significant amounts of ethyl acetate (11.8 times more than the control strain of Saccharomyces cerevisiae), while Torulaspora delbrueckii (YIT3) produces 3.2 times more aliphatic alcohols than the control strain. Rheofermentometric study in wheat dough demonstrates 29,6% lower carbon dioxide production by Wickerhamomyces anomalus (CBS S605T), and 64.5% lower production by Torulaspora delbrueckii (YIT3) as compared to control strain of Saccharomyces cerevisiae. Based on the data obtained, conclusions were drawn on the possibility of effective use of Wickerhamomyces anomalus (CBS S605T) and Torulaspora delbrueckii (YIT3) in sourdough breadmaking.

1 Introduction

Bread is one of the products that remains relevant for millenia as an important part of the diet for people all around the globe. According to Mordor Intelligence, the current global market for bakery products is estimated at $621.58 billion [1] and is one of the key markets in the food industry. In the context of growing competition, this determines the importance of finding new ways to develop in the market and the need to research innovative and unique products with specific properties.

Traditionally, *Saccharomyces cerevisiae* yeasts are used to ferment flour carbohydrates, producing ethanol and carbon dioxide that raises and leavens the dough. The by-products of this process – aldehydes, alcohols, esters and organic acids – form the flavor and aroma of bread [2]. However, other yeast species non-classical to industrial baking have attracted the attention of researchers. Some of them are known for their increased resistance to various

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environmental factors, unique flavor profile of secondary metabolites, and other metabolic features.

The study investigates two yeast strains – *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3) – in the context of their potential application in the baking industry. In order to obtain a reference point, commercial breadmaking strain of *Saccharomyces cerevisiae* ("Luxe", SAF-Neva LLC) has served as control.

Compared to *Saccharomyces cerevisiae,* the *Wickerhamomyces anomalus* yeasts are characterized by active aerobic and weak anaerobic metabolism. Under anaerobic conditions, the yeast *Wickerhamomyces anomalus* normally exhibits low rates of carbohydrate fermentation and low biomass yield [3].

One of the key features of *Wickerhamomyces anomalus* yeasts is their ability to produce killer toxins that allow them to inhibit the growth of certain microorganisms, particularly *Staphylococcus aureus* [4] and *Acinetobacter baumanii* [5]. For the investigated strain of *Wickerhamomyces anomalus,* CBS S605T*,* an antimicrobial activity against Bacillus subtilis has already been reported [6]. Such biocontrol strategy may serve as an alternative to the use of preservatives and makes the studies into the use of *Wickerhamomyces anomalus* in breadmaking highly relevant.

The *Torulaspora deblbrueckii* yeast species have also become a popular research object in recent years thanks to the unique flavor and aroma profile of their secondary metabolites, particularly when used in combination with *Saccharomyces cerevisiae,* as well as due to their high tolerance to osmotic stress and freezing. Members of this species are able to remain viable at -20°C for 4 months, while the viability of Saccharomyces cerevisiae under the same conditions drops by 80% in only 15 days of storage [7].

In a study dedicated to the use of *Torulaspora delbrueckii* combined with *Saccharomyces cerevisiae*, it was shown that this microbial combination allows to achieve ethyl acetate levels of up to 25 mg/L, which is about 6 times higher than results achieved with these cultures used separately, as well as sharp increase in the concentrations of various alcohols, including n-propanol, isobutanol, amyl and isoamyl alcohol, and beta-phenylethanol [8].

In relation to the joint use of these cultures in breadmaking, it is known that samples produced with combined culture of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* contained 3,7 times more n-hexanal, 2,2 times more isoamyl acetate, 56% more benzaldehyde and 29% more phenylethanol, which lead to product developing more herbal, floral and almond flavors [9].

2 Materials and methods

2.1 Characteristics of studied yeast strains

The yeast strains Wickerhamomyces anomalus (CBS S605T) and Torulaspora delbrueckii (YIT3) isolated as part of research work at ITMO University (Russian Science Foundation grant No. 23-26-00134) were used. The strains were sequenced using the Sanger method at the Genomic Technologies, Proteomics, and Cell Biology Center of the Federal State Research Institute of Agricultural Biology. Species identification was performed by comparing the obtained nucleotide sequences of genes with the sequences of reference strains of the key yeast species in the GenBank database (NCBI, USA).

Commercial breadmaking strain of Saccharomyces cerevisiae ("Luxe", SAF-Neva LLC) was used as a control sample

2.2 Screening test of biochemical properties

In the screening test, the growth of studied microorganisms was evaluated under a range of temperatures, acidity, osmotic pressures and ethanol contents.

