Metabolism of food phenolic acids by *Lactobacillus plantarum* CECT 748^{T}

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Abstract

Phenolic acids account for almost one third of the dietary phenols and are associated with organoleptic, nutritional and antioxidants properties of foods. This study was undertaken to assess the ability of *Lactobacillus plantarum* CECT 748^T to metabolize 19 food phenolic acids. From the hydroxycinnamic acids studied, only *p*-coumaric, caffeic, ferulic, and *m*-coumaric acid were metabolized by *L. plantarum*. Cultures of *L. plantarum* produce ethyl and vinyl derivatives from *p*-coumaric and caffeic acids, 4-vinyl guaiacol from ferulic acid, and 3-(3-hydroxyphenyl) propionic acid from *m*-coumaric acid. Among the hydroxybenzoic acids analysed, gallic acid and protocatechuic acid were decarboxylated to pyrogallol and catechol, respectively. Inducible enzymes seem to be involved, at least in *m*-coumaric and ferulic acid metabolism, since cell-free extracts from cultures grown in absence of these phenolic acids were unable to metabolize them. Further work is needed for the identification of the enzymes involved, since the knowledge of the metabolism of phenolic compounds is an important issue for the food industry.

Keywords: *Lactobacillus plantarum*; Hydroxycinnamic acids; hydroxybenzoic acids; decarboxylase; reductase; inducible enzymes.

1. Introduction

Vascular plants synthesize a diverse array of organic molecules, referred to as secondary metabolites. Phenolic acids are one such group of aromatic secondary plant metabolites widely spread throughout the plant kingdom. Phenolic acids have been associated with color, sensory qualities, and nutritional and antioxidant properties of foods (Shahidi & Naczk, 2003). Phenolic acids account for almost one third of the dietary phenols, and there is an increasing awareness and interest in the antioxidant behaviour and potential health benefits associated with these simple phenolic acids. It is their role as dietary antioxidants that have received the most attention in recent literature (Lodovici, Guglielmi, Meoni, & Dolara, 2001).

The term "phenolic acids", in general described phenols that possess one carboxylic acid functional group. The naturally occurring phenolics acids contain two distinguishing constitutive carbon frameworks: the hydroxycinnamic and the hydroxybenzoic structures. Hydroxybenzoic acids are components of complex structures such as hydrolysable tannins (gallotannins and ellagitannins). The hydroxycinnamic acids are more common than hydroxybenzoic acids and mainly include *p*-coumaric, caffeic, ferulic, and sinapic acids. These acids are rarely found in the free form, except in food that has undergone freezing, sterilization, or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid, and tartaric acid. Caffeic and quinic acid combine to form chlorogenic acid. Caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represent between 75% and 100% of the total hydroxycinnamic acid content of most fruits. Ferulic acid is the most abundant phenolic acid found in cereal grains (Shahidi & Naczk, 2003).

Lactobacillus plantarum is a lactic acid bacterial species that is most frequently encountered in the fermentation of plant materials where phenolic acids are abundant. These plant-fermentations include several food and feed products, like olives, must, and a variety of vegetable fermentations. It has been reported that *L. plantarum* is able to decarboxylate the hydroxycinnamic acids, *p*-coumaric and caffeic acids (Cavin, Andioc, Etievant, & Diviès, 1993). However, controversial results were obtained about decarboxylation of ferulic acid by *L. plantarum* strains (Cavin et al., 1993; van Beek & Priest, 2000; Barthelmebs, Diviès, & Cavin, 2001; Couto, Campos, Figueiredo, & How, 2006). Moreover, in this species the gene encoding a *p*-coumarate decarboxylase (PadA), having PAD activity (previously described as PDC activity), in this species has been cloned (Cavin, Barthelmebs, & Diviès, 1997a). The substrate specificity of the purified PadA enzyme was tested for ten hydroxycinnamic acids. The authors conclude that only the acids with a hydroxyl group *para* to the unsaturated side chain and with a substitution –H or –OH *meta* to the unsaturated side chain were metabolised (Cavin, Barthelmebs, Guzzo, van Beeumen, Samyn, Travers, & Diviès, 1997b).

As far as we known, there is no information about the ability of *L. plantarum* to metabolize hydroxybenzoic acids, as well as other phenolic acids frequently found in foods. Therefore, in this paper, we studied the degradation of 19 phenolic acids by *L. plantarum* and reported the identification of the degradation compounds obtained.

