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Proteolytic activity of *Lactobacillus* strains on soybean proteins

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ABSTRACT

The capacity of *Lactobacillus* strains for degrading soy proteins was evaluated. The hydrolytic action was monitored by SDS–PAGE and reverse phase-HPLC analyses. Peptidase activities were also determined using synthetic chromogenic substrates. All assayed strains were able to hydrolyze α'-subunit of β-conglycinin while for the other fractions the degree of degradation was strain-dependent. The pattern of peptides produced from soy proteins hydrolysis differed amongst the lactobacilli studied, suggesting different proteolytic enzyme specificities.

1. Introduction

Soy proteins stand out because of their high nutritional quality. The main soy proteins consist of β-conglycinin and glycinin. The first one is a trimer formed from various combinations of the three subunits (α′, α, and β, molecular weights 72, 68, and 52 kDa, respectively) while glycinin is made up of six subunits, each consisting of a basic polypeptide (B polypeptide) of approximately 20 kDa, and an acidic polypeptide (A polypeptide) of approximately 40 kDa which are linked by a disulfide bond (Christensen, Bruun, & Froekiaer, 2003).

In recent years there was a strong interest in soy protein for its health benefits (Liu & Zhao, 2010; Schalles & Montgomery, 2011). However, soy protein is considered the main allergen in children less than 3 years of age causing food hypersensitivity reactions even though the prevalence of soybean hypersensitivity in general population is lower than 0.5% (Besler, Helm, & Ogawa, 2000). As soy protein is increasingly used in a variety of foodstuffs, some predict that greater exposure will result in an increase of the hypersensitive population (Breiteneder & Ebner, 2000). Several reports have shown that glycinin is an important allergen in patients who have a food allergy to soybeans (Beardslee, Zeece, Sarath, & Markwell, 2000; Helm et al., 2000). All acidic glycinin chains of soybean were found to be the binding site of IgE from soy allergic patients (Pedersen & Djurtoft, 1989).

Some aspects of protein structure such as solubility, stability, size, degree of compaction of proteins folding are associated with allergenic effects (Aalberse, 2000) as well as functional properties (Friedman & Brandon, 2001; Lakemond, de Jongh, Hessing, Gruppen, & Voragen, 2000a,b; Rodríguez Niño, Carrera, Pizones, & Rodríguez Patino, 2005). A useful alternative to reduce the soy protein immunoreactivity is by increasing the solubility of the protein through enzymatic hydrolysis with proteases (Gibbs, Zougman, Masse, & Mulligan, 2004; Wang & Gonzalez de Mejia, 2005). The use of proteases in industrial processes is advantageous compared to chemical processes because it prevents side reactions, reduces environmental impact and increases the nutritional value of the protein. The enzyme-substrate specificity allows to obtain protein hydrolyzes with best features from the nutritional point of view. In addition, hydrolyzed proteins are more easily digested than the whole protein (Tavano, 2013).

Lactic acid bacteria (LAB), traditionally used in the manufacture of a wide range of vegetable and animal products, possess a proteolytic system composed of proteinases, peptidases and peptide transport systems which is vital to bacterial nutrition, but also contributes to the formation of flavor and texture of fermented products (Hebert, Raya, & Savoy de Giori, 2000; Pescuma, Hebert, Mozzi, & Font de Valdez, 2010). In a previous study, we demonstrated that some LABs, grown in a complex growth medium, were

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able to hydrolyze soy protein where α- and α'-subunit of β-conglycinin were the most degraded fractions (Aguirre, Garro, & Savoy de Giori, 2008). However, the proteinase activity of several LAB strains has been found to be modulated according to culture medium used for growth of LAB (Hebert et al., 2000). Proteolytic activity was repressed when the strains were grown in the peptide-rich medium such as MRS. This repression was not observed when a synthetic medium was used to grow LAB strains (Hebert et al., 2000; Meijer, Marugg, & Hugenholtz, 1996). While the proteolytic system of LAB in milk has been extensively studied from a nutritional and technological standpoint (Espeche Turbay, de Moreno de LeBlanc, Perdigón, Savoy de Giori, & Hebert, 2012; Liu, Bayjanov, Renckens, Nauta, & Siezen, 2010; Savijoki, Ingmer, & Varmanen, 2006), our current knowledge of this physiological trait of LAB in soybean is very scarce. Therefore, the aim of this work was to evaluate the ability of four strains of lactobacilli to hydrolyze the major soy proteins, β-conglycinin and glycinin, of a soy protein extract. A chemically defined medium was used to avoid interferences of other proteins present in complex media.

