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11 4 Aryl glycosidases from *Lactobacillus plantarum* increase antioxidant
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13 activity of phenolic compounds
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24 Abstract

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26 Glycosidases have great relevance in polyphenols, since their bioavailability is enhanced by
27 these enzymatic activities. Glycosylated phenolic compounds are deglycosylated either in
28 tissues or by the colonic microbiota. *Lactobacillus plantarum* is a widespread lactic acid
29 bacteria commonly found in many fermented food products and in the gastrointestinal tract,
30 some strains of which are employed as probiotics. The presence of glycosidase activities
31 was assayed in *L. plantarum* strains isolated from different origins. The analyzed strains
32 presented hydrolytic activity against α - and β -D-glucopyranoside and β -D
33 galactopyranoside. The latter activity **could be related to the presence of a β -galactosidase**
34 **encoding-gene** present in all the *L. plantarum* strains analyzed. Glycosylated food
35 phenolics, such as quercetin glucoside, phloridzin, esculin, salicin, and daidzin were
36 deglycosylated by cell cultures or by cell extracts from *L. plantarum* CECT 748^T as
37 demonstrated by HPLC analysis. In this study was clearly demonstrated for the first time
38 that the deglycosylation of specific aryl glycosides by *L. plantarum* was associated with an
39 increase in their antioxidant activity measured by DPPH and SOD methods. Therefore, in
40 addition to the improvement of their bioavailability, the presence of glycosidase activities
41 on *L. plantarum* strains increase the antioxidant activity of glycosylated phenolic
42 compounds.

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44 Keywords: *Lactobacillus plantarum*, aryl glycosides, glycosidases, antioxidant activity,
45 bioavailable.

1. Introduction

Phenolic compounds are traditionally associated with the sensory quality of fresh and processed plant foods. However, phenolic compounds are currently receiving much attention because of their beneficial health effects related to their antioxidant (Vinson, Zibik, Bose, Samman & Proch, 2005; Dehkharghanian, Lacroix & Vijayalakshmi, 2009), anti-inflammatory (Nichols & Katiyar, 2010), cardio-protective (Zern & Fernandez, 2005), cancer chemo-preventive (Nichols & Katiyar, 2010), and neuro-protective properties (Aquilano, Baldelli, Rotilio & Ciriolo, 2008). Consequently, polyphenols can be considered healthy food, and claim to have health promoting or disease-preventing properties, that is, a so-called functional food. Phenolic compounds are the most abundant antioxidants in our diets. Several thousands of natural polyphenols have been identified in plants, although only a more limited number are at significant levels in most human diets (Manach et al., 2004). The considerable diversity of their structures makes them different from other antioxidants. The chemical structure of polyphenols will affect their properties such as bioavailability, antioxidant activity, and their specific interactions with cell receptors and enzymes. Then, the transformation of polyphenols by glycosidases from intestinal microbiota could be important for polyphenol properties (Landete, 2012).

The absorption of food phenolics in humans, necessary for their beneficial effects, is determined primarily by their chemical structure, which depends on factors such as the degree of glycosylation, hydroxylation, acylation, conjugation with other phenolics, molecular size, degree of polymerization, and solubility (Karakaya, 2004). Glycosylated phenolic compounds, including tannins, ellagitaninns, lignans, isoflavones, flavonols and

anthocyanins are widespread among plant foods. Polyphenols are extensively deglycosylated either in tissues, once they are absorbed through the gut barrier, or, for the non-absorbed fraction and the fraction re-excreted in the bile, by the colonic microbiota. Therefore, the glycoside hydrolases responsible for the metabolism of phenolics play a central role in a large number of major biological processes (Sarry & Günata, 2004). The O-glycoside hydrolases (EC 3.2.1.x) are a widespread group of enzymes which catalyze the hydrolysis of the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety.

