SUPPLEMENTARY MATERIAL

Presence in *Lactobacillus plantarum* of an esterase active on a broad-range of phenolic esters

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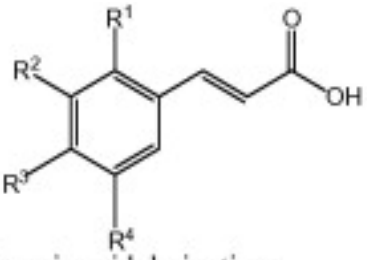
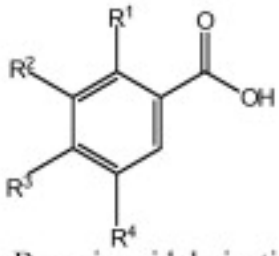




Substitution	Cinamic acid derivatives	Benzoic acid derivatives
R^1=OH	*o*-Coumaric acid	-
R^3=OH	*p*- Coumaric acid	*p*- Hydroxybenzoic acid
R^3=R^4=OH	Caffeic acid	Protocatechuic acid
R^2=OCH_3,R^3=OH	Ferulic acid	Vanillic acid
R^2=R^3=OCH_3	-	Veratric acid
R^2=R^3=R^4=OH	-	Gallic acid
R^1=R^4=OH	-	Gentisic acid
R^2=R^4=OCH_3, R^3=OH	Sinapic acid	Syringic acid

FIG S1. Chemical structures of some hydroxybenzoic and hydroxycinnamic acid derivatives

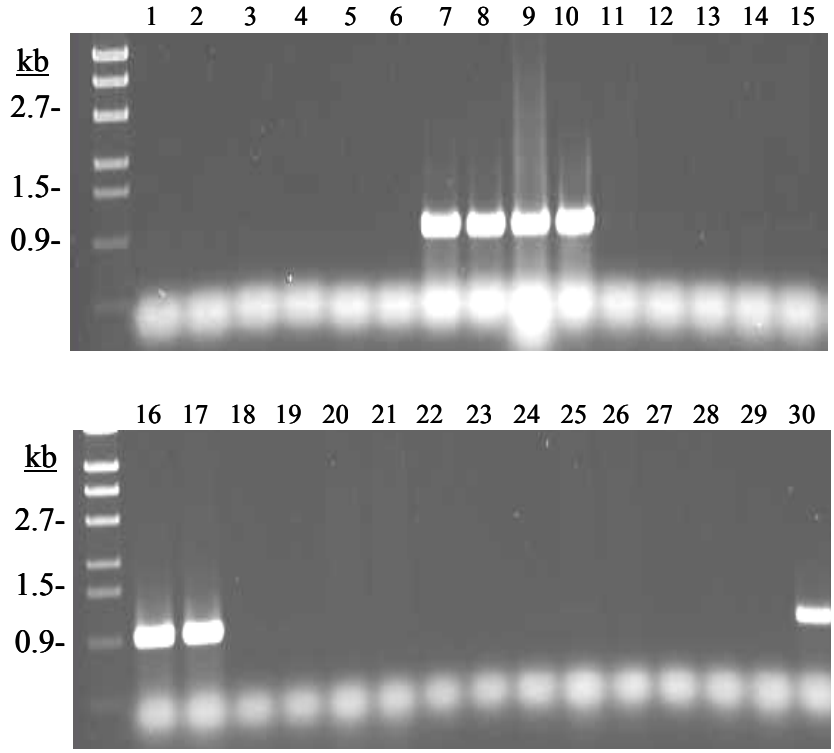
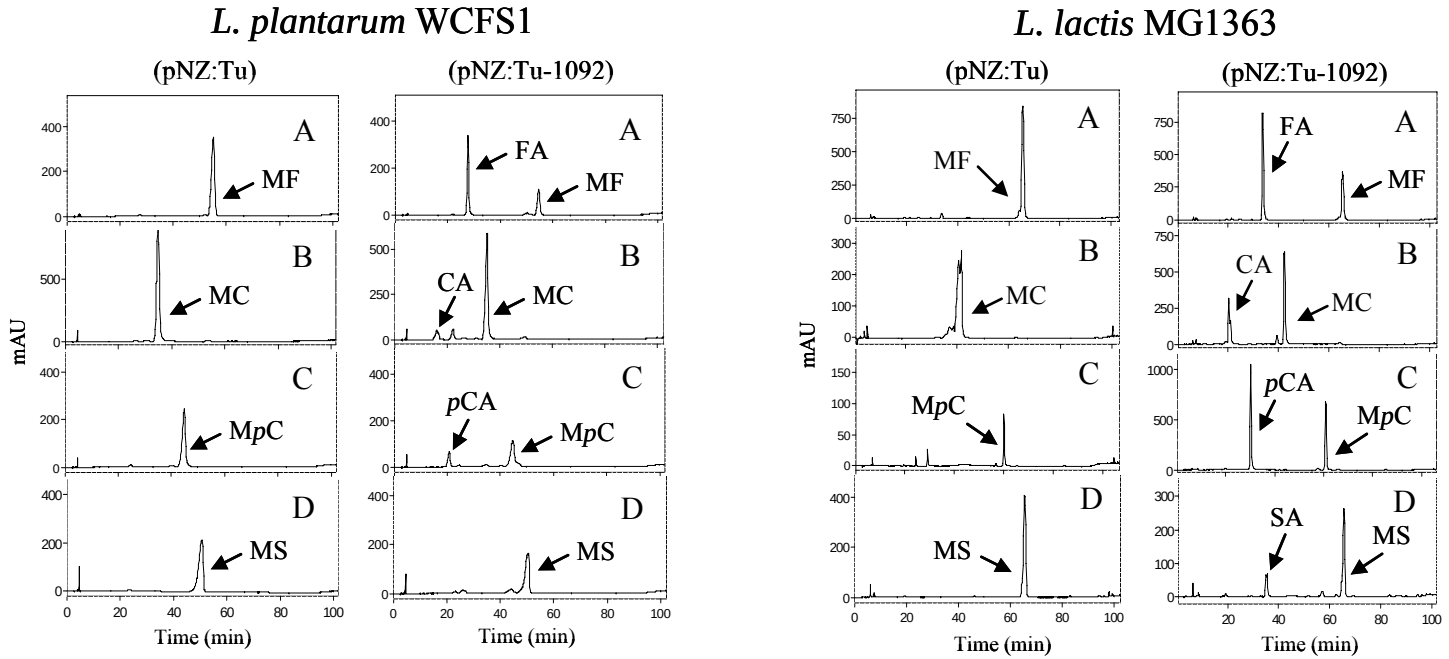


