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Isolation and identification of phytase-active yeasts from sourdoughs

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ABSTRACT

Identification of high phytase-active yeasts is necessary in order to find prominent candidates for the production of wholemeal bread with high content of bioavailable minerals. Tested yeasts were isolated from Danish and Lithuanian sourdoughs, since high phytase-active yeasts adapted to grow in sourdough matrix would be a good choice for bread industry. Isolated species were; *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Pichia occidentalis*, *Candida humilis* and *Kazachstania exigua*. Studies of phytase-positive isolates were carried out at conditions optimal for leavening of bread dough (pH 5.5 and 30 °C). All the tested yeasts isolated from sourdoughs exhibited phytase activities. The most prominent isolates for extracellular phytase production were found to be *S. cerevisiae* L1.12 with a specific extracellular activity of 10.6 U/10^{10} CFU, followed by *S. cerevisiae* L6.06 with a specific extracellular activity of 8.2 U/10^{10} CFU. Some other isolates of *S. cerevisiae*, one of *C. humilis*, and one of *P. kudriavzevii* also had high specific extracellular activities of *c.* 4–7 U/10^{10} CFU. These isolates may be potential candidates for improving mineral bioavailability in whole grain bread.

1. Introduction

Nowadays, whole grain bread is gaining popularity across the world due to increasing awareness of its nutritional benefits. Several epidemiological studies found that intake of whole grain food protects against certain type of cancers, cardiovascular disease, type 2 diabetes and obesity (Slavin, 2003), due to a wide range of nutrients and biologically active constituents, such as dietary fibre, vitamins and minerals (Spiegel et al., 2009). Whole grain food contains, however, considerable amounts of phytic acid, which is the most recognized and documented antinutritional factor that chelates divalent minerals such as iron and zinc (Brune, Rossanderhulten, Hallberg, Gleerup, & Sandberg, 1992; Sandberg & Svanberg, 1991). Phytic acid forms insoluble complexes with these minerals into phytate and thus reduces their bioavailability (Brune et al., 1992; Lopez, Leenhardt, Coudray, & Remesy, 2002). Efficient reduction of phytate can be achieved by enzymatic degradation during food processing, either by increasing the activity of endogenous phytase, or by addition of enzyme preparations (Sandberg et al., 1999). Several studies have shown that a reduction of the phytate content, based on enzymatic hydrolysis, causes an increased absorption of minerals, such as zinc, iron, calcium and magnesium (Brune et al., 1992; Reinhold, Faradji, Abadi, & Ismailbeigi, 1976).

Phytate degrading enzymes – phytases - are enzymes naturally found in cereals (Eeckhout & DePaepe, 1994) and microorganisms (Lambrechts, Boze, Moulin, & Galzy, 1992; Olstorpe, Schnurer, & Passoth, 2009; Ullah & Gibson, 1987). Wheat and rye exhibit high phytase activities with values ranging from 900 to 2886 U/kg dry matter in wheat and from 4100 to 6100 U/kg dry matter in rye (Eeckhout & DePaepe, 1994; Greiner & Egli, 2003). However, the activities in wheat were considered insufficient to notably improve the mineral bioavailability in whole grain wheat bread (Harland & Harland, 1980; Turk & Sandberg, 1992).

The amount of hydrolysed phytate in different bread types varied between 13 and 100% (Lopez et al., 2001) and depends on various factor, including phytase activity, temperature, pH, and fermentation time (Turk & Sandberg, 1992) A few studies have shown that the content of phytate decreasesby 25–60% of the initial level during wheat bread making from high extraction flour (Bartnik & Florysiak, 1988; Daniels & Fisher, 1981). On the other hand, phytate is fully degraded during commercial rye bread production due to the higher phytase activity in rye and long processing time as compared to wheat bread making (Nielsen, Damstrup, Dal Thomsen, Rasmussen, & Hansen, 2007).

Many studies have shown that the addition of sourdough during the bread making can reduce the phytate content in bread (Lopez et al., 2001; Turk, Carlsson, & Sandberg, 1996). Moreover, Mckenzieparnell and Davies (1986) reported that the highest levels of phytate remained in unleavened bread. Most wheat bread even whole wheat bread is produced with short fermentation times and

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the bread is leavened by use of baker's yeast instead of sourdough fermentation. The reason for this might be the time-consuming sourdough preparation.

Phytase activity in baker's yeast has been studied previously under bread dough leavening conditions (Harland & Harland, 1980; Turk et al., 1996; Turk, Sandberg, Carlsson, & Andlid, 2000). Harland and Harland (1980) showed that phytate hydrolysis may be increased up to 25% by adding the double amount of yeast. In contrast, very low phytase activity from baker's yeast was observed during wheat bread making in experiments of Turk et al. (1996).

To our knowledge, there are no high phytase-active yeasts available for bread industry today. Our objective therefore was to isolate and identify yeasts from different sourdoughs and to study their phytase activity under conditions optimal for leavening of wheat bread dough (30 °C and pH 5.5) in order to identify species and/or strains with high activities that might be used in the baking industry.

2. Materials and methods

2.1. Sourdough sampling

13 sourdoughs samples were collected from three Danish (A, B and C) and three Lithuanian (D, E and F) bakeries. Ten out of the 13 sourdoughs were made from rye flour, two from wheat flour, and one from spelt flour (Table 1). The two Danish rye sourdoughs (DS-1, DS-2) and the Lithuanian sourdough LS-2 were made from high extraction rye flour. Moreover, amylolytic enzymes were added to the sourdough LS-2.

The sourdough samples from the Danish bakeries were collected and delivered to our laboratory within 1 h, where they were stored at 4 °C until they were characterized (within 8 h).

