DIVERSITY AND PHYTATE-DEGRADING POTENTIAL OF YEAST MICROORGANISMS ISOLATED FROM SOURDOUGH

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ABSTRACT

Phytases, which perform the stepwise hydrolysis of phytic acid to myo-inositol and inorganic phosphate, are used worldwide to reduce phosphorus pollution and improve nutrition in monogastric animals and humans. Yeasts isolated from their natural environments represent rich and still underexplored sources of industrially valuable enzymes, including phytases; therefore, they are widely studied for the production of these enzymes. In this regard, thirteen yeast pure cultures were isolated from the microbial consortium of four types of sourdough obtained during the natural fermentation of different grain-based flours. Ten of the newly isolated yeast strains were selected as potential phytase producers based on their growth in liquid culture media with sodium phytate as the sole source of phosphorus. Using 18S rDNA and D1/D2 26S rDNA analyses, the species affiliation of the selected isolates was established. They referred to seven yeast species from 3 families, with the most significant representation of the family Saccharomycetaceae. Intracellular phytate-degrading activity was found in 8 isolates, the highest being in Nakaseomyces glabratus strain 7-4. The highest level of extracellular phytase was measured in Pichia membranifaciens strain 5-2. Both isolates showed significant antioxidant capacity higher than those of ascorbic acid.

Keywords: yeast, phytase, sourdough, feed additive, 18S rDNA, 26S rDNA.

INTRODUCTION

Phytases are a class of enzymes that catalyse the hydrolytic degradation of phytic acid to free inorganic phosphorus and lower molecular weight myo-inositol phosphate esters [1]. Among the phytate-degrading enzymes described so far, the most widespread are those belonging to the group of histidine-acid phosphatases (HAPs) (EC 3.1.3.8), which are found in both microorganisms and higher eukaryotes [2]. The addition of phytases to animal feed, on the one hand, increases the bioavailability of digestible phosphorus and minerals by breaking down phytic acid, thus removing its anti-nutrient effect and ensuring balanced nutrition. On the other hand, phytases, included as feed additives, lead to a reduction in the amount of undigested phytate in the manure, which significantly reduces the negative consequences for the environment [3, 4]. Microorganisms are a promising source of phytate-degrading enzymes. A significant number of bacterial and fungal species that produce phytases have been isolated from diverse environments [5, 6]. Phytate-degrading enzymes isolated from *Aspergillus niger*, *Peniophora lycci* and *Escherichia coli* are added to animal feed to improve the bioavailability of phosphorus and minerals [6 - 8]. Even though some of the already described microbial phytases have found industrial applications, they are still unable to meet all the requirements of the feed industry. The search for new phytases with high activity and stability at temperatures above 37°C and acidic pH, accompanied by low production cost, is the subject of increased scientific interest [9]. Yeasts are

good candidates for enzyme production due to their ease of cultivation, rapid growth, and genetic stability. Moreover, these eukaryotic microorganisms naturally inhabit the surface of phytic-acid-containing crops, vegetables, and other plants, suggesting the production of phytate-degrading enzymes from them. They could be isolated from various cereal-based fermented foods and beverages and are of great importance for preserving and improving the quality of food products due to their antioxidant and hydrolytic capacity. In the present study, we screened thirteen yeast cultures, newly isolated from different types of sourdough, for their extracellular and intracellular phytase production. The isolates with the highest phytase activity were taxonomically identified to a species level. The antioxidant capacity of the selected yeast microorganisms was also studied in relation to their potential industrial application in food and feed processing.

EXPERIMENTAL

Sourdough preparation

Four different types of cereal flour (wheat white flour (S5), rye flour (S6), wholegrain rye flour (S7) and white wheat flour type 500 (85 %) and type 1850 (15%) (S8)) were used as a raw material for spontaneous microbial fermentation and production of sourdough. Mixtures of 5 g flour and 5 mL prewarm distilled water were prepared and left for 24 hours at room temperature. The sourdough fermentation was performed for 7 days, and every 24 hours a new portion of flour and water was added to the mixture. The obtained fermented product was stored at 4ºC and used as a source for further analyses.

