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Phytate-degrading activity of probiotic bacteria exposed to simulated gastrointestinal fluids

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ABSTRACT

Phytate is considered an anti-nutrient, because it is not digestible for humans given our lack of phytase activity (PhyA). It has been hypothesized that Lactobacilli may act as carriers of phytate-degrading activity in the gastrointestinal tract. Therefore, the aim of this work was to assess the PhyA of nineteen probiotic bacteria exposed to simulated gastrointestinal fluids. Each culture was inoculated to three different growth media (MRS, MRS-MOPS and MRS-MOPS + AA). Cell-free extracts were used to test for PhyA assay. The highest PhyA ($P < 0.05$) was observed in MRS-MOPS, which was higher than MRS (control). A decrease in PhyA was observed in crude enzyme extracts (CEE) after exposure to gastrointestinal fluids. However, PhyA was higher at low gastric pH values. PhyA was greater in CEE obtained from bacteria that were previously exposed to simulated gastrointestinal conditions, than in those that were not exposed. CEE from Lc-L9 and Lc-12A strains showed the highest activities under intestinal conditions; while Lc-7R1 and Ls-B1917 were the highest under gastric conditions. The ability of specific probiotic bacteria to degrade phytate after exposure to simulated gastrointestinal conditions was demonstrated, which suggests that these bacteria may contribute to improve phytate degradation in humans during digestion.

1. Introduction

Phytate or phytic acid (PA) [*myo*-inositol hexa*kis*(dihydrogenphosphate)] is a naturally-occurring compound found in all seeds and possibly all cells of plants. It accumulates during the development until seed maturation and accounts for 60–90% of the total phosphorus content in cereals, legumes, nuts and oilseeds; it is the main form of phosphorus storage (Coelho & Benedito, 2008; De Angelis et al., 2003).

PA carries a strong negative charge and is capable to act as a strong chelator of cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}. PA can also complex the basic amino acid group of proteins, thus reducing the dietary bioavailability of these nutrients (Angel, Tamim, Applegate, Dhandu, & Ellestad, 2002). For this property, PA is considered as an anti-nutritional factor for humans and animals (Raghavendra & Halami, 2009).

Several strategies to reduce PA from foods have been considered, including some processing methods such as: cooking, germination, hydrothermal treatment, soaking, malting, and milling of cereals (Kumar, Sinha, Makkar, & Becker, 2010; Sandberg & Andlid, 2002). Current evidence suggests that phytases of the microorganisms used for fermentation may also contribute significantly to phytate degradation (Haros, Bielecka, Honke, & Sanz, 2008; Kumar et al., 2010). Additionally, recombinant *Lactobacillus* cultures with a phytase gene have been fed to livestock animals as a strategy to improve digestibility and reduce anti-nutritive effects of phytate (Askelson, Campasino, Lee, & Duong, 2014; Wang et al., 2014). Although this approach could represent an ideal animal feed additive in the future, different gastrointestinal factors that may affect the production of recombinant *Lactobacillus* phytase at intestinal level are still not entirely understood.

Additionally, some authors (Famularo, De Simone, Pandey, Sahu,

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& Minisola, 2005) have hypothesized that manipulating the endogenous digestive microbiota of subjects through the ingestion of specific lactic acid bacteria (LAB) might counteract the occurrence of the malabsorption syndrome dependent on the high PA content of their diet. In this respect, particular studies have screened the phytate-degrading activity of some probiotic bacteria (Khodaii et al., 2013), even at pH values similar to those of the human intestine, (Haros, Bielecka, & Sanz, 2005; Haros et al., 2008; Raghavendra & Halami, 2009). However, such studies have not considered the potential effect of digestive enzymes on the phytate-degrading activity of bacteria. Furthermore, it should be noted that many variables could influence the efficiency of phytase in the gastrointestinal tract including the type of phytase (*e.g.* 3- or 6-phytase), phytase origin (bacterial strain, fungal) pH optimum and the resistance of phytase to endogenous proteases (Dersjant-Li, Awati, Schulze, & Partridge, 2015). Hence, the purpose of this work was to investigate the phytate degrading activity of nineteen probiotic bacteria exposed to simulated gastrointestinal fluids that mimic human digestion.

2. Materials and methods

2.1. Probiotic bacteria culture

Table 1 lists the nineteen strains included in this study and their identification codes. All the freeze-dried strains were activated in MRS broth (De Man, Rogosa & Sharpe, Difco™, Becton-Dickinson, Le Pont de Claix, France) and maintained at −80 °C in 20% (v/v) glycerol.

