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# Production of vinyl derivatives from alkaline hydrolysates of corn cobs by recombinant *Escherichia coli* containing the phenolic acid decarboxylase from *Lactobacillus plantarum* CECT 748$^T$

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ABSTRACT

The enzyme PAD from *Lactobacillus plantarum* CECT 748$^T$ decarboxylates some cinnamic acids namely *p*-coumaric acid (*p*-CA), caffeic acid (CA), and ferulic acid (FA) into their corresponding 4-vinyl derivatives (4-VD): 4-vinyl phenol (4-VP), 4-vinyl catechol (4-VC), and 4-vinyl guaiacol (4-VG), respectively, which are valuable food additives mainly employed as flavouring agents. The gene encoding this enzyme was cloned and overexpressed in *Escherichia coli*. Recombinant *E. coli* cells overproducing *L. plantarum* PAD showed a preference to degrade mainly *p*-CA and CA. Sterilized liquors obtained after alkaline hydrolysis of corn cob or alkaline hydrolysis of the solid residue coming from acid hydrolysis of corn cob were employed as growth media in fermentations performed in shaker or bioreactor. The fermentative process allowed converting 2222.8 mg/L *p*-CA into 993.9 mg/L 4-VP. The process described here allowed the production with a high-yield of a valuable food additive from a by-product of the food industry.



## 1. Introduction

Corn cobs are an important by-product of corn industry as its annual generation is estimated to be approximately of 700 MM tons/year (Rivas et al., 2006), however large amounts remains unused as lignocellulosic waste or used as animal feed (Torre et al., 2008), in spite of ferulic acid (4-hydroxy-3-methoxycinnamic acid; FA) and *p*-coumaric acid (4-hydroxycinnamic acid; *p*-CA) are the major phenolic compounds identified in the cross-linking of primary and secondary cell walls of graminaceous plants, and in particular of cereals (Pan et al., 1998), representing together up to 1.5% by weight of cereal cell walls (Gasson et al., 1998). FA and *p*-CA are found covalently linked to polysaccharides by ester bonds and to components of lignin by ester or ether bonds (Scalbert et al., 1985). A considerable proportion of *p*-CA is known to be esterified with lignin, while ferulic acid is extensively etherified with lignin (Sun et al., 1998). The differences in stability of the ester and ether bonds allow a separation of ester and ether linked FA and *p*-CA (Xu et al., 2005). Once released, phenolic acids become substrates of phenolic acid decarboxylase (PAD) enzymes, which catalyse the formation of the corresponding 4-vinyl derivatives (Cavin et al., 1993), thus, nowadays, corn cobs has gained importance as raw material for obtaining added-value products (Rivas et al., 2003).

These 4-vinyl derivatives (4-VD) produced by PAD enzymes have important consequences in the aroma of wine and other fermented foods and beverages and are therefore approved as food additives (Rodríguez et al., 2007). Consequently, these vinyl derivatives are of importance in the flavouring and polymer industries (Matte et al., 2010). The activity of PAD can be considered to be a biological response of lactic acid bacteria to the chemical stress induced by phenolic acids at low pH, mainly *p*-coumaric, ferulic and caffeic acids (Rodríguez et al., 2007). However, although it is clear the ability of *L. plantarum* to decarboxylate *p*-CA and CA into 4VP and 4VC respectively, some controversial results have been observed about the decarboxylation of FA into 4VG by *L. plantarum* strains (Cavin et al., 1993; Van Beek and Priest, 2000; Bloem et al., 2006; Rodríguez et al., 2008a). To solve this question, the *L. plantarum* PAD enzyme was biochemically characterised (Rodríguez et al., 2008b). Their kinetic parameters revealed that at high substrate concentration, *p*-CA and CA were much more efficiently decarboxylated than FA.

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