Isolation of pure yeast cultures from sourdough

Enrichment of yeast cultures was performed for each sourdough sample (S5, S6, S7 and S8) (~10 g of each) aerobically at 28°C for 2 days, in 500 mL Erlenmeyer flasks containing 100 mL sterile YPD medium (20 g glucose, 10 g peptone and 10 g yeast extract per litre of distilled water, pH 6.3). Antibiotics Tetracycline and Streptomycin were added at a final concentration of 50 mg L^{-1} to inhibit bacterial growth. Ten-fold dilutions from the enriched microbial cultures S5, S6, S7 and S8 were inoculated onto YPD plates to isolate single colonies. The cultures were then incubated at 28ºC for 48 h. Morphologically, different types of colonies were selected after incubation, and pure cultures were obtained after at least three repeated cultivations on agar.

Taxonomic identification of the yeast isolates

Morphological characterization

The morphology of yeast colonies was observed on a solid YPD medium using a binocular magnifier. The morphology of yeast cells was observed by light microscopy.

Biochemical characterization

To taxonomically identify the newly isolated yeast microorganisms, a biochemical rapid identification test API 20 C Aux (bioMerieux) was performed according to the manufacturer's instructions. The results obtained were processed by apiweb@ software (bioMerieux).

Genetic characterization

Isolation of genomic DNA

The extraction of gDNA was performed according to the protocol described by Biss et al. [10].

PCR amplification and sequencing of 18S rDNA and D1/D2 region of 26S rDNA

Nearly the entire nucleotide sequence of the 18S rDNA gene (22 - 1771 nt; *Saccharomyces cerevisiae* numbering) was amplified from the extracted DNA for all yeast species using universal primers NS-1F (5`- GTAGTCATATGCTTGTCTC) and NS-8R (5`- TCCGCAGGTTCACCTACGGA) [11]. Domains 1 and 2 of the 26S r DNA gene (63 - 642 nt; *S. cerevisiae* numbering) for 2 yeast species were amplified using oligonucleotide primers NL - 1F (5`- GCATATCAATAAGCGGAGGAAAAG) and NL - 4R (5`- GGTCCGTGTTTCAAGACGG) as described by Kurtzman and Robnett [11]. The PCR reaction mixture (25 μL) contained: 1 to 10 ng of DNA, PCR Master Mix 2x (GENET BIO) containing 0.625 U Taq DNA Polymerase, and 400 nM of each primer. The reaction mixture was incubated in a Thermal Cycler (LKB) for an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60s, then a final extension step at 72°C for 5 min.

The amplified DNA fragments were sequenced in Macrogen Europe B.V, Netherlands. The same oligonucleotide primers were used for the sequencing. The obtained sequences were compared to the known

sequences in the GenBank database by using BLASTn search to determine their close relatives.

Screening of the yeast isolates for phytase production

The screening was performed on solid and liquid mineral medium (Phytase Screening Medium PSM), according to Palla et al. [12]. It contained (w/v): 1.0 % glucose, 0.4 % sodium phytate (Sigma, USA), 0.2 % $CaCl_2$, 0.5 % NH_4NO_3, 0.05 % KCl, 0.05 % $MgSO_4$, 0.001 % $FeSO_4$, 0.001 % $MnSO_4$ (pH 5.0). Phytase production by yeast isolates from S1, S2, S3, and S4 sourdough samples was determined qualitatively by the agar diffusion method. The culture broth of each yeast strain (0.1 mL) (grown on PSM for 48 h) was dropped into wells on PSM agar plates or a loop of 24 h pure yeast culture was stroked on the test media. After incubation for 72 h at 28°C, a specific two-step staining of the medium with an aqueous solution of $CoCl_2$ and subsequent soaking with a solution of 6.25 % $N_6H_{24}Mo_7O_{24}$ x $4H_2O$ and 0.42 % NH_4VO_3 (1:1) was applied as described by Bae et al. [13]. A commercial phytase enzyme preparation (Sigma-Aldrich) with a concentration of 0.4 U mL^{-1} was used in the experiments as a control. The positive phytase activity was shown as the presence of greenish zones of phytate degradation against yellow background.

The ability of the selected yeast isolates to degrade sodium phytate was detected also by their growth in the liquid PSM medium containing 0.4 % (w/v) sodium phytate as a sole source of phosphorus. Isolates were cultivated at 28°C for 48 h in flasks, and the growth was monitored by measuring the optical density at 600 nm (OD_{600nm}).

