POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

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Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 1, pp. 33-40

PHYTATE-DEGRADING ACTIVITY IN LACTIC ACID BACTERIA

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Key words: lactobacilli, phytate, phytate-degrading activity

The phytate-degrading and phosphatase activities of several *Lactobacillus* strains belonging to different species and isolated from different ecosystems were tested. The specific activities against phytate ($InsP_6$) varied from 0.024 to 0.531 U/mg protein, being the highest for *L. casei* DSM 20011. The studied strains hydrolysed *p*-nitrophenylphosphate at higher rates than phytate, except for *L. casei* DSM 20011 and *L. plantarum* W42. The ability of the different strains to hydrolyse $InsP_6$ and generate lower *myo*-inositol phosphates during growth was tested by HPLC. The $InsP_6$ hydrolysis was in the range from 0.0 to 8.83%, being the highest for *L. plantarum* W42 and *L. plantarum* 110, followed by *L. casei* 40W. The optimal pH and temperatures of phytate-degrading activity varied in the range from 5.0 to 7.5 and from 50 to 60°C, respectively. The incorporation of different types of carbon sources or inorganic phosphate to the growth medium modulated the synthesis of phytate-degrading enzymes in the studied strains.

Further studies should be carried out to provide progress in the understanding of the potential nutritional and technological roles of the most active strains in the elaboration of whole sour breads.

INTRODUCTION

Lactic acid fermentation of cereals is a long established processing method, being used in Asia and Africa for the production of foods like beverages, gruels, and porridge [Charalampopoulos *et al*., 2002]. In European countries, cereals, like wheat and rye are used for sourdough production, which is traditionally prepared by adding a prefermented sourdough to the dough [Charalampopoulos *et al*., 2002]. Artisan bread production, which often employs sourdough processes, provides a wide regional variety of breads and specialty products [De Vuyst *et al.*, 2005]. Yeast and lactic acid bacteria affect the characteristics of sourdoughs in different ways. Yeasts contribute to the leavening, whereas lactic acid bacteria play an important role in the acidification and structure of crumb and also contribute to the sensory quality [Gianotti *et al.*, 1997]. Whole wheat flours provide beneficial nutrients such as complex carbohydrates, proteins, vitamins, minerals and are a source of fiber. However, whole wheat flours also contain undesirable substances such as phytic acid and it salts [Fretzdorff & Brümmer, 1992]. The salts of phytic acid (*myo*-inositol hexaphosphate, $InsP_6$) or phytates are the primary source of inositol and the primary storage form of phosphate in plant seeds [Lassen *et al.*, 2001]. $InsP_6$ has been found to lower the absorption of minerals such as Ca^{2+} and Mg^{2+} as well as trace oligo-elements such as Fe^{2+} and Zn^{2+} by their chelation and precipitation with the six anionic phosphate groups [Leenhardt *et al.*, 2005]. In monogastric animals phytates act as an anti-nutrient by preventing the mineral uptake, since they have low levels of phytate-degrading enzymes in their digestive tracts [Wyss *et al.*, 1999; Lassen *et al.*, 2002]. Some mineral deficiencies are common in developing countries, but marginal mineral deficiencies also occur in developed countries, particularly in vulnerable human populations [Leenhardt *et al.*, 2005; Türk *et al.*, 2000]. The bioavailability of essential dietary minerals can be improved by reducing of the phytate content in foods and feeds [Türk *et al.*, 2000]. Due to these nutritional consequences, the degradation of phytate during food processing is desirable. Fermentation is widely used to improve the nutritional and functional qualities of food products [Fredrikson *et al.*, 2002; Bergqvist *et al.*, 2005]. Previous studies have shown that an advanced hydrolysis of phytate is achieved by increasing the fermentation time and/or decreasing the pH during whole wheat dough fermentation [Fretzdorff & Brümmer, 1992; Lopez *et al.*, 2001; Leenhardt *et al.*, 2005]. The sour whole bread quality depends basically on the starters and/or processing conditions, where the external factors like dough yield, temperature, fermentation time, oxygenation, addition of yeast and ingredients [Martínez-Anaya, 1994] could affect the quality of the final products as regards bread sensory attributes and crumb texture are concerned [Collar *et al.*, 1994; Matínez-Anaya *et al.*, 1994]. Most consumers dislike the crumb texture or the excessive acid taste of sourdough breads as resulting of long fermentations, which makes it difficult to produce breads with high specific volume, and the pungent bread aroma usually is not accepted [Salovaara & Spicher, 1987]. To obtain products with high quality and eliminate the adverse effects of $InsP_6$ during the

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sour whole dough fermentation is still a challenge for technologists. The aim of this study was to screen a wide number of lactobacilli strains isolated from different ecosystems in order to find phytate-degrading activities. The optimal conditions for phytate-degrading activities as well as the effects of the environmental factors on their synthesis have been studied, providing relevant information about their potential role in fermentation processes.

