Expression of genes involved in metabolism of phenolic compounds by *Lactobacillus pentosus* and its relevance for table-olive fermentations.

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24 **Abstract**

25 Genes with the potential to code for enzymes involved in phenolic compound metabolism were
26 detected in the genome of *Lactobacillus pentosus* IG1, isolated from a green olive fermentation.
27 Based on homology, these genes could code for a 6-P- β Glucosidase, two different Tannases, a
28 Gallate decarboxylase and a *p*-Coumaric decarboxylase. Expression of up to seven of these genes
29 was studied in *L. pentosus* IG1 (olive fermentation) and CECT4023^T (corn silage), including
30 responses upon exposure to relevant phenolic compounds and different olive extracts. Genes
31 potentially coding Tannase, Gallate decarboxylase and *p*-Coumaric acid decarboxylase
32 significantly increased their expression upon exposure to such compounds and extracts, although
33 it was strain dependent. In general, both the genetic organization and the characteristics of gene
34 expression resembled very much those described for *Lactobacillus plantarum*. In accordance to the
35 observed induced gene expression, metabolism of specific phenolic compounds was achieved by *L.*
36 *pentosus*. Thus, methyl gallate, gallic acid and the hydroxycinnamic acids *p*-coumaric, caffeic and
37 ferulic were metabolized. In addition, the amount of phenolics such as tyrosol, oleuropein, rutin and
38 verbascoside included in a minimal culture medium was noticeably reduced, again dependent on the
39 strain considered.

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41 Keywords: *Lactobacillus pentosus*; tannase; decarboxylase; glucosidase; olive fermentation;
42 phenolics.

43

44 Running title: Expression and activity of genes for phenolics metabolism in *L. pentosus*

45 **1. Introduction**

46 *Lactobacillus pentosus* is a lactic acid bacterium involved in a diverse range of food
47 fermentations (Abriouel et al., 2011) that have a great economic impact within food industry. This
48 is especially true for vegetable fermentations and, particularly, for table olive ones, where different
49 strains have been extensively used as starter cultures to improve quality and safety of this product
50 (Ruiz-Barba et al., 1994; de Castro et al., 2002; Panagou et al., 2008; Hurtado et al., 2010; Peres et
51 al., 2008; Ruiz-Barba and Jiménez-Díaz, 2012). Olives are not edible unless somehow submitted to
52 a debittering process which hydrolyzes their major phenolic compound: oleuropein. Different
53 procedures have been traditionally used to achieve this debittering step but just three of them are
54 used to produce economically important table olives: the Spanish-style, for green olives, and the
55 natural and oxidation procedures, for black olives (Garrido Fernández et al., 1995; Sánchez et al.,
56 2006; Rejano et al., 2010). Debittering of Spanish-style green olives is achieved through an alkali
57 treatment involving dilute solutions of NaOH as well as a washing step with water (Sánchez et al.,
58 2006; Rejano et al., 2010). Olives are finally covered with brine (10-12 % [w/v] NaCl) and let to
59 spontaneously ferment. Although at least three successive stages have been described, this
60 fermentation is dominated by strains of *L. pentosus* (de Castro et al., 2002; Rejano et al., 2010;
61 Ruiz-Barba and Jiménez-Díaz, 2012; Lucena-Padrós et al., 2014a and 2014b). Fermentation is
62 completed after two-three months, when all fermentable material has been metabolized to, mainly,
63 lactic acid and pH and free acidity are suitable for product preservation. As cited above, the
64 utilization of appropriate starters based on strains of *L. pentosus* are being used to guarantee the
65 results of this fermentation.

66 On the other hand, the phenolic content of the green olive fruits confers not only a bitter
67 taste but also an inhibitory effect on a range of bacteria, especially lactic acid bacteria (LAB) (Ruiz-
68 Barba and Jiménez-Díaz, 1989; Ruiz-Barba et al., 1990, 1991 and 1993; De Castro et al., 2005;
69 Medina et al., 2007, 2008 and 2009). Therefore, the alkali treatment and the washing steps are

critical for appropriate subsequent lactic acid fermentation. These steps are still carried out empirically in the production factories (called “*patios*”, in the Spanish argot), being guided by the expertise of skilled personnel. However, the intrinsic variability of the fruits, their physical origin, their maturation degree and size, the use of irrigation in the olive groves and other unpredictable parameters make this task a somehow risky issue. In fact, errors at this point have been proposed as the major cause of “stuck” fermentations or even spoilage of these fermentations at early stages, what may give rise to defects such as “gas pockets” or “alambrado” (Borbolla y Alcalá et al., 1959 and 1960; Rejano et al., 2010; Lanza, 2013). Gas pockets or “fish eye” are the result of the growth of gas-producing microorganisms, such as some yeast species and *Enterobacteriaceae*, under the olives cuticle (Vaughn et al., 1972; Lanza, 2013). Alambrado or “gated olives” result from the abnormal growth of microorganisms, especially Gram-negative bacteria, that produce an excess of CO₂ which accumulates within the olive pulp (Borbolla y Alcalá et al., 1959; Lanza, 2013).

Considering the above, it is clear that the use of starter cultures with increased capabilities to cope with the inhibitory phenolic compounds present in table olive fermentations would be an advantageous trait. In fact, several authors have described the selection of LAB, especially strains of *L. pentosus* and *Lactobacillus plantarum*, with enhanced capabilities to resist such inhibition in table olive fermentations (Ruiz-Barba and Jiménez-Díaz, 1989; Servilli et al., 2006; Ghabbour et al., 2011; among others). The examination of the genome of a *L. pentosus* strain isolated from Spanish-style green olive fermentation (Maldonado-Barragán et al., 2011) revealed the presence of several potential phenolic compound-degrading genes as deduced by their DNA and protein sequence homology (Table 1). The aim of this work was to study the actual expression of these genes in two selected strains of *L. pentosus* from different origins. This has been done in the presence or absence of a set of phenolic compounds, some of them characteristic of this table olive fermentation, as well as with the addition of different types of olive extracts, prepared as in the Spanish-style procedure, in order to detect gene-expression changes. In addition, we have evaluated

the capabilities of these strains to actually metabolize these phenolic compounds in a minimal culture medium. Finally, the presence of phenolic-compound degrading genes has been investigated in a range of wild-type *L. pentosus* strains isolated from Spanish-style green olive fermentations at different locations.