Intracellular and extracellular phytase activity assay

Phytase activity was assayed in a 1.075 mL total reaction mixture containing 0.2 % Phytic acid sodium salt (Sigma) in sodium-acetate buffer (0.05 M, pH 5.5) with 2 mM $CaCl_2$ at 30°C for 30 min. The reaction was terminated by adding 10 % trichloroacetic acid, and phosphorus liberated by the enzymatic action was measured after adding 0.75 mL colour reagent, prepared daily by mixing four volumes of 1.5 % (w/v) ammonium molybdate in 5.5 % (v/v) sulfuric acid solution and one volume of a 2.7 % $FeSO_4$ solution. The absorption was registered at 700 nm [13]. One unit of phytase activity is defined as the release of 1 μM inorganic orthophosphate per minute under the above conditions. The intracellular phytate-degrading activity was analysed in cell-free extracts obtained after cell disruption of the yeast isolates. For this purpose, yeast biomass was mixed with glass beads (size 0.1 mm) and 0.05 M potassium phosphate buffer, pH 7.8, in a ratio of 1:1:2 and subjected to disintegration in a Bullet Blender Storm homogeniser at 8000 rpm, three times for 5 min. Cell debris was removed by centrifugation at 2300 x g for 15 min at 4°C, and the resulting supernatant was clarified after centrifugation at 15500 x g for 20 min at 4°C. The cell-free homogenate was stored at -20°C and used for intracellular phytase assay. Extracellular phytase activity was analysed in culture broth obtained after 48 h cultivation in PSM medium (28°C), followed by centrifugation for 15 min at 2300 x g for cell removal.

Total antioxidant capacity of the selected yeast isolates

Cell-free extracts of the yeast strains, isolated from sourdough, were tested for their antioxidant capacity according to Kumaran and Karunakaran [14]. The ascorbic acid solution in methanol (0.09 % w/v) was used as a positive control. The antioxidant activities of the yeast strains were expressed as a number of equivalents of ascorbic acid (antioxidant activity = 1.0).

Data analysis

The analyses were performed in triplicate and the data used represent the mean values with Standard error of the mean (± SEM) of the three independent experiments. The statistical analysis was performed using MICROSOFT OFFICE 365 EXCEL 2020 software.

RESULTS AND DISCUSSION

Isolation and morphological characterization of yeast microorganisms from sourdough

A total of 13 yeast strains were isolated from four sourdough samples, S5, S6, S7 and S8, which were prepared in the laboratory as described above (Table 1). Four strains were isolated from S5 (5-1, 5-2, 5-3 and 5-4), two strains were isolated from S6 (6-1 and 6-2), 5 strains from sourdough 7 (7-1, 7-2, 7-3, 7-4 and 7-5) and two strains from sourdough 8 (8-2 and 8-3) (Table 1). The morphology of the yeast colonies was observed on

Table 1. Properties of yeast microorganisms, isolated from sourdough samples.