Among the glycosidases, β -glucosidases have been the subject of much work because of their importance in numerous biological processes and in biotechnological applications (Bhatia, Mishra & Bisaria, 2002), such as food detoxification (Birk, Bravdo & Shoseyov, 1996), biomass conversion (Pemberton, Brown & Emert, 1980), or flavour enhancement in beverages (Günata, Bayonove, Cordonnier, Arnaud, & Galzy, 1990). In addition, glucosidases are relevant enzymes in functional foods since biological properties of glucosylated polyphenols (e.g. soy isoflavones) are affected by glucosidase action (Zubik & Meydani, 2003; Krisch et al., 2012; de Silva et al., 2013).

Lactobacillus plantarum is commonly found in many fermented food products as well as in the human gastrointestinal tract. The aim of this work was to analyze the presence of aryl-glycosidase activities in *L. plantarum* and their influence on the antioxidant activity of glycosylated phenolic compounds.

2. Material and Methods

2.1. Strains and growth conditions

The *L. plantarum* strains used in this work are listed in Table 1. *L. plantarum* WCFS1 was kindly provided by M. Kleerebezem (NIZO Food Research, Kernhemseweg, The Netherlands). The strain is a single-colony isolate of *L. plantarum* NCIMB 8826 that was isolated from human saliva. The strain survives passage through the human stomach (Vesa, Pochart, & Marteau, 2000) and persist in the digestive tracts of mice and humans (Pavan, Desreumaux, & Mercenier, 2003). The strains *L. plantarum* CECT 220 (ATCC 8014), CECT 221 (ATCC 14431), CECT 223, CECT 224, CECT 748^T (ATCC 14917^T), CECT 749 (ATCC 10241), CECT 4185, and CECT 4645 were purchased from the Spanish Type Culture Collection (CECT). *L. plantarum* strains (*L. plantarum* RM28, RM31, RM34, RM35, RM38, RM39, RM40, RM41, RM71, RM72, and RM73) were isolated from wine samples (de las Rivas, Rodríguez, Curiel, Landete & Muñoz, 2009). *L. plantarum* strains are routinely grown in MRS medium adjusted to pH 6.5 and incubated at 30 °C. For deglycosylation assays, *L. plantarum* strains were cultivated in a modified basal and defined medium described previously for *L. plantarum* (Rozès & Peres, 1998). This defined medium was used to avoid the presence of phenolic compounds included in non-defined media.

2.2. Determination of glycosidase activities in *L. plantarum* cultures

Culture biomass was assessed for type and degree of glycosidase activity as described in previous work (Grimaldi, Bartowsky, & Jiranek, 2005) with some modifications. Briefly, *L. plantarum* strains grown in MRS overnight at 30 °C were harvested by centrifugation (20000 g, 5 min) and washed twice with 145 mM NaCl in distilled water. Assays (400 µl)

were performed in eppendorf tubes and comprised 200 μ l of 0.2 M McIlvane buffer (0.1 M citric acid and 0.2 M K_2HPO_4 , pH 5.0, unless otherwise stated) and 100 μ l of a suspension of the appropriate bacterial strain prepared in 145 mM NaCl and standardized to yield a 0.5 final OD₆₀₀ in the assay. **Substrate solutions (100 μ l) were then added to give a final concentration of 10 mM.** Different *p*-nitrophenyl (*p*-NP) derivatives were used as substrates, *p*-NB- β -D-glucopyranoside (*p*-NP- β -D-Glu), *p*-NP- α -D-glucopyranoside (*p*-NP- α -D-Glu), *p*-NP- β -D-galactopyranoside (*p*-NP- β -D-Gal), *p*-NP- β -D-fucopyranoside (*p*-NP- β -D-Fuc), *p*-NP- β -D-xylopyranoside (*p*-NP- β -D-Xyl), and *p*-NP- α -D-rhamnopyranoside (*p*-NP- α -D-Rham). Assays were incubated at 37 °C (unless otherwise stated) **for 1 h, stopped by the addition of 800 μ l of 0.5 M Na₂CO₃ and clarified by centrifugation (10000 g, 3 min). Supernatants (1000 μ l) were transferred to a cuvette (1 ml) and the absorbance at 400 nm was determined using a spectrophotometer (IQuant, Bio-Tek Instruments Inc., Winooski, VT, USA) set to automatic path-length correction. Blanks were prepared without bacterial cells but otherwise treated in the same manner. All reactions were performed in triplicate. One unit of glycosidase activity was defined as the amount of enzyme that released 1 μ mol of *p*-nitrophenol from substrate per minute under the conditions specified.**

