

Supplementary Material (Probiotic Characterization of Lactic Acid Bacteria Isolated from Traditional Pickles: A Functional and Safety Assessment)

Table S1. Primers used in the study*

Gene	Sequences (5'-3')	Annealing Temperature (°C)	Amplicon Size (bp*)	References
16S rRNA	8F: AGAGTTTGATCCTGGCTCAG 1541R: AAGGAGGTGATCCAGCCGCA	59.5	1500	[1]
<i>esp</i>	F: TTGCTAATGCTAGTCCACGACC R: GCGTCAACACTTGCAATTGCCGA	55	933	[2]
<i>efaAfs</i>	F: GACAGACCCTCACGAATA R: AGTTCATCATGCTGTAGTA	55	705	
<i>gelE</i>	F: ACCCCGTATCATTGGTTT R: ACGCATTGCTTTTCCATC	55	419	
<i>cylA</i>	F: TGGATGATAGTGATAGGAAGT R: TCTACAGTAAATCTTTTCGTCA	57	517	[3]
<i>hyl</i>	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	56	276	[4]
<i>asa</i>	F: GCACGCTATTACGAACTATGA R: AAGAAAGAACATCACCACGA	56	375	
<i>ace</i>	F: GAATTGAGCAAAAGTTCAATCG R: GTCTGTCTTTTCACTTGTTTC	55	1008	[5]
<i>agg</i>	F: AAGAAAAAGAAGTAGACCAAC R: AAACGGCAAGACAAGTAAATA	52	1553	[6]
<i>gad</i>	CoreF: CCTCGAGAAGCCGATCGCTTAGTTCG CoreR: TCATATTGACCGGTATAAGTGATGCCC	65	600	[7]
<i>gdh</i>	F: CCTTACAAGGGCGGCTTACG R: GCTGCCAATGCTGGTGGCGT	57	860	[8]
<i>L. brevis</i> specific gene	F: GCACAAGATGGCTCATGACGTTAAGACTAAGG R: GTCTAAGCTCGTATCAACCCACGGG	65	160	[9]
<i>L. plantarum</i> specific gene	F: CCGTTTATGCGGAACACCTA R: TCGGGATTACCAAACATCAC	56	318	[10]
<i>hdc</i>	F: GGNATNGTNWSNTAYGAYMGNGCNCA R: ATNGCDATNGCNSWCCANACNCCRTA	65	372	[11]
<i>tdc</i>	F: TGGYTNGTNCCNCARACNAARCAYTA R: ACRTARTCNACCATRTTRAARTCNNG	62	825	
<i>ldc</i>	F: CAYRTNCCNNGGNCAAYAA R: GGDATNCCNNGGNGGRTA	54.5	1185	
<i>odc</i>	F: GTNTTYAAYGCNGAYAARCANTAYTTYGT R: ATNGARTTNAGTTTCRCAYTTYTCNNG	61.5	1446	[12]
<i>epsA</i>	F: TAGTGACAACGGTTGTACTG R: GATCATTATGGACTGTCAC	52	800	[13]

<i>epsB</i>	F: CGTACGATTCGTACGACCAT R: TGACCAGTGACACTTGAAGC	57	1150
<i>gtf</i>	F: GAYAAYWSIAAYCCIRYIGTIC R: ADRTCICCRTARTAIAYIYKIG	54	660
<i>p-gtf</i>	F: TCATTTTATTCGTAAAACCTCAATTGAYGARYTNCC R: AATATTATTACGACCTSWNAYYTGCCA	60	189

* bp: base pair. *esp*: enterococcal surface protein, *efaAfs*: *Enterococcus faecalis* specific endocarditis antigen, *gelE*: gelatinase, *cylA*: cytolisin, *hyl*: hyaluronidase, *asa*: aggregation substance, *ace*: adhesion of collagen, *agg*: aggregation protein, *gad*: glutamic acid decarboxylase, *gdh*: glutamate dehydrogenase, *hdc*: histidine decarboxylase, *tdc*: tyrosine decarboxylase, *ldc*: lysine decarboxylase, *odc*: ornithine decarboxylase, *eps*: exopolysaccharide, *gtf*: glycosyltransferase.