2. Materials and methods

2.1. Bacterial strains and culture media

Bacterial strains used in this study were *L. pentosus* IG1, isolated from a Spanish-style green olive fermentation (Maldonado-Barragán et al., 2011), and *L. pentosus* CECT 4023^T (=NCDO 363^T) isolated from corn silage (Zanoni et al., 1987). In order to examine the distribution of genes related to phenolic compounds metabolism, a set of 49 wild-type strains of *L. pentosus* isolated from Spanish-style green olive fermentations at five different locations were used (Table S1). All *L. pentosus* strains were routinely grown in MRS broth or agar (Biokar Diagnostics, Beauvais, France) aerobically at 30 °C. For expression experiments, a modified Basal Medium (MBM) was used as previously described by Rozès and Peres (1998), substituting glucose by galactose as described by Jiménez et al. (2014) to avoid possible catabolite repression. Briefly, per litre: D(+)-galactose 2 g (Panreac, Castellar del Vallès, Spain); trisodium citrate dihydrate 0.5 g (Sigma-Aldrich, St. Louis, USA); D-L malic acid 5 g (Sigma-Aldrich); casamino acids 1 g (casein hydrolysate, Difco, Sparks, USA); yeast nitrogen base without amino acids 6.7 g (Difco); pH 6.0.

2.2. Detection of genes related to metabolism of phenolic compounds in *L. pentosus*

The published sequence of the *L. pentosus* IG1 genome (acc. no. FR874854; Maldonado-Barragán et al., 2011) was used as a template. Search of homologies of the deduced protein sequences and comparative genomics were performed using the bioinformatic tools BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and those provided by the CoGe platform (<https://genomevolution.org/coge/>). Multiple sequence alignments were performed using Multalin

(Corpet, 1988) at <http://multalin.toulouse.inra.fr/multalin/multalin.html>. In order to check the presence and distribution of genes related to phenolic-compound metabolism among wild-type strains of *L. pentosus*, PCRs were carried out with total DNA extracted from isolate colonies using the rapid chloroform method described by Ruiz-Barba et al. (2005). PCRs were performed using a T100 Thermal Cycler (Bio-Rad, Hercules, USA) with the PCR primer pairs described in Table S2 and an annealing temperature of 60 °C. For the putative genes LPENT_01513 and LPENT_01784, the corresponding qPCR primer pairs shown in Table S2 were used. The quality of the resulting amplicons was examined through agarose gel electrophoresis.

2.3. Gene expression experiments

2.3.1. *L. pentosus* cultures in the presence of phenolic compounds and extracts from Spanish-style-processed green olives

To assess the expression of relevant genes in the *L. pentosus* IG1 and *L. pentosus* CECT4023^T strains, 18-20 h cultures in MRS were adjusted to OD_{600nm} = 1.0, centrifuged (16000 g, 5 min), the pellets washed once in MBM and resuspended in the same volume of MBM. Different phenolic compounds were added to one-ml aliquots of these suspensions at a final concentration of 1 mM. These mixtures were incubated at 30 °C for 30 min before total RNA isolation (described in 2.3.2). The phenolic compounds used in these experiments were: gallic acid (Sigma-Aldrich), *p*-coumaric acid (Fluka, Buchs, Switzerland), caffeic acid (Fluka), ferulic acid (Fluka), tyrosol (Sigma-Aldrich), hydroxytyrosol (Extrasynthese, Genay, France), vanillin (Sigma-Aldrich), oleuropein (Extrasynthese), rutin (Extrasynthese), and verbascoside (Extrasynthese). These are phenolic compounds usually found in table olive fermenting brines and/or susceptible to be metabolized by some of the enzymatic activities putatively coded in the genome of *L. pentosus*. Experiments were carried out in duplicates.

For the preparation of different aqueous extracts of Spanish-style-processed green olives, the traditional procedure was followed (Rejano et al., 2010) using two different table-olive varieties, i.e.

145 “Gordal” and “Manzanilla”. It is known that these varieties differ in the concentration of phenolic
146 compounds that are inhibitory to LAB, being much higher in the Manzanilla variety than in the
147 Gordal one (Medina et al., 2010). Briefly, freshly collected green olives were treated with NaOH
148 (2-2.5 % w/v) for a period of 7-10 h, depending on the time it took the alkali to get into at least 1/2
149 (“short” treatment) or 2/3 (“normal” treatment) of the olive flesh. Olives of the Gordal variety were
150 submitted either to such a short (GII) or normal (GV) treatment, while olives of the Manzanilla
151 variety were submitted only to the normal one (M80). Treated olives were washed with tap water,
152 covered with distilled water and let in the fridge at 7 °C for different periods of time: GII and GV
153 remained for 45 d., while M80 was collected after 20 d. After these periods of cold aqueous
154 extraction, olives were removed and the pH of the aqueous phase was adjusted to 6.5 with HCl,
155 centrifuged and paper-filtered (paper 73 g/m², Filtros Anioia, Barcelona, Spain) to remove debris,
156 and finally sterilized by autoclaving at 115 °C for 10 min. These extracts were stored at -80 °C until
157 use. Their actual composition, in terms of major phenolic compounds, is shown in Table S3. To
158 examine the effect of these alkali-treated olive extracts on gene expression, *L. pentosus* IG1 and
159 CECT4023^T cell pellets were obtained as above, washed in MBM, resuspended in the same volume
160 of the different extracts (GII, GV or M80) and incubated at 30 °C for 30 min before total RNA
161 isolation. Experiments were carried out in duplicates.

162 **2.3.2. Total RNA isolation from cultures of *L. pentosus* strains**

163 After the exposure to either single phenolic compounds or alkali-treated olive extracts, *L.*
164 *pentosus* cells were collected by centrifugation and lysed. Cell lysis was achieved by incubation
165 with 100 µl of lysozyme (10 mg/ml; Sigma) for 15 min at 37 °C followed by the addition of 200 µl
166 lysis buffer (20% SDS, 50mM Tris, 20mM EDTA, pH 8). Immediately after cell lysis, 700 µl of
167 TRIsureTM (Bioline, London, UK) were added and total RNA was precipitated following
168 manufacturer’s instructions and finally dissolved in 50 µl of DEPC-treated deionized water. To
169 remove traces of contaminating DNA for subsequent cDNA synthesis, each total RNA sample was

170 treated with 10 µl of RNase-free DNase I (New England Biolabs, Ipswich, USA) for 1 h at 37°C.
171 The enzyme was finally removed and the RNA collected by total RNA precipitation with 250 µl of
172 TRIsure™ as described above. RNA concentration was determined with a NanoDrop™ 2000
173 (Thermo Scientific, Waltham, USA), while size and quality were assessed by electrophoresis in
174 agarose gels (1.5 %) through visual examination.

