

Title

Selection of *Lactiplantibacillus* strains for the production of fermented table olives

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Table S1. Reaction mixture and PCR-program for the amplification of partial 16S rRNA gene.

Reagents	Reaction mixture concentration (50 μ L)	Amplification program *
Deionized water	-	Initial denaturation: 95°C for 5 minutes
Reaction buffer (WonderTaq, Euroclone)	1x	
Primer forward 27F-ND	0.5 μ M	30 cycle of: denaturation (95°C, 45 sec.), annealing (54°C, 45 seconds) and extension (72°C, 45 seconds)
Primer reverse 1492R-ND-L	0.5 μ M	
Taq (WonderTaq, Euroclone)	0.025U/ μ L	
DNA	50 ng/ μ L	Final extension: 72°C for 7 minutes

* PCR was carried out in a T100™ Thermal Cycler Bio-Rad (Bio-Rad Laboratories Srl, Segrate, Milan, Italy); PCR products (1535 bp) were separated (90 min at 100 V) on 1.5% w/v agarose gel, stained with 0.05 μ L/mL of GelRed™ (10,000x in water; Botium Inc., Fremont, California) and visualized using GelDoc XR system (Bio-Rad Laboratories).

Table S2. List of genes involved in phenolic compound metabolism, used to verify the occurrence in publicly available genomes of *Lactiplantibacillus paraplantarum*, *Lpb. plantarum* and *Lpb. pentosus*.

Gene annotation	COG categories*	Strain	Accession n.	Locus tag (symbol)	Size (bp/aa)	References
β -glucosidase/6-phospho- β -glucosidase	G (COG 2723)	<i>Lpb. plantarum</i> WCFS1	CCC78348	lp_0906 (pbg2)	1503 bp/500 aa	[1]
aromatic acid carboxylase, subunit B	H (COG 0163)	<i>Lpb. plantarum</i> WCFS1	CCC77798	lp_0271 (<i>lpdB</i>)	564 bp/187 aa	[1, 2]
3-octaprenyl-4-hydroxybenzoate carboxy-lyase, UbiD family	H (COG 0043)	<i>Lpb. plantarum</i> WCFS1	CCC80016	lp_2945 (<i>lpdC</i>)	1473 bp/490 aa	[1-3]
aromatic acid carboxylase, subunit D	-	<i>Lpb. plantarum</i> WCFS1	CCC77799	lp_0272 (<i>lpdD</i>)	411 bp/136 aa	[1, 2]
phenolic acid decarboxylase	Q (COG 3479)	<i>Lpb. plantarum</i> WCFS1	CCC80619	lp_3665 (<i>padA</i>)	537 bp/178 aa	[1, 4]
transcriptional regulator of phenolic acid metabolism, PadR family	K (COG 1695)	<i>Lpb. plantarum</i> WCFS1	CCC80618	lp_3664 (<i>padR</i>)	546 bp/181 aa	[1]
tannase (tannin acyl hydrolase)	I (COG 0657)	<i>Lpb. plantarum</i> WCFS1	CCC80022	lp_2956 (<i>tanLp1</i> , <i>tanB_{Lp}</i>)	1410 bp/469 aa	[1-3, 5, 6]
hypothetical protein (with tannase-like activity)	-	<i>Lpb. plantarum</i> ATCC 14917	KRL35904	HMPREF0531_11477 (<i>tanA_{Lp}</i>)	1881 bp/626 aa	[1, 6-8]
carboxylesterase	Q (COG 1647)	<i>Lpb. plantarum</i> WCFS1	CCC78257	lp_0796	750 bp/249 aa	[5]
esterase	I (COG 0657)	<i>Lpb. plantarum</i> WCFS1	CCC80020	lp_2953	750 bp/249 aa	[2]
alpha/beta hydrolase	I (COG 0657)	<i>Lpb. plantarum</i> JDM1	WP_015825406	JDM1_1092	885 bp/295 aa	[6, 9]

* G: carbohydrate transport and metabolism; H: coenzyme transport and metabolism; Q: secondary metabolites biosynthesis, transport and catabolism; K: transcription; I: Lipid transport and metabolism. In bracket COG family.

Table S3. Exopolysaccharides (EPS) production and antimicrobial activity of strains.