2.3. Determination of glycosidase activities in *L. plantarum* cell extracts

To determine if *L. plantarum* possess enzymes with glycosidase activity, cell extracts containing all soluble proteins (supernatant) and cell-associated fractions (pellet) were prepared. *L. plantarum* strains (RM34, RM38, RM41, RM72 and CECT 4645) were grown in MRS medium at 30 °C until a late exponential phase. The cells were harvested by

centrifugation and washed three times with phosphate buffer (50 mM, pH 6.5), and subsequently resuspended in the same buffer for cell rupture. This suspension was disintegrated by using the French Press at 1500 psi pressure (Thermo FRENCH® Press, Thermo Electron, Waltham, MA, USA). The cell disruption steps were carried out on ice to ensure low temperature conditions required for most enzymes. The disintegrated cell suspension was centrifuged at 12000 g for 20 min at 4 °C. Protein concentration in the supernatant was determined using the Bio-Rad protein assay (Bio-Rad, München, Germany). From cell extracts obtained (supernatant and pellet), glycosidase activity analyses were performed as mentioned above. Glycosidic activities from cell extracts were measured as $\mu\text{mol } p\text{-nitrophenyl}$ released per mg of protein.

2.4. Presence of a β -galactosidase gene in *L. plantarum* strains

Genomic DNA was isolated from overnight *L. plantarum* cultures according to the method of Ruiz-Barba, Maldonado & Jiménez-Díaz (2005). The *lp_3629* gene was PCR amplified by using the specific primers 371 (5'-GTAGAGTTTCCGGAAGGCTTTG-3') and 372 (5'-TATCAAAACCCATTCCGTTCCCAAGC-3'). These primers were deduced from the **nucleotide sequences of a putative β -glucosidase gene (*lp_3629*)** previously identified on *L. plantarum* WCFS1 (GenBank accession number 004567). PCR reactions were performed **in 0.2 ml microcentrifuge tubes in a total volume of 25 μl containing 1 μl of template DNA** (approximately 100 ng), 20 mM Tris-HCl, pH 8.0, 50 mM KCl, 2.5 mM MgCl_2 , **200 μM** of each dNTP, **primer 371 (1 μM), primer 372 (1 μM)** and 1 U of AmpliTaq DNA polymerase. The reaction was performed in a GeneAmp PCR System 2400 (Perkin Elmer, Norwalk, CT, USA) using the following cycling parameters: initial 5 min denaturation at

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4 164 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C and 30 s at 72 °C. Amplified
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6 165 products were analyzed by 0.7 % agarose gel electrophoresis in TAE buffer (Sambrook,
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8 166 Fritsch, & Maniatis, 1989). The gel was stained with ethidium bromide and the bands were
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15 16 169 2.5. Deglycosylation of food phenolics by *L. plantarum* CECT 748^T 17 18 19 170

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21 171 *L. plantarum* CECT 748^T cultures and cell extracts were assayed for the deglycosylation of
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23 172 several glucosylated food phenolics, such as quercetin glucoside, phloridzin, esculin,
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25 173 daidzin, and salicin. For the deglycosylation assays by *L. plantarum* CECT 748^T culture,
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27 174 the sterilized defined basal medium was supplemented with the filter-sterilized glucosylated
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29 175 phenolic compound dissolved in ethanol at 10 mM final concentration. The media were
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31 176 inoculated with *L. plantarum* (1%) previously grown in modified basal media (Rozès &
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33 177 Peres, 1998) and incubated in darkness without shaking, at 30 °C for 10 days. Incubated
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35 178 media with cells and without phenolic compound and incubated media without cells and
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37 179 with phenolic compounds were used as controls.
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43 180 For the deglycosylation assay by cell extracts, extracts of *L. plantarum* CECT 748^T
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45 181 in phosphate buffer (25 mM, pH 6.5) were incubated during 1 or 18 h at 30 °C in the
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47 182 presence of the phenolic compound. As control, phosphate buffer containing the phenolic
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49 183 compound was incubated under the same conditions.
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54 55 185 2.6. HPLC analysis of the deglycosylation of food phenolics 56 57 58 186 59 60 61 62 63 64 65