175 **2.3.3. cDNA synthesis from total RNA samples and relative RT-qPCR**

176 cDNA was generated from 1 µg DNase I-treated RNA of each sample using random
177 hexamers and the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany), following the
178 manufacturer's instructions. Specific amplification of each gene was performed using the iQ™
179 SYBR® Green Supermix (Bio-Rad) and specific primers (Table S2), according to the
180 manufacturer's instructions, and the MyiQ2 Real-Time PCR Detection System (Bio-Rad).
181 Quantitation of the relative gene expression was done by means of the double delta Ct method
182 (Livak and Schmittgen, 2001) using 16S cDNA as the housekeeping gene. Primers were designed
183 using Fast PCR Professional 5.1.83 and Oligo Analyzer 3.1 programs. Each plate contained two
184 replicates of each cDNA sample. Specificity of the amplified products was verified by analysis of
185 the dissociation curves generated by the MyiQ2 Optical System Software (version 2.0; Bio-Rad)
186 based on the specific melting temperature for each amplicon. The results were based on two
187 independent experiments (i.e. 4 independent qPCR reactions). Calculation of gene expression levels
188 was carried out using the Rest 2009 v.1 Software (Qiagen).

189 **2.4. Metabolism of phenolic compounds by *L. pentosus***

190 In order to determine the ability of *L. pentosus* to degrade the single phenolic compounds,
191 including now methyl gallate (Sigma-Aldrich), 18-20 h cultures in MRS were processed as
192 described in section 2.3.1. One-ml aliquots containing independently each of the phenolic
193 compounds at 1 mM (final concentration) were incubated at 30 °C for 7 days. Samples were taken
194 at the start and at the end of the incubation period, when the presence and concentration of the

different phenolic compounds was determined through liquid chromatography as described in section 2.5. Uninoculated control experiments were set up in parallel to assess non-microbial degradation. Experiments were carried out in duplicates and the averaged percentages of reduction in the concentrations of the phenolic compounds were calculated.

2.5. HPLC analysis of phenolic compounds.

Chromatographic analysis of phenolic compounds was carried out by mixing 0.25 ml of sample, 0.25 ml of internal standard (2 mM syringic acid in water), and 0.5 ml of deionized water. The mixture was filtered through a 0.22 μ m pore size nylon filter, and an aliquot (20 μ l) was injected into the chromatograph. The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600 pump, a Waters column heater module, and a Waters 996 photodiode array detector operated with Empower2 software (Waters Inc., Mildford, USA). A 25 cm x 4.6 mm i. d., 5 μ m, Spherisob ODS-2 (Waters Inc.) column at a flow rate of 1 ml/min and a temperature of 35 °C, was used in all experiments. Separation was achieved by gradient elution using (A) water (pH 2.5 adjusted with 0.15% phosphoric acid) and (B) methanol. The initial composition was 90% A and 10% B. The concentration of B was increased to 30% over 10 min and was maintained for 20 min. Subsequently, B was raised to 40% over 10 min, maintained for 5 min, and then increased to 50%. Finally, B was increased to 60, 70, and 100% in 5-min periods. The initial conditions were reached in 10 min. Chromatograms were recorded at 280 nm. The evaluation of the concentration of each compound was performed using a regression curve with the relevant standard as a reference. Percentage of reduction in the phenolic concentration was calculated by comparison of the concentration before and after incubation in the presence of *L. pentosus*. Experiments and measurements were carried out in duplicates.

3. Results

219 3.1. Genome location and homology of genes involved in metabolism of phenolic 220 in *L. pentosus*

221 The relative position of ten genes potentially involved in metabolism of phenolic
222 compounds found across the genome of *L. pentosus* IG1 is shown in Fig. S1. Up to five *loci* were
223 identified which were related to putative 6-P- β glucosidase, Gallate decarboxylase, *p*-Coumaric acid
224 decarboxylase and two Tannases. Fig. 1 shows a genetic map of the organization of the *L. pentosus*
225 IG1 chromosomal regions harbouring the putative genes. This organization resembles that described
226 for *L. plantarum* WCFS1 (Reverón et al., 2017), except for the absence of the above mentioned
227 extracellular tannase (TanA). Similarly to *L. plantarum*, the gene encoding the putative subunit C of
228 the Gallate decarboxylase (LPENT_02396) is also separated from the other subunits B and D
229 (LPENT_01512 and 01513, respectively), although in the case of the strain IG1 the gap is just 1.05
230 Mb (Figs. 1 and S1) instead of 2.4 Mb as in WCFS1 (Reverón et al., 2017). In addition, the putative
231 tannase TanB (LPENT_02382) and the subunit C of the Gallate decarboxylase (LPENT_02396) are
232 located close in the chromosome, i.e. 17.2 Kb apart, although a bit further than in *L. plantarum* (6.5
233 Kb).

234 The homology of these ten genes is shown in Table 1. The highest similarity scores were
235 found with *L. plantarum*, which is among the species for which work on the actual expression and
236 activity of these genes has been done in the past. More precisely, the maximum similarity was
237 obtained with the strain *L. plantarum* WCFS1. The only exception to this was the similarity found
238 with an extracellular tannase activity coded by *tanA_{Lp}* which has been described only in a few *L.*
239 *plantarum* strains, such as in the type strain ATCC14917^T and NC8 (Jiménez et al., 2014), but not
240 in WCFS1. Likewise, homology with genes found specifically in the genomes of other *L. pentosus*
241 strains is shown in Table S4. Virtually identical genes were found in all three *L. pentosus* strains
242 whose genome sequence is currently available, except for the 6-P- β glucosidase gene. The presence
243 of seven of these genes, as assessed through PCR analysis (Table S2), was confirmed in all 51 *L.*

244 *pentosus* strains tested in this study with the exception of 6-P- β glucosidase, which could only be
245 detected in 53% of them (Table S1). No statistically significant relation was found between the
246 presence or not of this gene and the *patio* where the strain was isolated from. On the other hand, a
247 significant relation, although very weak (Cramér's V index 0.289), was found with the fermentation
248 stage when the *L. pentosus* strain was isolated. In this regard, more 6-P- β glucosidase-harboring
249 strains were isolated at the middle fermentation stage (Table S1), i.e. between the second and
250 seventh week after brining.

251 **3.2. Expression of relevant genes in the presence of phenolic compounds and extracts from** 252 **Spanish-style-processed green olives**

253 The *L. pentosus* IG1 and CECT4023^T relative transcription level of genes potentially
254 involved in phenolic compounds metabolism was measured in the presence of a set of relevant
255 phenolic compounds and it is shown in Table 2. Transcription of all seven genes was corroborated
256 through RT-qPCR analysis. After exposure to different phenolic compounds, only four genes
257 appeared to get a remarkable induction above the basal expression level: those for *p*-Coumaric acid
258 decarboxylase, Tannase I, Gallate decarboxylase and Tannase II, although differences between the
259 two strains tested were noteworthy (Table 2). The highest induction of these four genes was
260 observed after exposure of the strain CECT4023^T to gallic acid, while in the strain IG1 only the
261 Gallate decarboxylase gene was maximally induced (Table 2). On the other hand, the
262 hydroxycinnamic acids *p*-coumaric, caffeic and ferulic were also able to induce the putative *p*-
263 Coumaric acid decarboxylase gene at significant levels in both strains, except for ferulic acid in the
264 case of the strain CECT4023^T (Table 2).

