The use of *Lactobacillus plantarum* esterase genes: a biotechnological strategy to increase the bioavailability of dietary phenolic compounds in lactic acid bacteria

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**ABSTRACT**

In *Lactobacillus plantarum* the metabolism of hydroxybenzoic and hydroxycinnamic acid derivatives follows a similar two-step pathway, an esterase action followed by a decarboxylation. The *L. plantarum* esterase genes involved in these reactions have been cloned into pNZ8048 or pT1NX plasmids and transformed into technologically relevant lactic acid bacteria. None of the strains assayed can hydrolyse methyl gallate, a hydroxybenzoic ester. The presence of the *L. plantarum* tannase encoding genes (*tanALp* or *tanBLp*) on these bacteria conferred their detectable esterase (tannase) activity. Similarly, on hydroxycinnamic compounds, esterase activity for the hydrolysis of ferulic acid was acquired by lactic acid bacteria when *L. plantarum* esterase (JDM1_1092) was present. This study showed that the heterologous expression of *L. plantarum* esterase genes involved in the metabolism of phenolic acids allowed the production of healthy compounds which increase the bioavailability of these dietary compounds in food relevant lactic acid bacteria.

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**Introduction**

Lactic acid bacteria comprise a versatile group of microorganisms that are generally regarded as safe (GRAS) and have numerous applications in food fermentations. Lactic acid fermentation could be considered as a simple and valuable biotechnological process for maintaining and/or improving the safety nutritional, sensory and shelf-life properties of foods. Composition of microbiota and its development are important factors influencing fermentation and final product quality.

Phenolic compounds are important constituents of food products of plant origin. These compounds are related to the sensory characteristics of foods such as flavour, astringency and colour (Shahidi and Naczk 2003). In addition, the presence of phenolic compounds on the diet is beneficial to health due to their antioxidant, antiproliferative and anti-inflammatory properties (Cory et al. 2018). In this context, there has been a significant increase in the number of studies related to food fortification to different types of foods with phenolic compounds with functional properties and from natural sources. These products are of great interest to the food industry, as they can create differentiated products with high added value (Ribeiro et al. 2016; Carneiro de Barcelos 2020). In this way, for example, dairy products could play a key role since they are included in the diet of most of the world’s population (Cutrim and Sloboda 2018). However, not all the lactic acid bacteria found in dairy fermentations could survive in the presence of phenolic compounds as these compounds could exert an antibacterial effect on them. Moreover, the biotransformation of these phenolic compounds probably plays a major role in the biological activity of many of these compounds. Some food phenolics are transformed during fermentation by the fermentative microbiota. This conversion is often essential for...
absorption and modulates the biological activity of these dietary compounds (Selma et al. 2009).

*Lactobacillus plantarum* is the lactic acid bacterium that is the most frequently found in fermentations of food products of plant origin where phenolic compounds are abundant. This species is the model bacteria for the study of phenolic compound metabolism (Rodriguez et al. 2009). In this species the metabolism of phenolic acids has been elucidated. Phenolic acids, which account for almost one third of the dietary phenols, contain mainly two distinguishing constitutive carbon frameworks, the hydroxybenzoic and the hydroxycinnamic structures. The transformation of both carbon frameworks follows a similar two-step metabolism in *L. plantarum*, an initial esterase action followed by a decarboxylation. In relation to hydroxybenzoic esters, two esterase enzymes able to hydrolyse them have been described in *L. plantarum*. The *L. plantarum* TanA (tanAlp) (Jiménez et al. 2014) and *L. plantarum* TanB (tanBlp) (Iwamoto et al. 2008; Curiel et al. 2010) esterases, also known as tannases, which hydrolyse the ester bonds of gallic and protocatechuic acids. Subsequently, the hydroxybenzoic acids formed by tannase action are decarboxylated by a gallate decarboxylase enzyme (Lp_2945, LpdC or GalDC; Jiménez et al. 2013) (Figure 1(A)). Similarly, in hydroxycinnamic esters, the JDM1_1092 esterase is described in some *L. plantarum* strains (Esteban-Torres et al. 2015). Esterase action released free hydroxycinnamic acid from their naturally esterified forms, and some of these acids are subsequently decarboxylated by a phenolic acid decarboxylase enzyme (Lp_3665 or PAD; Cavin et al. 2006; Rodríguez et al. 2008; Figure 1(B)).