MATERIALS AND METHODS

Strains of bacteria and culture conditions

The strains listed in Table 1, which belong to different species of the genus *Lactobacillus*, were screened for phytase and phosphatase activities. The strains isolated from commercial fermented milks: *L. acidophilus* BS, *L. casei* 1K, *L. casei* 4OW; and from plant fermentations: *L. plantarum* 6OW, *L. plantarum* 110; *L. plantarum* JBPRS, *L. plantarum* W42 were from Collection of Animal Reproduction and Food Research of PAS, Olsztyn-Poland (IAR&FR PAS Collection). *Lactobacilli* were growth in modified MRS broth in which inorganic phosphate was replaced by 0.65 g/L sodium phytate (Sigma, St. Louis, MO) and 0.1 mol/L of 3-[N-Morpholino] propanesulfonic acid (MOPS, Sigma, St. Louis, MO) buffer [Morishita *et al.*, 1981] and the contents of yeast and meat extracts were reduced to 2 and 4 g/L, respectively [Palacios *et al.*, 2005], in order to achieve a low-phosphate growth condition that could promote the synthesis of the enzymes responsible for phytate--degrading activity. Thirty mL of this medium were inoculated at 1% (v/v) with 12-18-h old cultures previously propagated in the same conditions. Cultures were incubated at 37°C until the beginning of the stationary phase of growth was reached, which was monitored by measuring optical density at 600 nm. The cells were harvested by centrifugation (6.000 × *g*, 10 min, 4°C) and washed with 50 mmol/L Tris-HCl (pH 6.5). The cell pellets were finally suspended in 50 mmol/L sodium acetate-acetic acid (pH 5.5) and used for activity assays of cell-associated enzymes. The culture supernatant, after filtering through 22 μm Millipore filter (MILLEX-GV), was kept for inositol phosphates determination.

In order to evaluate the effect of the growth medium composition on phytate-degrading activity different concentrations of glucose (5, 10, 20 and 40 g/L) and different carbon sources (lactose, raffinose or FOS, fructo-oligosaccharides, WAKO PURE, Japan, grade of polymerization: 2-4) at 20 g/L, were studied. The effect of the presence of the inorganic phosphate (KH_2PO_4 2 g/L) was also studied. Phytate-degrading activity was assayed in cell suspensions of *L. casei* DSM 20011, *L. casei* 4OW, *L. fermentum* DSM 20052, *L. plantarum* JBPRS, *L. plantarum* W42, and *L. plantarum* 110 previously grown in the different culture media by the standard procedure. The viability and pH was also monitored during the incubation time. Bacteria counts were determined in MRS agar after incubation at 37°C for 48 h.

Phosphatase and phytase assays

Acid phosphatase activity was determined by monitoring the rate of hydrolysis of *p*-nitrophenyl phosphate [Haros *et al.*, 2005]. The reaction mixture consisted of 250 μL of 0.1 mol/L sodium acetate buffer, pH 5.5, containing 5 mmol/L *p*-nitrophenyl phosphate and 250 μL of enzyme sample. After incubation at 50°C for 15 min, the reaction was stopped by adding 500 μL of 1.0 mol/L NaOH. The *p*-nitrophenol released was measured at 405 nm. Phytase activity was determined by measuring the amount of liberated inorganic phosphate from sodium phytate. The reaction mixture consisted of 400 μL of 0.1 mol/L sodium acetate, pH 5.5, containing 1.2 mmol/L sodium phytate and 200 μL of enzyme sample. After incubation for 30 min at 50°C, the reaction was stopped by adding 100 μL of 2 g/L trichloroacetic acid [Haros *et al.*, 2001]. An aliquot was analysed to determine the liberated inorganic phosphate (Pi) by the ammonium molybdate method, at 405 nm [Tanner & Barnett, 1986].

One unit of phosphatase or phytase (U) was defined as 1.0 μmol of *p*-nitrophenol or 1.0 μmol of Pi liberated per hour at 50°C, respectively. In every case, phosphatase and phytase activities were detected in the cell suspensions whereas the levels of activities found in the extracellular medium (after filtering the supernatant with 22 μm Millipore filter, MILLEX-GV) were negligible.