Isolate code	Sourdough sample	Morphology of single colony	Morphology of vegetative cells	Nearest relative based on 18S rDNA sequence similarity
5-1	S5	Shape: folded, Margin: filiformed, Structure: fibrous, Profile: convex, Color: white, Glistening: no	Elongated cells, occurring singly, in pairs, or in chains	*Pichia membranifaciens* (LT854920.1), 99.74 %
5-2	S5	Shape: complex, Margin: Wavy, Structure: coarse-grained, Profile: convex, Color: white, Glistening: no	Small, ovoid, monopolar budding cells	*Pichia membranifaciens* (LT854920.1), 99.10 %
5-3	S5	Shape: round, Margin: Smooth, Structure: homogeneous, Profile: convex, Color: white, Glistening: yes	Round, monopolar budding cells	*Candida milleri* (*Kazachstania humilis*) (AB054677.1), 99.11 %
5-4	S5	Shape: round with a convex end, Finish: Smooth, Structure: fine-grained, Profile: convex, Color: orange, Glistening: yes	Elongated, oval, monopolar budding cells	ND
6-1	S6	Shape: round, Margin: Smooth, Structure: homogeneous, Profile: convex, Color: white, Glistening: no	Small, oval, monopolar budding cells	ND
6-2	S6	Shape: complex, Margin: irregular, Structure: fibrous, Profile: convex and toothed, Color: pink, Glistening: no	Ovoid, monopolar budding cells. Pseudohyphial structures are observed	*Pichia membranifaciens* (LT854920.1), 97.26 %
7-1	S7	Shape: Circular, Margin: regular, Structure: homogeneous, Profile: convex, Color: white, Glistening: yes	Small, ovoid, monopolar budding cells	*Kazachstania viticola* NRRL Y-27206, (NG_063240.1), 99.50 %
7-2	S7	Shape: round with a rhizoid end, Margin: hairlike, Structure: homogeneous, Profile: convex, Color: white, Glistening: no	Large elongated cells, sometimes observed in pairs.	*Pichia kudriavzevii,* (KP202859.1), 99.58 %
7-3	S7	Shape: round, Margin: Smooth, Structure: homogeneous, Profile: drop-shaped, Color: white, Glistening: weak	Small spherical, monopolar budding cells	*Wickerhamomyces anomalus* TEMFP3, (NG_062034.1), 87.49 %
7-4	S7	Shape: irregular, Margin: Wavy, Structure: homogeneous, Profile: bulging, Color: white	Small, spherical cells arranged singly, monopolar budding	*Nakaseomyces glabratus* ATCC 2001 (CP048130.1), 98.19 %
7-5	S7	Shape: circular, Margin: Wavy, Structure: homogeneous, Profile: bulging, Color: white, Glistening: yes	Small, spherical cells, monopolar budding	ND
8-2	S8	Shape: round with a convex centre, Margin: Smooth, Structure: homogeneous, Profile: bulging, Color: white, Glistening: light pearlescent	Round cells, singly located, monopolar and bipolar budding	*Saccharomyces cerevisiae* SC-125 (MW279237.1), 99.29 %
8-3	S8	Shape: round, Margin: Smooth, Structure: homogeneous, Profile: bulging, Color: white, Glistening: light pearlescent	Large, round to oval-shaped and budding cells	*Saccharomyces cerevisiae* ABO1 (MH938426.1), 99.18 %

ND, not determined

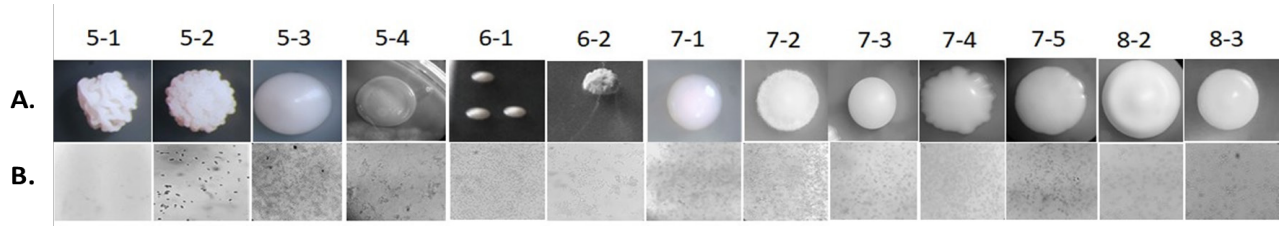

Fig. 1. **A.** Morphology of yeast colonies; **B.** Morphology of yeast cells.

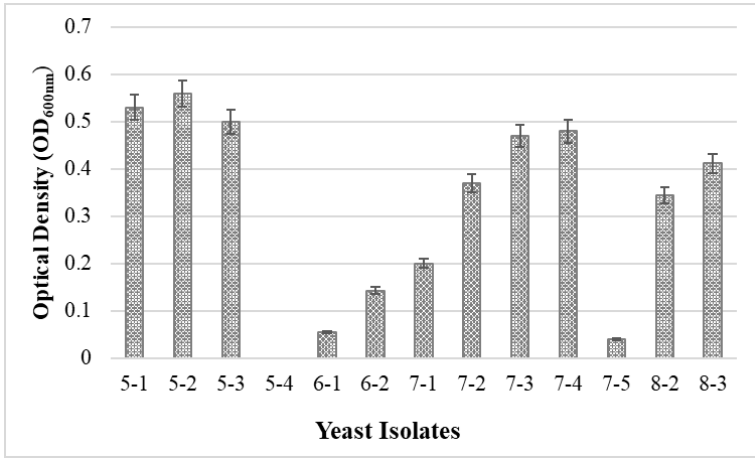

Fig. 2. Growth ($OD_{600\ nm}$) of yeast cultures in sodium phytate medium.