The metabolism of phenolic compounds described in *L. plantarum* is scarcely present in lactic acid bacteria belonging to other genera or species (Rodríguez et al. 2009). However, these lactic acid bacteria could possess great technological interest and be prevalent in the fermentation of specific food substrates, such as dairy products, that nowadays are fortified with phenolic compounds. Due to the antioxidant nature of phenolic compounds, they are used as food additives. Fortification of grape seed extract to yogurt had enhanced antioxidant activity (Chouchouli et al. 2013); similarly, phenolic compounds present in a chamomile extract when incorporated into cottage cheese enhanced its antioxidant activity and storage stability (Caleja et al. 2015). Therefore, the use of phenolic compounds in different dairy products showed beneficial effects by increasing its antioxidant activity. Moreover, as some phenolic compounds possessed antibacterial effect, the introduction of the ability to transform phenolic esters into their derivatives will increase bacterial survival and could play a relevant role in the biological activity of these compounds. Therefore, in this study we have explored the introduction in lactic acid bacteria of *L. plantarum* esterase genes involved in the metabolism of phenolic acid derivatives.

**Materials and methods**

**Materials and reagents**

Bacterial M17 culture broth was purchased from Scharlab (Senmanat, Spain) and MRS broth from Scharlau Chemie SA (Barcelona, Spain). AnaeroGen sachets were obtained from Oxoid Ltd (Basingstoke, UK).
Glucose, methyl gallate, gallic acid, methyl ferulate and ferulic acid were purchased from Sigma-Aldrich (St. Louis, MO). Ethyl acetate was obtained from Lab-Scand (Ireland). All other reagents used were of analytical grade, and the water was purified by a Milli-Q water purification system (Millipore, Bedford, MA).

**Bacterial strains and growth conditions**

*Lactococcus lactis* MG1363, was used as host microorganism for plasmid construction and *Streptococcus thermophilus* INIA 468 were grown at 30 °C in M17 broth supplemented with 0.5% glucose (GM17) under aerobic conditions. *Lactobacillus casei* BL23, *Lactobacillus reuteri* CECT 925 and *Enterococcus faecalis* INIA4 were routinely cultivated at 37 °C in MRS broth under anaerobic conditions (O2 < 0.1%; CO2 7–15%) in sealed jars using AnaeroGen sachets.

**Cloning of* L. plantarum* genes in lactic acid bacteria**

The selected *L. plantarum* esterase genes involved in the metabolism of phenolic compounds are showed in Table 1. Vector pT1NX or vectors derived from pNZ8048 plasmids were used to clone *L. plantarum* esterase genes. Vector pNZ8048 is a plasmid with broad host range replication in gram positive bacteria and a chloramphenicol resistance gene as selection marker (de Ruyter et al. 1996). Plasmid pT1NX is an acid bacteria vector derivatives used in this study, pNZ8048 and pT1NX are plasmid derivatives, the designed forward primer introduced a *Nco*I site upstream of the stop codon (Table 1).

To clone the *tanBLp* gene in the pT1.P1 plasmid, the forward F-pT1-tanB primer introduced a *Pst*I site downstream of the stop codon (Table 1). To clone the *tanBlp* gene in the pT1.P1 plasmid, the forward F-pT1-tanB primer introduced a *Pst*I site downstream of the stop codon (Table 1). The polymerase chain reaction products were digested with the two corresponding restriction enzymes and ligated into the pNZ8048 derivatives or pT1NX plasmids. The ligation mixtures were transformed into *L. lactis* MG1363 by electrophoration (Landete et al. 2014). The transformants were selected on 5 µg/ml chloramphenicol or erythromycin for pNZ8048 derivatives or pT1NX, respectively, and checked by restriction mapping and sequencing of the inserted fragment.

The constructed plasmids, pNZ8048 and pT1NX derivatives, were transformed into *L. casei* BL23, *L. reuteri* CECT 925, *S. thermophilus* INIA 468 and *E. faecalis* INIA4, as described previously (Landete et al. 2014).