To determine the optimum pH, phytase activity of the selected strains was determined at 50°C, in the pH range from 3.0-8.5 using as buffers sodium citrate/citric acid, sodium acetate/ acetic acid and tris-HCl. To determine the optimum temperature, phytase activity was measured at pH 5.5, in the temperature range from 37 to 70°C.

For comparisons of the levels of activities amongst different strains, data were expressed in Units per milligram of protein (Specific Activity).

Protein assay

For protein determinations, samples were diluted in 1 N NaOH, boiled for 5 min and centrifuged (8.000 × *g*, for 5 min). Protein concentrations were determined in the obtained supernatants by the micromethod of Biuret, using bovine albumin as a standard [Ohnishi & Barr, 1978].

Determination of inositol phosphates

The ability of the different strains to use phytate as a source of phosphate and to generate lower inositol phosphates ($InsP_3$-$InsP_5$) during growth was tested with HPLC according to Sandberg & Ahderinne [1986]. End-point determinations were carried out in the supernatant of cultures of the strains grown till stationary phase, in the conditions and medium described above. Briefly, 10 mL of supernatants were frozen-dried and redissolved in 15 mL of 0.025 mol/L HCl, transferred to mini-columns filled with resin Dowex AG 1-X8 (200-400 Mesh, BioRad, Hercules, CA, USA), from which inositol phosphates were eluted using 2 N HCl (5x4 mL), dried in air current at 40°C, and the dry residue was dissolved in the mobile phase and analysed using HPLC.

HPLC analyses were carried out using a Shimadzu chromatograph (pump LC-6A, system controller SCL-6B, Shimadzu Japan), a C-18 Chromasil column (5 μm, 4.6 by 150 mm, Barcelona, Spain) and refractometric detector (RID-6A, Shimadzu, Japan), 35°C oven temperature, methanol/0.05 mol/L formic acid (51:49, v/v) and 1.5 mL per 100 mL of tetrabutylamonium hydroxide as mobile phase.

The pH was adjusted to 4.3 by addition of 8 mol/L sulphuric acid. The mobile phase was filtered through a MF-Millipore filter (0.45 μm) under vacuum and degassed by sonication for 15 min. The flow rate was 0.4 mL/min. Retention times and peak areas were measured by a laboratory data system HP 3350 (Hewlett Packard LTD). Identification of the inositol phosphates was achieved by comparison with the mixture of standards obtained during hydrolysis of phytic acid sodium salt [Sandberg *et al.*, 1989] while a quantitative analysis was conduced using an external standard (sodium phytate, Sigma). Injections were made with a 20 μL loop.

Statistical analysis

Multiple sample comparison was statistically analysed with the Statgraphics Plus 5.0. Fisher's least significance difference (LSD) test was used to compare means at a 5% significance level.

RESULTS AND DISCUSSION

Screening for phosphatase and phytase activities

The phosphatase and phytase activities of several *Lactobacillus* strains belonging to different species and isolated from different ecosystems were tested in whole-cell suspensions (Table 1). The specific activities of the studied lactobacilli against phytate varied from 0.024 to 0.531 U/mg protein, being the lowest for *L. acidophilus* BS and the highest for *L. casei* DSM 20011. The specific activities against *p*-nitrophenylphosphate varied from 0.172 to 1.351 U/mg protein for *L. plantarum* W42 and *L. salivarius* subsp. *salicinius* DSM 20554, respectively. The studied strains hydrolysed *p*-nitrophenylphosphate at higher rates than phytate. The only exception was *L. casei* DSM 20011 and *L. plantarum* W42, which showed a phytase activity/phosphase activity ratio higher than 1.0 (Table 1). Palacios *et al.* [2005] showed that *Lactobacillus* strains had higher activity against *p*-nitrophenyl phosphate than phytate. The phytate-degrading activity of *Lactobacilli* seems to be due to a non-specific acid phosphatase [Palacios *et al.*, 2005; Zamudio *et al.*, 2001], which shows high hydrolysis rates with monophosphorylated compounds [Vohra & Satyanarayana, 2003]. However, phytase activity has been detected in *L. amylovorus* and *L. sanfranciscensis* [Screeramulu *et al.*, 1996; De Angelis *et al.*, 2003]. The activity of *L. sanfranciscensis* led to a 64-74% decrease of phytates in sour dough after 8 h of fermentation at 37°C. On the other hand, Lopez *et al.* [2000] reported that all the tested strains of lactic acid bacteria isolated from sourdough expressed phytase activity and were able to degrade ≈30% of phytate in only 2 h. Later, Reale *et al.* [2004] suggested the sourdough technology based on the use of lactic acid bacteria in the breadmaking process due to the phytase activity of lactobacillus strains.