The study used two-day cultures of the strains under investigation; the cultures were based on a liquid YEPD medium (1% yeast extract, 2% peptone, 2% glucose) and were cultivated at 26°C.

To investigate the possibility of culture growth at different temperatures (+4°C, +15°C, +25°C, +30°C, +35°C), surface seeding of suspensions of the studied yeasts on agarized YEPD medium (yeast extract - 1%, peptone - 2%, glucose - 2%, agar - 2%) was carried out.

To prepare mediums for the acidity resistance tests of the strains, a sulfuric acid solution with a concentration of 6 mol/L was used. 0.02 ml or 0.2 ml of sulfuric acid solution was added to a petri dish and poured into 20 ml of molten agarized YEPD medium. After solidification of the medium, suspensions were seeded on the surface. The results of yeast cultivation on the experimental nutrient media were compared with the growth of the culture on medium without acid addition [10].

The resistance to osmotic stress was tested by evaluating the growth of cultures on agarized YEPD medium with the addition of glycerol by 2.5%; 5.5%; 10% and 15% of the medium volume [10].

Ethanol resistance was assessed by evaluating the growth of cultures on YEPD agarized medium, with ethanol added by 4%; 6%; 8%; 10%; 12% and 16% of the volume [10].

In all cases, except for the study of microbial growth under various temperatures, the cultivation was carried out at 26°C for 72 hours. The results of cultivation were recorded after 48 hours and at the end of the experiment.

2.3 Determining the concentrations of volatile compounds

To determine the concentration of volatile compounds synthesized by the studied yeast cultures, yeast samples were cultivated in sterilized barley wort medium with a dry matter content of 10g/100ml at 30°C for 5 days. Wort distillate was obtained in accordance with GOST 32095-2013 [11]. The distillates were analyzed by the method of gas chromatography in accordance with GOST 32039-2013 [12] and GOST R 52363-2005 [13].

2.4 Determining the leavening ability

The total fermentation activity of studied yeast strains was measured using the modified Warburg method [14]. The time of the experiment was increased to 3 hours in order to adequately assess the activity of the studied strains and to increase the accuracy of measurements under conditions of reduced fermentation rate based on preliminary studies.

The leavening ability of studied yeast strains in a wheat dough was conducted using a Chopin F3 rheofermentometer (KPM Analytics, USA). The accumulation of yeast biomass was carried out in a sterilized barley wort medium with the dry matter content of 12g/100ml in 3 stages: on the first stage, the pure culture was inoculated into 30ml medium and cultivated at 30°C for 48 hours; on the second stage, the resulting culture was move into 300ml medium and cultivated under the same conditions; on the third stage, the resulting culture was moved into 3L medium and cultivated at 30°C for 120 hours, while regularly shaking the flask.

Separation of yeast biomass was conducted by centrifugation (4500 rpm, 4573g RCF, 20 minutes) using Rotanta 460 centrifuge (Hettich, Germany), followed by washing. The resulting precipitate was dried by the means of vacuum filtering on the Büchner funnel with

a paper filter. The moisture content of the resulting biomass was measured using Unibloc MOC63u automatic moisture analyzer (Shimadzu, Japan). The amount of biomass used for rheofermentometric analysis, as well as the amount of water added were adjusted accordingly.

The dough sample preparation was conducted in accordance with the device instruction manual. Analysis was carried out using the standard measurement mode provided by the device manufacturer, and lasted 3 hours at the temperature of 28°C. The mass of the dough sample was 315g, the yeast biomass calculated by dry matter was 1,75g. The results for the analysis were presented as a table containing the maximum value on gas release curve, time of peak gas release, time of pore formation, lost and retained volume of carbon dioxide, as well as the respective retention coefficient.

3 Results

During the first stage, the resistance of investigated microorganisms to various psysico-chemical condition during cultivation was evaluated. Table 1 describes conditions, under which the cultures of studied yeast strains demonsrate visible growth.

Table 1. Cultivation parameters resulting in growth of cultures of studied organisms.

Sample	Temperature range	Acid resistance, cm^3 of 6 mol/L H_2SO_4	Ethanol concentration, %	Osmotic resistance, % of glycerol
Saccharomyces cerevisiae ("Luxe", SAF-Neva LLC)	+25…+35	0.02	4…16	2.5…15
Wickerhamomyces anomalus (CBS S605T)	+15…+35	0.2	4...12	2.5…15
Torulaspora delbrueckii (YIT3)	+15…+35	0.02	4...12	2.5…15

On the next stage of the research, the concentrations of the main volatile metabolites, namely acetic aldehyde, benzaldehyde, aliphatic and aromatic alcohols (1-propanol, isobutanol, isoamyl alcohol, phenylethanol) and ethyl acetate, synthesized by the studied yeast strains on 10g/100ml sterilized barley wort medium, were determined. The results of the investigation is presented in Table 2.