**Enzymatic activity of* L. plantarum* esterase genes in lactic acid bacteria**

The activity of the *L. plantarum* esterase genes expressed in several lactic acid bacteria was assayed by incubating the bacteria in the presence of hydroxybenzoic (methyl gallate and gallic acid) and hydroxycinnamic (methyl ferulate and ferulic acid) derivative compounds (Curiel et al. 2010). Bacterial GM17 or MRS culture media containing a phenolic compound (1 mM final concentration) were inoculated with the corresponding bacterial culture and incubated for seven days at 30 °C in aerobic or anaerobic conditions in presence of chloramphenicol or erythromycin (5 µg/ml), depending of the plasmid carried. After incubation, the phenolic compounds present in the supernatants were extracted twice with one third of the reaction volume of ethyl acetate (Lab-Scan, Ireland) and analysed by HPLC-DAD (Bartolomé et al. 2000).

| Gene (locus) | L. plantarum strain source | Protein encoded | Accession | Primers (sequence 5′→3′) | Size (bp) |
|-------------|----------------------------|-----------------|-----------|--------------------------|----------|
| *tanA*<sub>lp</sub> (HMPREF0531_11477) | ATCC 1491<sup>7</sup> | Esterase/Tannase (tannin acylhydrolase) | ACGZ02000013 (26014−27894) | F-pNZ-tanA (TTCCATGGTGAGTAACCGATTGATTTTTGATG) | 1878 |
| *tanB*<sub>lp</sub> | WCFS1 | Esterase/Tannase (tannin acylhydrolase) | AL935263.2 (2630494−2631903) | F-pNZ-tanB (TTCCATGGTGAGTAACCGATTGATTTTTGATG) | 1407 |
| *est*<sub>1092</sub> (JDM 1092) | DSM 1055 | Esterase | CP001617.1 (1166740−1156627) | F-pNZ-1092 (TTCTAGATCATCAGGCCATATGTTCCTGCAA) | 885 |

*The NcoI, XbaI, and PsfI restriction sites in the primers are underlined.*
Extraction and high-performance liquid chromatography (HPLC) analysis of esterase activity

A Thermo chromatograph equipped with a P400 SpectraSystem pump, an AS3000 autosampler, and a UV6000LP photodiode array detector was used for esterase activity (Thermo Electron Corporation, Waltham, MA). 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; 90–120 min, washing 1.0 ml/min, and re-equilibration of the column under initial gradient conditions. Detection was performed by scanning from 220–380 nm. Samples were injected in duplicate onto the cartridge after being filtered through a 0.45 μm polyvinylidene fluoride 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 or by liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry.

Results and discussions

Biotransformation of hydroxybenzoic derivatives by lactic acid bacteria

Among food lactic acid bacteria, strains from the L. plantarum group are the only ones which possess esterase activity against tannins, one type or hydroxybenzoic esters (Nishitani and Osawa 2003; Nishitani et al. 2004; Vaquero et al. 2004). The biochemical pathway described for the degradation of tannins by L. plantarum implies the action of an esterase (tannase) followed by a gallate decarboxylase to decarboxylate the gallic acid formed by tannase action (Rodriguez et al. 2008). The L. plantarum WCFS1 gene encoding tannase (tanBlp or lp_2956) involved in tannin degradation has been identified (Iwamoto et al. 2008). Moreover, some L. plantarum strains, such L. plantarum ATCC 14917, possessed a second tannase gene (tanAlp or HMPREF0531_11477) also able to hydrolyse tannins (Figure 1(A)).

The presence of these L. plantarum esterase genes in bacteria present in food products containing phenolics could represent an advantage to their survival and an increase in the healthy properties of the fermented food product. In this context, L. lactis subsp. cremoris MG1363 was assayed to clone the L. plantarum esterase (tannase) genes. L. lactis MG1363 is the lactococcal strain most intensively studied throughout the world and it is the most widely used host strain for cloning and gene expression in L. lactis (Wegmann et al. 2007; Liñares et al. 2010). This strain displays a larger capability to grow on various sugars, especially those found in plant material, pointing to a plant associated biological niche for the ancestor of L. lactis MG1363. Two pNZ8084 expression vector derivatives, harbouring TuB or TuR promoters, were used to clone both esterase (tannase) genes described in L. plantarum strains (tanA lp or HMPREF0531_11477, and tanB lp or lp_2956) (Landete et al. 2014, 2015). After several unsuccessful attempts, the tanB lp gene could not be cloned into the pNZ.TuR vector. The recombinant plasmids obtained (pNZ.TuB.TanA, pNZ.TuR.TanA and pNZ.TuB. TanB) were transformed into L. lactis MG1363. L. lactis cells harbouring these plasmids were incubated in the presence of two related hydroxybenzoic compounds, gallic acid and methyl gallate, an acid and one of its esters (Figure 1(A)). Methyl gallate by the action of the L. plantarum tannase will be converted into gallic acid.