The ability of the different strains to hydrolyse $InsP_6$ and generate *myo*-inositols with lower numbers of phosphate groups ($InsP_3$-$InsP_5$) during growth was tested by HPLC (Table 1). The *myo*-inositol contents were determined in the culture supernatants of every strain grown until the stationary phase. The relative $InsP_6$ hydrolysis was in the range from 0.0 to 8.83% (Table 1). The highest degradation of $InsP_6$ was carried out by *L. plantarum* W42 (8.53%) and *L. plantarum* 110 (8.83%), followed by *L. casei* 40W (6.56%). *L. acidophilus* BS and *L. salivarius* subsp. *salivarius* DSM 20555 did not caused any phytate degradation during their growth at 37°C, even

TABLE 1. Relative phytase and phosphatase activities of several lactobacilli strains[a,d].

Strain	Origin	Phytase Activity (U/mg protein)	Phosphatase Activity (U/mg protein)	Ratio[b]	% Hydrolysis $InsP_6$[c]	% Generation		
						$InsP_5$[c]	$InsP_4$[c]	$InsP_3$[c]
L. acidophilus BS	Commercial fermented milk	0.02[a]	0.49[a]	0.05	0.00[a]	ND	ND	ND
L. casei DSM 20011	Cheese	0.53[d]	0.43[a]	1.22	1.22[b]	ND	ND	ND
L. casei 1K	Commercial fermented milk	0.15[b]	0.74[b]	0.20	0.48[a]	ND	ND	ND
L. casei 4OW	Unknown source	0.24[bc]	0.76[b]	0.31	6.56[cd]	ND	1.20[a]	1.49[a]
L. fermentum DSM 20052	Fermented beets	0.19[b]	0.46[a]	0.42	4.98[c]	ND	ND	ND
L. fermentum F-8	Unknown source	0.06[a]	0.36[a]	0.16	4.20[c]	ND	2.71[b]	1.33[a]
L. gasseri DSM 20243	Human	0.07[ab]	0.77[b]	0.09	3.43[bc]	ND	ND	ND
L. johnsonii DSM 10533	Human blood	0.04[a]	0.74[b]	0.05	4.52[bc]	ND	ND	0.88[a]
L. plantarum JBPRS	Plant origin	0.36[c]	0.75[b]	0.48	3.56[bc]	ND	ND	ND
L. plantarum W42	Plant origin	0.20[bc]	0.17[c]	1.18	8.53[d]	ND	2.40[b]	4.00[b]
L. plantarum 110	Fermented plant food	0.14[b]	0.22[ac]	0.63	8.83[d]	0.49	2.47[b]	1.10[a]
L. reuteri DSM 20016	Intestine of adult	0.17[b]	1.15[d]	0.15	2.91[b]	ND	ND	ND
L. rhamnosus DSM 20021	Lymph node	0.08[ab]	0.77[b]	0.11	3.02[bc]	ND	ND	ND
L. salivarius subsp. *salicinius* DSM 20554	Clinical source saliva	0.13[ab]	1.35[d]	0.09	1.47[b]	ND	ND	ND
L. salivarius subsp. *salivarius* DSM 20555	Clinical source saliva	0.07[ab]	0.82[b]	0.08	0.00[a]	ND	ND	ND

[a]Cultures were incubated at 37°C until the beginning of the stationary growth phase was reached (~12-18 hours) in modified MRS medium. Mean, n= 2 or 3. Values followed by the same letter in the same column are not significantly different at 95% confidence. [b]Ratio: Phytase Activity/Phosphatase Activity. [c]$InsP_3$ to $InsP_6$: *myo*-inositol phosphate containing 3-6 phosphates per inositol residues, [d]ND: not detected.

though produced some dephosphorylation of $InsP_6$ at 50°C, probably because usually the optimum of phytate-degrading activity in microorganism is between 50 and 70°C, being low at 37°C, depending on the strain.

L. casei 1K degraded only 0.48 of initial $InsP_6$ (Table 1). The $InsP_5$ that could have been formed in the first step of $InsP_6$ hydrolysis was detected only in *L. plantarum* 110 culture suspensions (Table 1). The lower inositol phosphates ($InsP_4$ and $InsP_3$) were detected in low concentrations in the medium inoculated with *L. casei* 40W, *L. fermentum* F-8, *L. johnsonii* DSM 10533, *L. plantarum* W42 and *L. plantarum* 110. *L. plantarum* W42 and *L. plantarum* 110 degraded similar $InsP_6$ amounts and generated similar $InsP_4$ quantities, but significantly differences ($p<0.05$) were found when compared with the generation of $InsP_3$ amounts, which could suggested that *L. plantarum* 110 hydrolysed more efficient $InsP_3$ than *L. plantarum* W42 (Table 1). On the other hand, *L. casei* 40W generated similar amount of $InsP_3$ as *L. fermentum* F-8. The accumulation of $InsP_4$ and $InsP_3$ in the growth media varied amongst strains regardless their ability to dephosphorylate the initial substrate ($InsP_6$).