2. Material and methods

2.1. Chemicals

98 The 19 phenolic acids analyzed in this study were seven hydroxycinnamic acids,
99 nine hydroxybenzoic acids, and three other food phenolic acids, such as phloretic acid
100 (Aldrich H524006), chlorogenic acid (Sigma C3878), and ellagic acid (Sigma E2250).
101 The hydroxycinnamic acids were: *p*-coumaric acid (Sigma C-9008), *o*-coumaric acid
102 (Fluka 28170), *m*-coumaric acid (Aldrich H23007), cinnamic acid (Aldrich C8, 085-7),
103 caffeic acid (Sigma C0625), ferulic acid (Sigma F3500), and sinapic acid (Sigma
104 D7927). The hydroxybenzoic acids assayed were: syringic acid (Fluka 86230), gallic
105 acid (Fluka 48630), salicylic acid (Merck 631), benzoic acid (Merck 6391513), gentisic
106 acid (Aldrich 149357), veratric acid (Fluka 94872), *p*-hydroxybenzoic acid (Fluka
107 54630), protocatechuic acid (Sigma P5630), and vanillic acid (Fluka 94770).
108 The phenolic acid derivatives 4-ethyl phenol (Fluka 04700), 4-ethyl catechol
109 (Lancaster A12048), 4-ethyl guaiacol (Aldrich W 24,360-4-K), 4-vinyl phenol
110 (Lancaster L10902), 4-vinyl guaiacol (Lancaster A13194), pyrogallol (Merck 612),
111 catechol (Sigma C9510), and 3-(3-hydroxyphenyl) propionic acid (Lancaster L01279),
112 were used as standard for the identification of the degradation compounds.
113
114 *2.2. Bacterial strain and growth conditions*
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116 *L. plantarum* CECT 748^{T} (ATCC 14917, DSMZ 20174) isolated from pickled
117 cabbage was purchased from the Spanish Type Culture Collection. This strain was
118 selected as it represents the type strain of this species.
119 The bacterium was cultivated in a modified basal medium described previously
120 for *L. plantarum* (Rozès & Peres, 1998). The basal medium has the following
121 composition: glucose, 2.0 g/l; trisodium citrate dihydrate (SO 0200, Scharlau), 0.5 g/l;
122 D-, L-malic acid (AC 1420, Scharlau), 5.0 g/l; casamino acids (223050, BD), 1.0 g/l;

123 yeast nitrogen base without amino acids (239210, BD), 6.7 g/l; pH adjusted to 5.5. The
124 basal media was modified by the replacement of glucose by galactose (216310, Difco).
125 This defined medium was used to avoid the presence of phenolic compounds included
126 in non-defined media. For the degradation assays, the sterilized modified basal media
127 was supplemented at 1mM final concentration with the phenolic compound filter
128 sterilized. The *L. plantarum* inoculated media were incubated at 30 ºC, in darkness,
129 under microaerophilic conditions, without shaking, for 10 days. Long incubation period
130 was used to find the dead-end products of phenolic acid degradation. Incubated media
131 with cells and without phenolic compound and incubated media without cells and with
132 phenolic compounds were used as controls. From the supernantants, the phenolic
133 products were extracted twice with one third of the reaction volume of ethyl acetate
134 (Lab-Scan, Ireland).
135
136 *2.3. Degradation of phenolic acids by cell-free extract*
137
138 In order to prepare cell-free extracts, *L. plantarum* CECT 748^{T} strain was growth
139 in MRS media (Difco, France) under microaerobic conditions at 30 ºC until a late
140 exponential phase was reached. The cells were harvested by centrifugation and washed
141 three times with phosphate buffer (50 mM, pH 6.5), and subsequently resuspended in
142 the same buffer for cell rupture. Bacterial cells were disintegrated twice by using the
143 French Press at 1500 psi pressure (Thermo Electron). The disintegrated cell suspension
144 was centrifuged at 12000*g* for 20 min at 4 ºC in order to sediment cell debris. The
145 supernatant containing the soluble proteins was filtered aseptically using sterile filters of
146 0.2 μm pore size (Sarstedt, Germany).