From the L. lactis MG1363 tannase derivative strains constructed, only those harbouring pNZ.TuR.TanA plasmid, exhibited tannase activity as it was able to partially hydrolyse the methyl gallate present in the media into gallic acid (Table 2; Figure 2(A)). Contrarily, L. lactis cells harbouring recombinant plasmids pNZ.TuB.TanB and pNZ.TuB.TanA did not exhibit tannase activity (Table 2). Therefore, as no tanB lp activity was found, pT1NX plasmid was used for the expression of the tanB lp (lp_2956) tannase gene. On this occasion, pT1NX.TanB plasmid was successfully constructed, and the recombinant L. lactis strains harbouring it were able to produce gallic acid from methyl gallate (Table 2; Figure 2(A)).

As L. plantarum TanA lp and TanB lp tannases were functional in L. lactis MG1363 strain when cloned into pNZ.TuR.TanA and pT1NX.TanB plasmids, respectively, these plasmids were selected to transform lactic acid bacteria belonging to different species and genera such as L. casei BL23, L. reuteri CECT 925, S. thermophilus INIA 468 and E. faecalis INIA4. These bacterial species were chosen due to their relevant role in food fermentations. L. casei is a remarkably adaptive species and may be isolated from raw and fermented dairy products, fresh and fermented plant products, and in the reproductive and intestinal tracts.
of humans and other animals. Strain BL23 has been widely used for genetic, physiological and biochemical studies (Maze et al. 2010). When *L. casei* BL23 was transformed with pNZ.TuR.TanA and pT1NX.TanB plasmids, appreciable but limited tannase activity was observed and gallic acid could be identified (Figure 2(B); Table 2).

Strains from the *L. reuteri* species are present in the human gut and are important probiotic organisms (Saulnier et al. 2011). *L. reuteri* CECT 925$^T$ (JCM 1112$^T$) strain, a probiotic bacteria (Morita et al. 2008), when harbours pNZ.TuR.TanA or pT1NX.TanB plasmids exhibited tannase activity in methyl gallate containing media, as compared to untransformed cells (Figure 2(C); Table 2).

*S. thermophilus* is considered the second most important species of industrial lactic acid bacteria after *L. lactis*. Beyond the dairy environment, *S. thermophilus* strains have also been isolated from plant sources (Michaylova et al. 2007). In this work, *S. thermophilus* INIA468 was selected due to its high aminopeptidase activity when was used as thermophilic starter during cheese manufacture (Joosten et al. 1996). When the *L. plantarum* tannase genes were cloned into *S. thermophilus* INIA468, hydrolysis of methyl gallate was observed and the concomitant presence of its product, gallic acid (Figure 2(D); Table 2). Tannase activity is particularly relevant in a fermenting-milk bacteria, such as *S. thermophilus*, when the milk contained pomegranate juice, with high content of gallotannins (Pena et al. 2020).

As observed in Figure 2, *L. plantarum* tannase genes were more efficient when they were cloned into *L. lactis* strains, since gallic acid was clearly observed as an apparent peak in the chromatograms. In the other lactic acid bacteria assayed, the presence of gallic acid was not so evident and it was clearly identified by comparing its retention time and spectral data with the standard from commercial supplier.