2. Materials and methods

2.1. Microorganisms, media and growth conditions

Lactobacillus (*L.*) *paracasei* subsp. *paracasei* CRL 207, *Lactobacillus delbrueckii* subsp. *lactis* CRL 581, *Lactobacillus helveticus* CRL 1062, and *Lactobacillus reuteri* CRL 1099 belonging to the culture collection (CRL) of the Centro de Referencia para Lactobacilos (CERELA-CONICET, Tucumán, Argentina) were used in this study. The strains were previously selected by their proteolytic activity on soymilk (Aguirre et al., 2008). Working cultures of the lactobacilli strains were propagated twice in MRS broth at 37 °C for 16 h. To eliminate carryover nutrients, cells were harvested by centrifugation at 8000 *g* for 10 min; washed twice in sterile 0.85% (w/v) saline solution, and suspended in this solution to the original volume. This cell suspension was used to inoculate a chemically defined medium (CDM) previously described by Hebert et al. (2000) at an initial optimal density at 560 nm (OD_{560}) of 0.08–0.10. After reaching exponential growth phase (OD_{560} = 0.5–0.6), cells were harvested by centrifugation (10,000 *g*, 10 min, 4 °C), washed twice with saline solution containing 10 mmol L^{-1} $CaCl_2$ (pH 7.0) and suspended in 100 mmol L^{-1} phosphate buffer (pH 7.0) to give a final concentration of 30% of wet weight (w/v). These cell suspensions constitute non-proliferating cells systems. They were maintained on ice and immediately used for further assays.

2.2. Soy proteins extract

Soybean proteins were extracted according to the method of Aguirre et al. (2008). Briefly, defatted commercial soybean flour (20 g) was extracted once with 400 ml of 30 mmol L^{-1} Tris-HCl buffer (pH 8.0) containing 10 mmol L^{-1} 2-mercaptoethanol at room temperature with agitation for 1 h and then centrifuged at 15,000 *g* for 20 min at 4 °C. The resulting supernatant (soybean protein extract, SPE), a glycinin and β-conglycinin-rich fraction, was sterilized through 0.22 μm filters (Millipore, Bedford, MA). The SPE was immediately kept at −20 °C until use.

2.3. Hydrolysis of soy proteins extract by non-proliferating cells

To evaluate the ability of LAB strains for hydrolyzing soy proteins extract, a non-proliferating cell system, prepared as described above, was used. Cell suspensions of each microorganism were kept 30 min at 37 °C for amino acid starvation and then incubated at a cell-protein ratio of 2:1 (v/v) using SPE as protein source. The cell-protein mixtures were incubated for 6 h at 37 °C, then centrifuged (10,000 *g* for 10 min at 4 °C) and stored at −20 °C for further analysis. Non-proliferating cells suspension without adding SPE and SPE without the addition of non-proliferating cells, incubated for the same period, were used as controls.

2.4. Gel electrophoresis

Soy protein degradation, analyzed in the supernatants, was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Aguirre et al., 2008). Coomassie Brilliant Blue R-250 was used to visualize the proteins bands. The gels were scanned and analyzed with QuantiScan software (Biosoft International, Ferguson, MO, USA). Soy proteins hydrolysis was expressed as a percentage of disappearance of the protein fraction bands with respect to the control (non-inoculated soy proteins). The protein concentration was determined according to Bradford (1976) using bovine serum albumin as standard.

2.5. Peptide analysis

Peptides released were also analyzed by reversed-phase high-performance liquid chromatography (RP–HPLC) according to Aguirre et al. (2008) using an ISCO System HPLC (ISCO, Lincoln; NE, USA) with a UV detector fitted with a Spherisorb ODS2 column (C_{18}, 4.6 × 250 mm, 5 μm) ISCO (Lincoln; NE, USA.). The solvents and operating conditions were same as described previously (Aguirre et al., 2008).