To determine if uninduced *L. plantarum* cells possess enzymes able to metabolize phenolic acids, the cell-free extract was incubated in presence of each phenolic acid at 1 mM final concentration. *L. plantarum* cell-free extract in phosphate buffer (25 mM, pH 6.5) containing approximately 1 mg of total protein, was incubated during 20 h at 30 ºC in presence of each phenolic acid. As control, phosphate buffer containing the phenolic acid was incubated under the same conditions. The reaction products were extracted twice with ethyl acetate (Lab-Scan, Ireland).

2.4. HPLC-DAD analysis

A Thermo (Thermo Electron Corporation, Waltham, Massachussetts, USA) chromatographic system equipped with a P400 SpectraSystem pump, and AS3000 autosampler, and a UV6000LP photodiode array detector was 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 C_{18} cartridge (25 cm x 4.0 mm i.d.; 4.6µm particle size) at room temperature. The elution program was 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. Detection was performed by scanning from 220 to 380 nm (Bartolomé, Peña-Neira, & Gómez-Cordovés, 2000). Samples were injected in duplicate onto the cartridge after being filtered through a 0.45 µm polyvinylidene difluoride (PVDF) filter (Teknokroma, Spain).

The identification of degradation intermediates was carried out by comparing the retention times and spectral data of each peak with those of standards from commercial suppliers or by LC-DAD/ESI-MS.

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173 *2.5. High-Performance Liquid Chromatography-Diode Array Detector-Electrospray*

174 *Mass Spectrometry (HPLC-DAD/ESI-MS)*

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176 A Hewlett-Packard series 1100 (Palo Alto, CA) chromatographic system

177 equipped with a diode array detector (DAD) and a quadrupole mass spectrometer

178 (Hewlett-Packard series 1100 MSD) with an electrospray interface was used. Separation

179 was performed on a reversed-phase Waters Nova-Pak C18 column at room temperature.

180 The elution programme described above was applied. DAD detection was performed

181 from 220 to 380 nm, with 0.7 ml/min. The ESI parameters were as follows: drying gas

182 (N2) flow and temperature, 10 L/min at 340 ºC; nebulizer pressure , 40 psi; capillary

183 voltage, 4000 V. The ESI was operated in negative mode, scanning from *m/z* 100 to

184 3000 using the following fragmentator voltage gradient: 100 V from 0 to 200 *m/z* and

185 200 V from 200 to 3000 *m/z*.

186

187 **3. Results and discussion**

188

189 *3.1. Hydroxycinnamic acids degradation by* L. plantarum

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191 There is a great variety of hydroxycinnamic acids in foods. The biosynthesis of

192 this diversity of hydroxycinnamic acids has been studied. In plants, phenylalanine

193 ammonia lyase (PAL) catalyzes the release of ammonia from phenyl-alanine and leads

194 to the formation of a carbon-carbon double bond, yielding *trans*-cinnamic acid. In some

195 plants and grasses tyrosine is converted into 4-hydroxycinnamic via the action tyrosine

196 ammonia lyase (TAL). Introduction of a hydroxyl group into the *para* position of the

197 phenyl ring of cinnamic acid proceeds via catalysis by monooxygenase utilizing
198 cytochrome P_{450} as the oxygen binding site. The *p*-coumaric acid formed may be
199 hydroxylated further in position 3 and 5 by hydroxylases and possibly methylated via
200 O-methyl transferase with S-adenosylmethionine as methyl donor; this leads to the
201 formation of caffeic, ferulic and sinapic acids. These compounds posses a phenyl ring
202 and a C3 side chain and serve as precursors for the synthesis of lignins and many other
203 compounds.

204 Studies were limited to seven commercially available hydroxycinnamic acids. In
205 order to known if *L. plantarum* has the ability to degrade these acids two procedures
206 were carried out. First, *L. plantarum* cultures were grown for 10 days in presence of
207 each hydroxycinnamic acid at 1mM final concentration. So, if *L. plantarum* cells are
208 able to metabolise the hydroxycinnamic acid, the dead-end degradation products could
209 be detected in the culture media. In addition, cell-free extracts containing all the soluble
210 proteins were incubated at 37 ºC during 20 h in presence of 1 mM of each commercial
211 hydroxycinnamic acid. Since the soluble proteins were present in phosphate buffer (50
212 mM, pH 6.5), control samples were prepared in this buffer and incubated in the same
213 conditions. By using this second approach, information about induction of the involved
214 enzymes could be obtained, since in the extracts are only present the proteins that were
215 synthesized in the absence of the corresponding hydroxycinnamic acid.