265 The relative transcription level of the above mentioned genes after incubation of the two *L.*
266 *pentosus* strains tested in different extracts from Spanish-style-processed green olives is shown in
267 Table 3. In this case, only the putative gene for the *p*-Coumaric acid decarboxylase exhibited
268 substantial induction in both strains, although only in the presence of olive extract GII (Table 3).

269 3.3. Metabolism of phenolic compounds by *L. pentosus* cultures

270 Reduction in the concentration of selected phenolic compounds in the presence of cultures
271 of the two *L. pentosus* strains tested in this study is shown in Fig. 2. Differences in the activity of
272 each strain were remarkable, being the strain IG1 generally more active than CECT4023^T, with the
273 exception of the rutin assay. Methyl gallate and gallic acid were completely metabolized by both
274 strains, while very little or not significant activity was observed on hydroxytyrosol and vanillin.
275 Only the strain IG1 showed activity on tyrosol and verbascoside, while both strains showed activity
276 on oleuropein and the hydroxycinnamic acids, i.e. *p*-coumaric, caffeic and ferulic acids.
277 Chromatograms of representative phenolic compounds incubated in the presence of *L. pentosus* IG1
278 at time 0 and 7 days are shown in Fig. S2.

279

280 4. Discussion

281 Olive fermentation brines represent a hostile environment for LAB colonization due to the
282 presence of a plethora of phenolic compounds which could inhibit their growth. Therefore, the
283 ability to enzymatically degrade these compounds could be a selective trait allowing enhanced
284 survival of specific strains. Current genome sequences availability allows for *in silico* identification
285 of genes coding for proteins with relevant biochemical activities. Thus, we detected in the genome
286 of an *L. pentosus* strain isolated from a Spanish-style green olive fermentation the presence of a
287 putative gene set that could be involved in phenolic compounds metabolism. These genes have the
288 potential to encode enzymes with described hydrolase, esterase and carboxylase activities on a
289 range of phenolic compounds. This finding offered an opportunity to explore their actual
290 functionality with a view to enhancing the survival and growth rate of selected *L. pentosus* strains
291 used as inoculants in these food fermentations.

292 Analyses of available *L. pentosus* genomes indicate that the genes involved in phenolic
293 compound metabolism, as well as their genetic organization, are very similar to what has been

described in *L. plantarum*. However, differences can be found among different strains. For instance, all 51 *L. pentosus* strains tested in this study had the potential of an extracellular Tannase activity coded by a gene homologous to *tanA_{Lp}* while this gene is only present in a few *L. plantarum* strains (Jiménez et al., 2014). Besides, a 6-P-β glucosidase gene homologous to *pbg2* in *L. plantarum* was harbored just by 53 % of those *L. pentosus* strains. On the other hand, as deduced from the genome of *L. pentosus* IG1 and other strains that have been sequenced to date (Table S4), this species appears to be the only bacterium, along with *L. plantarum* (Reverón et al., 2017), where genes encoding the different subunits (B-D and C) of the putative Gallate decarboxylase are physically apart in the chromosome.

Our experiments showed that up to seven genes potentially related to phenolic compound metabolism are actually being expressed in *L. pentosus* IG1 and CECT4023^T, at least at a basal level. Furthermore, induction of the expression of some of these genes occurred upon the presence in the culture medium of specific phenolic compounds, although differences between the two strains tested existed. Thus, the expression of LPENT_02382 (Tannase I), homologous to *tanB_{Lp}* in *L. plantarum*, was induced in the presence of gallic acid, although only in the strain CECT4023^T. In contrast, none or very little induction was shown in *L. plantarum* WCFS1, where *tanB_{Lp}* codes for a well characterized intracellular tannase (Reverón et al., 2017). Significant induction by gallic acid in the strain CECT4023^T also occurred with LPENT_02868 (Tannase II), homologous to *tanA_{Lp}* in *L. plantarum* which codes for an extracellular tannase (Jiménez et al., 2014). Again, the expression of *tanA_{Lp}* in *L. plantarum* was reported as not being inducible, at least by its canonical substrate methyl gallate (Jiménez et al., 2014). Apparently both putative enzymes possess all the elements to work in *L. pentosus* as they do in *L. plantarum*, for both Tannase I and II, exhibited essential motifs in their predicted amino acid sequences (Fig. S3), including the residues involved in the catalytic triad and the galloyl binding interactions (Ren et al., 2013) as it was previously shown for *L. plantarum* (Jiménez et al., 2014). The activity of these enzymes was actually checked by culturing

319 both *L. pentosus* strains from this study in the presence of methyl gallate. As a result, all methyl
320 gallate was metabolized by both strains after 7 days (Fig. 2), strongly suggesting that Tannase
321 activity is actually present in *L. pentosus*, being most probable accomplished by the product/s of
322 LPENT_02382 and/or LPENT_02868. The fact that in the strain CECT4023^T such activity appears
323 to be clearly inducible by gallic acid, in contrast to the situation in *L. plantarum*, is a matter of
324 future research.

325 Expression of LPENT_02396, the putative gene coding for the catalytic subunit C of a
326 Gallate decarboxylase in *L. pentosus* IG1, showed strong induction upon the presence of gallic acid
327 in the culture medium in both strains tested (Table 2). This is in accordance to the results described
328 by Reverón et al. (2017) in *L. plantarum* WCFS1. In addition, all the *L. pentosus* strains tested
329 harbored a potential transport protein that, in the strain IG1, shows 97 % identity (99 % similarity)
330 to GacP of *L. plantarum* WCFS1 (Table 1). Reverón et al. (2017) have recently demonstrated that
331 GacP, a gallic acid permease, is essential for full Gallate decarboxylase activity in *L. plantarum*
332 WCFS1, probably acting as an antiporter protein for the uptake of gallic acid and the coupled
333 excretion of pyrogallol to the culture medium. Gallic acid was indeed completely metabolized by
334 both *L. pentosus* strains in this study, suggesting the functionality of their putative Gallate
335 decarboxylases (Fig. 2).