2.6. Preparation of cell-free extracts

Cell cultures were grown in CDM as described previously, washed twice with 100 mmol L^{-1} sodium phosphate buffer (pH 7.0) and resuspended to a final OD_{560} of approximately 10 in the same buffer. Cells were disrupted with two volumes of glass beads (0.1 mm diameter, Biospec Products, Bartlesville, OK, USA) in a mini bed beater-8 (Biospec Products) for 10 min (with 1 min interruption on ice after each minute) at maximum speed. Glass beads, cell debris and unbroken cells were removed by centrifugation (10,000 *g*, 5 min, 4 °C) and the supernatant fluid was used as cell-free extract. Cell-free extracts were maintained on ice and immediately used for enzymatic assays.

2.7. Enzyme assays

2.7.1. Aminopeptidase (AP) activity

AP activity was measured by using chromogenic substrates *p*-nitroanilide (*p*-NA) derivatives of L-anomers of lysine, proline, or glutamine as described by Rollan, De Angelis, Gobbetti, and de Valdez (2005). The released *p*-nitroaniline was spectrophotometrically measured in the supernatant at 410 nm in a VersaMax Tunable Microplate Reader (Molecular Devices; Sunnyvale, CA, USA). One unit (U) of enzyme was defined as the amount of enzyme required to release 1 μmol of *p*-nitroaniline per minute under the assay conditions. Specific activity was expressed as units of aminopeptidase per milligram of protein.

2.7.2. X-prolyl-dipeptidyl aminopeptidase (PDA) activity

PDA activity was measured on Leu–Pro–*p*-NA as substrate as previously described (Rollan et al., 2005). The enzyme units were defined as before.

2.7.3. Dipeptidase (DP), tripeptidase (TP) and endopeptidase (EP) activities

DP activity was measured on Leu–Leu, Leu–Pro, and Pro–Leu, TP activity on Leu–Leu–Leu, **DL**-Leu–Gly-**DL**-Phe and Leu–Gly–Gly, and EP activity on *N*-succinyl **L**-phenyl-alanine-*p*-NA and N-glutaryl **L**-phenyl-alanine-*p*-NA as substrates. The hydrolysis of the synthetic substrates was carried out at 37 °C following the procedure described by Rollan et al. (2005). Enzyme activity was determined by reading at 505 nm in a VersaMax Tunable Microplate Reader (Molecular Devices; Sunnyvale, CA, USA). One enzyme unit (U) was defined as the amount of enzyme required to release 1 μmol of amino acid per minute. The specific activity was defined as enzyme units per milligram of protein.

2.8. Statistical analysis

All assays were carried out in triplicate, and results were expressed as mean values with standard deviations. Data were compared by one-way analysis of variance (ANOVA) followed by Dunnett *t*-test. The statistical analyses were performed with the Minitab-12 software (Minitab Inc., State College, PA, USA) and differences were considered significant at $P < 0.05$.

3. Results

3.1. Hydrolysis of SPE by LAB proteolytic activity: Electrophoretic analysis

Cell lysis was not observed throughout the incubation period since no aminopeptidase activity, used as a lysis marker, was detected. All LAB strains showed different protein degradation degrees on SPE, reaching values between 10 and 90% at 6 h of incubation (Table 1). β-Conglicinin, one of the major protein, was the preferred substrate for *L. delbrueckii* subsp. *lactis* CRL 581, *L. helveticus* CRL 1062, and *L. reuteri* CRL 1099. *L. paracasei* subsp. *paracasei* CRL 207 hydrolyzed, in the same extent, both main fractions (β-conglycinin and glycinin). Hydrolysis of β-conglycinin subunits α′- (67 kDa), α- (63 kDa), and β- (48 kDa) was strain-dependent. *L. reuteri* CRL 1099 showed the highest percentage of degradation for α′- (90%) and α- (80%) fractions. In general, α'-fraction was more susceptible to hydrolysis (1.12–1.38-fold) than α-fraction while the β subunit was degraded to a lesser extent. Regarding glycinin, most of the strains hydrolyzed in a greater degree the acidic fraction (AS, 40 kDa) than the basic fraction (BS, 20 kDa). *L. reuteri* CRL 1099 was able to increase (3.7 times) the acidic fraction hydrolysis respect to the basic fraction. In contrast to that found for CRL 1062 that preferentially degraded the basic fraction (1.47 times). No β-conglycinin or glycinin breakdown was detected without adding non-proliferating cells even after 8 h of incubation, indicating that protein degradation was because of the proteolytic activity displayed by the LAB strains.