The reaction products from the deglycosylation reactions were extracted twice with one-third of the reaction volume of ethyl acetate (Labscan, Gliwice, Poland). The solvent fractions were filtered through a 0.45 µm-pore-size polyvinylidene fluoride filter (PVDF) (Teknokroma, Barcelona, Spain) and subsequently injected onto the column and analyzed by HPLC. A Thermo (Thermo Electron) chromatograph equipped with a P400 SpectraSystem pump, and AS3000 autosampler, and a UV6000LP photodiode array detector were used. A gradient of solvent A (water/acetic acid, 98:2, v/v) and solvent B (water/acetonitrile/acetic acid, 78:20:2, v/v/v) was applied to a reversed-phase Nova-pack C₁₈ (25 cm x 4.0 mm i.d.) 4.6 µm particle size, cartridge at room temperature as follows: 0–55 min, 80% B linear, 1.1 ml/min; 55–57 min, 90% B linear, 1.2 ml/min; 57–70 min, 90% B isocratic, 1.2 ml/min; 70–80 min, 95% B linear, 1.2 ml/min; 80–90 min, 100% linear, 1.2 ml/min; 100–120 min, washing 1.0 ml/min, and reequilibration of the column under initial gradient conditions. Detection was performed by scanning from 220 to 380 nm. Samples **were injected onto the cartridge after being filtered through a 0.45 µm PVDF filter. The** identification of degradation compounds was carried out by comparing the retention times and spectral data of each peak with those of standards from commercial suppliers.

2.7. Determination of antioxidant activity by DPPH and SOD methods

The free radical scavenging capacity of the phenolic compounds was determined using the method of Brand-Williams, Cuvelier and Berset (1995) with some modification. A 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol was prepared and to 2 ml of this solution was added 0.1 ml of each one of the compounds studied. This solution was incubated with *L. plantarum* CECT 748^T cells at 37 °C during 1 h, and the absorbance

change at 515 nm was measured 10 min later with a spectrophotometer (Uvikon 940, Kontron, Zurich, Switzerland). Solution incubated with *L. plantarum* CECT 748^T but without the phenolic compound added, and incubated media without *L. plantarum* CECT 748^T cells but containing the phenolic compound were used as controls. Measurements were performed in triplicate. Inhibition of coloration was expressed as a percentage, scavenging activity (%) was measured as $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of the sample.

Superoxide radicals were generated by reaction of the hypoxanthine-xanthine oxidase (SOD) system. The assay mixture contained, in a final volume of 1 ml, 50 mM saline phosphate buffer pH 7.8, 25 mM solution of xanthine, 0.24 mM nitro blue tetrazolium chloride (NBT) and 24 mU xanthine oxidase (specific activity 1 U/mg⁻¹ of protein), and 1 mM of glycosylated phenolic substrate. The substrates were incubated in presence of *L. plantarum* CECT 748^T during 1 h at 37 °C before the addition of the xanthine oxidase enzyme. The inhibition of xanthine oxidase was measured in a spectrophotometer (550 nm) during 30 sec and 3 min. Incubated media with *L. plantarum* CECT 748^T and without phenolic compound, and incubated media without *L. plantarum* CECT 748^T and with phenolic compounds were used as controls. The results were expressed as percentage of enzyme activity inhibition. Measurements were performed in triplicate. Scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of the sample.

