

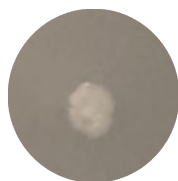
Supplementary material

Biotransformation of camu-camu galloylated ellagitannins by *Lactiplantibacillus plantarum* with extracellular tannase activity

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Strains showing Est_1092 activity
L. plantarum ATCC 8014



Strain lacking Est_1092 activity
L. plantarum WCFS1

Figure S1. Detection of Est_1092 activity in *L. plantarum* strains using the method developed by Donaghy et al. ¹. The enzymatic activity is evidenced by a surrounding clear halo.

Table S1. Identification of gallic acid derivatives, ellagic acid derivatives and ellagitannins in camu-camu extract and fermentation samples by UPLC-QToF

Class	Compound	Common name	Retention time (min)	[M-H] ⁻ , <i>m/z</i> detected	Mass accuracy (ppm)	MS2 fragment. <i>m/z</i> (relative intensity)	ID level _a
Gallic acid and derivatives	Pyrogallol		3.70	125.0235	-3.2	95,0138 (100); 79,0191 (54)	1
	Gallic acid		4.41	169.0141	-0.6	125,0243 (100); 79,0185 (17)	1
Ellagic acid derivatives	Valoneic acid dilactone		6.82	469.0048	-0.2	425.0137 (100); 299.9898 (59); 407.0026 (51)	2
	Ellagic acid hexoside		8.96	463.0514	-0.9	300.9982 (100); 299.9914 (83)	2
	Ellagic acid pentoside		9.93	433.0416	0.9	299.9909 (100)	2
	Ellagic acid desoxyhexoside		10.18	447.0574	1.1	299.9908 (100)	2
	Ellagic acid		10.56	300.9994	1.3	229.0138 (100)	1
	Ellagic acid acetyl-rhamnoside – 1		11.67	489.0673	-0.3	299.9908 (100)	2
	Ellagic acid acetyl-rhamnoside – 2		11.81	489.0670	-0.9	299.9908 (100)	2
	Ellagic acid glycoside – 1		12.76	719.2175	-1.7	300.9996 (100); 275.0196 (5)	2
	Ellagic acid glycoside – 2		12.95	719.2200	1.8	300.9994 (100); 275.0189 (4)	2
	Ellagic acid glycoside – 3		13.33	719.2167	-2.8	300.9991 (100); 275.0186 (4)	2
	Ellagic acid glycoside – 4		13.48	719.2174	-1.8	300.9992 (100); 275.0200 (4)	2
Ellagitannins	Vescalagin		5.81	933.0636	-0.4	915.0575 (100); 300.9990 (43); 493.0054 (38); 467.0251 (35); 569.0616 (24); 249.0398 (23); 871.0693 (22); 613.0490 (21); 275.0196 (19); 889.0867 (4); 631.0604 (3)	1
	Castalagin		6.56	933.0641	0.1	631.0570 (100); 300.9989 (42); 425.0146 (39); 569.0553 (20); 467.0238 (13); 249.0398 (12); 275.0198 (12); 915.0534 (8); 889.0748 (8); 871.0630 (6)	2
	Di-HHDP-glucose – 1	Peduncalagin	6.16	783.0686	0.0	300.9987 (100); 275.0169 (39); 481.0637 (6); 249.0381 (3)	2
	Di-HHDP-glucose – 2	Peduncalagin	7.15	783.0674	-1.5	300.9986 (100); 275.0190 (50); 249.0394 (8); 481.0625 (6)	2
	HHDP-galloyl-glucose		7.93	633.0728	-0.8	300.9983 (100); 275.0189 (12); 463.0528 (4); 249.0392 (4)	2
	Di-HHDP-galloyl-glucose – 1	Casuarictin / Potentillin	7.88	935.0814	1.9	917.0685 (100); 249.0390 (86); 275.0184 (66); 301.0000 (24); 573.0515 (24); 633.0737 (8)	2
	Di-HHDP-galloyl-glucose – 2	Casuarictin / Potentillin	8.15	935.0800	0.4	275.0193 (100); 299.0192 (70); 633.0735 (69); 300.9987 (47); 249.0400 (26); 783.0661 (7)	2
	Di-HHDP-galloyl-glucose – 3	Casuarictin / Potentillin	9.46	935.0802	0.6	300.9989 (100); 633.0731 (18); 275.0191 (17); 249.0396 (4); 783.0650 (2)	2
	Di-galloyl-HHDP-glucose	Tellimagrandin I	8.46	785.0851	1.0	300.9981 (100); 275.0184 (38); 249.0384 (25); 169.0141 (13); 483.0787 (7); 633.0682 (4)	2

Tri-galloyl-HHDP-glucose	Tellimagrandin II	9.88	937.0960	0.7	300.9987 (100); 275.0196 (17); 169.0134 (12); 465.0677 (11); 767.0751 (11); 249.0390 (10); 741.1017 (3)	2
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^a Identification levels were established according to Sumner et al ². Level 1 identifications were validated by comparing RT and MS2 fragmentation spectra with an authentic standard, while level 2 identifications were proposed according to exact mass and MS2 fragmentation spectra compared to Fracassetti et al ³.