Table S2. Composition, origin and fermentation status of pickle samples

Sample No	General Composition	City of Origin	Fermentation Status
1	Cabbage , pepper, tomato, unripe melon, Armenian cucumber, parsley	Erzurum	Fermented
2	Turnip, mint	Erzurum	Fermentation started
3	Cabbage, pepper, tomato	Istanbul	Fermented
4	Cabbage	Erzurum	Fermented
5	Cabbage	Erzurum	1 week old
6	Cabbage	Erzurum	1 week old
7	Cabbage	Denizli	Fermented
8	Bean	Denizli	Fermentation started
9	Cabbage, pepper, tomato	Erzurum	Fermented
10	Cabbage, pepper, tomato	Artvin	Fermented
11	Bean	Erzurum	Fermented
12	Turnip	Erzurum	Fermented
13	Cabbage, pepper, tomato	Ankara	Fermented
14	Armenian cucumber, cucumber	Ardahan	Fermented
15	Cabbage, pepper	Rize	Fermented
16	Cucumber	Rize	Fermented
17	Armenian cucumber, pepper, cucumber, cabbage, tomato	İzmir	Fermented
18	Armenian cucumber, pepper	Hatay	Fermented
19	Tomato	Artvin	Fermented
20	Cabbage	Erzurum	Fermented
21	Pepper	Artvin	Fermentation started
22	Pepper	Giresun	Fermentation started
23	Bean	Giresun	Fermentation started
24	Bean	Artvin	Fermentation started
25	Bean	Tokat	Fresh
26	Cabbage	Gümüşhane	Fermented
27	Cabbage, pepper, tomato	Diyarbakır	Fermentation started
28	Tomato	Samsun	Fermented
29	Armenian cucumber, pepper	Adana	Fermentation started
30	Red cabbage, tomato, pepper	Erzincan	Fermented
31	Cabbage, tomato, pepper	Erzincan	Fermentation started
32	Pepper	Samsun	Fermentation started
33	Bean	Samsun	Fermented
34	Cabbage, pepper, tomato	Tokat	Fermented

Sample No	General Composition	City of Origin	Fermentation Status
35	Cabbage, pepper	Gümüşhane	Fermentation started
36	Cucumber	Samsun	Fermented
37	Turnip, pepper	Istanbul	Fermented
38	Cabbage	Istanbul	Fermented
39	Cabbage	Erzurum	Fermented
40	Turnip	Erzurum	Fermented
41	Cabbage, pepper	Ağrı	Fermented
42	Cabbage	Iğdır	Fermentation started
43	Cabbage	Erzurum	Fermented
44	Cabbage	Manisa	Fermentation started
45	Turnip	Erzurum	Fermented
46	Cabbage	Bayburt	Fermented
47	Bean	Erzurum	Fermented
48	Mixed	Mersin	Fermented
49	Cabbage	Erzurum	Fermented
50	Bean, red cabbage, gherkin	Erzurum	Fermented
51	Cabbage	Malatya	Fermentation started
52	Turnip	Erzurum	Fermented
53	Cabbage, bean, tomato	Elazığ	Fermented