3. Results

3.1. Glycosidase activities in *L. plantarum* strains

L. plantarum strains were assayed against *p*-nitrophenyl forms of some key glycosides relevant in food products, such as *p*-NP- β -D-Glu, *p*-NP- α -D-Glu, *p*-NP- β -D-Gal, *p*-NP- β -D-Fuc, *p*-NP- β -D-Xyl, and *p*-NP- α -D-Rham. All analyzed strains possessed high detectable β -glucosidase, α -glucosidase and β -galactosidase activities against *p*-NP- β -D-Glu, *p*-NP- α -D-Glu and *p*-NP- β -D-Gal, respectively (Table 1). The absorbance values showed glycosidase activities higher than 100 units in all cases. However, *L. plantarum* strains did not show detectable activity against *p*-NP- β -D-Fuc, *p*-NP- β -D-Xyl and *p*-NP- α -D-Rham (Table 1).

These glycosidase activities were also assayed in cell extracts (supernatant and pellet) from five strains of *L. plantarum* (Table 2). High β -glucosidase, α -glucosidase and β -galactosidase activities from the five strains were observed in the pellet fraction. Similarly, the five strains showed high α -glucosidase activity in the supernatant. However, the β -glucosidase and β -galactosidase activities from the supernatant were low or null in the five strains studied. **Table 2 also shows that** β -galactosidase activity was the lower glycosidic activity showed by all the *L. plantarum* strains analyzed. In relation to the subcellular location of glycosidase activities, the obtained results clearly showed that β -glucosidase and β -galactosidase activities seems to be membrane-bound, **whereas** α -glucosidase activity appeared to be soluble as well as membrane-bound in the insoluble fraction.

All the assayed strains showed β -galactosidase activity on their insoluble fraction. In agreement with this result, all strains amplified the *lp_3629* gene, **encoding a** β -galactosidase enzyme (Figure 1). The analyzed strains produced a 1400 bp amplicon when

DNA extracted from them was used as template in PCR reactions with oligonucleotides 371 and 372 to amplify *lp_3629* gene. These results indicated the widespread presence of a β -galactosidase enzyme in *L. plantarum*.

3.2. β -glucosidase activity against glycosylated food phenolics

Deglycosylation assays detected by HPLC showed that *L. plantarum* CECT 748^T cultures transformed food aryl glycosides (quercetin glucoside, phloridzin, esculin, daidzin, and salicin) into their corresponding aryl aglycones (quercetin, phloretin, esculetin, daidzein, and saligenin) (Figure 2). However, when *L. plantarum* extracts were used, deglycosylation was only observed after prolonged incubation times (18 h). All the assayed glycosylated phenolic compounds were transformed to their aglycones by extract, except quercetin glucoside which remains glycosylated after incubation (Figure 2).

3.3. Antioxidant activity of food phenolics deglycosylated by *L. plantarum* CECT 748^T

Antioxidant activity was determined by DPPH and SOD methods in food aryl glycosides, before and after their incubation with *L. plantarum* CECT 748^T. As observed in Figure 3, the antioxidant activity increased after incubation with *L. plantarum* CECT 748^T in the substrates assayed (quercetin glucoside, phloridzin, and esculin). The deglycosylation of these compounds yield quercetin, phloretin, and esculetin, respectively. By using the DPPH assay, it could be deduced that the antioxidant activity of quercetin increased almost 200% in relation to the antioxidant activity showed by their corresponding glycoside, quercetin

glucoside. The increase showed by esculetin and phloretin is 75 and 60% as compared to
esculin and phloridzin, respectively.

By the SOD method, the antioxidant activity of esculin after *L. plantarum*
incubation increased 70%, similar to the increase detected by the DPPH method. However,
by the SOD assay, quercetin glucoside and phloridzin showed only a low increase (lower
than 10%) on their antioxidant activity when incubated with *L. plantarum*.

Figure 3 clearly shows that aglycones from the food phenolics assayed exhibited
higher antioxidant activity than their corresponding glycosylated forms. Therefore, these
results suggested that *L. plantarum* incubation is an appropriate method to increase the
antioxidant activity of glycosylated food phenolics.

