

1 **Strain diversity of plant-associated *Lactiplantibacillus plantarum***

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18

19 **Abstract**

20 The intraspecific phenotypic and genetic diversity of *Lactiplantibacillus plantarum*
21 (formerly *Lactobacillus plantarum*) was examined for five strains isolated from fermented olives
22 and eight strains from cactus fruit, fermented tomatoes, teff injera, wheat boza, and wheat
23 sourdough starter sources. Carbohydrate utilization and stress tolerance characteristics showed
24 that the olive isolates grew more robustly in galactose and raffinose, showed higher tolerance to
25 12% v/v EtOH, and exhibited a greater capacity to inhibit an olive spoilage strain of
26 *Saccharomyces cerevisiae* than *L. plantarum* from the other plant sources. Certain traits were
27 variable between fermented olive isolates such as the capacity for biofilm formation and survival
28 at pH 2 or 50 °C. By comparison, all *L. plantarum* from fruit sources grew better at a pH of 3.5
29 than the strains from fermented grains. Multi-locus sequence typing and genome sequencing
30 indicated that strains from the same source type tended to be genetically related. Comparative
31 genomics was unable to resolve strain differences, with the exception of the most phenotypically
32 impaired and robust isolates. The findings show that *L. plantarum* is adapted for growth on
33 specific plants or plant food types, but that intraspecific variation may be important for
34 ecological fitness of *L. plantarum* within individual habitats.

35 Introduction

36 Certain LAB required for food fermentations are recognized for their genetic and
37 phenotypic diversity and have been classified as “nomadic” or “generalist” because of their
38 broad habitat range (Duar *et al.*, 2017; Choi *et al.*, 2018; Yu *et al.*, 2020). *Lactiplantibacillus*
39 *plantarum* (formerly *Lactobacillus plantarum* (Zheng *et al.*, 2020)) is included among those
40 nomadic LAB (Duar *et al.*, 2017) and is known for its significant intraspecific versatility
41 (Molenaar *et al.*, 2005; Siezen *et al.*, 2010; Martino *et al.*, 2016). *L. plantarum* is frequently
42 isolated from fresh and fermented plant, meat, and dairy foods and is an inhabitant of the
43 gastrointestinal and vaginal tracts of humans and animals (Delgado *et al.*, 2005; Aquilanti *et al.*,
44 2007; Di Cagno *et al.*, 2008; Yang *et al.*, 2010; Ciocia *et al.*, 2013; Jose *et al.*, 2015; Zago *et al.*,
45 2017; Parichehreh *et al.*, 2018; Barache *et al.*, 2020). This species is essential for the production
46 of numerous fermented foods (e.g., fermented olives, sauerkraut, salami, and sourdough), and
47 certain strains are effective probiotics (Marco, 2010; Seddik *et al.*, 2017; Crakes *et al.*, 2019).
48 Consistent with its host and environmental range, *L. plantarum* strains have larger genomes
49 compared with LAB with narrow host ranges and also carry strain-specific genes, often located
50 on lifestyle adaptation islands (Molenaar *et al.*, 2005; Sun *et al.*, 2015; Zheng *et al.*, 2015; Duar
51 *et al.*, 2017; Salvetti *et al.*, 2018;).

52 Despite the robust growth of *L. plantarum* in different host-associated and food
53 environments, *L. plantarum* genomes and cell properties have thus far shown limited correlations
54 with isolation source across disparate habitats (Siezen *et al.*, 2010; Martino *et al.*, 2016). These
55 findings indicate that either intraspecific variation of *L. plantarum* within individual sources is
56 fortuitous and members of this species have not evolved for growth in specific habitats (Martino
57 *et al.*, 2016), or that this observed variation is the result of adaptive evolution of the *L. plantarum*

58 species within certain habitats with the outcome of maximizing co-occurrence by niche
59 complementarity (Bolnick *et al.*, 2011; Ehlers *et al.*, 2016).

60 To begin to address these two hypotheses, we examined the intraspecies variation of a
61 collection of *L. plantarum* strains isolated from fermented olives and other plant food types. *L.*
62 *plantarum* is typically highly abundant in olive fermentations (Hurtado *et al.*, 2012).
63 Assessments of the population sizes of individual *L. plantarum* strains in olive fermentations
64 over time have shown how these fermentations are highly dynamic, likely undergoing succession
65 processes at both the species and strain levels (Zaragoza *et al.*, 2017). These findings are notable
66 because although LAB have received considerable attention for their contributions to plant
67 fermentations, the diversity, abundance and importance of *L. plantarum* and other LAB in plant
68 microbiomes are not well understood (Yu *et al.*, 2020). It has been found that LAB in
69 spontaneous (wild) plant food fermentations are subject to dispersal and selection constraints
70 (Miller *et al.*, 2019). However, adaptations expressed by these bacteria that are specific to plant
71 environments and interactions between the same or highly-related LAB species remain to be
72 determined.