Figure 2. HPLC chromatograms of a hydroxybenzoic ester (methyl gallate) hydrolysis by *L. plantarum* tannase genes in recombinant lactic acid bacteria. Chromatograms are shown of supernatants from lactic acid bacteria harbouring pNZ.TuR.TanA or pT1NX.TanB plasmids grown in the presence of methyl gallate (1 mM) for seven days. HPLC chromatograms from the control strains are also indicated. The strains assayed were (A) *Lactococcus lactis* MG 1363, (B) *Lactobacillus casei* BL23, (C) *Lactobacillus reuteri* CECT 925, (D) *Streptococcus thermophilus* INIA 468 and (E) *Enterococcus faecalis* INIA4. Chromatograms were recorded at 280 nm. MG: methyl gallate; GA: gallic acid; PG: pyrogallol.
Table 2. Hydroxybenzoic metabolism in LAB strains and their derivatives harbouring *L. plantarum* esterase (tannase) genes.

| Strain                  | Gene                  | Plasmid                | Ester hydrolysis | Decarboxylation |
|-------------------------|-----------------------|------------------------|------------------|-----------------|
| *Lactococcus lactis* MG1363 | None                  | pNZ8048                | -                | -               |
|                         | HMPREF0531_11477      | pNZ.TuB.TanA           | -                | -               |
|                         | HMPREF0531_11477      | pNZ.TuR.TanA           | +                | -               |
|                         | lp_2956               | pNZ.TuB.TanB           | -                | -               |
|                         | None                  | pNZ8084                | -                | -               |
|                         | lp_2956               | pNZ.TuR.TanB           | +                | -               |
| Lactobacillus casei BL23 | None                  | pNZ8084                | -                | -               |
|                         | HMPREF0531_11477      | pNZ.TuR.TanA           | +                | -               |
|                         | None                  | pNZ8084                | -                | -               |
|                         | lp_2956               | pNZ.TuR.TanB           | +                | -               |
| Lactobacillus reuteri CECT 925 | None                  | pNZ8084                | -                | -               |
|                         | HMPREF0531_11477      | pNZ.TuR.TanA           | +                | -               |
|                         | None                  | pNZ8084                | -                | -               |
|                         | lp_2956               | pNZ.TuR.TanB           | +                | -               |
| Streptococcus thermophilus INIA 468 | None                  | pNZ8084                | -                | -               |
|                         | HMPREF0531_11477      | pNZ.TuR.TanA           | +                | -               |
|                         | None                  | pNZ8084                | -                | -               |
|                         | lp_2956               | pNZ.TuR.TanB           | +                | -               |
| Enterococcus faecalis INIA4 | None                  | pNZ8084                | -                | +               |
|                         | HMPREF0531_11477      | pNZ.TuR.TanA           | +                | +               |
|                         | None                  | pNZ8084                | -                | +               |
|                         | lp_2956               | pNZ.TuR.TanB           | +                | +               |

The last lactic acid bacteria strain assayed belonged to the *E. faecalis* species, a natural inhabitant of the mammalian gastrointestinal tract which is also found in soil, sewage, water and food, while others are associated with nosocomial infections (Kim and Marco 2014). In this study, the enterocin 4 *E. faecalis* INIA4 producer strain was used as host for the expression *L. plantarum* esterase genes (Joosten et al. 1996). *L. plantarum* tannase genes carried by pNZ.TuR.TanA and pT1NX.TanB plasmids were functional in *E. faecalis* when recombinant strains were grown in the presence of methyl gallate (Figure 2(E); Table 2). It is noteworthy that pyrogallol was observed from methyl gallate in *E. faecium* cultures harbouring *L. plantarum* tannases. Pyrogallol is produced by the decarboxylation of gallic acid, therefore *E. faecalis* INIA4 should possess an enzyme able to carry this decarboxylation (Figure S1). A different behaviour was noticed in both plasmids, whereas a fully hydrolysis of methyl gallate was observed in pNZ.TuR.TanA culture, only a partial methyl gallate hydrolysis and a noticeable amount of pyrogallol was observed in the pT1NX.TanB culture (Figure 2(E)). This result indicates that in *E. faecalis* INIA4 there is a more efficient methyl gallate hydrolysis by pNZ.TuR.TanA than by pT1NX.TanB recombinant plasmids.

