

Figure S1. Depiction of the inhibition zone achieved by each tested culture-free supernatant, including the well diameter: < 9.2 mm (white), 9.2 mm ≤ inhibition zone < 15.0 mm (green), 15.0 mm ≤ inhibition zone < 25.0 mm (red), ≥ 25.0 mm (blue).

Tested bacterial species: K.m. = *Kluyveromyces marxianus* CNTA.1649, P.f. = *Pseudomonas fluorescens* CNTA 571, L.p. = *Lentilactobacillus parabuchneri* DSMZ 5987, R.m. = *Rothia mucilaginosa* DSMZ 18184, P.a. = *Pseudomonas aeruginosa* CECT 110T, L.m. = *Listeria monocytogenes* CECT 7467, B.c.193 = *Bacillus cereus* CECT 193, B.c.503 = *Bacillus cereus* CNTA 503, S.T. = *Salmonella* Typhimurium CECT 443, S.a. = *Staphylococcus aureus* CECT 976, E.c. = *Escherichia coli* DSMZ 19206.

A dendrogram showing the taxonomic hierarchical relationship between the different tested strains is also shown. 1 = *Lactiplantibacillus plantarum*, 2 = *Lactiplantibacillus pentosus*, 3 = *Lactiplantibacillus paraplantarum*, 4 = *Pediococcus acidilactici*, 5 = *Pediococcus pentosaceus*, 6 = *Pediococcus parvulus*, 7 = *Lacticaseibacillus paracasei*, 8 = *Lacticaseibacillus casei*, 9 = *Lacticaseibacillus rhamnosus*, 10 = *Lacticaseibacillus manihotivorans*, 11 = *Lacticaseibacillus saniviri*, 12 = *Levilactobacillus brevis*, 13 = *Leuconostoc mesenteroides*, 14 = *Leuconostoc pseudomenteroides*, 15 = *Leuconostoc citreum*, 16 = *Leuconostoc suiucom*, 17 = *Limosilactobacillus fermentum*, 18 = *Limosilactobacillus reuteri*, 19 = *Lentilactobacillus parabuchneri*, 20 = *Lentilactobacillus buchneri*, 21 = *Latilactobacillus curvatus*, 22 = *Latilactobacillus sakei*, 23 = *Lactobacillus delbrueckii*, 24 = *Lactobacillus johnsonii*, 25 = *Lactobacillus acidophilus*, 26 = *Weissella confusa*, 27 = *Weissella cibaria*, 28 = *Loigolactobacillus coryniformis*, 29 = *Companilactobacillus alimentarius*, 30 = *Liquorilactobacillus nagelii*, 31 = *Oenococcus oeni*, 32 = *Ligilactobacillus salivarius*, 33 = *Furfurilactobacillus rossiae*, 34 = *Streptococcus thermophilus*, 35 = *Streptococcus salivarius*, 36 = *Lactococcus lactis*, 37 = *Lactococcus cremoris*, 38 = *Enterococcus faecalis*, 39 = *Enterococcus faecium*, 40 = *Enterococcus mundtii*, 41 = *Bacillus subtilis*, 42 = *Bifidobacterium animalis* subsp. *lactis*, 43 = *Bifidobacterium pseudocatenulatum*, 44 = *Bifidobacterium longum*.