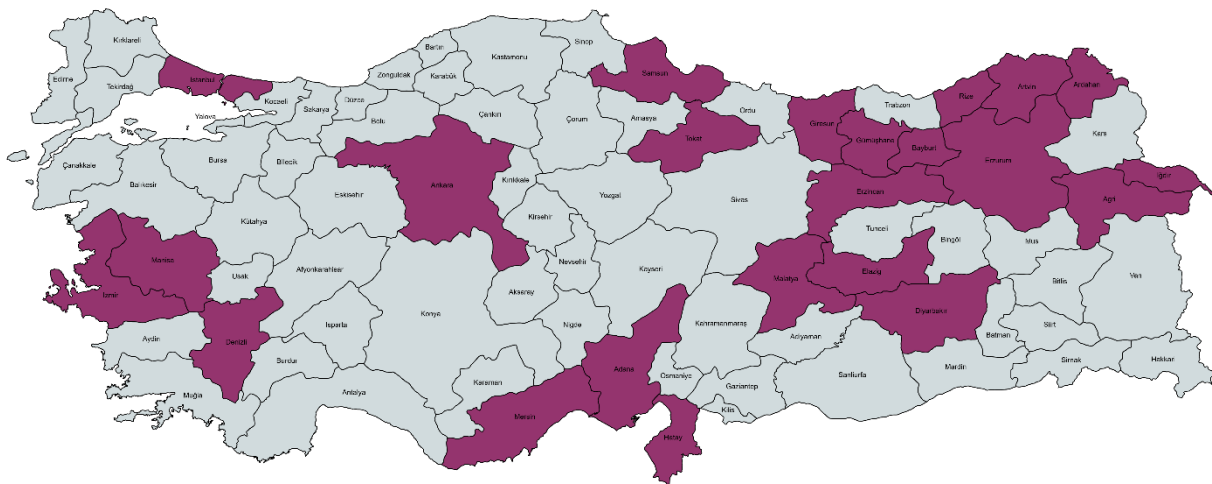


Figure S1. Map of Türkiye showing the cities where pickle samples were taken

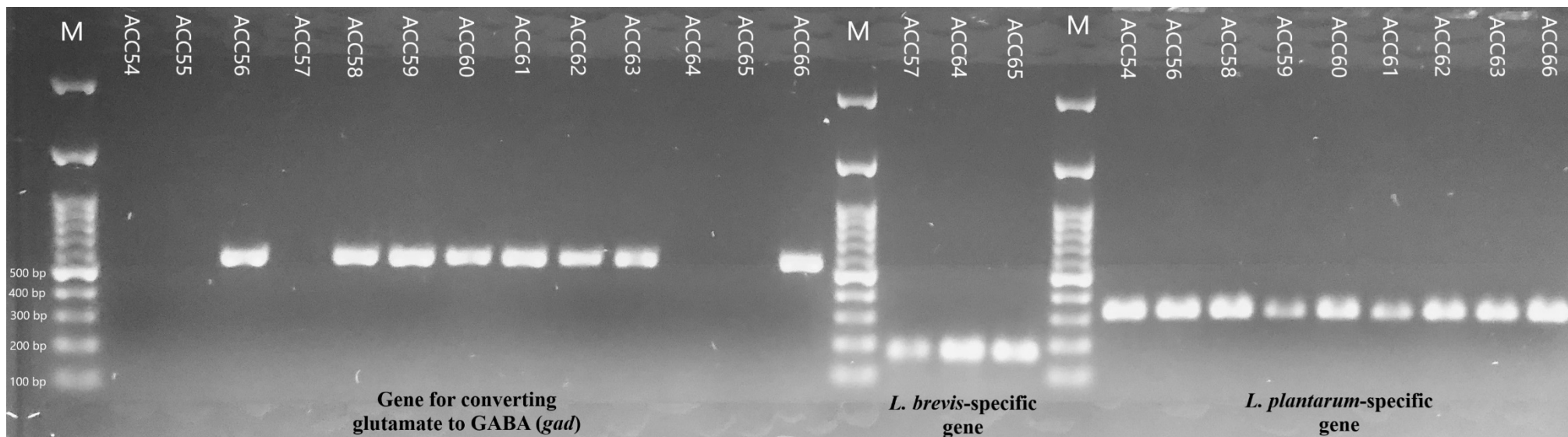


Figure S2. Agarose gel image of pickle-derived lactic acid bacteria obtained throughout the study. M: marker, bp: base pair, GABA: gamma-aminobutyric acid.

References

1. Devereux R, Willis SG. Amplification of ribosomal RNA sequences. In Akkermans ADL, Van Elsas JD, De Bruijn FJ, editors. *Molecular Microbial Ecology Manual*. Berlin:Springer; 1995. p. 277-87.
2. Gómez NC, Ramiro JMP, Quecan BXV, de Melo Franco BDG. Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 biofilm formation. *Front Microbiol*. 2016;7:863.
3. Padilla C, Lobos O. Virulence, bacteriocin genes, and antibacterial susceptibility in *Enterococcus faecalis* strains isolated from water wells for human consumption. *SpringerPlus*. 2013;2:43.
4. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, et al. Development of a multiplex PCR for the detection of *asa1*, *gelE*, *cylA*, *esp*, and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *J Clin Microbiol*. 2004;42:4473-9.
5. Martín-Platero AM, Valdivia E, Maqueda M, Martínez-Bueno M. Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses. *Int J Food Microbiol*. 2009;132:24-32.
6. Eaton TJ, Gasson MJ. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol*. 2001;67:1628-35.
7. Siragusa S, De Angelis M, Di Cagno R, Rizzello CG, Coda R, Gobbetti M. Synthesis of gamma-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl Environ Microbiol*. 2007;73:7283-90.
8. De Angelis M, Calasso M, Di Cagno R, Siragusa S, Minervini F, Gobbetti M. NADP-glutamate dehydrogenase activity in nonstarter lactic acid bacteria: Effects of temperature, pH, and NaCl on enzyme activity and expression. *J Appl Microbiol*. 2010;109:1763-74.
9. Herbel SR, Lauzat B, von Nickisch-Rosenegk M, Kuhn M, Murugaiyan J, Wieler LH, et al. Species-specific quantification of probiotic *Lactobacilli* in yoghurt by quantitative real-time PCR. *J Appl Microbiol*. 2013;115:1402-10.
10. Mousavizadeh M, Mehrabian S, Ashrafi F, Ebrahimi MT. Biochemical study and molecular identification of bacteria with probiotic potential in traditional yogurt, kefir, and curd and their antimicrobial effect on *Escherichia coli* in Mazandaran Province of Iran. *Pak J Med Health Sci*. 2020;14:1443-8.
11. De Las Rivas B, Marcobal A, Carrascosa AV, Muñoz R. PCR detection of foodborne bacteria producing the biogenic amines histamine, tyramine, putrescine, and cadaverine. *J Food Protect*. 2006;69:2509-14.
12. Botthoulath V, Upaichit A, Thumarat U. Identification and in vitro assessment of potential probiotic characteristics and antibacterial effects of *Lactobacillus plantarum* subsp. *plantarum* SK119, a bacteriocinogenic strain isolated from Thai fermented pork sausage. *J Food Sci Technol*. 2018;55:2774-85.
13. Palomba S, Cavella S, Torrieri E, Piccolo A, Mazzei P, Blaiotta G, et al. Polyphasic screening, homopolysaccharide composition, and viscoelastic behavior of wheat sourdough from a *Leuconostoc lactis* and *Lactobacillus curvatus* exopolysaccharide-producing starter culture. *Appl Environ Microbiol*. 2012;78:2737-47.