a solid YPD medium, and the following features were observed - shape, edge, structure, profile, colour, and glistening (Fig. 1A). The shape, size, and presence/lack of budding of the vegetative cells were also defined. The thirteen isolated yeast strains exhibited different colony and cell morphology (Table 1, Fig. 1B).

Screening of the isolates for growth in phytate mineral medium

The growth of the isolated thirteen pure yeast cultures in a liquid medium containing sodium phytate as the sole source of phosphorus was evaluated based on their $OD_{600\ nm}$ values. (Fig. 2).

Ten isolates showed very good growth after 48 hours of cultivation, with OD_{600nm} varying between 0.143 - 0.560. One isolate (5-4) showed no growth under the selected culture conditions. Two more strains - 6-1 and 7-5 were registered with very poor growth (OD_{600nm} < 0.1). Strains 5-1, 5-2, and 5-3, followed by 7-4, 7-3, 8-3, 7-2 and 8-2, showed high optical density values, indicating that they efficiently uptake the substrate from the PSM medium and expression of probable phytase activity. The presence of the enzyme phytase breaks down the phytate and provides the phosphorus necessary for growth and metabolic processes in those yeast strains [15].

Biochemical and genetic characterization of the potential phytase-producing yeast cultures

The 10 yeast strains, showing good growth in the phytate mineral medium, were further taxonomically characterized by 18S rRNA gene analysis. After amplification and sequencing of the 18S rRNA gene for each isolate, and performed phylogenetic analysis, the ten isolates were affiliated to seven yeast species from 3 families (Table 1): *Pichiaceae (Pichia membranifaciens* (3 strains) and *Pichia kudriavzevii* (1 strain); *Saccharomycetaceae* - *Candida milleri*, reclassified as *Kazachstania humilis* [16] (1 strain), *Kazachstania viticola* (1 strain), *Nakaseomyces glabratus* (1 strain) and *Saccharomyces cerevisiae* (2 strains) and *Phaffomycetaceae* (*Wickerhamomyces anomalus*) (1 strain). Eight of the isolates showed more than 98 % similarity to the closest representative

in the NCBI database (Table 1), allowing their species identification. In two of the isolates - 6-2 and 7-3, however, a lower percentage of identity of the 18S rRNA gene sequence (97.26 and 87.49 %) with the most closely related organisms in the database was found; therefore, additional biochemical and genetic (Domains 1 and 2 (63-642 nt) of 26S rRNA gene) analyses were performed.

The yeast isolate 7-3 showed 87.49 % similarity in the 18S rRNA gene to that of the yeast species *Wickerhamomyces anomalus*. According to the NCBI database, this species was originally described as *Saccharomyces anomalus* E.C. Hansen, based on morphological characteristics [17]. Later, the application of the polyphasic approach in taxonomic identification led to a number of subsequent reclassifications of this species - *Endomyces anomalus*, *Hansenula anomala*, *Pichia anomala*, *Willia anomala*, *Candida beverwijkiae* and *Candida pelliculosa*. Since 2008, the species has been assigned to the newly described genus *Wickerhamomyces* [17]. This genus belongs to the order *Saccharomycetales*, family *Phaffomycetaceae*. Since the strains of *W. anomalus* show quite considerable morphological and physiological variation, the species has a large number of synonyms. A distinguishing biochemical characteristic of *Wickerhamomyces anomalus* is its ability to degrade cellobiose and ferment sugars, including xylose [18]. Performed biochemical analysis of isolate 7-3, confirmed the ability of this strain to ferment a number of sugars, including xylose, and to degrade cellobiose (Table 2). Studies by other authors regarding the biotechnological application of *W. anomalus* have shown the ability of this species to synthesize cell-bound phytase [19]. Palla et al. reported the isolation of *W. anomalus* as the dominant species during the natural fermentation of different whole-grain flours [20]. The belonging of isolate 7-3 to this yeast species was confirmed also by phylogenetic analysis based on the partial sequencing of 26S rRNA gene. Isolate 7-3 was determined to share 98.86 % sequence similarity of its 26S rRNA gene D1/D2 domain to the *Wickerhamomyces anomalus* strain TEMFP3 (MH481638.1).