216 Among the seven hydroxycinnamic acids assayed, only *p*-coumaric and caffeic
217 acids were metabolized by cell cultures as well as by cell-extracts of *L. plantarum*
218 CECT 748^T. Figure 1A showed the HPLC chromatograms obtained with *p*-coumaric
219 acid. As compared to the control, we could observe that in cell-free extracts, a
220 proportion of *p*-coumaric acid was decarboxylated, and vinyl phenol was obtained (Fig.
221 1A, 3). However, supernatants obtained from cell cultures showed the presence of

vinyl- and ethyl phenol, resulting from the decarboxylation, and decarboxylation plus reduction of *p*-coumaric acid (Fig. 1A, 2). Previously, it was reported that in *L. plantarum* LPNC8 strain no *p*-coumaric acid degradation was detected in the uninduced cell extracts (Barthelmebs, Diviès, & Cavin, 2000). However, no information was provided about the reaction time used.

A similar situation was observed in the caffeic acid sample (Fig. 1B). Cell-extracts were able to fully decarboxylate the caffeic acid present in the reaction (Fig. 1B, 3), whereas in the supernatants from the cultures, the products of the decarboxylation (4-vinyl catechol) as well as the decarboxylation plus reduction (4-ethyl catechol) of caffeic acid were identified (Fig. 1B, 2). The caffeic acid derivative, 4-vinyl catechol was identified by LC-DAD/ESI-MS. The degradation peak, at retention time 37 min, was identified as 4-vinyl catechol by its molecular ion, $[M\text{-}H^-]$ *m/z* 135 (data not shown).

From these results we could conclude that uninduced cell-free extracts contained decarboxylases able to decarboxylate *p*-coumaric and caffeic acids. In fact, a *p*-coumaric acid decarboxylase (PadA), able to metabolize *p*-coumaric and caffeic acid, was purified, and its corresponding gene was cloned and heterologously expressed (Cavin et al., 1997a). However, culture induction is needed to synthesize the reductase involved in the conversion of the vinyl derivatives to the corresponding ethyl derivatives. It has been previously suggested the presence in *L. plantarum* of a phenolic acid reductase activity (named PAR) induced by *p*-coumaric and ferulic acid in the presence of glucose (Barthelmebs et al., 2000).

Unlike *p*-coumaric and caffeic acid, ferulic and *m*-coumaric acids were found to be metabolized only by *L. plantarum* cell cultures (Fig. 2A); however, in both acids, no degradation was observed by cell-free extracts (data not shown). These results indicated

247 that the enzymes involved in their metabolism need to be synthesized after their
248 induction by the presence of the corresponding phenolic acid.
249 As showed in Fig. 2A, 1 ferulic acid was decarboxylated to vinyl guaiacol, as
250 determined by comparing its retention time and spectral data with the commercial
251 standard (Fig. 2B, 1). Controversial results were obtained in relation to the
252 decarboxylation of ferulic acid. Cavin et al. (1997b) reported that only *p*-coumaric and
253 caffeic acids were metabolised by the *L. plantarum* purified *p*-coumaric acid
254 decarboxylase (PdaA), and they concluded that the absence of detectable ferulic acid
255 decarboxylase activity of the purified PdaA confirmed the existence of another phenolic
256 acid decarboxylase, which was able to decarboxylate ferulic acid and was induced by
257 ferulic acid only. However, later, the same authors reported that purified PadA appeared
258 to decarboxylate ferulic acid in vitro (Barthelmebs et al., 2000) and therefore, they
259 suggests that the PDC activity present in *L. plantarum* should be renamed PAD due to
260 its decarboxylase activity on *p*-coumaric, ferulic, and caffeic acids (Barthelmebs et al.,
261 2001)
262 So far, the observed decarboxylation of *p*-coumaric, ferulic and caffeic acids
263 could be due to the activity of the PadA enzyme. However, purified PadA enzyme was
264 unable to decarboxylate *m*-coumaric acid (Cavin et al., 1997b). We have observed *m*-
265 coumaric acid degradation (Fig. 2A, 2), with the production of a degradation
266 intermediate showing a retention time of 28.8 min (Fig. 2A, 2) and UV absorbance
267 maxima at 236/272 nm (Fig. 2B, 2) as determined by using a diode array detector. In
268 order to identify the compound obtained, LC-DAD/ESI-MS was applied to the sample.
269 The compound eluted at a retention time of 28.8 min, was identified by its molecular
270 ion, [M-H$^-$] *m/z* 165, as 3-(3-hydroxyphenyl) propionic acid (HPPA) (data not shown).
271 Later, HPPA was additionally identified by comparison with the commercial