EPS production				Antimicrobial activity	
Strains	G-MRS ^a	M-MRS ^a	S-MRS ^a	<i>Y. lipolytica</i> ^b	halo ^c
B15 (Lpl)	+	+	+	CNRZ1890 (Lpl)	23.0
38AA (Lpl)	+	+	+	WCFS1 (Lpl)	18.1
B7N23 (Lpl)	+	+	+	MT2D6S (Lpl)	18.1
MTD12L (Lpl)	+	+	+	MT2D7S (Lpl)	17.9
MTNTA3S (Lpl)	+	+	+	MTC13L (Lpl)	17.2
MT2D6S (Lpl)	+	+	+	1513 (Lpl)	13.1
MT2D7S (Lpl)	+	+	+	872 (Lpl)	13.4
MTC13L (Lpl)	+	+	+	4TP (Lpe)	19.0
MT2S (Lpl)	+	+	+	954 (Lpl)	20.3
MTF13S (Lpl)	+	+	+	4TG (Lpe)	15.0
MTF1L (Lpl)	+	+	+	O4 (Lpl)	8.6
MTF28L (Lpl)	+	+	+	O19 (Lpe)	12.1
MTF9L (Lpl)	+	+	+	O20 (Lpe)	14.5
2TP (Lpe)	+	+	+	OM53 (Lpe)	11.6
5TP (Lpe)	+	+	+	OM52 (Lpe)	10.5
O4 (Lpl)	+	+	+		
O18 (Lpe)	-	+	-		
O19 (Lple)	+	+	+		
OM50 (Lpe)	-	-	+		
OM53 (Lpe)	-	-	+		

^a G-MRS, M-MRS, S-MRS: MRS with 20 g/L maltose (M-MRS), or 20 g/L glucose (G-MRS), or 50 g/L sucrose (S-MRS) as carbon source (+, EPS-positive strains; -, EPS-negative strains); ^b *Y. lipolytica*: inhibitory activity of strains against *Yarrowia lipolytica* YL-12 (Deferred Antagonism Assay [10]); ^c halo: the size of inhibition zone (mm) was measured with a caliper. In brackets: Lpl, *Lpb. plantarum*; Lpe, *Lpb. pentosus*.