3.2. Peptide profiles from SPE hydrolysis: RP-HPLC analysis

Analysis by SDS–PAGE and RP–HPLC revealed different peptide profiles from SPE hydrolysis according to the strain tested (Fig. 1). The SPE chromatogram showed seven important peaks eluting at different retention times (RT) (Fig. 1a): 3.40 min and 4.70 min (peaks 1–2) and RT of 13.65 min and 15.04 min (peaks 3–4) corresponding to the majority of β-conglycinin fractions. The other three peaks (5–7) had RTs of 25.87 min, 29.19 min and 33.12 min, respectively, related to glycinin. Slight changes were observed in the peptide profiles of control samples when the SPE was mixed with different non-proliferating cells suspensions at the initial time (Fig. 1b) with respect to SPE; this control was similar for each strain of LAB used (one representative pattern is shown in the figure). The peptide profiles for each of these SPE hydrolyzates after 6 h of incubation were different with several peaks decreasing in size and new peaks being generated (Fig. 1c–f). In general, the peptides released were hydrophilic but some hydrophobic characteristics were also observed.

The action of non proliferating cells of *L. paracasei* subsp. *paracasei* CRL 207 on SPE after 6 h generated peaks eluting between 3.00 and 5.50 min of retention time, while other peaks related to glycinin (25.87–33.12 min) were almost fully hydrolyzed (Fig. 1c).

SPE degradation by *L. delbrueckii* subsp. *lactis* CRL 581 (Fig 1d) released different peptides, mostly of them at the beginning of the chromatogram (2.60–5.30 min) while two peptides, one of them less hydrophilic (15 min) and other hydrophobic (33 min) were also observed.

Small amounts of peptides within a broad range (6–25 min) were released by *L. helveticus* CRL 1062 (Fig. 1e) and *L. reuteri* CRL 1099 (Fig. 1f). CRL 1099 activity on SPE, corresponding to glycinin fraction, was not as relevant but also contributed to release of new peptides in the hydrophobic area (in the region from 25 to 40 min, Fig. 1f).

3.3. Peptidase activities

The peptidase activities of LAB cell-free extracts are shown in Table 2. Aminopeptidase activity was, in general, highest when leucine (Leu-*p*NA) or lysine (Lys-*p*NA) was present at the N-terminal position with respect to alanine (Ala-*p*NA), glycine (Gly-*p*NA), methionine (Met-*p*NA), glutamic acid (Glu-*p*NA), proline (P-*p*NA) or

Table 1
Degradation of soy protein extract by non-proliferating cells of *Lactobacillus* (*L.*) *paracasei* subsp. *paracasei* CRL 207, *L. delbrueckii* subsp. *lactis* CRL 581, *L. helveticus* CRL 1062, and *L. reuteri* CRL 1099. Mean values in each column having different letters are significantly different ($p < 0.05$). The letter 'a' represents the highest value.

Strains	Soy protein extract (% hydrolyzed)					
	β-Conglycinin			Glycinin		Total average
	α′	α	β	AS	BS	
CRL 207	82.0 ± 5.0b	58.0 ± 3.0c	63.0 ± 4.0a	80.0 ± 4.5a	57.0 ± 3.8a	68.0 ± 4.1a
CRL 581	80.0 ± 3.0b	68.0 ± 4.0b	60.0 ± 5.0a	62.0 ± 3.8b	51.0 ± 3.8 ab	64.0 ± 3.9a
CRL 1062	65.0 ± 3.0c	58.0 ± 3.0c	51.0 ± 4.0b	40.0 ± 4.5c	59.0 ± 3.8a	55.0 ± 3.7 ab
CRL 1099	90.0 ± 3.0a	80.0 ± 4.0a	25.0 ± 5.0c	37.0 ± 3.8c	10.0 ± 3.8c	48.0 ± 3.9b
Total average	79.0 ± 3.5b	66.0 ± 3.5b	49.0 ± 4.5b	55.0 ± 4.2b	44.0 ± 3.8b	

α, α′, β: main fractions of β-conglycinin.
AS: acidic fraction of glycinin.
BS: basic fraction of glycinin.
% hydrolyzed: hydrolysis of each main fraction of soy protein extract (SPE) treated with non-proliferating cells of different lactobacilli strains, during 6 h at 37 °C, with respect to those SPE without treatment.