FIG S2. PCR amplification of Est_1092 encoding gene from several *L. plantarum* strains. Chromosomal DNA from the following *L. plantarum* strains was used for PCR amplification: NC8 (1), ATCC 14971^T (2), DSM 16365^T (3), DSM 12028 (4), WCFS1 (5), LPT 57/1 (6), CECT 220 (7), CECT 221 (8), CECT 223 (9), CECT 224 (10), CECT 749 (11), CECT 4185 (12), CECT 4645 (13), RM28 (14), RM31 (15), RM35 (16), DSM 1055 (17), DSM 2648 (18), DSM 10492 (19), DSM 12028 (20), DSM 13273 (21), DSM 20246 (22), RM34 (23), RM38 (24), RM39 (25), RM40 (26), RM41 (27), RM71 (28), RM72 (29), and RM73 (30). PCR fragments were subject to agarose gel electrophoresis and stained with Gel Red. Left lane, λ-EcoT14I digest (Takara). Numbers indicated some of the molecular sizes (in kb).



F1G S3. HPLC analysis of hydroxycinnamic ester degradation by *L. plantarum* WCFS1 or *L. lactis* MG1363 cultures harbouring pNZ:Tu-1092 plasmid. Culture media containing 1 mM methyl ferulate (A), methyl caffeate (B), methyl *p*-coumarate (C) or methyl sinapinate (D) was inoculated and incubated at 30 ºC for 7 days. A media inoculated with *L. plantarum* WCFS1 or *L. lactis* MG1363 harbouring pNZ:Tu control plasmid was incubated in the same conditions. Detection was performed at 280 nm. The methyl ferulate (MF), methyl caffeate (MC), methyl *p*-coumarate (M*p*C) or methyl sinapinate (MS), ferulic acid (FA), caffeic acid (CA), *p*-coumaric acid (*p*CA), and sinapic acid (SA) detected are indicated. The chromatograms were recorded at 280 nm. AU, arbitrary units.