336 A putative *p*-Coumaric acid decarboxylase is encoded by LPENT_01783 in *L. pentosus* IG1.
337 The expression of this gene was induced mainly by gallic and hydroxycinnamic acids, although to a
338 different extent depending on the tested strain (Table 2). A homologous enzyme, PadA, has been
339 described in *L. plantarum* (Cavin et al., 1997a and 1997b; Rodríguez et al., 2008a), where
340 apparently no induction was necessary to metabolize *p*-coumaric and caffeic acids but it was in the
341 case of ferulic acid (Rodríguez et al., 2008b). Both *L. pentosus* tested strains appeared to metabolize
342 the three hydroxycinnamic acids used as substrates (Fig. 2). However, the strain IG1 showed a
343 remarkably higher activity on *p*-coumaric and caffeic acids. Whether or not induction of

344 LPENT_01783 by specific phenolic acids is necessary appears to be a matter related to the assayed
345 strain and it actually posed a controversial issue in the past for the *L. plantarum* species (Rodríguez
346 et al., 2008b). The structure of the active site and decarboxylation catalytic mechanism of the *p*-
347 Coumaric acid decarboxylase in *L. plantarum* has been well described by Rodríguez et al. (2010).
348 Alignment of the amino acid sequences of various *L. pentosus* and *L. plantarum* PadAs show that
349 conserved motifs are present in all the checked strains with the exception of the His89 one, which is
350 substituted by Asn in all *L. pentosus* strains (Fig. S4). Histidine at this position has been shown to
351 be involved in the hydrogen bonding interactions and salt bridges identified in the interface between
352 the monomers that constitute mature PadA in *L. plantarum* (Rodríguez et al., 2010). Further
353 research is necessary to elucidate whether this change is translated into differences in substrate
354 specificity or enzyme activity in *L. pentosus* strains. Transcriptional regulation of *padA* in *L.*
355 *plantarum* has been described to be mediated through PadR, a transcriptional repressor (Gury et al.,
356 2004). In *L. pentosus*, a protein showing 86 % identity (92 % similarity) with PadR of *L. plantarum*
357 WCFS1 has been found to be potentially coded by a gene (LPENT_02398 in IG1) that is also
358 divergently oriented to *padA* (Fig. 1). The expression of this gene was not affected by any of the
359 phenolic compounds tested, suggesting that a mechanism involving inactivation of PadR after the
360 addition of hydroxycinnamic acids through a specific mediator is probably present as it is the case
361 in *L. plantarum* (Gury et al., 2004; Rodríguez et al., 2009).

362 In *L. pentosus* IG1, a putative 6-P- β glucosidase is coded by LPENT_00991, sharing high
363 similarity with a homologous gene (*pbg2*) in *L. plantarum* WCFS1 (Table 1). Such a gene is not
364 present, or at least it does not shown significant similitude, in other *L. pentosus* strains whose
365 genome sequence has been reported to date (Table S4). It has not been detected either in the type
366 strain for *L. pentosus*, CECT4023^T. In fact, only 53 % of the strains isolated from olive
367 fermentations harbored it (Table S1). The activity of this enzyme on phenolic compounds has been
368 described in the past, especially on oleuropein, the major phenolic compound of green olives, in

369 both *L. plantarum* and *L. pentosus* (Ciafardini et al., 1994; Marsilio et al., 1996; Ghabbour et al.,
370 2011). *L. pentosus* IG1, 6-P- β glucosidase transcription level did not appear to be influenced by any
371 specific phenolic compound in the culture medium (Table 2), and such activity cannot be carried
372 out by an inexistent 6-P- β glucosidase in CECT4023^T. However, both strains were able to
373 metabolize *ca.* 50 % of the oleuropein under the conditions of the assay (Fig. 2). This metabolism
374 could be explained by the enzyme basal activity, at least in the IG1 strain, and by the tannases
375 (Yuan et al., 2015) and esterases (Rodríguez et al., 2009; Kaltsa et al., 2015) activities that may play
376 a role in this metabolic pathway as described by the cited authors.

377 The rest of phenolic compounds whose influence on gene transcription was studied here
378 showed no significant results (Table 2). Metabolism of these compounds was again very dependent
379 on the strain tested, although both strains showed significant activity on rutin (Fig. 2). Putative α -L-
380 rhamnosidases, potentially able to hydrolyze rutin, have been found in the genomes of *L. pentosus*,
381 for instance LPENT_00969 in the strain IG1. In contrast, only *L. pentosus* IG1 degraded to some
382 extent tyrosol and verbascoside (Fig. 2). Laccases have been described in *L. plantarum* whose
383 activity could degrade tyrosol and other phenylethanoids. In *L. pentosus* IG1 the putative gene
384 LPENT_01433 codes for a protein 95 % similar to a laccase described in *L. plantarum* J16 by
385 Callejón et al. (2016). Finally, the low activity shown by IG1 on verbascoside could be due to the
386 action of non-specific esterases.

387 Finally, in good accordance to the results obtained with single phenolic compounds, only the
388 olive extract containing *p*-coumaric acid, i.e. olive extract GII, significantly induced the
389 expression of the putative *p*-Coumaric acid decarboxylase coded by LPENT_01783 in both tested
390 strains (Table 3). This extract was obtained after a “short” alkali treatment which is reflected in its
391 particular phenolic composition (Table S3). It is clear that *L. pentosus* is able to switch its
392 enzymatic machinery on to cope with a changing environment including inhibitory phenolic
393 compounds. This is of great importance in a still empirical process such as the alkali treatment and

394 subsequent water washes on green olives which is not always successful at neutralizing and
395 removing inhibitory compounds such as phenolics.

396

397 **4. Conclusions**

398 *L. pentosus* harbors and expresses genes involved in phenolic compound metabolism. Thus,
399 6-P- β glucosidase, Tannase, Gallate decarboxylase and *p*-Coumaric decarboxylase were expressed
400 to a different extent depending on the strain considered. Both the genetic organization and the
401 characteristics of gene expression resembled very much those described for *L. plantarum*. Induction
402 of gene transcription upon exposure to specific phenolics was observed for genes coding Tannase,
403 Gallate decarboxylase and *p*-Coumaric acid decarboxylase enzymes. Accordingly to the observed
404 gene up regulation, metabolism of specific phenolic compounds was achieved. More specifically,
405 methyl gallate, gallic acid and hydroxycinnamic acids such as *p*-coumaric, caffeic and ferulic were
406 metabolized. Phenolic composition of olive brines is very variable along the fermentation time and
407 also across olive varieties, table olive seasons, local processing protocols, etc. Therefore,
408 microorganisms thriving in olive fermentations must adapt their metabolism to these changing
409 conditions. An advantageous response to the changing environment composition challenge is the
410 regulated expression of enzymes involved in the metabolism of potential inhibitory compounds
411 such as phenolics. Genetic composition in terms of relevant genes able to express a range of
412 phenolic compound-degrading enzymes should be taken into consideration when selecting *L.*
413 *pentosus* strains for successful starters of olive fermentations.

414

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Table 1. Homology of ORFs related to phenolic compound metabolism found in the chromosome of *Lactobacillus pentosus* IG1.