2.2. Crude cell-free enzyme extracts preparation

Working cultures were prepared from frozen stock by two transfers in MRS broth. Both subculture steps involved 0.1% inoculum with incubation at 37 °C for 12 h and 8 h, respectively. An inoculum of 1% (v/v) of each actively grown culture was added into 5 mL of either MRS broth (pH 6.2), or modified MRS broth (MRS-MOPS) in which inorganic phosphate was replaced by sodium phytate (0.65 g/L, Sigma), 0.1 M 3-[N-morpholino] propanesulphonic acid (MOPS, Sigma) was added, and the contents of glucose, yeast extract and meat extract were reduced to 10, 2 and 4 g/L, respectively (Palacios, Haros, Rosell, & Sanz, 2008). Additionally, MRS-MOPS broth added with arginine and glycine (10 g/L), and supplemented with lactose (10 g/L) instead of glucose (MRS-MOPS + AA) was inoculated with each active culture (Palacios & Haros, 2005). All inoculated media were incubated without shaking during 20 h at 37 °C. After the incubation period, the cells were removed by centrifugation (3214 g, 10 min, 10 °C), and the clear supernatant fluid was filter sterilized (0.22 μm) and stored at −20 °C until used for the enzyme activity assay.

Using a crude enzyme extract (CEE) was a practical approach since a crude enzyme preparation as well as an enzyme present in a food matrix appears to be more stable than the corresponding highly purified enzyme (Greiner & Konietzny, 2006).

2.3. Preparation of simulated saliva, gastric and intestinal juice

To simulate *in vivo* solution of saliva, a sterile electrolyte solution (6.2 g/L NaCl, 2.2 g/L KCl, 0.22 g/L $CaCl_2$, 1.2 g/L $NaHCO_3$) was prepared. Then, human lysozyme (Sigma) was added to a final concentration of 0.01% (Fernández de Palencia, López, Corbí, Peláez, & Requena, 2008). Simulated gastric juice was prepared by suspending pepsin (3 g/L, Sigma) in sterile NaCl solution (5% w/v) and the pH was adjusted to 1.0 and 2.0 with concentrated HCl. Meanwhile, simulated small intestine juice was prepared by suspending pancreatin (1 g/L, Sigma) and bile salts (0.3% w/v, Sigma) in sterile NaCl solution (5% w/v) and the pH was adjusted to 6.0 and 7.0 with concentrated HCl or 0.1 N NaOH, as needed (Kos, Suskovic, Goreta, & Matosic, 2000).

2.4. Exposition of CEE to simulated gastrointestinal fluids

Susceptibility of phytase inactivation by simulated gastric or intestinal juice was determined by pre-incubating 50 μL of crude enzyme preparation in 50 μL of simulated gastric (pH 1 and 2) or intestinal juice (pH 6 and 7) for 60 min at 37 °C. After the incubation period, sodium phytate (substrate) was directly added to the reaction mixture to assay the PhyA as described below. A negative control was prepared without substrate added after the incubation period.

2.5. Exposure of bacterial strains to simulated human gastrointestinal fluids

In order to determine the ability of bacteria to produce phytases once they were exposed to simulated gastrointestinal fluids,

Table 1
Probiotic bacteria used in this study.

Strain	Code	Provider/Source
L. reuteri NRRL 14171	Lr-14171	Prof. Garcia's culture collection, (Instituto Tecnológico de Veracruz, Mexico)
B. bifidum NCFB 2715	Bb-2715	
L. acidophillus NRRL B-4495	La-B4495	Prof. Lopez-Malo's culture collection, (Universidad de las Américas, Mexico)
L. casei NRRL B-1922	Lc-B1922	
L. fermentum NRRL B-1932	Lf-B1932	
L. plantarum NRRL B-4496	Lp-B4496	
L. rhamnosus NRRL B-442	Lr-B442	
L. sakei NRRL B-1917	Ls-B1917	
L. casei DPC3968	Lc-3968	
L. casei L30	Lc-L30	Prof. James Steele's culture collection, Department of Food Science (University of Wisconsin; Madison, WI, USA)
L. casei L9	Lc-L9	
L. casei ATCC334	Lc-334	
L. casei 12A	Lc-12A	
L. casei 21/1	Lc-21/1	
L. casei 7R1	Lc-7R1	
B. lactis BB12	Bl-Bb12	Yogurt Albert Heijn® (Oostzaan, Netherlands)
L. casei CRL431	Lc-CRL431	
L. helveticus R0052	Lc-R0052	Maramor Chocolates™ (Columbus, OH, USA)
B. longum R0175	Bl-R0175	