Although *L. plantarum* gallate decarboxylase is composed by three protein subunits, however only the expression of the *L. plantarum* C subunit (*lpdC*) was enough to confer gallate decarboxylase activity to *E. coli* cells (Jiménez et al. 2013). In order to confirm the possible existence of an enzyme exhibiting gallate decarboxylase activity in *E. faecalis* INIA4, a search for proteins similar to *LpdC* in the protein database was performed. The search revealed that a high number of *Enterococcus faecium* strains possessed a protein 73.88% identical to *L. plantarum* LpdC (accession Q3Y2U5 from *E. faecium* ATCC BAA-472). However, only few *E. faecalis* strains could also possess a protein 73.27% identical to the *L. plantarum* LpdC protein (accession S4D0H4 from *E. faecalis* 13-SD-W-01). Both, enterococcal proteins are 89.82% identical among them (Figure S2). Therefore, similarly to *E. faecalis* 13-SD-W-01 and considering the results observed in this study, *E. faecalis* INIA4 should possess, at least, the protein equivalent to the catalytic subunit of the *L. plantarum* gallate decarboxylase (LpdC). This protein could confer gallate decarboxylase activity to *E. faecalis* INIA4 cells as observed when they are cultured in the presence of gallic acid (Figure S1) or when they harboured a *L. plantarum* tannase gene and are grown in the presence of methyl gallate (Figure 2(E); Table 2). A previous study performed in lactic acid bacteria revealed that, in contrast to tannase activity, gallate decarboxylase activity is common among lactic acid bacteria (Jiménez et al. 2013). Strains from the species *E. faecium*, *L. brevis*, *L. pentosus*, *L. plantarum* and *O. oeni* were able to decarboxylate gallic; however, *L. hilgardii*, *L. mesenteroides*, *L. sakei* and *P. pentosaceus* strains did not decarboxylate it (Jiménez et al. 2013). Taking into account the results obtained in this study, at least some *E. faecalis* strains, could also be included among the lactic acid bacteria possessing gallate decarboxylase activity, since *E.*
faecalis INIA4 and all its derivatives constructed in this study exhibited gallate decarboxylase activity when gallic acid was included in the culture media (Figure 2(E), Figure S1; Table 2).

From these results it could be concluded that none of the lactic acid bacteria strains assayed possessed hydroxybenzoic esterase (tannase) activity. The scarcity of this enzymatic activity among lactic acid bacteria has been previously reported (Nishitani and Osawa 2003; Osawa et al. 2000; Nishitani et al. 2004; Vaquero et al. 2004). Therefore, in this study has been demonstrated that both L. plantarum tannase genes could be functional in lactic acid bacteria. The presence of a tannase gene could provide a selective advantage to these species for life in foods or environments where compounds of plant origin are abundant. By the presence of tannase activity on these bacteria, they should be able to transform them and does not depend on other microorganisms for the initial degradation of these compounds.

The obtained results indicated that the presence in lactic acid bacteria of L. plantarum esterase genes involved in hydroxybenzoic metabolism could play an important role when tannins are present in foods and in the intestine, having the capability of degrading and detoxifying these constituents into simpler and more bioavailable compounds.

### Biotransformation of hydroxycinnamic derivatives by lactic acid bacteria

In plant food substrates, a high proportion of hydroxycinnamic acids are found esterified. The biochemical pathway for the degradation of cell wall hydroxycinnamates starts with the action of esterases, which releases free hydroxycinnamic acids from their naturally esterified forms. Particularly, feruloyl esterases are the enzymes involved in the release of hydroxycinnamic acids from plant cell walls. Some L. plantarum strains possess an esterase (JDM1_1092 or est_1092) which confers feruloyl esterase activity to cell cultures (Esteban-Torres et al. 2015; Figure 1(B)). After esterase action, some hydroxycinnamic acids are subsequently metabolised by L. plantarum strains. The main transformation of hydroxycinnamic acids is their decarboxylation, carried by the phenolic acid decarboxylase enzyme (PAD), encoded by the lp_3665 gene (Cavin et al. 2006; Rodriguez et al. 2008; Figure 1(B)).

Similarly, to hydroxybenzoic derivatives, L. lactis subsp. cremoris MG1363 was used as host strain for cloning and gene expression of the L. plantarum esterase gene involved in the metabolism of hydroxycinnamic acids. For the cloning of the JDM1_1092 gene only the pNZ.TuR derivative plasmid was used (Table 3). The L. lactis transformed cells were grown in the presence of methyl ferulate, a hydroxycinnamic ester. When L. lactis strain harboured pNZ.TuR.1092 plasmid exhibited esterase activity and was able to hydrolyse the methyl ferulate present in the media into ferulic acid (Table 3; Figure 3(A)).

