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Peptides with angiotensin I converting enzyme (ACE) inhibitory activity generated from porcine skeletal muscle proteins by the action of meat-borne *Lactobacillus*☆

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ABSTRACT

Angiotensin I converting enzyme (ACE) inhibitory activity of peptides derived from the hydrolysis of sarcoplasmic and myofibrillar porcine proteins by the action of *Lactobacillus sakei* CRL1862 and *Lactobacillus curvatus* CRL705 (whole cells + cell free extracts) was investigated at 30 °C for 36 h. The protein hydrolysates were subjected to RP-HPLC in order to fractionate the extracts for further evaluation of ACE inhibitory activity. Bioactive fractions were only found from the hydrolysis of sarcoplasmic proteins by both assayed lactobacilli strains. Identification of peptides contained in the bioactive fractions was carried out by tandem mass spectrometry using a nanoLC-ESI-QTOF instrument and the mascot search engine. From the four most active fractions obtained, a total of eighteen and fifty peptides were characterized from *L. sakei* CRL1862 and *L. curvatus* CRL705 protein hydrolysates, respectively. The sequence FISNHAY was generated by the proteolytic activity of the two lactobacilli species. Sequence similarity analyses between the peptides identified in this study and those previously identified as ACE inhibitory peptides and detailed in the BIOPEP database were outlined. Results suggest that meat-borne *Lactobacillus* were able to generate peptides with ACE inhibitory activity, highlighting their potential to be used in the development of functional fermented products.

Biological significance
The results of this study would enable the obtention of porcine functional foods by applying lactic acid bacteria generating bioactive peptides. ACE inhibitory peptides obtained by the hydrolytic action of *L. curvatus* CRL705 and *L. sakei* CRL1862 on sarcoplasmic proteins were analyzed. Among them, the peptide FISNHAY exhibited the highest activity and its sequence has not yet been reported.

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1. Introduction

Food proteins have long been recognized for their nutritional and functional properties. Nutritional properties are associated with their amino acid content in conjunction with the physiological utilization of specific amino acids upon digestion and absorption [1,2]. On the other hand, the functional properties of proteins are related to their contribution to physiochemical and sensory properties of foods [3]. In recent years, a considerable amount of research has also focused on the release of bioactive peptides which are encrypted within the primary structure of food proteins, in view to investigate such peptides as functional food ingredients aimed at health maintenance.

A variety of biologically active peptides derived from food proteins have been identified [4]. Inhibitors of angiotensin I converting enzyme (ACE) have attracted particular attention for their ability to prevent hypertension. Angiotensin I converting enzyme is a dipeptidyl carboxypeptidase [EC 3.4.15.1] that not only catalyzes conversion of angiotensin I to angiotensin II causing hypertension, but also inactivates bradykinin, a vasodilatory peptide [5]. Consequently, those compounds capable to inactivate the activity of this enzyme would generate the opposite effect, i.e. a decrease of blood pressure. Many ACE inhibitor peptides have been isolated from enzymatically digested food proteins such as casein, whey proteins, plant proteins [6] and muscle sources [7]. On the other hand, proteolytic release of bioactive sequences by lactic acid bacteria (LAB) has been debated recently due to the great advantages of using food grade microorganisms to enrich foods with bioactive substances [8]. As reported in the literature, potent ACE inhibitory peptides were produced during milk fermentation or casein proteolysis by lactic acid bacteria proteases [8–10] however this effect has been poorly documented from meat proteins. Although, a study has been performed on marine proteins, particularly during fermentation of shrimp (*Acetes chinensis*) with *Lactobacillus fermentum* SM605 in which three ACE inhibitory peptides were identified [11].

In this work, *L. curvatus* CRL705 and *L. sakei* CRL1862 isolated from traditional Argentinean sausages [12,13] were used as proteolytic enzyme sources to evaluate the generation of ACE inhibitory peptides from porcine skeletal muscle proteins Muscle foods serve as interesting substrates for investigation into the potential presence of bioactive peptides, specifically ACE inhibitory peptides to design new healthy meat products.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus curvatus CRL705 and *Lactobacillus sakei* CRL1862 strains from CERELA culture collection, previously isolated from traditional sausages [12,13], were used to generate bioactive peptides. The strains were routinely grown in MRS broth (Britania, Argentina) at 30 °C for 18 h and then maintained at −80 °C in 15% (vol/vol) glycerol. Counts were carried out using Plate Count Agar for total mesophiles and MRS agar for LAB at 30 °C during 48 h.

2.2. Preparation of *L. curvatus* CRL705 and *L. sakei* CRL1862 cell suspensions and meat protein extracts

Whole-cell (WC) suspensions (150 ml) from *L. sakei* CRL1862 and *L. curvatus* CRL705 grown to logarithmic phase were collected by centrifugation (10,000 rpm, 20 min, 4 °C), washed twice in 20 mM phosphate buffer (pH 7.0), and resuspended in the same buffer (20% of initial volume). The cell free extracts (CFE) were obtained by the procedure described by Sanz et al. [14]. Meat protein extracts were obtained from porcine *longissimus dorsi* sarcoplasmic and myofibrillar proteins according to Fadda et al. [15]. The protein contents of sarcoplasmic and myofibrillar extracts determined by the method of Bradford [16] using bovine serum albumin as standard were 2.50 and 0.60 mg/ml, respectively. For both extracts, sterility was confirmed by determining the absence of bacterial growth on Plate Count Agar (Merck, Argentina) incubated overnight at 30 °C.

2.3. Protein hydrolysis and peptide analysis

For each meat protein extract (sarcoplasmic or myofibrillar) a combination (1:1) of CFE + WC suspension of *L. curvatus* CRL705 or *L. sakei* CRL1862, added separately, was used as proteolytic enzyme source. The reaction mixture consisted of 3 ml of each suspension (WC and CFE) aseptically added to 30 ml of protein extract. The mixtures were incubated at 30 °C in a shaken water bath and sampled at time 0 h and after 96 h for further analyses. In each case, control samples without the addition of any bacterial enzymes were assayed simultaneously. The extracts with different treatments were centrifuged for 20 min at 14,000 rpm, and the supernatants were collected for the experiments.

The analysis of generated peptides was performed by RP-HPLC as follows: 4 ml of each sarcoplasmic or myofibrillar extract was deproteinized with 10 ml of methanol, centrifuged (10,000 rpm, 10 min, 4 °C), supernatants concentrated by evaporation to dryness and resuspended in deionized water. Peptide extracts were analyzed at day 0 and after 96 h of incubation, using a 1050 high-performance liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a photodiode array detector and manual injector. A 4.6 × 250 mm Symmetry C18 column (Waters, Milford, MA, USA) was used. The mobile phase consisted of solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile–water–trifluoroacetic acid [60:40:0.085, vol/vol/vol]). The elution was performed as follows: an isocratic phase in 1% solvent B for 5 min, followed by a linear gradient from 1 to 100% solvent B for 55 min, at a flow rate of 1.0 ml/min at 40 °C. Peptides were detected at 214 nm.

2.4. Assay for ACE inhibitory activity

The fractions were collected, dried and re-dissolved in 70 μl of 150 mM Tris-base buffer (pH 8.3). The ACE inhibitory activity of each fraction was measured according to procedures described previously [17]. This assay is based on the ability of ACE to hydrolyze the internally quenched fluorescent substrate o-aminobenzoylglycyl-p-nitro-L-phenylalanyl-L-proline (Abz-Gly-Phe-(NO_2)-Pro). A sample solution (50 μl) was mixed with 50 μl of 150 mM Tris-base buffer (pH 8.3) containing