4. Discussion

The bioavailability of food glycosylated polyphenols is improved by glycosidase
action. Several studies have been carried out to analyze the ability of lactic acid bacteria to
produce glycosidase enzymes. Grimaldi, McLean & Jiranec (2000) and Grimaldi et al.
(2005) observed as *Lactobacillus* spp., *Pediococcus* spp. and *Oenococcus oeni* isolated
from wine possess variable β -and α -glucosidase activities. Also, β -glucosidase activities
were found in *Lactobacillus* and *Enterococcus* strains isolated from the faeces of healthy
children and adults (Mroczynsk & Libudzisz, 2010). In *L. plantarum* strains, β -glucosidase
activity have previously been described (Fernández, Margolles, Suárez, & Mayo, 1999;
Sestelo, Poza, & Villa, 2004). However, the knowledge on other glycosidase activities is
scarce. In this work, for the first time, the glycosidase activity against seven model

substrates was assayed in *L. plantarum* strains isolated from various origins. All the strains exhibited α - and β -D-glucosidase, and β -D-galactosidase activities. No detectable β -D-fucosidase, β -D-xylosidase, and β -D-rhamnosidase activity was found, in spite that the presence of α -L-rhamnosidases has been described in some *L. plantarum* strains (Ávila, Jaquet, Moine, Requena, Peláez, Arigoni & Jankovic, 2009).

To our knowledge, there are no report describing the genetic and biochemical identification of α - and β -D-glucosidase enzymes in *L. plantarum* strains. Contrarily, the identification of β -galactosidases has previously been reported in *L. plantarum*. Fernández et al. (1999) described the duplication of a β -galactosidase gene in some *L. plantarum* strains. Moreover, Spano, Rinaldi, Ugliano, Moio, Beneduce and Massa (2005) characterized a gene codifying a putative β -glucosidase enzyme from a *L. plantarum* strain isolated from wine. Later, Acebrón, Curiel, de las Rivas, Muñoz and Mancheño (2009) proved experimentally that the putative β -glucosidase (Lp_3629 in *L. plantarum* WCFS1) encodes a functional β -galactosidase, but not a β -glucosidase, as formerly described. Currently, Lp_3629 protein is reannotated as β -galactosidase (Siezen, Francke, Renckens, Boekhorst, Wells, Kleerebezem, & van Hijum, 2012). Several putative transmembrane domains were found in Lp_3629 amino acid sequence (Spano et al., 2005). The presence of putative transmembrane domains may suggest that Lp_3629 is membrane-bound in *L. plantarum*. This location is in agreement with the results found in this study as β -galactosidase activity, as well as β -glucosidase, was only present in the membrane-bound fraction. Contrarily, α -glucosidase, was present in both, the soluble and in the insoluble fractions of cell extracts. As far as we know, in this study it have been demonstrated for the first time that the widespread β -galactosidase activity could be related with the widespread presence on Lp_3629 protein among *L. plantarum* strains. Moreover, Spano et al. (2005)

reported that similar proteins to Lp_3629 are widely present among several species of lactic acid bacteria.

Aryl glycosidase activity in *L. plantarum* have previously been described. Oleuropein, an secoiridoid glucoside commonly found in leaves of the olive tree as well as in olives, was found to be hydrolyzed by the β -glucosidase activity present in *L. plantarum* strains (Ciafardini, Marsilio, Lanza, & Pozzi, 1994). **Later, the β -glucosidase BglT from *L. plantarum*** was shown to be active on *p*-NP- β -D-glucoside and salicin, as revealed by enzymatic assays; however, no activity was detected on the arylglucoside arbutin (Marasco, Salatiello, De Felice, & Sacco, 2000).