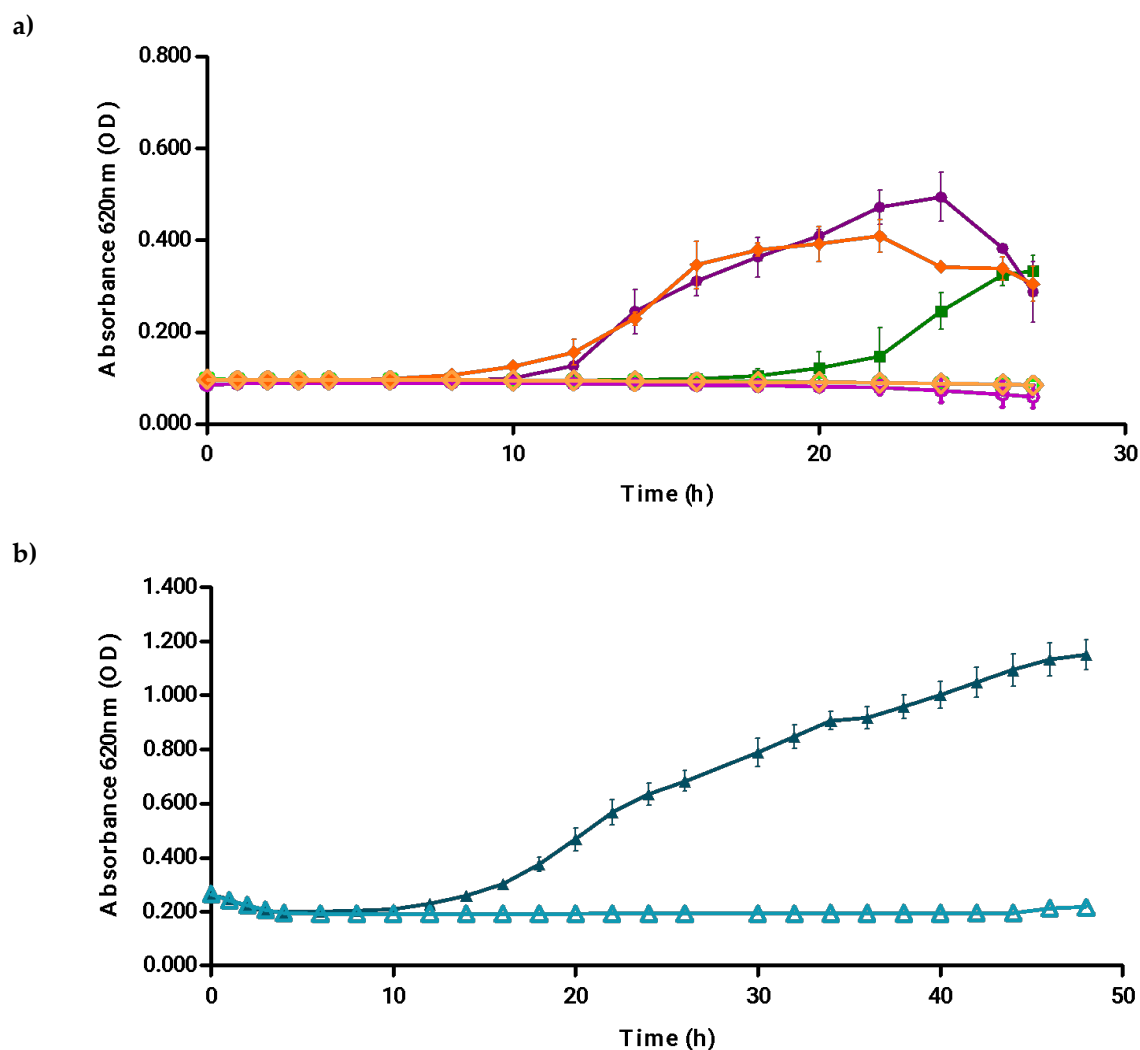


Figure S2. Growth over time of the selected indicator microorganisms *Listeria monocytogenes* (a) or *Lactilactobacillus parabuchneri* (b) measured by absorbance at 620 nm in the presence of cell-free supernatant (CFS) at the maximum non-inhibitory concentration and the minimum inhibitory concentration of each strain: (a) *Pediococcus acidilactici* CNTA 1059 at 1.25% (■) and 2.5% (□), *Lactiplantibacillus plantarum* at 2.5% (●) and 5% (○) and *Bacillus subtilis* at 0.625% (◆) and 1.25% (◇); (b) *Lactilactobacillus parabuchneri* CNTA 1374 at 0% (▲) and 0.15% (△) concentration. Data are mean \pm standard deviations (error bars) obtained from at least three independent experiments.