compound. Microbial degradation of *m*-coumaric acid has been only scarcely reported. As early as 1968, it was reported that cells of the wood-destroying fungi *Sporobolomyces roseus* were able to convert *m*-coumaric acid into *m*-hydroxybenzoic acid, but the latter compound, which accumulated in the medium, was not further metabolized (Moore, Subba Rao, & Towers, 1968). Later, it was reported that the bacteria *Clostridium glycolicum* transformed *m*-coumaric acid to HPPA by reducing the double bond of the side chain (Chamkha, Labat, Patel, & García, 2001). Therefore, it seems that *C. glycolicum* and *L. plantarum* shared a similar mechanism for the degradation of *m*-coumaric acid.

3.2. Hydroxybenzoic acids degradation by L. plantarum

In plants, benzoic acids derivatives are produced via the loss of a two-carbon moiety from cinnamic acids. Salicylic acid is a benzoic acid derivative that acts as a signal substance. After infection or UV irradiation, many plants increase their salicylic acid content, which may induce the biosynthesis of defence substances. Aspirin, the acetyl ester of salicylic acid, was first isolated from the bark of the willow tree. Similar to hydroxycinnamic acids, hydroxylation and possibly methylation of hydroxybenzoic acid leads to the formation of dihydroxybenzoic acid (protocatechuic acid), vanillic acid, syringic acid and gallic acid. Hydroxybenzoic acids are commonly present in the bound form in foods and are often the component of a complex structure like lignins and hydrolysable tannins.

Among the hydroxybenzoic acids assayed, only gallic and protocatechuic acids were metabolized by both cell cultures and cell-free extracts from *L. plantarum* CECT 748^{T}. Fig. 1C showed the HPLC chromatograms obtained with gallic acid. As compared

297 to the control, we could observe that in the cell-free extracts, a proportion of gallic acid
298 was decarboxylated, and pyrogallol was obtained (Fig. 1C, 3). However, in the
299 supernatants obtained from cell cultures only pyrogallol was detected (Fig. 1C, 2).
300 These results are in agreement with a previous study suggesting the occurrence of a
301 gallate decarboxylase activity in *L. plantarum* (Osawa, Kuroiso, Goto, & Shimizu,
302 2000).

303 Protocatechuic acid was completely decarboxylated to catechol by cultures of *L.*
304 *plantarum* grown in presence of this hydroxybenzoic acid (Fig. 1D, 2). However, and
305 similarly to gallic acid, cell-free extracts produced catechol but non-decarboxylated
306 protocatechuic acid was also detected (Fig. 1D, 3). As early as 1971, Whiting and
307 Coggins reported that *L. plantarum* cells grown in a medium containing protocatechuic
308 acid completely metabolised it to catechol, and there was no indication of a further
309 metabolism of catechol (Whiting & Coggins,1971). Both results seems to indicate that
310 catechol is a dead-end product of protocatechuate degradation in *L. plantarum* cultures.
311 No information is available about the *L. plantarum* enzyme involved in the
312 protocatechuic acid decarboxylation. As far as we known, enzyme possessing
313 protocatechuic acid decarboxylase activity had only been reported in *Clostridium*
314 *hydrobenzoicum* (He & Wiegel, 1996).

315 Recently, hydroxybenzoic acid derivatives (including gallic acid, and
316 protocatechuic acid) had been proposed as minor polyphenols that could serve as
317 characteristic indices for discrimination of varietal red wines (Kallithraka, Mamalos, &
318 Makris, 2007). This choice was based on the consideration that these components are in
319 general chemically and microbiologically stable; thus, they could be viewed as indices
320 for a reliable differentiation. However, after the results obtained in this study, caution
321 should be taken in relation to this proposal, since *L. plantarum* is frequently associated

to malolactic fermentation in wines and, as reported in this work, it is able to metabolize some of the hydroxybenzoic acids chosen for the discrimination of varietal red wines.