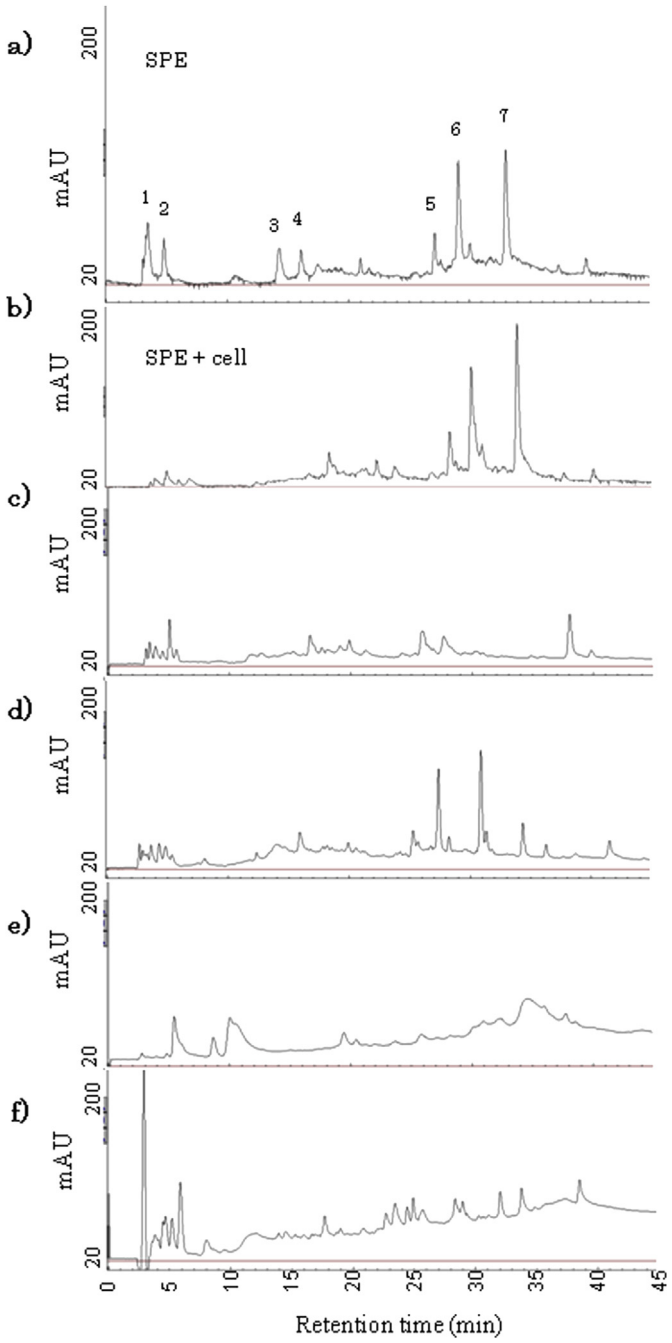

Fig. 1. Reverse-phase HPLC patterns of soluble peptides present in soy protein extract (SPE) treated with lactic acid bacteria (LAB) enzymes. Controls: (**a**) SPE; (**b**) SPE and non-proliferating cells of LAB at 0 h. Samples, SPE treated with whole-cell suspensions of LAB at 6 h; (**c**) *L. paracasei subsp. paracasei* CRL 207; (**d**) *L. delbrueckii* subsp. *lactis* CRL 581; (**e**) *L. helveticus* CRL 1062; (**f**) *L. reuteri* CRL 1099.

valine (Val-*p*NA). This enzyme had high affinity for positively charged amino acids such as Lys or for hydrophobic amino acids such as Leu showing low activity towards Glu- and Pro-*p*NA. Activity values for *L. paracasei* subsp. *paracasei* CRL 207 were 1.7–11.7–45.8 (for Leu-*p*NA) and 1.2–12.4–22.8 (for Lys-*p*NA) fold higher than those detected for *L. helveticus* CRL 1062, *L. reuteri* CRL 1099 and *L. delbrueckii* subsp. *lactis* CRL 581, respectively.

All strains showed a wide spectrum of dipeptidase activities values; *L. paracasei* subsp. *paracasei* CRL 207 showed the highest (713.6 μmol min^{-1} mg^{-1} protein) (for Leu-Leu-*p*NA), while the lowest (6.2 μmol min^{-1} mg^{-1} protein) (for Leu-Pro-*p*NA) corresponded to *L. reuteri* CRL 1099. Respect to tripeptidase activities, LAB were active against all substrates assayed chosen according to the major amino acid residues present in the soy proteins being *L paracasei* subsp. *paracasei* CRL 207 more active, with only CRL 207 displayed the highest EP activity for both substrates N-succinyl-phenyl-alanine-*p*-NA and N-glutaryl-phenyl-alanine-*p*-NA.