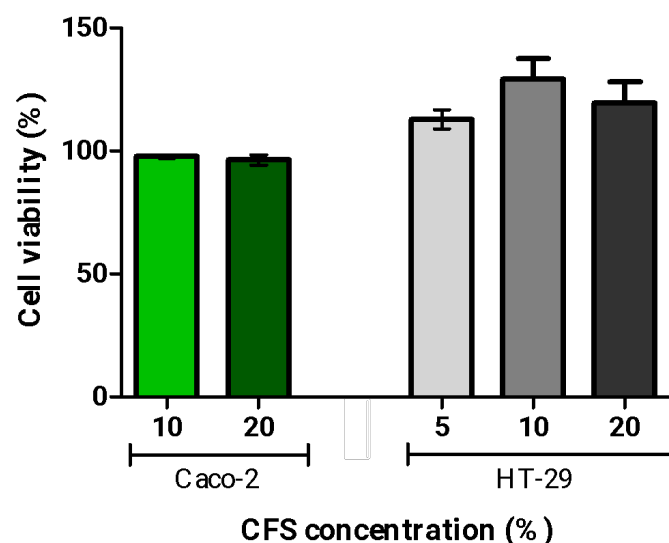


Figure S3. The cytotoxic effect of *Bacillus subtilis* CNTA 517 cell-free supernatant (CFS) on the cell viability of Caco-2 cell line (green colors) at different concentrations of CFS (10% and 20%) and on HT-29 cell line's (grey colors) viability at the CFS concentrations of 5%, 10% and 20%. Data are means \pm standard deviations (error bars) obtained from at least three independent experiments.

Table S1. Potential protective culture species from CNTA collection employed for the preliminary screening: number of strains of each species and origin of the isolation.

Potential protective culture species (number of strains)		
<i>Bacillus subtilis</i> (16) (a, b)	<i>Lacticaseibacillus</i> spp. (24) (a, c, d, e, f, g, h)	<i>Lentilactobacillus</i> spp. (10) (c, d, e, g)
<i>Bifidobacterium longum</i> (1) (c)	<i>Ligilactobacillus</i> spp. (4) (c)	<i>Streptococcus</i> spp. (5) (c, g)
<i>Bifidobacterium bifidum</i> (1) (d)	<i>Limosilactobacillus</i> spp. (14) (a, c, d, f, g, h)	<i>Lactococcus</i> spp. (4) (a, c, g)
<i>Bifidobacterium animalis</i> subsp. <i>Lactis</i> (7) (e)	<i>Loigolactobacillus</i> spp. (5) (a)	<i>Enterococcus</i> spp. (7) (a, g, i)
<i>Bifidobacterium pseudocatenolatum</i> (2) (e)	<i>Latilactobacillus</i> spp. (8) (a, h, i)	<i>Oenococcus</i> sp. (1) (f)
<i>Lactiplantibacillus</i> spp. (39) (a, c, d, f, g, h)	<i>Companilactobacillus</i> spp. (4) (h)	<i>Pediococcus</i> spp. (35) (a, d, e, f, h, i)
<i>Levilactobacillus</i> spp. (20) (a, c, d, f, g, h)	<i>Liquorilactobacillus</i> spp. (2) (a)	<i>Leuconostoc</i> spp. (19) (a, c, d, f, h, i)
<i>Lactobacillus</i> spp. (7) (a, c)	<i>Furfurilactobacillus</i> sp. (1) (c)	<i>Weisella</i> spp. (12) (d, f)

Origin of the isolation: a) Non-fermented vegetable product/subproduct, b) wasted paint, c) type or private collection, d) cryovial contamination, e) human feces, f) fermented vegetable product, g) dairy products, h) chorizo, i) cereals

Table S2. Potential protective culture microorganisms' growth conditions.