The yeast strain 6-2 showed 18S rRNA sequence similarity below 98 % to the nearest phylogenetic relative *Pichia membranifaciens*. It was distinguished among other isolated yeast strains by its pink colonies on solid medium and elongated cells that occur singly, in pairs, or in chains forming a pseudomycelium. The cells of *Pichia membranifaciens*, like those of isolate 6-2, are elongated and form filamentous structures - pseudohyphae. However, there is a difference in the morphology of the colonies, which for the species *Pichia membranifaciens* are described as yellowish and smooth [21]. Yeasts of this species are most often isolated from various cereals and plants and performed alcoholic fermentation. Its ability to ferment sugars to ethanol with high capacity makes it a promising and affordable sustainable biological solution to the global water and energy crisis [22]. The anamorphic species of *Pichia membranifaciens* are *Candida valida* and *Pichia manchuria* [23]. Biochemical profiling of isolate 6-2

Table 2. Biochemical characteristics of yeast isolates.

API 20 C AUX V4.0 Tests	Isolate 7-3 (S7)	Isolate 6-2 (S6)	API 20 C AUX V4.0 Tests	Isolate 7-3 (S7)	Isolate 6-2 (S6)
D-Glucose	+	+	Metyl-α-D-Glucopyranoside	+	-
Glycerol	+	-	N-Acetyl-Glucosamin	+	+
Calcium 2-keto-gluconate	-	-	D-Cellobiose	+	-
L-Arabinose	-	-	D-Lactose	+	-
D-Xylose	+	+	D-Maltose	+	-
Adonitol	-	-	D-Saccharose	+	-
Xylitol	-	+	D-Trehalose	+	-
D-Galactose	+	-	D-Mellezitose	+	-
Inositol	-	-	D-Raffinose	+	-
D-sorbitol	+	-			

revealed the strain's ability to ferment a limited range of sugars such as glucose, xylose, xylitol and N-acetyl glucosamine (Table 2). Processing of the obtained results with apiweb software showed 98% similarity of isolate 6-2 with *Candiba boidini*. The assignment of yeast species to genera and families was primarily based on vegetative cell morphology, mating type, and physiologic-biochemical characteristics when applying fermentation and growth tests commonly used in yeast systematics. The application of molecular methods based on gene sequence analyses to yeast systematics shows a discrepancy between data from phenotypic and genotypic analyses [11]. To confirm the species identity of isolate 6-2, additional analyses of the D1/D2 sequence of 26S rRNA gene were performed. The obtained high percent similarity of 98.74 % to the nearest *Pichia membranifaciens* strain MUT<ITA>:6351 (MT151656.1), proved the belonging of the 6-2 isolate to this yeast species.

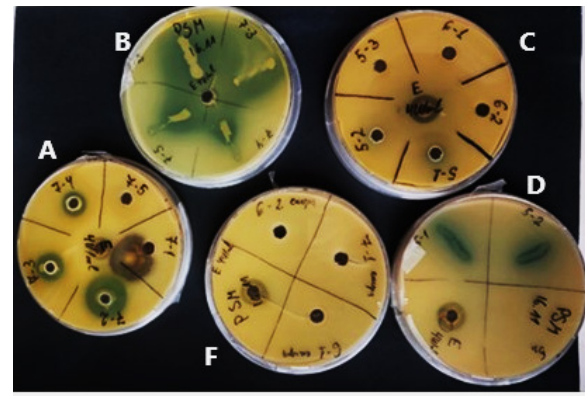

Fig. 3. A qualitative method for detection of extracellular phytase. Petri Plates A, C and F: 0.1 mL of culture liquids were dropped into wells on phytate agar medium. The supernatants were obtained after 48 hours of cultivation of the selected yeast strains in liquid PSM medium and subsequent centrifugation of the culture broth for removing the cells. Petri plates B and D: Strains, inoculated by stroke method.