Locus tag ¹	Size (bp)	Predicted protein (aas)	Annotation ¹	% Protein identity (sim ²)	<i>L. plantarum</i> strain ³	Predicted function	Locus tag (gene)	Accession no.
LPENT_00991	1509	502	β-glucosidase	83 (90)	WCFS1	β-glucosidase	lp_0906 (<i>pbg2</i>)	CCC78348
LPENT_01512	411	136	putative uncharacterized protein	85 (93)	WCFS1	Gallate decarboxylase (subunit D)	lp_0272 (<i>lpdD</i>)	CCC77799
LPENT_01513	564	187	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	98 (98)	WCFS1	Gallate decarboxylase (subunit B)	lp_0271 (<i>lpdB</i>)	YP_004888312
LPENT_01783	537	178	phenolic acid decarboxylase	88 (96)	WCFS1	p-Coumaric acid decarboxylase	lp_3665 (<i>padA</i>)	YP_004891133
LPENT_01784	555	184	regulator of phenolic acid metabolism PadR	88 (93)	WCFS1	transcriptional regulator of phenolic acid metabolism	lp_3664 (<i>padR</i>)	YP_004891132
LPENT_02382	1413	470	tannase	73 (84)	WCFS1	Tannase	lp_2956 (<i>tanB_{Lp}</i>)	YP_004890536
LPENT_02396	1473	490	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	98 (99)	WCFS1	Gallate decarboxylase (subunit C)	lp_2945 (<i>lpdC</i>)	YP_004890530
LPENT_02397	1380	459	transport protein	97 (99)	WCFS1	cation transport protein	lp_2943 (<i>gacP</i>)	YP_004890529
LPENT_02398	918	305	transcription regulator	86 (92)	WCFS1	transcriptional regulator (LysR family)	lp_2942 (<i>tanR</i>)	YP_004890528
LPENT_02868	1884	627	tannase	91 (95)	ATCC 14917 ^T	Tannase ⁴	HMPREF0531_11477 (<i>tanA_{Lp}</i>) ⁵	KRL35904

¹According to *L. pentosus* IG1 genome (accession no. FR874854); ²In brackets, similarity; ³Highest homology scores among species for which work has been done on the actual expression and activity genes were found with *L. plantarum*; ⁴This tannase is not present in *L. plantarum* WCFS1; ⁵ According to Jiménez et al., 2014.

Table 2. Relative transcriptional expression of genes putatively involved in phenolic compound degradation by two strains of *Lactobacillus pentosus* in the presence of different phenolic compounds.

Phenolic compound	<i>L. pentosus</i> strain ²	Gene/Enzyme						
		LPENT_0991 ¹ β-glucosidase	LPENT_01513 Gallate decarboxylase (subunit B)	LPENT_01783 p-coumaric acid decarboxylase	LPENT_01784 PadR	LPENT_02382 Tannase I	LPENT_02396 Gallate decarboxylase (subunit C)	LPENT_02868 Tannase II
gallic acid	CECT	- ³	nd ⁴	20.8*	nd	29.9*	19.0	30.6*
	IG1	0.9	nd	0.1	nd	1.1	32.6	0.6
p-coumaric acid	CECT	-	1.0	10.1	1.7	1.2	1.6	0.8
	IG1	1.8	1.6	19.8	1.9	1.8	2.1	2.2
caffeic acid	CECT	-	1.1	10.4	0.9	1.7	1.4	0.6
	IG1	0.7	1.4	5.2	0.5	1.3	1.6	1.1
ferulic acid	CECT	-	2.6	0.8	1.4	1.1	1.2	0.7
	IG1	0.9	2.4	6.5*	1.0	1.1	2.1	1.9
tyrosol	CECT	-	1.5	1.9	1.1	1.0	3.4	0.9
	IG1	1.0	1.7	0.9	0.7	1.0	2.0	1.4
hydroxytyrosol	CECT	-	0.5	0.4	1.0	0.3	0.4	0.5
	IG1	1.6	0.9	0.6	1.5	0.4	0.5	0.6
vanillin	CECT	-	1.5	1.7	0.8	1.1	3.5	1.0
	IG1	1.0	1.4	1.1	0.5	1.0	2.5	1.8
oleuropein	CECT	-	1.1	1.1	0.9	0.4	0.6	0.9
	IG1	0.7	1.3	0.7	0.9	0.6	0.7	0.6
rutin	CECT	-	2.3	0.7*	2.4	0.9	1.1*	0.5
	IG1	1.0	2.3	2.0	2.4	2.1	2.1	1.6
verbascoside	CECT	-	2.1	1.4	2.2	0.8	0.8	1.0
	IG1	1.3	2.7	1.4	2.5	1.2	1.5	1.5

¹According to *Lactobacillus pentosus* IG1 genome annotation (accession no. FR874854).

²CECT: *L. pentosus* CECT4023^T; IG1: *L. pentosus* IG1.

³-, the gene is not present in this strain.

⁴Not determined.

*Significant difference (p<0.05) in the expression levels between both strains tested.

Light-, medium- and dark-green cells highlight 2- to 10-fold, 10- to 20-fold, and >20-fold increases in the relative transcriptional expression, respectively, over that registered in the absence of phenolic compounds.

Table 3. Relative transcriptional expression of genes potentially involved in phenolic compound degradation by two strains of *Lactobacillus pentosus* in the presence of different extracts from Spanish-style-processed green olives.

Olive extract ²	<i>L. pentosus</i> strain ³	Gene/Enzyme						
		LPENT_0991 ¹ β-glucosidase	LPENT_01513 Gallate decarboxylase (subunit B)	LPENT_01783 <i>p</i> -Coumaric decarboxylase	LPENT_01784 PadR	LPENT_02382 Tannase I	LPENT_02396 Gallate decarboxylase (subunit C)	LPENT_02868 Tannase II
GII	CECT	- ⁴	0.5	59.6*	3.2*	0.6	0.1	0.8
	IG1	0.4	0.8	41.7*	3.3*	0.6	0.1	1.1
GV	CECT	-	0.4	0.8	0.4	0.7	0.2	0.9
	IG1	1.0	0.6	0.4	0.7	0.6	0.1	1.3
M80	CECT	-	0.2	0.3	0.2 [†]	0.3	0.4	0.3
	IG1	0.7	0.6	0.9	1.2 [†]	0.8	0.6	0.8

¹According to *Lactobacillus pentosus* IG1 genome annotation (accession no. FR874854).

²Aqueous extract of Spanish-style-processed green olives.

³CECT: *L. pentosus* CECT4023^T; IG1: *L. pentosus* IG1.

⁴The gene is not present in this strain.

*Significant difference (p<0.05) between the expression level in the presence of extract GII and the other two extracts.