Potential protective culture	Growth conditions			
	Medium	Temperature (° C)	Oxygen conditions	Time (h)
Lactic Acid Bacteria	MRSb*	30	Anaerobic	24
Lactic Acid Bacteria	MRSb*	37	Anaerobic	24
<i>Bifidobacterium</i> sp.	MRSb*	37	Anaerobic	24
<i>Bacillus subtilis</i>	TSB-YE**	37	Aerobic	24

*MRSb: Man, Rogosa and Sharpe Broth (Merck GmbH, Darmstadt, Germany)

**TSB-YE: Tryptic Soy Broth – Yeast Extract (Scharlau, Barcelona, Spain)

Table S3. Pathogenic and spoilage microorganisms chosen as indicator strains and growth conditions.

Pathogenic/ spoilage microorganisms	Growth conditions			
	Medium	Temperature (° C)	Oxygen conditions	Time (h)
<i>Listeria monocytogenes</i> CECT 7467	TSB-YE*	37	Aerobic	18
<i>Escherichia coli</i> O157:H7 DSMZ 19206	TSB-YE*	37	Aerobic	18
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium CECT 443	TSB-YE*	37	Aerobic	18
<i>Staphylococcus aureus</i> CECT 976	TSB-YE*	37	Aerobic	18
<i>Bacillus cereus</i> CECT 193	TSB-YE*	37	Aerobic	18
<i>Pseudomonas fluorescens</i> CNTA 571	PDA**	25	Aerobic	24
<i>Rhodotorula mucilaginosa</i> DSMZ 18184	PDA**	25	Aerobic	24
<i>Kluyveromyces marxianus</i> CNTA 1649	PDA**	25	Aerobic	24
<i>Lentilactobacillus parabuchneri</i> DSMZ 5987	MRSa***	37	Anaerobic	24

*TSB-YE: Tryptic Soy Broth – Yeast Extract (Scharlau, Barcelona, Spain) (Tunçer and Karaçam, 2020)

**PDA: Potato Dextrose Broth (Scharlau, Barcelona, Spain)

***MRSa: Man, Rogosa and Sharpe Agar (Merck GmbH, Darmstadt, Germany)

Table S4. Antimicrobial activity of the cell-free supernatants (CFS) against pathogenic and spoilage strains with a well diameter of 7.20 mm. A bold typescript indicates the halo size achieved in the selected indicator microorganism.

Indicator strain	Halo diameter (mm)			
	<i>Pediococcus acidilactici</i> CNTA 1059	<i>Lactiplantibacillus plantarum</i> CNTA 600	<i>Levilactobacillus brevis</i> CNTA 1374	<i>Bacillus subtilis</i> CNTA 517
<i>Listeria monocytogenes</i> CECT 7467	24.53 ± 0.21	22.35 ± 0.38	7.20 ± 0.00	28.52 ± 0.08
<i>Staphylococcus aureus</i> CECT 976	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00	19.36 ± 0.07
<i>Salmonella</i> Typhimurium CECT 443	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00
<i>Escherichia coli</i> DSMZ 19206	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00
<i>Bacillus cereus</i> CECT 193	7.20 ± 0.00	13.36 ± 0.67	7.20 ± 0.00	7.20 ± 0.00
<i>Pseudomonas fluorescens</i> CNTA 571	7.20 ± 0.00	10.78 ± 0.05	7.20 ± 0.00	7.20 ± 0.00
<i>Rhodotorula mucilaginosa</i> DSMZ 18184	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00	7.20 ± 0.00
<i>Lentilactobacillus parabuchneri</i> DSMZ 5987	7.20 ± 0.00	7.20 ± 0.00	32.20 ± 0.04	7.20 ± 0.00
<i>Kluyveromyces marxianus</i> CNTA 1649	7.20 ± 0.00	14.14 ± 0.12	7.20 ± 0.00	7.20 ± 0.00

Table S5. Antibiotic resistance genes identified in *Bacillus subtilis* CNTA 517 after screening of contigs after whole genome sequencing for antimicrobial resistance genes using the CARD database (results from the Resfinder database are not shown to avoid duplicities).