Table S2. *L. plantarum* isolates screened for *est_1092* gene and feruloyl esterase activity

Culture Collection	Isolate	Isolation source	Country of origin	<i>est_1092</i> gene	Feruloyl esterase activity
Probi AB	6A	Human rectum	Sweden	-	-
	12C	Human, oral	Sweden	-	-
	12D	Human, oral	Sweden	-	-
	32A	Human, oral	Sweden	-	-
	35C	Human, oral	Sweden	-	-
	36D	Human, oral	Sweden	-	-
	37C	Human, oral	Sweden	-	-
	39-26	Fermented sorghum	Sweden	-	-
	39-27	Sorghum	Sweden	-	-
	42A	Human rectum	Sweden	-	-
	49-26	Fermented sorghum	Sweden	-	-
	49-27	Fermented sorghum	Sweden	-	-
	56-12	Fermented sorghum	Sweden	+	+
	56-24	Fermented sorghum	Sweden	+	+
	59-12	Fermented sorghum	Sweden	+	+
	62C	Human, oral	Sweden	-	-
	67B	Human rectum	Sweden	-	-
	74-27	Fermented sorghum	Sweden	-	-
	74-29	Fermented sorghum	Sweden	-	-
	78B	Human, oral	Sweden	-	-
	81D	Human, oral	Sweden	-	-
	86C	Human, oral	Sweden	-	-
	88C	Human, oral	Sweden	-	-
	90-29	Fermented sorghum	Sweden	-	-
	90-26	Fermented sorghum	Sweden	-	-
	962	Unknown	Sweden	-	-
	E12	Human feces	United Kingdom	-	-
	I123	Human feces	Italy	-	-
	I154	Human feces	Italy	-	-
	I177	Human feces	Italy	-	-
	S 71	Human feces	Sweden	-	-
	S114	Human feces	Sweden	-	-
S126	Human feces	Sweden	-	-	
S144	Human feces	Sweden	-	-	
S177	Human feces	Sweden	-	-	
S204	Human feces	Sweden	-	-	
S265	Human feces	Sweden	-	-	
S268	Human feces	Sweden	-	-	
Roy, Kennang, and Gagnon	RKG 1-473	Corn silage	Canada	-	-
	RKG 1-500	Bovine raw milk	Canada	-	-
	RKG 2-219	Corn silage	Canada	-	-
	RKG 2-690	Grass silage	Canada	-	-

**Table S3. Pairwise genome comparison of the novel Est_1092
L. plantarum producing strains**

<i>L. plantarum</i> strains				
	ATCC 8014	PROBI 56-12	PROBI 56-24	PROBI 59-12
	ANI and (aligned nucleotides) (%)			
ATCC 8014	*	99.11 (91.44)	99.12 (91.40)	99.08 (91.50)
PROBI 56-12	99.11 (88.50)	*	99.97 (98.38)	99.72 (94.06)
PROBI 56-24	99.11 (89.22)	99.97 (99.15)	*	99.78 (94.46)
PROBI 59-12	99.08 (90.80)	99.72 (96.61)	99.78 (96.31)	*

Alignment performed with Mummer at
<https://jspecies.ribohost.com/jspeciesws/>

**Table S4. Multiple sequence alignment of the genomic features encoding selected PAZymes in the Est_1092+
L. plantarum strains, performed in BV-BRC**

Genomic feature	<i>L. plantarum</i> strain	Length (NT)	Length (AA)	ALN Length	Identity	Query cover	Subject cover	Hit from	Hit to	Score	E value
Est_1092	PROBI 56-12	888	295	295	100	100	100	1	295	619	0
	PROBI 56-24	888	295	295	100	100	100	1	295	619	0
	PROBI 59-12	888	295	295	100	100	100	1	295	619	0
LpdB	PROBI 56-12	609	202	202	100	100	93	16	202	386	2.00E-137
	PROBI 56-24	609	202	202	100	100	93	16	202	386	2.00E-137
	PROBI 59-12	609	202	202	100	100	93	16	202	386	2.00E-137
LpdC	PROBI 56-12	1473	490	490	100	100	100	1	490	1008	0
	PROBI 56-24	1473	490	490	100	100	100	1	490	1008	0
	PROBI 59-12	1473	490	490	100	100	100	1	490	1008	0

