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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 antioxidant properties of foods. This study was undertaken to assess the ability of *Lactobacillus plantarum* CECT 748^T to metabolize 19 food phenolic acids. Among the hydroxycinnamic acids studied, only *p*-coumaric, caffeic, ferulic and *m*-coumaric acids were metabolized by *L. plantarum*. Cultures of *L. plantarum* produced 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 the 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 colour, 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 has received the most attention in recent literature (Lodovici, Guglielmi, Meoni, & Dolara, 2001).

The term "phenolic acids", in general, describe 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 are 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 acids combine to form chlorogenic acid. Caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represents 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).

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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, e.g. olives, must and a variety of vegetable fermentation products. 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 (Barthelmebs, Diviès, & Cavin, 2001; Cavin et al., 1993; Couto, Campos, Figueiredo, & Hogg, 2006; van Beek & Priest, 2000). 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 of —H or —OH *meta* to the unsaturated side chain were metabolised (Cavin et al., 1997b).

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

2. Material and methods

2.1. Chemicals

The 19 phenolic acids analyzed in this study were seven hydroxycinnamic acids, nine hydroxybenzoic acids, and three other food phenolic acids, such as phloretic acid (Aldrich H524006), chlorogenic acid (Sigma C3878) and ellagic acid (Sigma E2250). The hydroxycinnamic acids were: *p*-coumaric acid (Sigma C-9008), *o*-coumaric acid (Fluka 28170), *m*-coumaric acid (Aldrich H23007), cinnamic acid (Aldrich C8, 085-7), caffeic acid (Sigma C0625), ferulic acid (Sigma F3500) and sinapic acid (Sigma D7927). The hydroxybenzoic acids assayed were: syringic acid (Fluka 86230), gallic acid (Fluka 48630), salicylic acid (Merck 631), benzoic acid (Merck 6391513), gentisic acid (Aldrich 149357), veratric acid (Fluka 94872), *p*-hydroxybenzoic acid (Fluka 54630), protocatechuic acid (Sigma P5630), and vanillic acid (Fluka 94770).

The phenolic acid derivatives, 4-ethyl phenol (Fluka 04700), 4-ethyl catechol (Lancaster A12048), 4-ethyl guaiacol (Aldrich W 24,360-4-K), 4-vinyl phenol (Lancaster L10902), 4-vinyl guaiacol (Lancaster A13194), pyrogallol (Merck 612), catechol (Sigma C9510) and 3-(3-hydroxyphenyl) propionic acid (Lancaster L01279), were used as standards for the identification of the degradation compounds.

2.2. Bacterial strain and growth conditions

L. plantarum CECT 748^T (ATCC 14917, DSMZ 20174), isolated from pickled cabbage, was purchased from the Spanish Type Culture Collection. This strain was selected as it represents the type strain of this species.

The bacterium was cultivated in a modified basal medium described previously for *L. plantarum* (Rozès & Peres, 1998). The basal medium has the following composition: glucose, 2.0 g/l; trisodium citrate dihydrate (SO 0200, Scharlau), 0.5 g/l; D-, L-malic acid (AC 1420, Scharlau), 5.0 g/l; casamino acids (223050, BD), 1.0 g/l; yeast nitrogen base without amino acids (239210, BD), 6.7 g/l; pH was adjusted to 5.5. The basal medium was modified by the replacement of glucose by galactose (216310, Difco). This defined medium was used to avoid the presence of phenolic compounds included in non-defined media. For the degradation assays, the sterilized modified basal medium was supplemented at 1 mM final concentration with the phenolic compound filter-sterilized. The *L. plantarum* inoculated media were incubated at 30 °C, in darkness, under microaerophilic conditions, without shaking, for 10 days. A long incubation period was used to find the dead-end products of phenolic acid degradation. Incubated media with cells and without phenolic compound and incubated media without cells and with phenolic compounds were used as controls. From the supernantants, the phenolic products were extracted twice with one third of the reaction volume of ethyl acetate (Lab-Scan, Ireland).

2.3. Degradation of phenolic acids by cell-free extract

In order to prepare cell-free extracts, *L. plantarum* CECT 748^T strain was grown in MRS media (Difco, France) under microaerobic conditions at 30 °C until a late exponential phase was reached. The cells were harvested by centrifugation and washed three times with phosphate buffer (50 mM, pH 6.5), and subsequently resuspended in the same buffer for cell rupture. Bacterial cells were disintegrated twice by using the French Press at 1500 psi pressure (Thermo Electron). The disintegrated cell suspension was centrifuged at 12000*g* for 20 min at 4 °C in order to sediment cell debris. The supernatant containing the soluble proteins was filtered aseptically using sterile filters of 0.2 μm pore size (Sarstedt, Germany).

To determine whether uninduced *L. plantarum* cells possessed enzymes able to metabolize phenolic acids, the cell-free extract was incubated in the 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 °C in the 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).