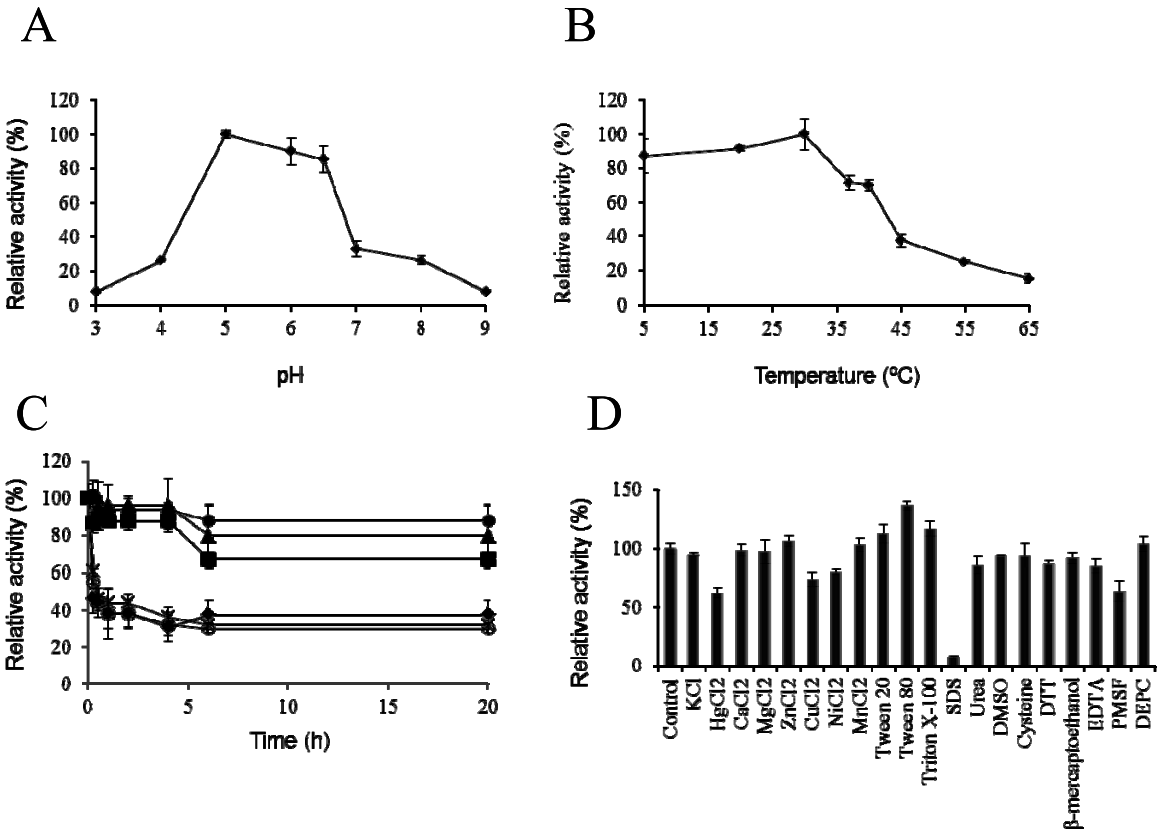


FIG S4. Some biochemical properties of esterase Est_1092 from *L. plantarum* DSM 1055. (A) Relative activity versus pH. (B) Relative activity of Est_1092 versus temperature. (C) Thermal stability of Est_1092 after preincubation at 20 ºC (filled circle), 30 ºC (filled triangle), 37 ºC (filled square), 45 ºC filled diamond), 55 ºC (cross), and 65 ºC (empty circle) in 50 mM McIlvaine buffer (pH 5.0). (D) Relative activity of Est_1092 after incubation with 1 mM concentrations of different additives. The activity of the enzyme in the absence of additives was defined as 100%. The experiments were done in triplicate. The mean value and the standard error are shown.