Intracellular and extracellular phytate-degrading activity in the selected 10 yeast isolates

The 10 yeast strains, selected on the basis of their good growth on liquid phytate medium, were tested for extracellular phytate-degrading activity on agar medium containing the specific substrate (Fig. 3). Clearly visible halos formed as a result of phytate degradation by extracellular phytase, were observed after specific two-step staining [13]. The application of this staining method makes it possible to distinguish the effect of the phytase from that of acids, produced by some yeast strains. The results from the performed qualitative analysis revealed that 30 % of the isolates (7-2, 7-3, 7-4) formed clearly visible halos (Petri plate A). Weak degradation of phytate (poorly visible zones) was observed by the culture broths of *Pichia membranifaciens* strains 5-1 and 5-2 (Petri Plate C). Bigger halos of phytic acid degradation were detected around the strokes of the same strains (Petri Plate D).

All ten yeast strains were quantitatively tested for the presence of intracellular and extracellular enzyme activity, although the qualitative analysis showed the presence of extracellular phytase activity in only 5 of the isolates. The measurement of the intracellular phytate-degrading activity revealed that three of the yeast cultures - *Kazachstania humilis* (5-3), *Pichia membranifaciens* (6-2) and *Nakaseomyces glabratus* (7-4) showed significantly higher intracellular enzyme activity than the other studied strains (Fig. 4). It varied between 0.57 - 0.74 U mL^{-1}. In the isolates 5-1 and 5-2 (*Pichia membranifaciens*), *Kazachstania viticola* (7-1), *Pichia kudriavzevii* (7-2) and *Wickerhamomyces anomalus* (7-3), the intracellular phytate-degrading activity was in the range between 0.11 - 0.51 U mL^{-1}. No phytase activity was registered for the intracellular extracts of strains 8-2 and 8-3, which were identified as *Saccharomyces cerevisiae*, based on 18S rDNA analysis.

The spectrophotometric assay of the extracellular phytase production showed the highest level of the enzyme in *Pichia membranifaciens* isolate 5.2 - 0.09 U mL^{-1} (Fig. 5). Other authors also reported the production of phytase from this species [24, 25]. For strains *Pichia kudriavzevii* 7-2, *Wickerhamomyces anomalus* 7-3 and *Nakaseomyces glabratus* strain 7-4 the detected extracellular activity was about 0.05 - 0.06 U mL^{-1}. In 4 yeast strains (*Pichia membranifaciens* 6-2, *Kazachstania viticola* 7-1, *S. cerevisiae* strains 8-2 and 8-3) no phytase activity was measured in the tested culture broth, although they were selected on the basis of good growth in phytate selective medium. *Saccharomyces cerevisiae* strains have earlier been reported to have phytase activity, which was not confirmed by our results [26]. Similar results for the presence of growth but lack

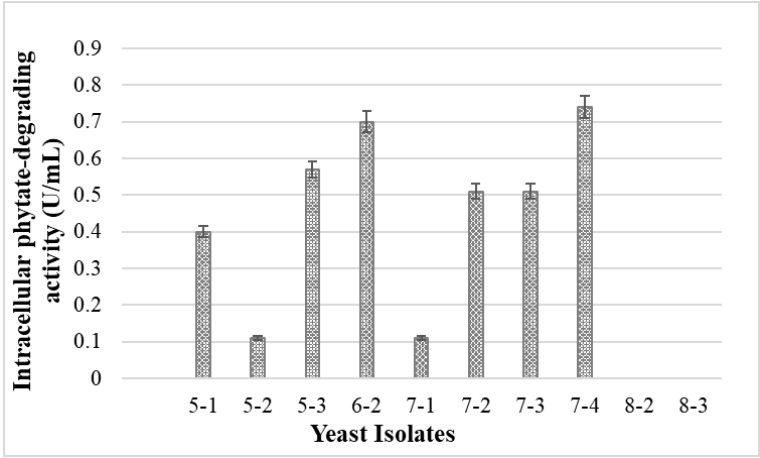

Fig. 4. Intracellular phytase activity of the tested yeast cultures.