L. casei DSM 20011, *L. casei* 40W, *L. fermentum* DSM 20052, *L. plantarum* JBPRS, *L. plantarum* W42 and *L. plantarum* 110 showed the highest phytase activities, the highest phytase/phosphatase activity ratios and the highest percentage of $InsP_6$ hydrolysis, and, therefore, they were selected for further investigations.

Effect of pH and temperature on phytate-degrading activity

The effects of pH on phytate-degrading activity of the lactobacilli were determined in order to know their putative role during food processing. The phytate-degrading activity of *L. casei* 40W was optimal at pH 6.5 (Figure 1A). This strain showed activity at acid pH (4.0-7.0), whereas the activity dropped dramatically at pH higher than 7.0. *L. fermentum* DSM 20052 showed a similar behaviour, with maximal activity at pH 6.5 (Figure 1A). *L. casei* DSM 20011 phytate-degrading activity had a rather broad pH optimum, displaying more than 70% of its maximal specific activity between pH 3.5 and pH 8.5 (Figure 1A). This strain also showed two pH optima at 5.5 and 7.0, suggesting that two different enzymes could contribute to phytate degradation. Similar behaviour was previously described by Tomschy *et al.* [2002] in *Aspergillus niger* NRRL 3135, which showed two optimal pHs, one at 2.5 and the other at pH 5.0-5.5. The optimal pH for phytate-degrading activities of *L. plantarum* W42 and *L. plantarum* JBPRS were similar (6.0-6.5); whereas *L. plantarum* 110 showed its optimum at slightly alkaline pH (7.5) (Figure 1B). *L. plantarum* NRRL B-4496 was reported to have optimal activity at pH 5.5, whereas other species of this genus such, as *L. pentosus* CECT4023 and *L. sanfranciscensis* CB1, showed their optimum at pH 5.0 and 4.0, respectively [Zamudio *et al.*; 2001; Palacios *et al.*, 2005; De Angelis *et al.*, 2003]. The isolated enzyme of *L. plantarum* JBPRS retained above 60% of its optimal activity in the pH ranged from 4.5 to 7.5, which suggests that this strain and *L. casei* DSM 20011 could have high phytate-degrading activity during cereal fermentation process (5.7-4.5), [Palacios *et al*., 2007].

The effects of temperature on phytate-degrading activities of the tested strains were also determined (data not shown). Enzyme activity of *L. casei* 40W, *L. fermentum* DSM 20052, *L. plantarum* JBPRS and *L. plantarum* 110 was optimal at 50°C, while that of *L. casei* DSM 20011 and *L. plantarum* W42 was at 60°C. Only *L. plantarum* 110 showed high activity at 37°C (67.9%), the rest of the strains only retained their activity between 3.3% (*L. fermentum* DSM 20052) and 20.6% (*L. plantarum* W42). In general, the optimal temperatures of phytate-degrading enzymes vary from 35 to 77°C, whereas the optimal temperatures from bacterial phytase are comprised between 50 and 70°C [Konietzny & Greiner, 2002; Vohra & Satyanarayana, 2003; Oh *et al.*, 2004]. The optimal temperatures of the activity of the studied strains are within the typical range found for phytase activities of bacteria.

Effect of the composition of the growth medium on phytate-degrading activity

The type and concentration of carbon source are one of the known nutritional factors that regulate the synthesis of bacterial phytases [Vohra & Satyanarayana, 2003]. The con-