73 *L. plantarum* was isolated from olive fermentations (AJ11, BGM55, BGM37, BGM40,
74 and EL11), tomato fermentations (T2.5 and WS1.1), teff injera fermentations (W1.1, B1.1, and
75 B1.3), wheat sourdough starter (K4), wheat boza (8.1), and prickly pear cactus fruit (1B1)
76 (**Table 1**). Some isolates were collected from the same source either at the same time (strain
77 B1.1 and B1.3) or on different days over the course of fermentation (strains AJ11, BGM37, and
78 BGM40). The strains were selected without considering special criteria or selective pressure. A
79 reference strain from saliva (NCIMB8826R) was used for comparison. To investigate their
80 phenotypic range, the *L. plantarum* strains were evaluated for growth on a variety of plant-

81 associated carbohydrates and during exposure to high NaCl (4% (v/v)), ethanol (EtOH) (8% and
82 12% (v/v)), or surfactant (sodium dodecyl sulfate (SDS, 0.03% (w/v)) stress. The isolates were
83 measured for the capacity to grow at a low pH (pH 3.5) as well as survive (pH 2) and tolerate a
84 high temperature (50 °C) incubation. Biofilm formation and growth inhibition of *Saccharomyces*
85 *cerevisiae* UCDFST 09-448, a pectinolytic spoilage yeast (Golomb *et al.*, 2013), were also
86 tested. Lastly, to establish the genetic basis for the observed strain differences, multi-locus
87 sequence typing (MLST) and comparative genomics were performed.

88

89 **Results**

90 **Strain differentiation and phylogenetic analysis.** The isolates were identified as *L. plantarum*
91 by 16S rRNA gene sequence analysis and differentiated from the closely-related species
92 *Lactiplantibacillus pentosus* (formerly *Lactobacillus pentosus* (Zheng *et al.*, 2020)) and
93 *Lactiplantibacillus paraplantarum* (formerly *Lactobacillus paraplantarum* (Zheng *et al.*, 2020))
94 by multiplex PCR targeting *recA* (Torriani *et al.*, 2001).

95 The strains were also found to have unique allelic MLST sequence types (ST) (**Table**
96 **S1**), thus confirming that they are genetically distinct and not derived from the same clonal
97 populations. Among the eight genes tested by MLST, between 6 (*uvrC*) and 12 (*pyrG*) different
98 alleles were found (**Table S1**). Phylogenetic analysis of the ST showed that the *L. plantarum*
99 strains clustered into two clades (**Fig. 1A**). The isolates from fermented olives were contained in
100 one clade, suggesting they are more closely related to each other and to the teff injera strain B1.3
101 than those retrieved from other sources. The two other strains from teff injera (B1.1 and W1.1)
102 clustered together in the other clade which also contained NCIMB8826R and the strains isolated
103 from wheat boza, sourdough, cactus fruit, and fermented tomatoes (**Fig. 1A**). When examined in

104 a MLST phylogenetic tree containing 264 other *L. plantarum* strains (**Fig. S1**), the *L. plantarum*
105 isolates collected from fermented olives remained clustered closely together, whereas the others
106 were distributed across the tree.

107

108 **Carbohydrate utilization capacities.** The capacity of the *L. plantarum* strains to use different
109 sugars for growth was measured using MRS, a complete medium commonly used for cultivation
110 of LAB (De Man et al., 1960). To exclude metabolizable carbon sources, the MRS was modified
111 (mMRS) to remove beef extract and dextrose. In mMRS containing glucose, maltose, or sucrose,
112 all *L. plantarum* strains except B1.3 (teff injera) and 8.1 (wheat boza) were found to have robust
113 growth according to area under the curve (AUC) rankings (**Fig. 2, Fig. 3, and Table S2**). Those
114 strains which grew robustly reached maximum OD₆₀₀ values within 12 h (**Fig. 3 and Table S3**)
115 and displayed growth rates ranging from a low of $0.31 \pm 0.01 \text{ h}^{-1}$ (strain W1.1 (teff injera) in
116 maltose) to a high of $0.45 \pm 0.01 \text{ h}^{-1}$ (BGM37 (fermented olives) in glucose) (**Table S4**). By
117 comparison, the growth rate of B1.3 was lower in glucose ($0.20 \pm 0.00 \text{ h}^{-1}$) and maltose ($0.15 \pm$
118 0.00 h^{-1}) compared to the other strains (**Fig. 3 and Table S4**). In mMRS-sucrose, both B1.3 and
119 8.1 exhibited poor growth (**Fig. 2, Fig. 3, and Table S2**).