Increase in the antioxidant activity of fermented food products containing glycosylated polyphenols have previously been described. Dehulled and cooked grains of five millet varieties subjected to in vitro enzymatic digestion and microbial fermentation produced and increase in the antioxidant activity and bioavailability of the millet grain phenolics (Chandrasekara and Shahidi, 2012). In addition, fermentation of soymilk by *Lactobacillus rhamnosus* CRL981 enhanced antioxidant activity and increased isoflavone aglycone content (Marazza et al., 2012). Pyo, Lee and Lee (2005) demonstrated that the antioxidant activity of soybean increase with lactic acid fermentation due to the increased content of aglycones. Despite the existence of reports describing the increase in the antioxidant activity of fermented glycosylated phenolics, in this study was clearly demonstrated for the first time that the deglycosylation of specific aryl glycosides by *L. plantarum* was associated with an increase in their antioxidant activity. In this work, *L. plantarum* CECT 748^T was able to efficiently biotransform arylglucosides to their bioactive aglycones, thus could be used as functional starter culture to increase the antioxidant activity of plant foods during fermentation. Despite that differences were observed among

the two antioxidant detection methods used, the results obtained in this work showed that the action of *L. plantarum* increase the antioxidant activity of food aryl glucosides. Thus, plant extracts and preparations with *L. plantarum* may be regarded as effective natural and functional dietary food supplement due to their remarkable content of bioactive aglycones and to their significant radical scavenging capacity.

It is generally accepted that the bioavailability of phenolics is rather low and the values of the relative urinary excretion of the intake range from 0.3% for anthocyanins to 43% for isoflavones such as daidzin (Manach, Williamson, Morand, Scalbert & Rémésy, 2005). This demonstrates the great variability in the bioavailability of the different polyphenols. This bioavailability can be even lower when the food polyphenols have a large molecular weight, as is the case of hydrolyzable and condensed tannins and complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids. The content of these complex phenolics in food generally is higher than that of simpler phenolics, and these complex molecules have been underestimated in many studies mainly due to analytical problems. The microbiota metabolizes these complex polyphenols into smaller molecules which are generally better absorbed in the intestine (Selma, Espin & Tomás-Barberan, 2009). This indicates that the systemic effects of polyphenols can be modulated by the microbial metabolism.

The hydrolysis of glycosides results in metabolites that are potentially more biologically active than the parent compounds (Selma, Espin, & Tomás-Barberan, 2009). Furthermore, the glucosides are known to be less bioactive than their respective aglycones (Mariusz, Jun Yamakoshi, & Yukihiro, 1999). It is well known that almost all soy isoflavones exist as glycosides, which are less estrogenic than their respective aglycones, in soy and unfermented soy foods. Isoflavone glycosides are not absorbed intact across the

enterocyte of healthy adults because of their higher hydrophilicity and molecular weights (Chang & Nair, 1995; Hur, Lay, Beger, Freeman, & Rafii, 2000). Their bioavailability **requires the conversion of glycosides to aglycones via the action of β -glycosidase** from tissue or bacteria that colonize the small intestine for uptake to the peripheral circulation (Setchell, Brown, Zimmer-Nechemias, Bradhear, Wolfe, Kirschner, & Heubi, 2002; Setchell, Brown, & Lydeking-Olsen, 2002). Glucosidases of intestinal microbiota, such those from *L. plantarum*, could hydrolyze the glucoside isoflavones to aglycones and promote their absorption (Zubik & Meydani, 2003). Therefore, *L. plantarum* and other bacteria possessing glucosidase activity, are relevant in the production of compounds with better absorption, better bioavailability, increased antioxidant activity.

In summary, aryl β -glucosides are abundant in food environments that naturally inhabit *L. plantarum* and **the production of aryl β -glucosidases by *L. plantarum*** originates an increase in the functionally (antioxidant activity and bioavailability) of glycosilated phenolic compounds.

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Tables.

Table 1. *L. plantarum* glycosidic activities against six synthetic glycosidic substrates.