[†]Significant difference (p<0.05) of the expression level between strain CECT4023^T and IG1.

605 **Legends of the Figures**

606 **Figure 1.** Genetic map showing the organization of the *Lactobacillus pentosus* IG1 chromosomal
607 regions harboring putative Gallate decarboxylase-, *p*-Coumaric acid decarboxylase and Tannase-
608 encoding genes (GenBank accession no. FR874854.1, positions 1588874 to 1594426, 1912878 to
609 1919970, 2622184 to 2651235, and 3193247 to 3199635, respectively). Gap sizes between
610 chromosomal fragments are indicated (Mb). Locus tags for every coding DNA sequence are
611 indicated (LPENT_). Filled arrows indicate genes putatively involved in phenolic compound
612 metabolism. Below these genes, their putative products are named in terms of their homologous
613 ones described for *Lactobacillus plantarum*.

614

615 **Figure 2.** Reduction percentages of phenolic compounds after 7-day incubation in the presence of
616 *Lactobacillus pentosus* IG1 (solid bars) or *L. pentosus* CECT4023^T (open bars).

Figure 1. Carrasco et al.

Figure 2. Carrasco et al.

Table S1. Presence of the β -glucosidase gene in wild-type strains of *Lactobacillus pentosus* isolated from Spanish-style green olive fermentations at different table olive fermenting yards (*patios*).

Strain ¹	β -glucosidase gene	Fermentation stage ²	Strain	β -glucosidase gene	Fermentation stage
A12.1	+	I	G32.10	+	II
A12.5	-	I	G37.2	+	II
A14.1	-	I	G39.2	+	II
A14.4	+	I	J1.7	-	I
A14.44.2	+	I	J15.6	-	I
A14.47	-	I	J21.6	+	II
A15.1	-	I	J22.42	+	II
A4.41	+	I	J25.41	-	II
A5.1	+	I	J25.42	-	II
A5.41	-	I	J26.3	-	II
F10.12	-	II	J33.41	+	II
F10.4	+	II	J34.42	-	II
F11.6	+	II	J6.7	-	I
F12.1	-	II	P1.5	+	II
F12.2	-	II	P11.3	-	II
F4.1	+	II	P11.5	+	II
F6.2	+	II	P12.11	+	II
F7.1	+	II	P14.12	+	II
F9.2	+	II	P2.12	+	II
G11.41	-	I	P2.2	+	II
G12.10	-	I	P3.1	-	II
G13.5	-	I	P8.2	-	II
G15.8	+	I	LPCO10 ³	+	-
G16.9	+	I	IG1	+	-
G17.41	-	I	CECT4023 ^T	-	-
G20.5	-	I			

¹*Patio* code: A: Aceitunas Los Dos S.L. (Almendra); F: Aceitunas Francisca Moreno S.L. (Pilas); G: Goya en España S.A.U. (Alcalá de Guadaira); J: Jolca S.A. (Huévar); P: Olive Aljarafe S.L. (Pilas). All these *patios* are located in the province of Sevilla, southwestern Spain.

²I and II, initial and middle fermentation stages, respectively.

³Starter strain for table olive fermentation, used as a reference (Jiménez-Díaz et al., 1993).

Table S2. PCR and qPCR primers used in this study.

Gene	locus tag ¹	PCR primer	Sequence (5'-3')	qPCR primer	Sequence (5'-3')
β -glucosidase	LPENT_00991	PCR-Glu-F	TTCGTCAGCGGGATTAGTTG	qPCR-Glu-F	GTCGATAACGCAAACAGTCAG
		PCR-Glu-R	CCACTCACTCTCAGGCAAATAC	qPCR-Glu-R	CGCAAATCAAGTGGAAGGTG
Gallate decarboxylase (sub B)	LPENT_01513			qPCR-Carbox-F	TGGTCGGATGGTTATAAAAGGC
				qPCR-Carbox-R	TCAAGGAGCAACGTAACTCG
p-coumaric acid decarboxylase	LPENT_01783	PCR-Pcu-F	CCTCGGCACCCACTTTATTT	qPCR-Pcu-F	CAACTTTGTAAATGCCGGGTG
		PCR-Pcu-R	TGGAACGTGACCGTGATTTC	qPCR-Pcu-R	TGGGAATATGAATGGTACGCG
Transcriptional repressor PadR	LPENT_01784			qPCR-PadR-R	ACCCATGAAATCACCGTCAG
				qPCR-PadR-F	TCCTTAGTGGCCGTCAAATC
Tannase I	LPENT_02382	PCR-Tna1-F	GGTTGACGCCTGAACAAATTC	qPCR-Tan1-F	GCCTTGAAAACCGACTATTTGG
		PCR-Tna1-R	CATTACCACTAGCACCCATCAA	qPCR-Tan1-R	TGTGTTCTGTGAGAATGACG
Gallate decarboxylase (sub C)	LPENT_02396	PCR-Gal-F	TGGTTTGGATCCTGCCATTAC	qPCR-Gal-F	CCAATGCCAATCACGATCAAC
		PCR-Gal-R	CCCTTCATCCGCTTCATTATCC	qPCR-Gal-R	ACCAGCAACCCCTAATTCTG
Tannase II	LPENT_02868	PCR-Tna2-F	CGATACCAATCGGGTCTTCAC	qPCR-Tan2-F	CGTCGAGTCAAAGTGGTTAGC
		PCR-Tna2-R	CACCACTCGGACCTTCATTAC	qPCR-Tan2-R	GATGTGGGTGCTTTTGATGG
16S <i>rDNA</i>		PCR-16S-F	ATGGGTAGCAAACAGGATTAG	qPCR-16S-F	CAAGCGTTGTCCGGATTATTG
		PCR-16S-R	CCGAAGTGAAGTGGCTTTA	qPCR-16S-R	CCAGTTTCCGATGCACTTCT

¹According to *Lactobacillus pentosus* IG1 genome (acc. no. FR874854; Maldonado-Barragán et al., 2011)

Table S3. Composition of the different extracts of Spanish-style-processed green olives used in this study in terms of major phenolic compounds.

Extract ¹	phenolic compound					
	<i>p</i> -coumaric acid (mM)	Tyrosol (mM)	Hy-4-glucoside ² (mM)	Hydroxytyrosol (mM)	Comsegoloside (mM)	Hy-acetate ³ (mM)
GII	0.194 (0.015) ⁴	0.870 (0.004)	0.719 (0.008)	nd ⁵	nd	nd
GV	nd	0.534 (0.023)	nd	0.606 (0.013)	nd	nd
M80	nd	1.095 (0.007)	1.347 (0.151)	4.425 (0.021)	0.023 (0.001)	0.392 (0.002)

¹ Aqueous extracts of Spanish-style-processed green olives

² Hydroxytyrosol-4-glucoside

³ Hydroxytyrosol-acetate

⁴ In brackets, standard deviation

⁵ Not detected

Table S4. Homology of ORFs related to phenolic compounds metabolism found in the chromosome of *Lactobacillus pentosus* IG1 with other *L. pentosus* strains whose genome has been sequenced.