4. Discussion

Proteolysis is considered one of the most important biochemical processes involved in the manufacture of many fermented products (Savijoki et al., 2006) the proteolytic system of LAB participation to the organoleptic characteristics of the final product being significant. Also, proteolysis could contribute to preventing allergenic problems frequent in children under 3 years of age due to reduction of immunoreactivity of certain soy protein fractions (Besler et al., 2000).

This work was conducted using a CDM to allow counteracting the generation of interfering protein/peptide bands present in complex media such as MRS broth. In addition, some LAB proteolytic enzymes were repressed when the strains are grown in complex (rich peptide) media (e.g. MRS); this repression was not observed when a CDM was used to grow LAB strains (Hebert et al., 2000; Meijer et al., 1996). The ability of the four LAB strains to breakdown the main soy proteins, β-conglycinin and glycinin, was evaluated in CDM by using a non-proliferating cell system. In this system, LAB strains were able to degrade the major soy proteins up to 6 h of incubation time, being the degradation strain-dependent. In general, β-conglicinin was degraded by all strains; likely due to the structural characteristics of the substrate allowing bacterial proteinase easy access to the cleavage sites. In this respect, Barac, Jovanovic, Stanojevic, and Pesic (2006) also observed easier enzymatic degradation of β-conglycinin that glycinin because the latter protein has a compact structure stabilized by disulfide bonds and hydrophobic and electrostatic interactions. The high activity of the proteinase of *L. paracasei* subsp. *paracasei* on glycinin could be

Table 2
Peptidase activities of cell free extracts of *Lactobacillus* (*L.*) *paracasei* subsp. *paracasei* CRL 207, *L. delbrueckii* subsp. *lactis* CRL 581, *L. helveticus* CRL 1062, and *L. reuteri* CRL 1099, grown on CDM. Mean values in each row having different letters have significantly different ($p < 0.05$). The letter 'a' represents the highest value.

Substrate/Strains	Peptidase activity (μmol min^{-1} mg $protein^{-1}$)			
	CRL 207	CRL 581	CRL 1062	CRL 1099
Aminopeptidases				
Ala-*p*NA	35.6 ± 5.7a	1.5 ± 0.2c	23.4 ± 6.0a	4.2 ± 0.8b
Glu-*p*NA (PepA)	18.3 ± 5.3a	1.6 ± 0.2c	1.5 ± 0.9c	6.3 ± 1.8b
Gly-*p*NA	9.3 ± 2.2a	1.1 ± 0.1c	5.7 ± 0.3b	8.6 ± 2.2a
Leu-*p*NA (PepL)	164.9 ± 47.9a	3.6 ± 0.4d	96.6 ± 4.7b	14.0 ± 1.8c
Lys-*p*NA	258.2 ± 51.9a	11.3 ± 2.2c	212.7 ± 9.1a	20.8 ± 1.9b
Met-*p*NA	51.5 ± 14.2a	1.2 ± 0.1d	21.7 ± 1.7b	5.6 ± 1.0c
Pro-*p*NA (PepI)	8.1 ± 2.9a	1.6 ± 0.2c	3.6 ± 0.6b	0.5 ± 0.2d
Val-*p*NA	9.9 ± 2.5a	0.6 ± 0.1d	1.4 ± 0.3c	2.9 ± 0.7b
Dipeptidases				
Leu–Leu (PepV)	713.6 ± 33.0a	258.4 ± 11.9c	348.5 ± 39.2b	123.2 ± 8.5d
Leu–Pro (PepQ)	309.1 ± 22.0a	114.5 ± 8.1b	119.3 ± 46.0b	6.2 ± 2.4c
Pro–Leu (PepR)	35.8 ± 6.1b	48.5 ± 8.2b	116.1 ± 20.2a	25.5 ± 3.2c
Tripeptidases				
Leu-Leu-Leu (TPa)	2232.3 ± 160a	283.8 ± 5.7c	462.6 ± 19.5b	250.9 ± 11.5d
Leu-Gly-Gly (TPb)	138.7 ± 9.7c	107.2 ± 7.4d	191.0 ± 13.5a	159.5 ± 11.2b
Leu-Gly-Phe (TPc)	621.3 ± 43.7a	55.8 ± 3.9d	197.4 ± 13.9b	109.0 ± 7.7c
Endopeptidases				
Succ-Phe-*p*NA	14.5 ± 4.1a	1.1 ± 0.1c	0.9 ± 0.3c	2.2 ± 0.5bc
Glu-Phe-*p*NA	6.2 ± 1.4a	0.8 ± 0.1b	1.0 ± 0.1b	1.3 ± 0.3b