Table S5. Multiple sequence alignment of the TanB genomic feature in the Est_1092 and TanA-producing *L. plantarum* strains, performed in BV-BRC

<i>L. plantarum</i> strain	Length (NT)	Length (AA)	ALN Length	Identity	Query cover	Subject cover	Hit from	Hit to	Score	E value
PROBI S126	1410	469	469	100	100	100	1	469	963	0
PROBI S204	1410	469	469	99	100	100	1	469	960	0
PROBI 5612	1410	469	469	100	100	100	1	469	961	0
PROBI 5624	1410	469	469	100	100	100	1	469	961	0
PROBI 5912	1410	469	469	100	100	100	1	469	961	0
RKG 1-473	1410	469	469	99	100	100	1	469	960	0
RKG 1-500	1410	469	469	99	100	100	1	469	960	0
RKG 2-219	1410	469	469	99	100	100	1	469	960	0
RKG 2-690	1410	469	469	99	100	100	1	469	957	0

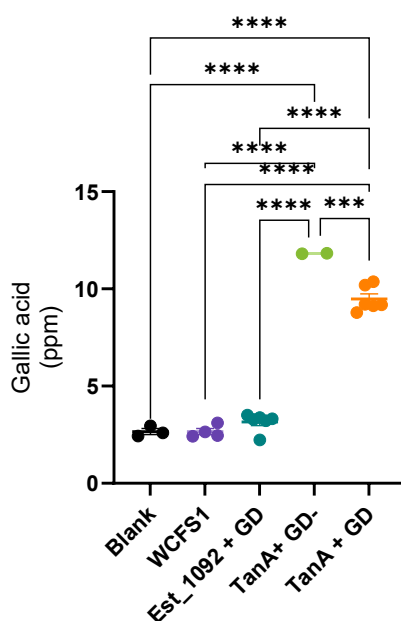


Fig. S2 Gallic acid at the end of the fermentation of *L. plantarum* strains with different enzymatic capacities. Each point represents the mean value of three independent experiments with one *L. plantarum* strain. Lines show mean values, and error bars indicate the standard error. Statistical significance was determined by a One-Way ANOVA analysis with Tukey-Kramer multiple comparisons, where * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$). Blank, uninoculated medium; GD+, strains producing gallate decarboxylase; GD-, strains lacking gallate decarboxylase.