3.3. Degradation of other phenolic acids by L. plantarum

As mentioned above, the metabolism of three food phenolic acids, such as phloretic, chlorogenic, and ellagic acid, was also studied. Ellagic and chlorogenic acids were no detected by the chromatographic method used in this study. Phloretic acid was not metabolized by cell cultures as well as by the cell-free extracts (data not shown). As explained above, PadA from *L. plantarum* was purified, and its corresponding gene was cloned and heterologously expressed (Cavin et al., 1997a). The substrate specificity of the purified enzyme was tested for several phenolic acids, and found that phloretic acid was not decarboxylate by this enzyme. Later, a *L. plantarum* mutant strain deficient in PDC activity, *L. plantarum* LPD1, was constructed (Barthelmebs et al. 2000). In LPD1 mutant, in cells induced with *p*-coumaric acid, this acid was metabolized but vinyl phenol was not the product of the reaction. Instead, phloretic acid or ethyl phenol appeared to be produced, based on the UV spectrum. Phloretic acid was not further degraded, similarly to the results found in this work.

To improve our understanding of phenolic acid degradation by *L. plantarum*, further work on the identification of the involved enzymes is required. Table 1 summarizes the results obtained in this work. These results indicate that *L. plantarum* is able to degrade some hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, and *m*-coumaric acid) and some hydroxybenzoic acids (gallic and protocatechuic acid). The reactions involved in their metabolism are decarboxylation and reduction of the

phenolic acid. A phenolic acid decarboxylase (PadA) had been characterized previously in *L. plantarum*. This enzyme, only decarboxylate *p*-coumaric, caffeic, and ferulic acid, from the hydroxycinnamic acids assayed, and does not decarboxylate *m*-coumaric acid. However, no information is available about the decarboxylation of hydroxybenzoic acids by this enzyme. Therefore, additional information is needed in relation to the substrate specificity of this decarboxylase; and, at least, and additional enzyme, the reductase (o reductases) involved in the formation of ethyl derivatives from their corresponding vinyls, and in the reduction of *m*-coumaric acid to HPPA, need to be identified. For the food industry, the knowledge of the enzymes involved in the metabolism of compounds possessing an important role in food quality is of great interest.

Acknowledgments

This work was supported by grants AGL2005-000470 (CICYT), FUN-C-FOOD Consolider 25506 (MEC), RM03-002 (INIA) and S-0505/AGR-0153 (CAM). We thank Dra. C. Gómez-Cordovés for her help in the HPLC analysis. The technical assistance of M. V. Santamaría is greatly appreciated. H. Rodríguez was a recipient of a predoctoral fellowship from the I3P-CSIC. J. M. Landete was a recipient of a postdoctoral fellowship from the MEC.

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Figure captions

Fig. 1. HPLC chromatograms of the degradation of *p*-coumaric, caffeic, gallic, and protocatechuic acids by *Lactobacillus plantarum*. Chromatograms of supernatants from *L. plantarum* CECT 748^T grown for 10 days in presence of *p*-coumaric (A), caffeic (B), gallic (C) and protocatechuic acid (D) (2) or from cell-free extracts after 20 h incubation in presence of the same phenolic acids (3) are shown. The HPLC chromatograms from the control samples are also indicated (1). The chromatograms were recorded at 280 nm. pCA, *p*-coumaric acid; CA, caffeic acid; GA, gallic acid; PA, protocatechuic acid; VP, vinyl phenol; EP, ethyl phenol; VC, vinyl catechol, EC, ethyl catechol; P, pyrogallol; C, catechol.

Fig. 2. HPLC chromatograms showing the degradation of the hydroxycinnamic acids, *m*-coumaric and ferulic acids, by *L. plantarum* cultures. (A) Chromatograms of supernatants from *L. plantarum* CECT 748^T grown for 10 days in presence of ferulic acid (FA) (1) or *m*-coumaric acid (mCA) (2). Chromatograms were recorded at 280 nm. (B) Comparison between spectra of the compounds identified and the standards: vinyl guaiacol (VG), and 3-(3-hydroxyphenyl) propionic acid (HPPA).

(Table 1. Rodríguez, Landete, de las Rivas & Muñoz)

Table 1. Metabolism of phenolic acids by *L. plantarum* CECT 748^{T}.