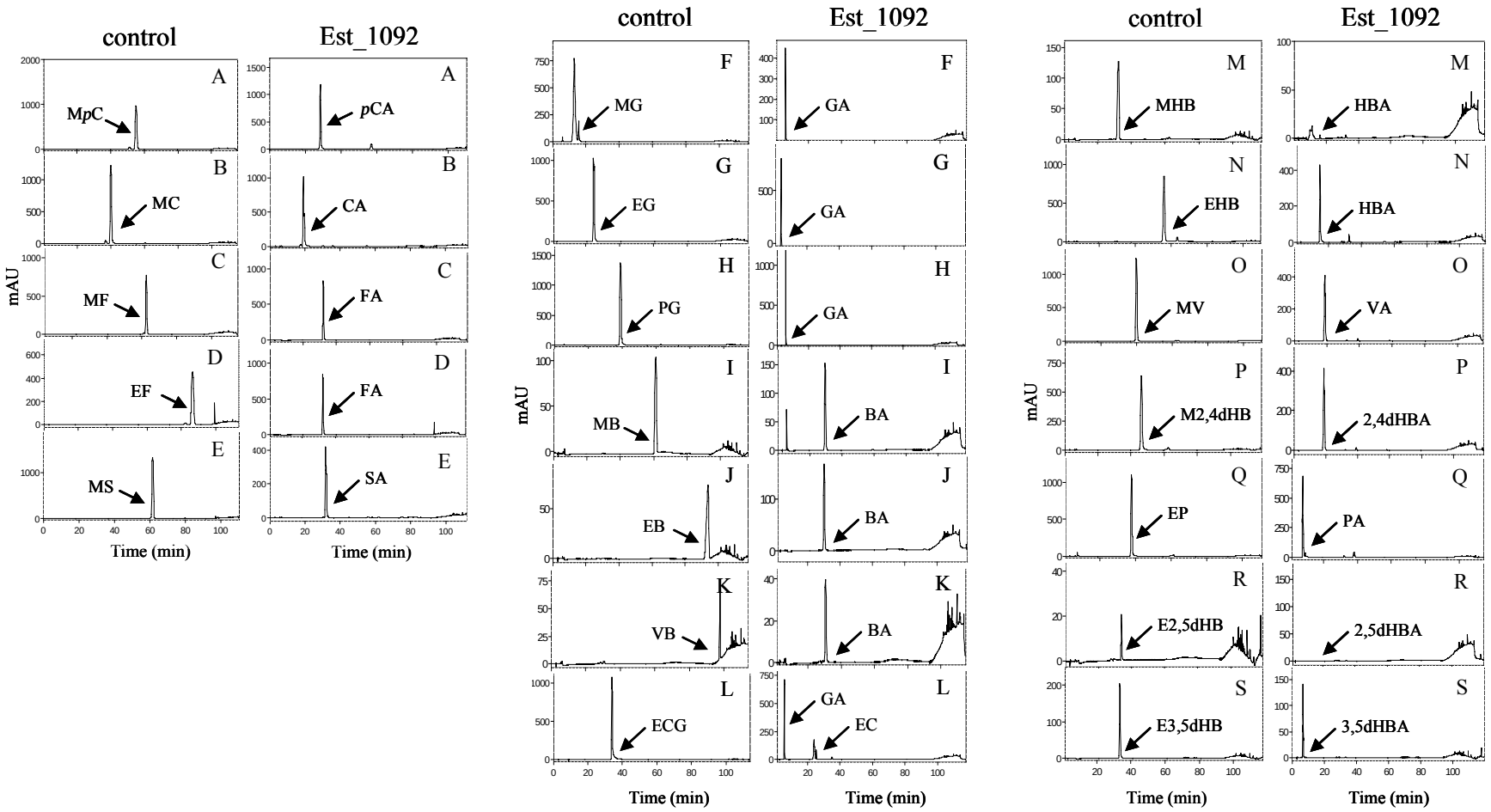


FIG S5. HPLC analysis of the esterase activity of Est_1092 protein from *L. plantarum* DSM 1055 against phenolic esters. Esterase activity of purified Est_1092 protein compared with control reactions on which the enzyme was omitted. HPLC chromatograms of Est_1092 incubated in 50 mM McIlvaine buffer pH 5.0 and 1 mM of hydroxycinnamic esters [methyl *p*-coumarate (M*p*C) (A), methyl caffeate (MC) (B), methyl