related to their physicochemical characteristics that would allow it to interact more easily with the substrate facilitating its hydrolysis. The fraction α' of β-conglycinin and the acidic fraction (AS) of glycinin were more susceptible to hydrolysis by most of the strains. These results indicate that the proteinase of the studied LAB act mainly on the hydrophilic fraction (α' of β-conglycinin and AS-glycinin) that is more exposed in the native structure of β-conglycinin and glycinin.

Unlike acid polypeptide (approximately 37–43 kDa) of glycinin, So and Kim (2001) reported that the basic polypeptide is notoriously resistant to enzymatic hydrolysis. This differential susceptibility to hydrolysis of the acid fraction compared with the basic polypeptides was probably due to a greater hydrophobicity and compactness of the basic polypeptides of glycinin with respect to the acid polypeptides (Kang, Rhee, & Park, 1988). Moreover, in the system of non-proliferating cells in CDM, the protein hydrolyzing ability of the strains was optimized with respect to obtained with growing cells in MRS (Aguirre et al., 2008); a higher LAB protease activity showed their potential to diminish AS-glycinin concentrations (one of the major allergens of soybean) resulting more active *L. paracasei* subsp. *paracasei*. Song, Frias, Martinez-Villaluenga, Vidal-Valdeverde, and Gonzalez de Mejia (2008) found a greater number of small peptides (<30 kDa) and less immunoreactive in a soybean inoculated with a *L. plantarum* strain as compared with natural fermentation. According to these authors, the microbial hydrolysis of soy protein can change the structure of the allergens and therefore interfere with the antigen–antibody complex. *L. reuteri* was more active (10 fold) to hydrolyze α' of β-conglycinin as compared with those found in MRS (Aguirre et al., 2008). While, *L. helveticus* was capable of degrading (40%) AS-glycinin fraction, contrary to the results obtained for hydrolysis in MRS. This fact could be due to low molecular weight peptides present in complex media that play important roles in the medium-dependent regulation of proteinase activity (Hebert, Raya, & Savoy de Giori, 2002).

In general, SPE degradation by LAB released more hydrophilic peptides than those observed for the hydrolysis of SPE by papain (Wu, Hettiarachchy, & Qi, 1998). The peptide patterns produced from SPE hydrolysis differed among the LAB strains used, suggesting different proteinase specificities. It is noteworthy that hydrophilic peptides are normally correlated with desirable fermented soy flavors (Feng, Chen, Li, & Ren, 2013). In this regards, *L. paracasei* subsp. *paracasei* CRL 207 exerted the major effect over the main soy protein fractions releasing only hydrophilic peptides and fully hydrolyzed those related with glycinin fraction. The *Lactobacillus* AP evaluated had several features in common with other AP from different *Lactobacillus* species showing that Lys-*p*NA or Leu-*p*NA was the preferred substrate. These observations also have been reported for others LAB (Rollan & Font de Valdez, 2001). DP or TP activities were relatively high when Leucyl di- or tripeptides were used as substrates. The higher DP activity found on Leu–Leu rather than on Leu–Pro, and the preferential hydrolysis of dipeptides containing Leu, was similar to those found for *L. delbrueckii* subsp. *lactis* CRL 581 and *L. helveticus* CRL 1062 (Hebert et al., 2002) and for *L. plantarum* CRL 759 and CRL 778 (Rollan et al., 2005).

The potentiality of four lactobacilli strains to hydrolyze the major soy proteins, especially AS-glycinin (considered one of immunoreactive proteins) was shown in this study. These LAB strains could be used as adjunct cultures to improve soy protein digestibility allowing their inclusion in human diet. According to Zuo, Chen, and Zou (2005) conglycinin digestion represents an important mechanism for the peptides that show important physiological functions in addition to their nutritional importance. Therefore, the characterization of β-conglycinin hydrolyzates obtained by the action of the proteolytic system of LAB, elucidation of the relationship between peptide structure and activity awaits future study.

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