Phenolic acid	Compound produced	Enzyme involved
p-Coumaric acid	4-vinyl phenol	PadA decarboxylase
	4-ethyl phenol	Reductase
Caffeic acid	4-vinyl catechol	PadA decarboxylase
	4-ethyl catechol	Reductase
Ferulic acid	4-vinyl guaiacol	PadA decarboxylase
	4-ethyl guaiacol	Reductase
m-Coumaric acid	3-(3-hydroxyphenyl) propionic acid)	Reductase
Gallic acid	Pyrogallol	Decarboxylase
Protocatechuic acid	Catechol	Decarboxylase

Figure 1

Figure 1 (Rodríguez, Landete, de las Rivas & Muñoz)

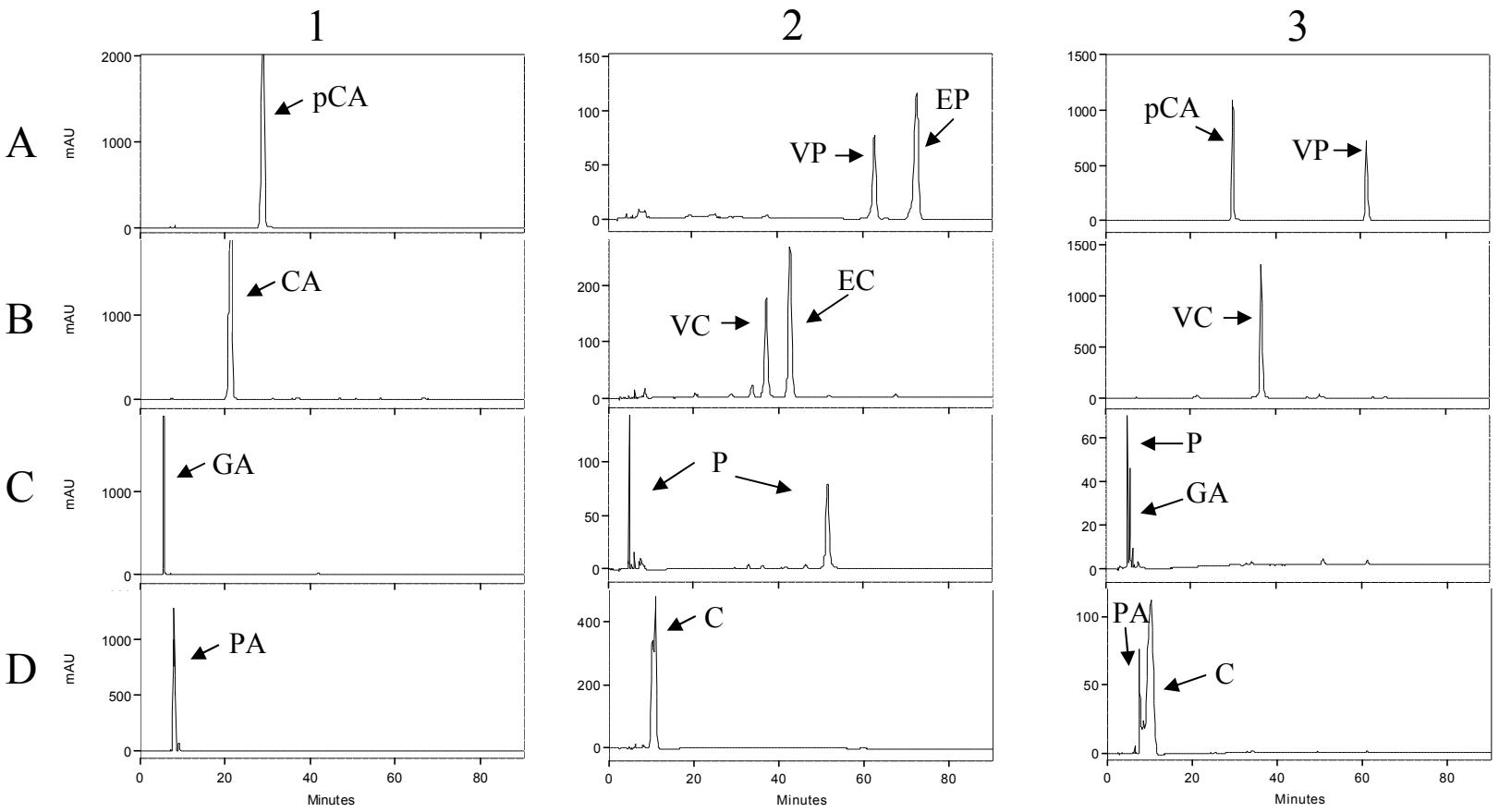

Figure 2

Figure 2 (Rodríguez, Landete, de las Rivas & Muñoz)