Locus tag ¹	Size (bp)	Predicted protein (aas)	Annotation ¹	% Protein identity (sim ²)	<i>L. pentosus</i> strain	Annotation	Locus tag (gene)	Accession no.
LPENT_00991	1509	502	β-glucosidase	46 (63) 45 (63) 43 (59)	KCA1 MP10 DSM 20314	β-glucosidase = ³ =	KCA1_2875 LPE_02502 FD24_GL002568	EIW12476 CCB83451 KRK26223
LPENT_01512	411	136	putative uncharacterized protein	100 (100) 99 (99) 99 (99)	KCA1 MP10 DSM 20314	aromatic acid carboxylyase(subD) putative uncharacterized protein hypothetical protein	KCA1_0243 LPE_01834 FD24_GL001762	EIW15307 CCB82807 KRK22669
LPENT_01513	564	187	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	100 (100) 99 (100) 99 (100)	DSM 20314 MP10 KCA1	3-octaprenyl-4-hydroxybenzoate carboxy-lyase = aromatic acid carboxylyase(subB)	FD24_GL001763 LPE_01835 KCA1_0242	KRK22670 FR871811 EIW15306
LPENT_01783	537	178	phenolic acid decarboxylase	99 (100) 99 (100) 99 (99)	MP10 DSM 20314 KCA1	Phenolic acid decarboxylase = =	LPE_01270 FD24_GL000907 KCA1_3004	CCB82257 KRK23698 EIW12605
LPENT_01784	555	184	regulator of phenolic acid metabolism <i>PadR</i>	100 (100) 100 (100) 99 (99)	MP10 DSM 20314 KCA1	regulator of phenolic acid metabolism <i>PadR</i> Transcriptional repressor <i>PadR</i> =	LPE_01271 FD24_GL000908 KCA1_3003	CCB82258 KRK23699 EIW12604
LPENT_02382	1413	470	tannase	97 (98) 97 (98) 94 (97)	MP10 DSM 20314 KCA1	Tannase = =	LPE_02253 FD24_GL000461 KCA1_242	CCB83221 KRK24099 EIW12931
LPENT_02396	1473	490	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	100 (100) 99 (100) 99 (99)	DSM 20314 KCA1 MP10	3-octaprenyl-4-hydroxybenzoate carboxy-lyase (<i>UbiD</i> family) =	FD24_GL000447 KCA1_2410 LPE_02263	KRK24087 EIW12921 CCB83231
LPENT_02397	1380	459	transport protein	100 (100) 100 (100) 99 (99)	MP10 DSM20314 KCA1	(cation) transport protein = =	LPE_02264 FD24_GL000446 KCA1_2409	CCB83232 KRK24086 EIW12920
LPENT_02398	918	305	transcription regulator	99 (99) 99 (99) 96 (96)	MP10 DSM20314 KCA1	transcription regulator (<i>LysR</i> family) =	LPE_02265 FD24_GL000445 KCA1_2408	CCB83233 KRK24085 EIW12919
LPENT_02868	1884	627	tannase	99 (100) 99 (100) 29 (47)	MP10 DSM 20314 KCA1	Tannase = =	LPE_00166 FD24_GL001393 KCA1_2422	CCB81159 KRK26597 EIW12931

¹According to *Lactobacillus pentosus* IG1 genome (accession no. FR874854); ²In brackets, similarity; ³=, the same annotation as immediately above.

Supplementary material – Figure legend

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Figure S1. *Lactobacillus pentosus* IG1 chromosomal map showing the relative positions of genes related to phenolic compound metabolism.

Figure S2. HPLC chromatograms of representative phenolic compounds incubated for 7 days (t=7 d) at 30 °C in Modified Basal Medium (MBM) inoculated with *Lactobacillus pentosus* IG1. Panels at the left side show the corresponding chromatograms at the start of the experiment (t=0 d). Chromatograms were recorded at 280 nm. IS: internal standard (syringic acid).

Figure S3. Amino acid sequence alignment of bacterial Tannases showing closest homology to the Tannases found in the genome of *L. pentosus* IG1. Sequence code: LPENT_02382 and LPENT_02868, Tannase I and II, respectively, of *L. pentosus* IG1; TanBLp, Tannase of *L. plantarum* WCFS1 (acc. no. YP_004890536); TanALp, Tannase of *L. plantarum* ATCC14917^T (acc. no. KRL35904); TanASl, Tannase of *Staphylococcus lugdunensis* (acc. no. BAF03594); TanASg, Tannase of *Streptococcus gallolyticus* (acc. no. ZP_07464373). Color code: red, high consensus; blue, low consensus; black, neutral. Red triangles, residues of the catalytic triad; blue triangles, residues involved in galloyl binding interactions, as described by Ren et al. (2013) and Jiménez et al. (2014).

Figure S4. Amino acid sequence alignment of phenolic acid decarboxylases (PadA) found in *L. plantarum* and *L. pentosus* strains. Sequence code: LPENT_01783 from *L.*

pentosus IG1 (acc.no. CCC17087); LPE_01270 from *L. pentosus* MP-10 (acc. no. CCB82257); FD24_GL000907, from *L. pentosus* DSM20314 (acc. no. KRK23698); KCA1_3004 from *L. pentosus* KCA1 (acc. no. EIW12605); PadALp_WCFS1 from *L. plantarum* WCFS1 (acc. no. YP_004891133); and PadALp_LPCHL2 from *L. plantarum* LPCHL2 (acc. no. AAC45282). Underlined amino acids indicate highly conserved positions among bacterial PadAs as reported by Rodríguez et al. (2010). The position of the conserved residue His89 is indicated.

Figure S1. Carrasco et al.

Figure S2. Carrasco et al.

Figure 1. Multiple sequence alignment of the deduced amino acid sequences of the *LPENT* proteins from *Leishmania* species. The alignment is shown in blocks of 100 residues, with positions 1 to 130, 131 to 260, 261 to 390, 391 to 520, and 521 to 647. The sequences are color-coded: red for conserved residues, blue for residues that are conserved in at least one species, and black for non-conserved residues. The sequences are: *LPENT_02382* (TanBLp), *LPENT_02868* (TanALp), *LPENT_02868* (TanAS1), and *LPENT_02868* (TanASg). The alignment shows high conservation of the protein structure across the different species, with some variations in the C-terminal region.

Figure S4. Carrasco et al.