Table 2. The results of determining mass concentrations of volatile compounds in the distillates of wort fermented by the cultures of studied yeast strains.

Determined substance	Mass concentration of the compound, mg/L		
	***Saccharomyces cerevisiae* ("Luxe", SAF-Neva LLC)**	***Wickerhamomyces anomalus* (CBS S605T)**	***Torulaspora delbrueckii* (YIT3)**
Acetic aldehyde	9.1±1.4	13.4±2.0	58.7±8.8
Fusel alcohol	Total – 119	Total – 38.1	Total – 380, including 2-propanol – 34.8±5.2
1-propanol	15.8±2.4	6.7±1.3	61.3±9.2
Isobutyl alcohol	21.0±3.2	8.2±1.2	69.1±10.4
Isoamyl alcohol	82.6±12.4	23.3±3.5	215±32
Phenylethanol	35.8±3.8	9.2±1.4	48.4±7.3
Benzaldehyde	1.37±0.21	0.63±0.09	3.84±0.58
Ethyl acetate	6.7±1.0	87±13	38.9±5.8

On the next stage of research, an investigation into leavening ability of studied strains was conducted. The results of the analysis are presented in Table 3.

Table 3. Analysis of carbohydrate fermentation intensity by the cultures of studied yeast strains by modified Warburg method.

Sample	Volume of CO_2 released, ml			
	Glucose	Fructose	Maltose	Sucrose
Saccharomyces cerevisiae ("Luxe", SAF-Neva LLC)	10	10	10	10
Wickerhamomyces anomalus (CBS S605T)	3	3	1	3
Torulaspora delbrueckii (YIT3)	3	2	1	1

The results presented in Table 3 confirm the ability of *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3) to ferment glucose, fructose, maltose and sucrose similarly to *Saccharomyces cerevisiae* ("Luxe", SAF-Neva LLC), albeit less rapidly.

Finally, the leavening ability of the studied cultures in wheat dough was determined. The results of the rheofermentometric analysis are demonstrated in Table 4.

Table 4. Results of rheofermentometric analysis.

Metric	Yeast strain studied		
	***Saccharomyces cerevisiae* ("Luxe", SAF-Neva LLC)**	***Wickerhamomyces anomalus* (CBS S605T)**	***Torulaspora delbrueckii* (YIT3)**
Gas release curve maximum, mm	66.2	45.9	27.2
Time of peak gas release, hh:mm:ss	02:01:30	02:30:00	03:00:00
Time of pore formation, hh:mm:ss	01:09:00	02:06:00	02:57:00
Volume of CO_2 produced, ml	1438	1012	510
CO_2 retention coefficient, %	81.1	94.7	99.3

4 Discussion

The investigation into the influence of physicochemical conditions of the medium on the growth of the studied microorganisms showed that both studied strains demonstrate growth in the temperature range of +15...+35 ºC. They have moderate (*Torulaspora delbrueckii* YIT3) to high (*Wickerhamomyces anomalus* CBS *S605T) acid* resistance, and are able to withstand significant osmotic pressure of the cultivation medium (growth of both organisms was observed on media with the addition of 2.5% to 15% glycerol). The obtained results indicate the possibility of using yeasts *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3) in the composition of dough.

The results of gas chromatography have confirmed existing data on the possible influence of used strains on flavor and aroma of bread, with *Wickerhamomyces anomalus* (CBS S605T) demonstrating 11,8 times higher ethyl acetate output, and *Torulaspora delbrueckii* (YIT3) producing 3,2 times more aliphatic alcohols compared to the control strain.

Based on the research by modified Warburg method a conclusion was drawn that all studied yeast strains are able to ferment glucose, fructose, maltose and sucrose; however,

the fermentation occurs less rapidly compared to the control strain of *Saccharomyces cerevisiae* ("Luxe", SAF-Neva LLC).

Rheofermentometric analysis confirms the data obtained and demonstrates that studied strains of *Wickerhamomyces anomalus* and *Torulaspora delbrueckii* both have lower leavening ability in wheat dough compared to control sample of *Saccharomyces cerevisae* ("Luxe", SAF-Neva LLC), thereby requiring more time to form the required structure of the crumb. As such, the yeast strains studied can be recommended to research as part of sourdough formulations alongside *Saccharomyces cerevisiae*, which should become the focus of future research.

5 Conclusion

The research conducted has shown that *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3) yeasts can be recommended to use in sourdough formulations. Additional research is required to determine their role in sourdough compositions and, thereby, the conditions for their use in breadmaking.

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