The sourdough samples from the Lithuanian bakeries were collected and promptly frozen, and stored at −20 °C until shipped to our laboratory. Afterwards, these sourdoughs were refreshed according to the bakery recipes and characterized.

2.2. pH and total titratable acidity (TTA)

Ten grams of each sourdough samples were suspended in 90 ml sterile ultrapure water and homogenized in a stomacher (STOMACHER 400, VWR Bie & Berntsen, Herlev, Denmark) at normal speed for 1 min. The pH value was recorded with a Knick Portamess pH meter (Elscolab, Heiloo, Nederland). Subsequently, the suspension was titrated with 0.1 mol/L NaOH to a final pH of 8.5 ± 0.1. The TTA was expressed as the amount (mL) of 0.1 mol/L NaOH required to neutralize 10 g of sample. Two independent measurements were performed for each sample.

2.3. Isolation of yeasts and enumeration

Ten grams of each sourdough samples were suspended in sterile 90 mL saline peptone solution (NaCl, 8.5 g/L; Na_2HPO_4, 0.3 g/L; peptone, 1.0 g/L; pH 5.5) and homogenized in a stomacher at normal speed for 1 min. Further, decimal dilutions were prepared and 0.1 mL of each were spread onto YPG agar (D-(+)-glucose, 10 g/L; yeast extract, 3 g/L; peptone 5 g/L; pH 5.5) supplemented with 50 mg/L chlortetracycline and 100 mg/L chloramphenicol to inhibit bacterial growth. Incubation was carried out at 30 °C for 72 h. Two independent counts of colony forming units (CFU) were performed for each sample. Further, 20 colonies were randomly selected from plates with 50–200 colonies, re-cultivated in 10 mL YPG broth at 30 °C for 24 h, and purified by streaking onto YPG agar. Purified yeast isolates for long-term storage were stored at −80 °C in YPG broth containing 2.75 mol/L glycerol.

2.4. DNA extraction, molecular typing and identification

A total of 221 isolates were initially grouped according to their rep-PCR (repetitive DNA sequence-based polymerase chain reaction) fingerprinting patterns as described below. Initially, DNA was extracted using InstaGene DNA extraction kit (Bio-Rad Laboratories, Sundbyberg, Sweden) following the instructions of the manufacturer. The rep-PCR reaction was carried out in a 25 μL volume containing 2.5 μL 10 × PCR reaction buffer (Fermentas, Vilnius, Lithuania), 0.2 μL *Taq* polymerase (5 U/μL, Fermentas), 1.5 mmol/L $MgCl_2$ (Fermentas), 200 μmol/L of dNTP-mix (Fermentas), 0.8 μmol/L of primer GTG_5 (DNA Technologies, Aarhus, Denmark), 1.5 μL of DNA template, and sterile MilliQ water to adjust to 25 μL (Nielsen, Teniola, et al., 2007). The reaction was performed in an automatic thermal cycler (GeneAmp PCR System 9700, Perkin-Elmer) and was basically carried out as described by Nielsen, Teniola, et al. (2007) with slight changes: 5 min of initial denaturation at 94 °C, 30 cycles of 94 °C for 30 s, 45 °C for 60 s, 65 °C for 8 min followed by a final elongation step of 65 °C for otherwise 16 min. The PCR products were separated by 1.5% agarose gel

Table 1
Main characteristics of sourdoughs.

Bakeries	Sourdough sample	Flour type	Fermentation temperature, °C	pH	TTA, mL[a]	Dry matter, %	log CFU/g sample
Danish bakeries							
A	DS-1	Rye	32	3.75 ± 0.01	13.3 ± 0.1	36.1 ± 0.1	7.3 ± 0
B	DS-2	Rye	21	3.80 ± 0.00	20.1 ± 1.3	47.4 ± 0.1	6.9 ± 0.1
C	DS-3	Wheat	22	3.80 ± 0.01	7.7 ± 0.4	34.8 ± 0.5	7.8 ± 0
	DS-4	Spelt	22	3.65 ± 0.00	13.9 ± 1.3	35.9 ± 1.2	6.9 ± 0.1
Lithuanian bakeries							
D	LS-1	Rye	28–32	4.07 ± 0.01	14.9 ± 0.1	55.1 ± 0	7.4 ± 0
	LS-2	Rye	43–45	3.37 ± 0.01	28.1 ± 0.3	33.3 ± 1.4	Nd[b]
E	LS-3	Rye	24–26	4.20 ± 0.01	10.0 ± 0.3	49.1 ± 0	7.9 ± 0
	LS-4	Rye	24–26	4.17 ± 0.01	11.3 ± 0.1	38.1 ± 0	7.4 ± 0
F	LS-5	Wheat	34–35	3.60 ± 0.01	7.5 ± 0.2	18.7 ± 0	Nd
	LS-6	Rye	34–35	3.47 ± 0.01	18.3 ± 0.3	26.0 ± 0	7.7 ± 0
	LS-7	Rye	34–35	3.46 ± 0.01	11.9 ± 0.1	30.0 ± 0	7.5 ± 0
	LS-8	Rye	34–35	3.43 ± 0.00	11.0 ± 0.1	27.8 ± 0.2	7.5 ± 0
	LS-9	Rye	34–35	3.91 ± 0.01	10.9 ± 0.1	25.5 ± 0.1	6.7 ± 0.1

Results are expressed as the mean of two replicated measurements and standard deviation (±SD).
[a] the TTA was expressed as the amount (mL) of 0.1 mol/L NaOH required to neutralize 10 g of sample.
[b] not detected.