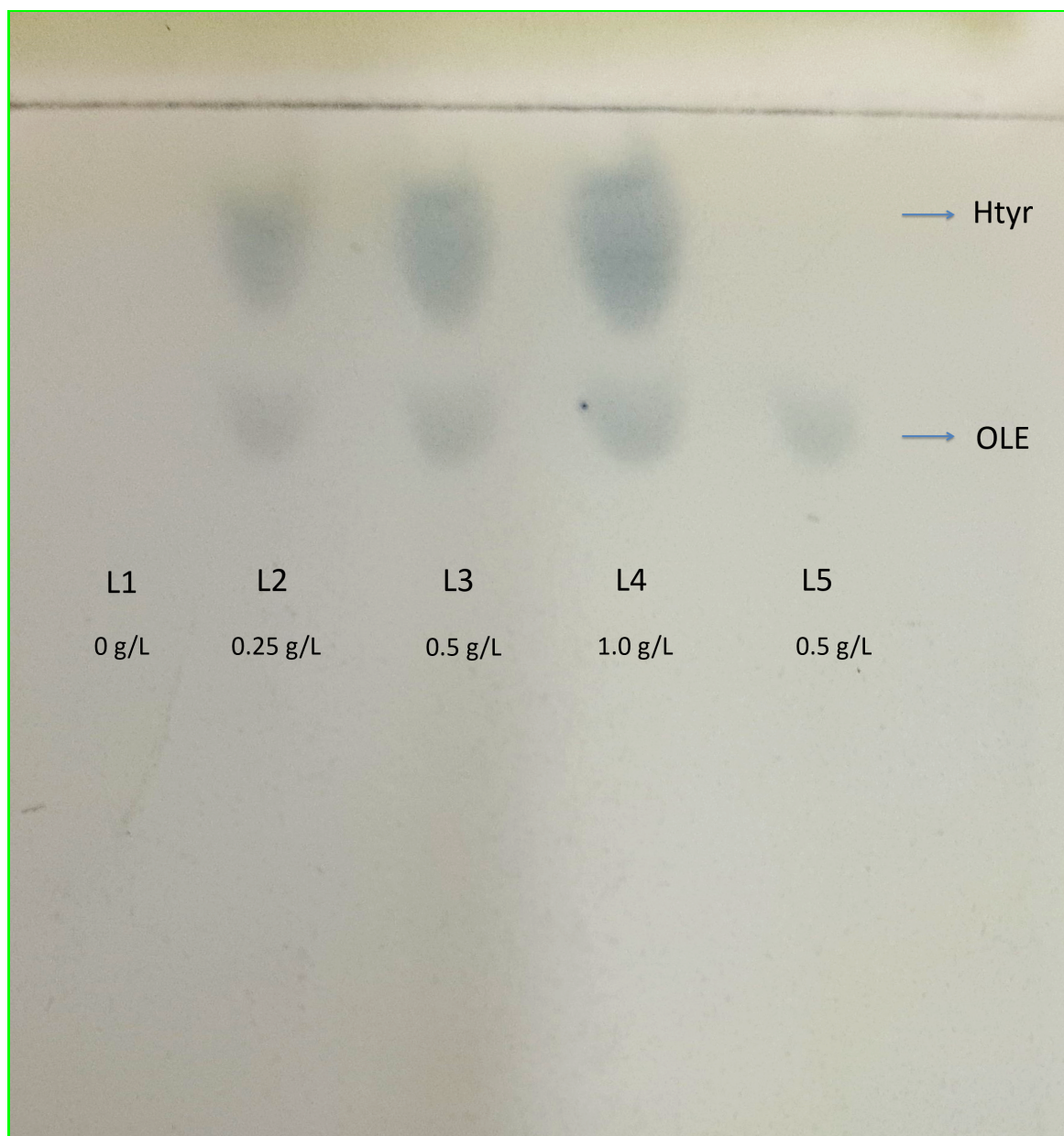


Figure S1. Thin Layer Chromatography (TLC) of MRS samples ([11]; *section 2.3*) unsupplemented (lane 1, L1) and supplemented with 0.25 g/L of oleuropein (OLE) and hydroxytyrosol (Htyr; L2), or with 0.5 g/L of OLE and Htyr (L3) or with 1 g/L of OLE and Htyr (L4) or only with 0.5 g/L of OLE (L5).

Table S4. Qualitative evaluation of oleuropein degradation and hydroxytyrosol formation using Thin Layer Chromatography (TLC).

Strains	Oleuropein (OLE) TLC spot		Hydroxytyrosol (Htyr) TLC spot	
	presence	absence	presence	absence
<u>Lpb. paraplantarum</u> B7N26 <u>Lpb. plantarum</u> WCFS1, ISLCPT68, PA20S, PE2S, B7N23, MT2A11S, B161, O1, O13; <i>Lpb. plantarum</i> subsp. <i>plantarum</i> B15, C17, 1069, DKO22, S85, UBS3, P1.5, MTNTA3S, DCU101, 38AA, FSM170, S12, MT2D6S, 954, UT2.1, US3.1, S2A19LPa; <i>Lpb. plantarum</i> subsp. <i>argenteratensis</i> CNRZ1890, DK36, FSL170, MTC13L <u>Lpb. pentosus</u> 2TP, 4TP, 4TG, 5TP, P13.3, OM24, OM53, OM52, OM62, OM50, OM13, OM14, OM35, O12, O17, O18, O19, O20, O24	OLE degradation ability		Htyr formation ability	
<u>Lpb. paraplantarum</u> MTG30L, MTG8L <u>Lpb. plantarum</u> MT2D3S, O4; <i>Lpb. plantarum</i> subsp. <i>plantarum</i> NCIMB8826, MTD12L, MT2D20S, ISLCPT57, MT2D7S, MT2D25L, MT2S, MTF13S, MTF1L, MTF28L, MTF9L, LM3; <i>Lpb. plantarum</i> subsp. <i>argenteratensis</i> NCIMB12120 <u>Lpb. pentosus</u> LPL, O5, O11, O15	OLE degradation ability			Htyr formation inability
<u>Lpb. paraplantarum</u> F10 <u>Lpb. plantarum</u> subsp. <i>plantarum</i> 1505, 1513, 872, 1089, NCFB340		OLE degradation inability		Htyr formation inability