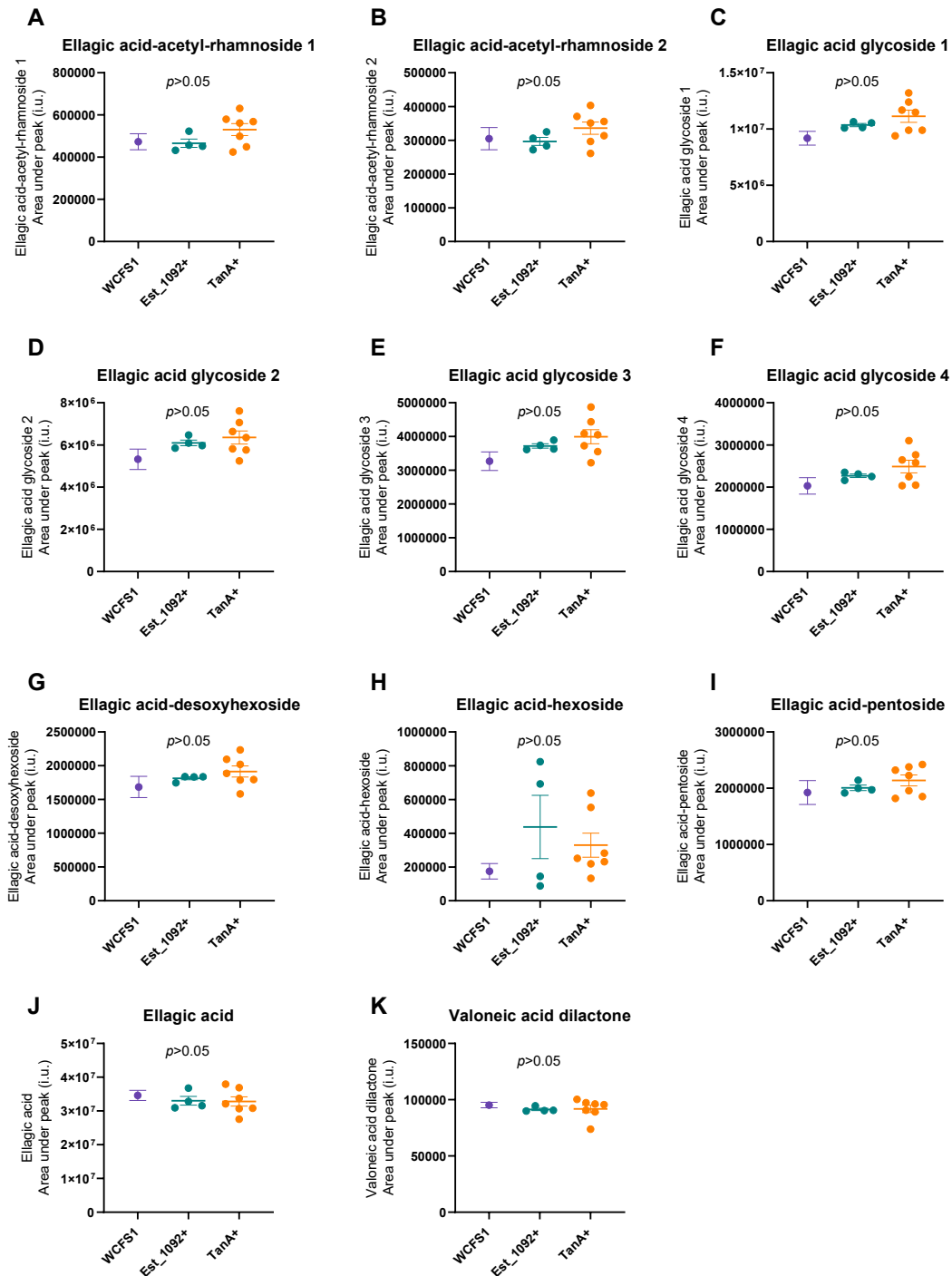


Fig. S3 Non-ester camu-camu phenolic compounds at the end of the fermentation of *L. plantarum* strains with different tannase capacities.

Each point represents the mean value of three independent experiments with one *L. plantarum* strain. Lines show mean values, and error bars indicate the standard error. In purple, WCFS1 (Est_1092 and TanA lacking reference strain); in blue, Est_1092+ strains (ATCC 8014, PROBI 56-12, PROBI 56-24, PROBI 59-12); in orange, TanA+ strains (ATCC 14917, PROBI S204, PROBI S126, RKG 1-473, RKG 1-500, RKG 2-219, and RKG 2-690). Statistical significance was determined by a One-Way ANOVA analysis with Tukey-Kramer multiple comparisons, where * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$). Statistical significance was determined by a One-Way ANOVA. If data were not distributed normally, the Kruskal–Wallis test by ranks was applied (ellagic acid desoxyhexoside, ellagic acid hexoside, valoneic acid dilactone).

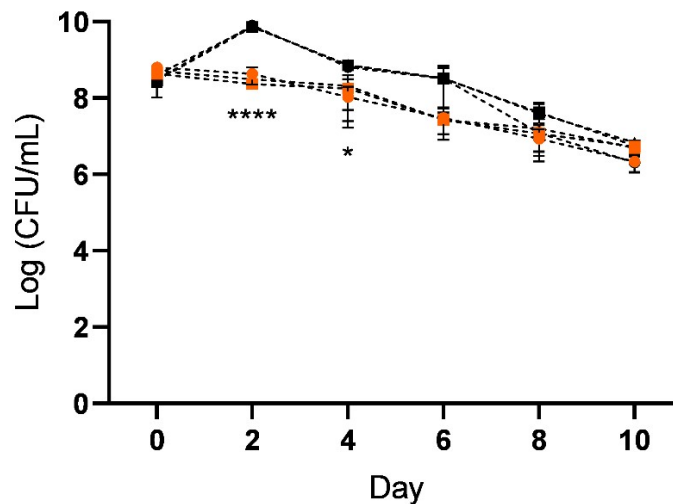


Fig. S4 Effects of the camu-camu extract on the growth of *L. plantarum* strains. Black symbols denote counts obtained during the growth of the strains in the control medium (without polyphenols), while orange symbols indicate the counts obtained during the growth of the strains in the camu-camu supplemented medium. *L. plantarum* WCFS1 ●, ○; Est_1092+ *L. plantarum* strains, ■, □; TanA+ *L. plantarum* strains, ▲, △. Values at each time point are the average of at least two replicates. The mean and the standard deviation are shown. A Two-Way ANOVA followed by Šidák correction was used to compare the growth of the strains in the camu-camu supplemented medium with their growth in the control medium at each fermentation time. * $p \leq 0.05$, **** $p \leq 0.0001$. CFU, colony-forming units.

References

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