Gene	% Coverage	% Identity	Resistance
<i>Bacillus_subtilis_mprF</i>	100.00	97.90	Cationic peptides (eg defensins)
<i>vmlR</i>	100.00	96.53	Lincosamide, streptogramin, pleuromutilin, virginiamycin
<i>tet(L)</i>	99.93	80.04	Tetracycline
<i>blt</i>	100.00	99.00	Fluoroquinolone, acridine dye
<i>aadK</i>	99.77	98.83	Aminoglycoside
<i>bmr</i>	100.00	98.97	Fluoroquinolone, nucleoside, phenicol, acridine dye
<i>ykkD</i>	100.00	99.69	Aminoglycoside, phenicol, tetracycline
<i>ykkC</i>	100.00	99.70	Aminoglycoside, phenicol, tetracycline
<i>tmrB</i>	100.00	97.98	Tunicamycin (nucleoside)
<i>lmrB</i>	100.00	99.51	Lincosamide
<i>mphK</i>	100.00	99.13	Macrolide
<i>rphB</i>	97.78	80.33	Rifamycin

Table S6. Antimicrobial activity of the cell-free supernatants (CFS) after a heat treatment of 110 °C and 121 °C at different times, considering the 0.00 mm as 7.20 mm (no halo).

Temperature (°C)	CFS	Halo diameter (mean ± SD mm)				
		0 min	10 min	60 min	90 min	120 min
110	<i>P. acidilactici</i> CNTA 1059	16.73 ± 0.29	15.59 ± 0.33	12.95 ± 0.37	11.93 ± 0.93	9.26 ± 0.93
	<i>L. plantarum</i> CNTA 600	15.13 ± 0.38	12.25 ± 0.19	11.47 ± 0.32	8.08 ± 0.03	4.66 ± 0.61
	<i>L. brevis</i> CNTA 1374	25.00 ± 0.04	22.54 ± 1.48	20.11 ± 0.64	19.75 ± 0.63	17.70 ± 0.20
	<i>B. subtilis</i> CNTA 517	21.78 ± 0.08	17.03 ± 0.61	11.42 ± 1.09	3.75 ± 0.45	0.00 ± 0.00
121	<i>P. acidilactici</i> CNTA 1059	16.73 ± 0.29	14.06 ± 0.42	11.70 ± 0.05	7.99 ± 0.19	2.98 ± 0.73
	<i>L. plantarum</i> CNTA 600	15.13 ± 0.38	10.49 ± 0.60	6.89 ± 0.02	2.97 ± 0.39	0.00 ± 0.00
	<i>L. brevis</i> CNTA 1374	25.00 ± 0.04	22.38 ± 0.45	18.63 ± 0.32	15.53 ± 0.20	13.83 ± 0.47
	<i>B. subtilis</i> CNTA 517	21.78 ± 0.08	16.07 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table S7. Lost antimicrobial activity of the cell-free supernatants (CFS) after heat treatments at 110 °C and 121 °C.

CFS of	Temperature (°C)	Equation	R ²
<i>Pediococcus acidilactici</i> CNTA 1059	110	$0.3432x + 1.333$	0.98
	121	$0.6105x + 1.9034$	0.95
<i>Lactiplantibacillus plantarum</i> CNTA 600	110	$0.4975x + 3.9658$	0.91
	121	$0.7545x + 10.853$	0.96
<i>Levilactobacillus brevis</i> CNTA 1374	110	$0.2098x + 4.1638$	0.92
	121	$0.3579x + 3.6514$	0.98
<i>Bacillus subtilis</i> CNTA 517	110	$0.7987x + 5.7009$	0.98
	121	$1.6050x + 4.6284$	0.99