Strains	Origin	<i>p</i> -NP- β - <i>D</i> -Fuc ^a	<i>p</i> -NP- β - <i>D</i> -Xyl ^b	<i>p</i> -NP- β - <i>D</i> -Glu ^c	<i>p</i> -NP- α - <i>D</i> -Glu ^d	<i>p</i> -NP- β - <i>D</i> -Rham ^e	<i>p</i> -NP- β - <i>D</i> -Gal ^f
WCFS1	Human saliva	-	-	+	+	-	+
CECT 220	Corn silage	-	-	+	+	-	+
CECT 221	Grass silage	-	-	+	+	-	+
CECT 223	ND ^g	-	-	+	+	-	+
CECT 224	ND	-	-	+	+	-	+
CECT 748 ^T	Pickled cabbage	-	-	+	+	-	+
CECT 749	Pickled cabbage	-	-	+	+	-	+
CECT 4185	Ensilage	-	-	+	+	-	+
CECT 4645	Cheese	-	-	+	+	-	+
RM 28	Wine	-	-	+	+	-	+
RM 31	Wine	-	-	+	+	-	+
RM 34	Wine	-	-	+	+	-	+
RM 35	Wine	-	-	+	+	-	+
RM 38	Wine	-	-	+	+	-	+
RM 39	Wine	-	-	+	+	-	+
RM 40	Wine	-	-	+	+	-	+
RM 41	Wine	-	-	+	+	-	+
RM 71	Wine	-	-	+	+	-	+
RM 72	Wine	-	-	+	+	-	+
RM 73	Wine	-	-	+	+	-	+

Substrates analyzed: ^a *p*-NP- β -*D*-fucopyranoside (*p*-NP- β -*D*-Fuc), ^b *p*-NP- β -*D*-xylopyranoside (*p*-NP- β -*D*-Xyl), ^c *p*-NP- β -*D*-glucopyranoside (*p*-NP- β -*D*-Glu), ^d *p*-NP- α -*D*-glucopyranoside (*p*-NP- α -*D*-Glu), ^e *p*-NP- α -*D*-rhamnopyranoside (*p*-NP- α -*D*-Rham), and ^f *p*-NP- β -*D*-galactopyranoside (*p*-NP- β -*D*-Gal). ^g ND, no data available.

Table 2. Glycosidic activities in *L. plantarum* cell extracts, in soluble fraction or supernatant (S), and in insoluble fraction or pellet (P), against six synthetic glycosidic substrates. Glycosidic activity was measured as μM p-nitrophenyl/mg of protein.

Activity	RM34		RM38		RM41		RM72		CECT 4645	
	S	P	S	P	S	P	S	P	S	P
<i>p</i> -NP- B -D-Fuc	0	0	0	0	0	0	0	0	0	0
<i>p</i> -NP- B -D-Xyl	0	0	0	0	0	0	0	0	0	0
<i>p</i> -NP- B -D-Glu	17	502	0	325	0	524	0	260	0	610
<i>p</i> -NP- α -D-Glu	116	432	590	412	611	480	313	525	36	458
<i>p</i> -NP- B -D-Rham	0	0	0	0	0	0	10	0	0	0
<i>p</i> -NP- B -D-Gal	0	21	0	160	42	195	16	12	21	237

Figures legends

Figure 1. **PCR detection of Lp_3629 β -galactosidase** in *L. plantarum* strains. *Lb. plantarum* WCFS1 (a) CECT 220 (b), CECT 221 (c), CECT 223 (d), CECT 748^T (e), RM28 (f), RM31 (g), RM34 (h), RM40 (i), and RM71 (j). A DNA marker standard (*Eco*RI and *Hind*III digested λ DNA) **was included in the left**. The amplicon size (1400 bp) is indicated by an arrow.

Figure 2. *L. plantarum* deglycosilation of aryl glucosides by HPLC analysis. Chromatograms of supernatants from *L. plantarum* CECT CECT 748^T culture grown for 10 days in the presence of esculin (A), phloridzin (B), quercetin-glucoside (C) and p-NP- β -D-glucoside (D), or from cell extracts after 20 h incubation in the presence of the same aryl glucosides are shown. The HPLC chromatograms from the control samples are also indicated. The chromatograms were recorded at 280 nm.

Figure 3. Variation in the antioxidant activity of aryl-glucosides after incubation with *L. plantarum* CECT 748^T as measured by DPPH (grey) and SOD (black) methods. Values represent the means of three independent experiments; error bars represent the standard deviations.

Figure 1.