Plasmid pNZ.TuR.1092 was used to transform lactic acid bacteria from other species assayed. L. casei BL23, L. reuteri CECT 925, S. thermophilus INIA 468 and E. faecalis INIA4 cultures transformed with pNZ.TuR.1092 and grown in the presence of methyl ferulate exhibited an identical behaviour than L. lactis. The presence of pNZ.TuR.1092 plasmid confers them the ability to hydrolyse methyl ferulate into ferulic acid (Table 3; Figure 3(B–E)). All these strains showed a partial hydrolysis of the methyl ferulate present in the media. Although the strains assayed in this study were not able to hydrolyse ferulic acid, feruloyl esterase activity had been reported in some lactic acid bacteria such as L. fermentum (Deng et al. 2019; Fritsch et al. 2017; Xu et al. 2017), L. crispatus (Xu et al. 2019), L. acidophilus (Fritsch et al. 2017; Xu et al. 2017) or L. helveticus (Song and Baik 2017) species, among others.

In L. plantarum strains after esterase action, some hydroxycinnamic acids are subsequently decarboxylated by the action of the phenolic acid decarboxylase
enzyme (PAD), encoded by the lp_3665 gene (Cavin et al. 2006; Rodríguez et al. 2008). The decarboxylation of these hydroxycinnamic acids originates their 4-vinyl derivatives (e.g. vinylguaiacol from ferulic acid) that are considered food additives and are approved as flavouring agents (JECFA 2001). To known if the lactic acid bacteria assayed in this study possessed hydroxycinnamic acid decarboxylase activity, they were also grown in media containing ferulic acid. After incubation, the supernatants were assayed for the presence of vinyl guaiacol, originated by ferulic acid decarboxylation. Vinyl guaiacol was detected only in the L. lactis MG1363 cultures (Table 3; Figure S3), indicating that this strain possessed phenolic acid decarboxylase activity able to decarboxylate the ferulic acid present on the culture media. As far as we known, this is the first report describing the presence of phenolic acid decarboxylase activity on L. lactis strains. As the complete genome of the L. lactis MG1363 strain was available, a search for a protein similar to L. plantarum PAD enzyme was performed. The search revealed that L. lactis MG1363 possessed a protein annotated as PadC (accession A2RN76) which is 74.17% identical to L. plantarum PDC, therefore confirming the presence of decarboxylase activity on this strain (Figure S4). Among lactic acid bacteria, PAD activity has been previously described in L. plantarum (Cavin et al. 1993; Rodríguez et al. 2008), P. pentosaceus (Barthelmebs et al. 2000) and L. brevis (de las Rivas et al. 2009, Curiel et al. 2010, Landete et al. 2010).

Hydroxycinnamic acids are found covalently attached to the plant cell wall by esters and amides bonds (Faulds and Williamson 1999). Enzymes
capable of cleaving hydroxycinnamates esters (esterases) released ferulic and/or p-coumaric acid from plant cell walls which, in their free form, become substrates of phenolic acid decarboxylase (PAD) enzymes, which convert these compounds into their vinyl phenol derivatives (Cavin et al. 2006; Rodríguez et al. 2008). Decarboxylation of ferulic acid to 4-vinyl guaiacol is a potentially profitable value addition to the starting material if the ferulic acid can be extracted efficiently from its bound polysaccharides (Mathew and Abraham 2006; Rosazza et al. 1995).

Conclusion

The genetic manipulation of lactic acid bacterial strains has great potential in the development of functional products, with potential positive impact on the food industries. As example, L. lactis are used extensively in food fermentations being an important industrial microorganism. Its economic importance combined with a long history of safe usage has prompted the development of technology for genetic and metabolic engineering. Another relevant lactic acid bacteria species is S. thermophilus, which has potential as probiotic, is extensively used for the manufacture of several important fermented dairy foods, including yoghurt and some cheese varieties (Delorme 2008). The genetic manipulation of these relevant lactic acid bacteria species (e.g. L. lactis or S. thermophilus, among others) have great potential in the development of functional products, with potential positive impact on the food industries. In this work, for the first time, L. plantarum esterase genes involved in the metabolism of phenolic compounds have been transformed into different lactic acid bacterial strains, conferring them the ability to transform some phenolic compounds, therefore increasing their metabolic potential. Nevertheless, safety and legal issues must be considered regarding genetically modified microorganisms.

Disclosure statement

No potential conflict of interest was reported by the authors.

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