ferulate (MF) (C), ethyl ferulate (EF) (D), and methyl sinapinate (MS) (E)] or benzoic acids [methyl gallate (MG) (F), ethyl gallate (EG) (G), propyl gallate (PG) (H), methyl benzoate (MB) (I), ethyl benzoate (EB) (J), vinyl benzoate (VB) (K), methyl 4-hydroxybenzoate (M4HB)(M), ethyl 4-hydroxybenzoate (EHB) (N), methyl vanillate (MV) (O), methyl 2,4-dihydroxybenzoate (M2,4dHB) (P), ethyl protocatechuate (EP) (Q), methyl 2,5-dihydroxyenzoate (M2,5dHB) (R), and ethyl 3,5-dihydroxibenzoate (E3,5dHB) (S)] and epicatechin gallate (ECG) (L). The *p*-coumaric acid (*p*CA), caffeic acid (CA), ferulic acid (FA), salicylic acid (SA), gallic acid (GA), benzoic acid (BA), hydroxybenzoic acid (HBA), vanillic acid (VA), 2,4-dihydroxybenzoic acid (2,4dHBA), protocatechuic acid (PA), 2,5-dihydroxybenzoic acid (2,5dHBA), 3,5-dihydroxybenzoic acid (3,5dHBA), and epicatechin (EC) detected are indicated. The chromatograms were recorded at 280 nm.

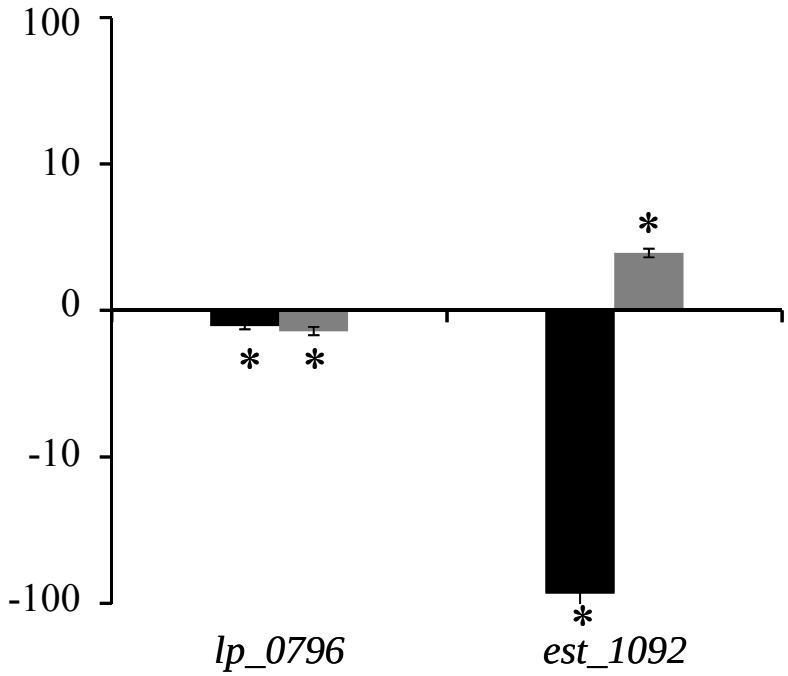


FIG S6. Comparison of the relative expression levels of *lp_0796* and *est_1092* from *L. plantarum* DSM 1055 in response to 30 mM methyl gallate or methyl ferulate exposure. Relative expression levels were calculated with the 7500 Fast System relative quantification software using *L. plantarum rRNA16S* gene as endogenous gene and the growth in the absence of methyl gallate or methyl ferulate as growth condition calibrator. Expression level under methyl gallate and methyl ferulate exposure is represented by black and grey bars, respectively.