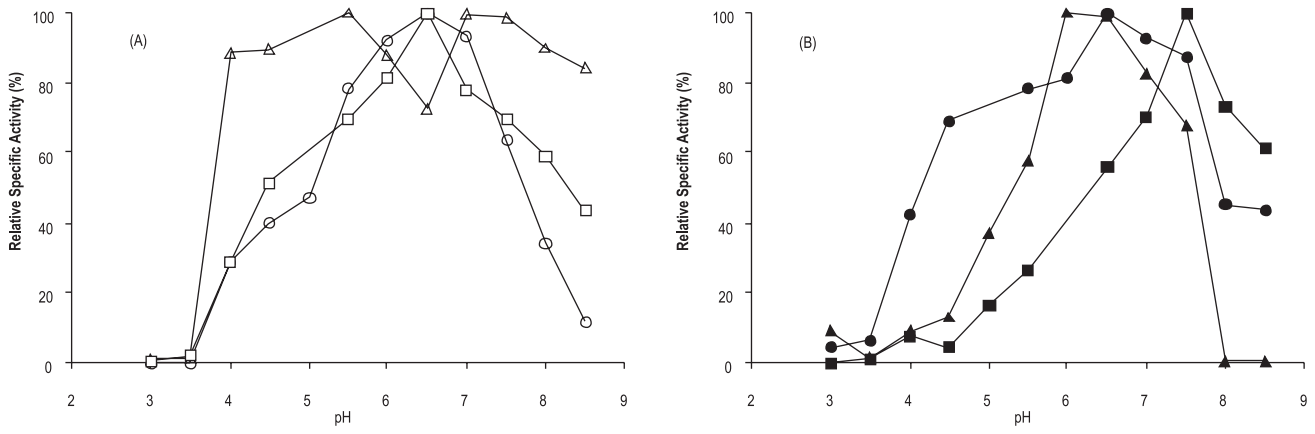

FIGURE 1. Effects of pH on phytate-degrading activities from *Lactobacillus* strains. Activities were determined in cell suspensions obtained from cultures incubated at 37°C until the stationary phase of growth was reached (18-20 h). The data are expressed as percentages of the maximal activity obtained at optimum pH. (A) *L. casei* DSM 20011 (△); *L. casei* 40W (○); *L. fermentum* DSM 20052 (□). (B) *L. plantarum* W42 (▲); *L. plantarum* JBPRS (●); *L. plantarum* 110 (■).

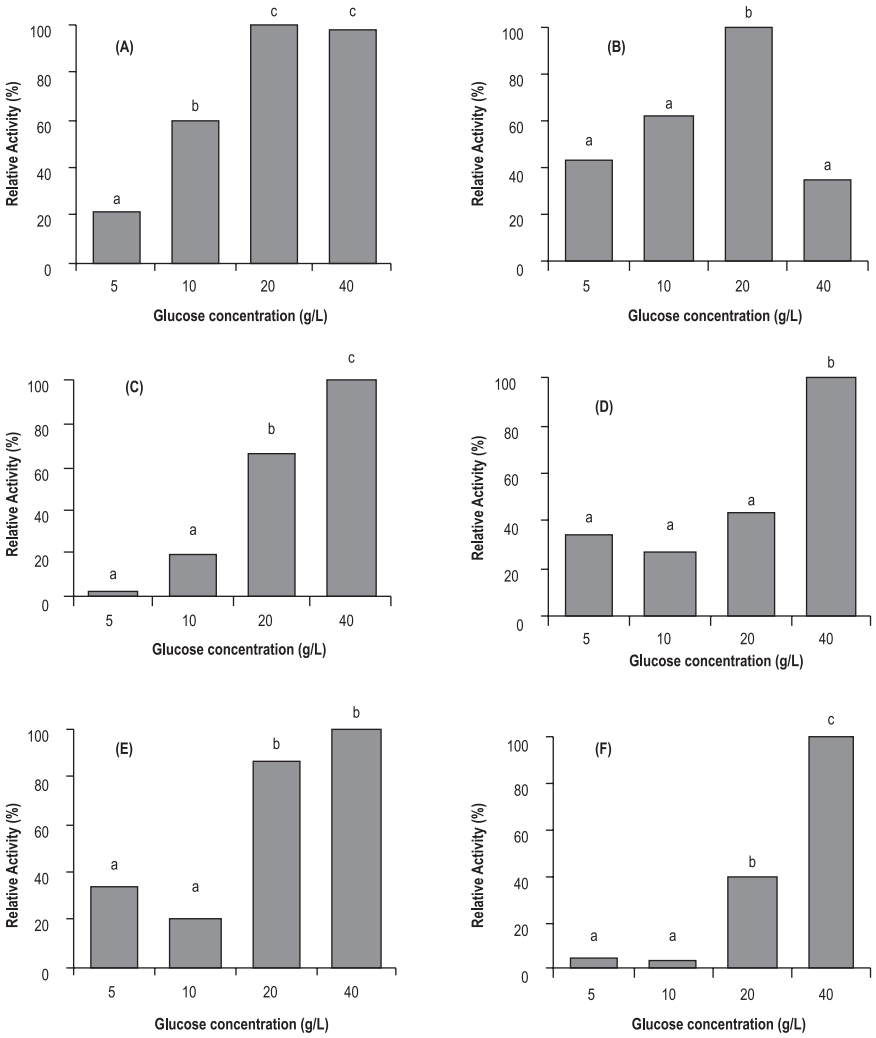

FIGURE 2. Effects of glucose concentration in the growth medium on specific phytate-degrading activity from *Lactobacillus* strains. Activities were determined in cell suspensions obtained from cultures incubated at 37°C until the stationary phase of growth was reached. (A) *L. casei* DSM 20011; (B) *L. casei* 40W; (C) *L. fermentum* DSM 20052; (D) *L. plantarum* JBPRS; (E) *L. plantarum* W42; (F) *L. plantarum* 110. Different letters indicate significant difference ($p<0.05$).