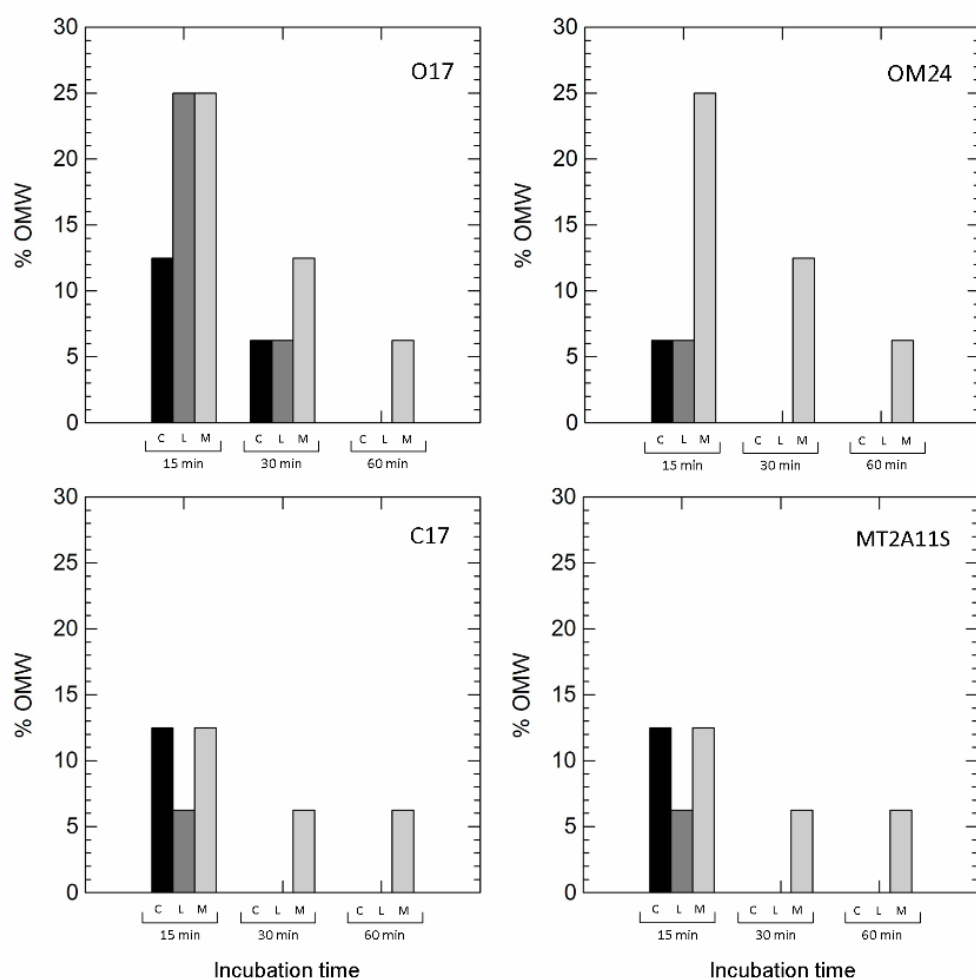


Figure S2. Survival of *Lpb. pentosus* O17, OM24 and *Lpb. plantarum* C17, MT2A11S to different concentrations (from 50% to 6.12% v/v) of *Leccino*, *Coratina* and *Cima di Melfi* OMWs after 15, 30, 60 and 120 minutes of incubation at 30°C.

Table S5. Occurrence analysis of genes involved in degradation and metabolism of phenolic compounds in *Lactiplantibacillus pentosus* genomes.

Strains	Sequencing status	Isolation source	<i>pbg</i> ^a	<i>padA</i> ^b	<i>padR</i> ^c	Carboxyl-esterase	<i>lpdB</i> ^d	<i>lpdC</i> ^e	<i>lpdD</i> ^f	Esterase	<i>tanA</i> ^g	<i>tanB</i> ^h
<i>Lpb. pentosus</i> 1.2.11, 1.2.13, 1.2.7, 1.8.18, 1.8.6, 14.2.16, 14.2.3, 14.8.42, 3.2.36, 3.2.37, 3.8.24, 3.8.45, 7.2.11, 7.2.15, 7.2.20, 7.2.23, 7.8.2, 7.8.46, LA0445, MU0445	Permanent draft	Cucumber fermentation										
<i>Lpb. pentosus</i> ZFM222	Finished	Fermented vegetables	1	1	1	1	1	1	0	1	1	1
<i>Lpb. pentosus</i> SLC13	Finished	Mustard pickles										
<i>Lpb. pentosus</i> BGM48	Finished	Olive fermentation										
<i>Lpb. pentosus</i> 3.2.8	Permanent draft	Cucumber fermentation	1	1	1	1	1	1	1	1	1	1
<i>Lpb. pentosus</i> 1.8.9	Permanent Draft	Cucumber fermentation	1	0	1	1	1	1	0	1	1	1
<i>Lpb. pentosus</i> DSM 20314	Finished	Corn silage	1	1	1	1	1	1	0	1	1	0
<i>Lpb. pentosus</i> FL0421	Permanent draft	Temperate deciduous forest biome soil	1	1	1	1	1	1	0	1	0	0
<i>Lpb. pentosus</i> KCA1	Permanent draft	Vagina of a healthy Nigerian woman	1	1	1	1	1	1	0	1	0	1
<i>Lpb. pentosus</i> O17	Draft (this study)	Brine from treated table olives (<i>Cerignola</i> cv.)	1	1	1	0	0	1	0	1	1	1

^a *pbg*: β -glucosidase; ^b *padA*: p-coumaric acid decarboxylase; ^c *padR*: Transcriptional regulator PadR; ^d *lpdB*: Gallate decarboxylase subunit B; ^e *lpdC*: Gallate decarboxylase subunit C;

^f *lpdD*: Gallate decarboxylase subunit D; ^g *tanA*: Tannase subunit A; ^h *tanB*: Tannase subunit B. 1: gene presence; 0: gene absence.

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