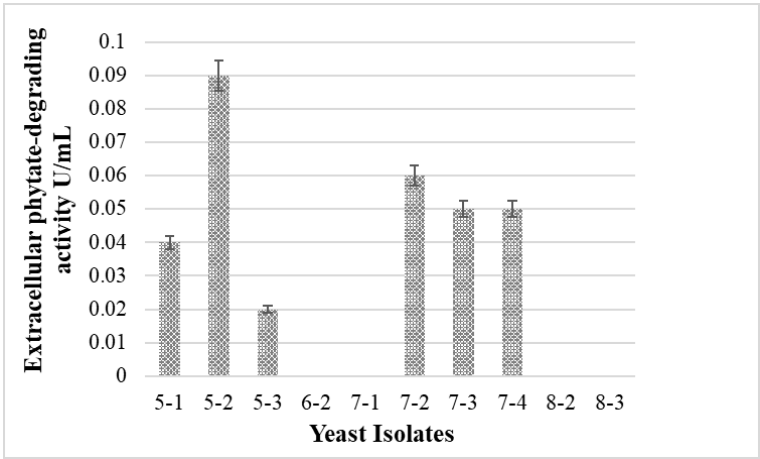

Fig. 5. Level of extracellular phytase in supernatants of the tested yeast isolates.

of phytase activity or very low levels of the synthesized enzyme were reported by other authors [27]. In this case, the growth was probably due to the uptake of free phosphorus, which was found, albeit in very low concentrations, in the commercial phytate preparations. Another possible explanation is cell growth at the expense of reserve phosphorus in the inoculum.

Antioxidant capacity of yeast cultures isolated from sourdough

Due to the fact that reactive oxygen species are at the base of many human diseases, such as cancer, diabetes, autoimmune diseases, etc., in the last few years, the interest in the antioxidant properties of foods has greatly increased. Recently, yeasts have been shown to enhance bioactive components in fermented food products through the biosynthesis of enzymes and metabolites such as glutathione, citric acid, coenzyme Q, torulahoidin, tocopherols, riboflavin (vitamin B2), cytochrome C, etc, which can act as antioxidants thus affecting the antioxidant properties of foods, in particular bakery products [20]. In this regard, the antioxidant capacity of the determined most active phytate-degrading yeast cultures was investigated. (Table 3). The study revealed that all the tested strains reduced Mo (VI) to Mo (V) in different proportions, with the level of antioxidant activities varied between 0.42 - 1.5. Furthermore, two of the seven tested yeast strains - 7-4 and 5-2, showed higher antioxidant capacity than the ascorbic acid. Among the remaining five yeast strains, the intracellular extract of *Pichia membranifaciens* strain 5-1 showed the same antioxidant activity as ascorbic acid, while isolates 7-2 and 7-3 were characterized by 8 and 12 % lower reducing power than the positive

Table 3. Antioxidant activity of yeast culture isolated from different sourdough samples.

Yeast culture	Antioxidant activity*
Pichia membranifaciens 5-1	1.0
Pichia membranifaciens 5-2	1.31
Kazachstania humilis 5-3	0.57
Pichia membranifaciens 6-2	0.42
Pichia kudriavzevii 7-2	0.92
Wickerhamomyces anomalus 7-3	0.88
Nakaseomyces glabratus 7-4	1.5
Ascorbic acid	1.0

**AA is compared with the activity of ascorbic acid = 1.0 as a control*

control. The data obtained suggest that the studied yeast microorganisms, in addition to their phytate-degrading capacity, which has a positive effect on increasing the bioavailability of various minerals, could also serve as a source of natural antioxidants added as starter cultures to foods.

CONCLUSIONS

An increasing number of studies highlight the important role of yeast metabolism on sourdough functional and nutritional characteristics. Therefore, the evaluation of the diversity and important functional properties of these microorganisms is of increased scientific interest. Newly isolated yeast microorganisms offer new metabolic genes and metabolites, that could serve to develop new biotechnological products. In regard to this, our study revealed the diversity, phytate-degrading and antioxidant potential of yeast microorganisms, newly isolated from a sourdough microbiota. Four different types of sourdough were obtained in the laboratory by spontaneous microbial fermentation of 4 Bulgarian grain-based flours. The results obtained showed a different yeast species composition in the 4 investigated sourdough samples. Some of the isolates are perspective producers of industrially important phytate-degrading enzymes and bioactive metabolites with antioxidant properties.

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