centration of glucose as the sole carbon source in the growth medium had an important effect on phytate-degrading enzyme production (Figure 2). The strains of the species *L. casei* showed the highest specific phytate-degrading activity when the glucose concentration in the growth medium was of 2%, whereas an increase in twice the glucose concentration dramatically decreased the enzyme production in *L. casei* 40W without modifying significantly the growth yield (1.11×10^9 CFU/mL, $p<0.05$). A reduction in the glucose concentration also caused a decrease in the enzyme production, but not in the growth yield ($0.82\text{-}1.14 \times 10^9$ CFU/mL, $p<0.05$). *L. casei* DSM 20011 also decreased the enzyme production when decrease the glucose concentration, being the CFU/mL at 5 g/L of glucose significantly lower ($p<0.05$). On the other hand, when the glucose amount was 40 g/L the production of the enzyme was not significantly different than that obtained at 20 g/L. According to Vohra & Satyanarayana [2003] glucose has been the most preferred substrate for phytase production, being 10 g/L the optimal concentration for *Lactobacillus amylovorus* [Sreeramulu *et al.*, 1996]. Palacios *et al.* [2005] reported that the enzyme production was maximal for *L. pentosus* CECT 4023 when glucose was at a level of 5 g/L. Opposite behaviour was found in the current work, since the tested strains showed their maximal enzyme production at the highest glucose concentrations (20-40 g/L). *L. fermentum* and the strains of *L. plantarum* showed their maximal production when glucose was at 40 g/L in the growth medium while the activity production dropped when the glucose was decreased to 5 g/L (Figure 2C-F), whereas the growth yield did not show any significantly difference (results not shown).

The effects of carbon sources alternative to glucose in the growth yields and specific phytase activities were also analysed (Table 2). The incorporation of lactose instead of glucose in the growth medium caused a significant reduction in the phytate-degrading activity in *L. casei* DSM 20011 (30.4%), *L. plantarum* W42 (19.8%) and *L. plantarum* 110 (15.5%), while growth yield was not significantly reduced in the last two strains ($p<0.05$). The specific relative phytate-degrading activity was significantly lower ($p<0.05$) when including raffinose as sole carbon source in *L. casei* DSM 20011, *L. plantarum* JBPRS, *L. plantarum* W42 and *L. plantarum* 110, whereas the growth yield did not show significant differences ($p<0.05$)

TABLE 2. Effects of the composition of the growth medium on relative specific phytate-degrading activity from lactic acid bacteria[a,b].

Strain	Parameter	Glucose 20 g/L	Lactose 20 g/L	Raffinose 20 g/L	FOS[c] 20 g/L	K_2HPO_4 2 g/L
L. casei	Relative Specific Phytase Activity (%)	100.0[a]	69.6[b]	5.85[d]	22.1[c]	81.0[b]
DSM 20011	Log CFU/mL	8.15[a]	9.03[b]	8.00[a]	9.25[b]	8.38[a]
L. casei	Relative Specific Phytase Activity (%)	100.0[b]	105.2[b]	232.3[a]	92.3[b]	35.5[c]
40W	Log CFU/mL	9.20[a]	9.26[a]	7.17[b]	9.26[a]	9.26[a]
L. fermentum	Relative Specific Phytase Activity (%)	100.0[b]	105.5[b]	191.8[a]	37.3[c]	48.0[c]
DSM 20052	Log CFU/mL	9.40[a]	9.23[a]	9.41[a]	9.29[a]	9.35[a]
L. plantarum	Relative Specific Phytase Activity (%)	100.0[b]	110.0[ab]	86.3[b]	141.3[a]	51.3[c]
JBPRS	Log CFU/mL	9.21[a]	9.02[a]	9.16[a]	9.08[a]	9.24[a]
L. plantarum	Relative Specific Phytase Activity (%)	100.0[a]	80.1[b]	9.45[d]	0.98e	43.9[c]
W42	Log CFU/mL	9.05[a]	9.27[a]	9.32[a]	9.26[a]	9.17[a]
L. plantarum	Relative Specific Phytase Activity (%)	100.0[a]	84.7[a]	41.0[b]	17.5[bc]	2.62[c]
110	Log CFU/mL	8.95[a]	8.85[a]	7.92[b]	8.86[a]	8.85[a]

[a]Cultures were incubated at 37°C until the stationary growth phase was reached (~14-18 hours) in modified MRS medium. [b]Mean, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence. [c]Fructo-oligosaccharides.

with the exception of *L. plantarum* 110 (Table 2). Contrarily, *L. casei* 40W and *L. fermentum* DSM 20052 showed a considerable increase ($p<0.05$) in the production of phytate-degrading enzyme in the presence of raffinose, even when the growth yield was significantly lower ($p<0.05$), (Table 2).

The specific phytate-degrading activity was significantly lower when using the complex oligosaccharide FOS instead glucose, with the exception of *L. casei* 40W and *L. plantarum* JBPRS. The first one showed a similar behaviour as when grown on glucose medium whereas the last one showed an increased enzyme production. In any case differences were not observed in the growth yields (Table 2).

When the strains were grown in the presence of inorganic phosphate the activities against phytate were markedly reduced, while the growth yields were not affected (Table 2). This result suggests that phytate-degrading enzyme synthesis is inhibited by free phosphate or the synthesis of bacterial phosphatases is stimulated due to the regulation of the gene expression in response to phosphate limiting conditions as it was described in *bacilli* [Hullet, 1996].

According to Shieh & Ware [1968] the phosphorus available in the medium controls the synthesis of the phytate-degrading enzymes by microorganism. In contrast, synthesis of phytase is generally induced when limiting concentrations of phosphorous are present in the growth medium in yeast, moulds and bacteria [Nayini & Markakis, 1984; Thaller *et al.*, 1997; Vohra & Satyanarayana, 2003].

The incorporation of different types of carbon sources or inorganic phosphate to the growth medium seemed to modulate the synthesis of phytate-degrading enzyme in the studied strains.

CONCLUSIONS

In summary, most of the studied strains have higher hydrolytic activity on mono-phosphates but could also contribute, to some extent, to phytate degradation. They have been demonstrated to possess phytate-degrading activity with different biochemical regulation properties, which suggests that they could contribute to phytate degradation during food processing.

Further studies should be carried out to provide progress in the understanding of the potential nutritional and technological roles of the most active strains in the elaboration of whole sour breads.

ACKNOWLEDGEMENTS

The authors thank Danuta Rostek (Department of Food Microbiology, Institute of Animal Reproduction and Food Research of the Polish, Olsztyn, Poland) for excellent technical assistance. This work was supported by Centre of Excellence (CENEXFOOD-EU, ICA-CT-2000-70017) and Ramón y Cajal Project (Ministerio de Ciencia y Tecnología-Spain).

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Received March 2007. Revision received and accepted June 2007.

AKTYWNOŚĆ BAKTERII FERMENTACJI MLEKOWEJ W DEGRADOWANIU FITYNIANÓW

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Zbadano zdolność do degradacji fitynianów i aktywność fosfatazy szczepów *Lactobacillus* wyizolowanych z różnych źródeł i należących do różnych gatunków. Specyficzna aktywność wobec fitynianu (Ins P_6) była zróżnicowana w szerokim przedziale 0,024-0,531 U/mg białka, a najwyższą aktywnością charakteryzował się szczep *L. casei* DSM 20011. Badane szczepy hydrolizowały p-nitrofenylofosforan w wyższym stopniu niż fitynian, z wyjątkiem *L. casei* DSM 20011 i *L. plantarum* W42. Zdolność szczepów do hydrolizy $InsP_6$ i generowania prostszych fosforanów *myo*-inozitolu podczas wzrostu określano metodą HPLC. $InsP_6$ był hydrolizowany w zakresie od 0,0 do 8,83, a najwyższy poziom hydrolizy stwierdzono w hodowli *L. plantarum* W42 i *L. plantarum* 110, a następnie *L. casei* 40W. Warunki hodowli, w których badane szczepy wykazywały najwyższą aktywność degradacji, były następujące: poziom pH 5,0-7,5 i temperatura 50-60°C. Zastosowanie w podłożu wzrostowym różnych źródeł węgla i fosforu nieorganicznego powodowało modulację syntezy enzymów degradujących fityniany.

Przyszłe badania powinny być prowadzone w kierunku pogłębienia wiedzy o potencjalnie odżywczej i technologicznej roli najbardziej aktywnych szczepów *Lactobacillus* w produkcji pełnoziarnistego chleba produkowanego na zakwasie.