120 All strains grew moderately to robustly when galactose was provided as the sole carbon
121 source in mMRS (**Fig. 2, Fig. 3, and Table S2**). Growth rates ranged from a low of 0.16 ± 0.003
122 h^{-1} (B1.3 (teff injera)) to a high of $0.42 \pm 0.01 \text{ h}^{-1}$ (BGM37 (fermented olives)) (**Table S4**). Final
123 OD₆₀₀ values measured after 24 h incubation ranged from 2.58 ± 0.05 (BGM40 (fermented
124 olives)) to 3.62 ± 0.03 (BGM37) (**Table S3**). Because incubation in glucose-containing MRS
125 prior to exposure to mMRS-galactose might result in carbon catabolite repression (Kremling *et*
126 *al.*, 2015), several strains with only moderate growth in that culture medium (AJ11, BGM40, and

127 EL11 (fermented olives), 8.1 (wheat boza), B1.3 (teff injera), and T2.5 (fermented tomatoes))
128 were inoculated in succession into mMRS-galactose. However, prior exposure to mMRS-
129 galactose did not result in higher AUC values (data not shown).

130 In mMRS with raffinose, all five *L. plantarum* strains isolated from fermented olives
131 (BGM37, BGM55, BGM40, AJ11, and EL11) exhibited either moderate or robust growth (**Fig.**
132 **2, Fig. 3, and Table S2, S3, and S4**). Although strain W1.1 (teff injera) also grew robustly, the
133 other strains isolated from teff and wheat fermentations (8.1, B1.1, and B1.3) and both strains
134 isolated from fermented tomatoes (T2.5 and WS1.1) displayed limited or poor growth (**Fig. 2,**
135 **Fig. 3 and Table S2, S3, and S4**). To address whether the poor growth of those isolates was due
136 to carbon-catabolite repression, serial passage in mMRS-raffinose was performed. Notably,
137 growth of four out of the five strains (B1.1, 8.1, T2.5, and WS1.1) was improved by successive
138 cultivation in mMRS-raffinose (**Fig. S2**).

139 When fructose was provided, all *L. plantarum* isolates except for strain 8.1 (wheat boza)
140 exhibited either moderate or robust growth (**Fig. 2, Fig. 3, and Table S2**). Similar to incubation
141 in glucose and galactose, strain BGM37 (fermented olives) reached the highest OD₆₀₀ (OD₆₀₀ =
142 3.44 ± 0.03) (**Table S3**). Remarkably, growth of B1.3 (teff injera) was improved in mMRS-
143 fructose compared to the other sugars tested, as demonstrated by a higher growth rate (**Table S4**)
144 and final OD₆₀₀ (**Table S3**). Similar to the lack of effect on AUC values found after successive
145 passage in the presence of mMRS-galactose, no significant differences in growth were found for
146 any of the 14 strains after multiple passages in mMRS-fructose (data not shown).

147 Growth of *L. plantarum* was poor in mMRS containing xylose, ribose, or arabinose. Only
148 four olive-associated strains (AJ11, BGM55, BGM37, and BGM40) and NCIMB8826R grew in
149 the presence of mMRS-ribose or mMRS-arabinose and none grew in mMRS-xylose (**Fig. 2, Fig.**

150 **3, and Table S2, S3, and S4**). After 38 h in mMRS-ribose, the OD₆₀₀ values for those strains
151 ranged from a low of 1.36 ± 0.15 (AJ11 (fermented olives)) to a high of 2.93 ± 0.17 (BGM37
152 (fermented olives)) (**Fig. 3 and Table S3**). In mMRS with arabinose, only NCIMB8826R and
153 BGM37 grew, reaching an OD₆₀₀ of 1.94 ± 1.56 and 2.99 ± 0.14 , respectively (**Fig. 3 and Table**
154 **S3**). To investigate whether growth could be improved by prior exposure to those pentose sugars,
155 strains AJ11, BGM37, 8.1, and NCIMB8826R were incubated with successive passages in
156 mMRS-ribose or mMRS-arabinose. This resulted in shorter lag phase times and higher final
157 OD₆₀₀ values for AJ11, BGM37, and NCIMB8826R in both media (**Fig. S3 and Fig. S4**). By
158 comparison, no difference in growth was observed for strain 8.1 (wheat boza) in mMRS-ribose
159 or mMRS-arabinose irrespective of the adaptation period (**Fig. S3 and Fig. S4**).

160
161 **Growth in the presence of EtOH.** Because mMRS-glucose resulted in robust growth of the
162 majority of *L. plantarum* strains investigated here, that culture medium was used for
163 investigation of stress tolerance properties. In mMRS-glucose containing 8% (v/v) (174 mM)
164 ethanol (EtOH), the AUCs for all strains except B1.3 (teff injera) were either moderate or robust
165 (**Fig. 2, Fig. 4, and Table S2**). Although lag phase times were longer (data not shown) and
166 growth rates were reduced when EtOH was included in the culture medium (**Table S5**), the
167 growth curves of six strains (AJ11, BGM37, and EL11 (fermented olives), 8.1 (wheat boza),
168 B1.1 (teff injera), and 1B1 (cactus fruit)) were still regarded as robust according to AUC
169 assessments (**Fig. 2**). Surprisingly, two strains, BGM37 (fermented olives) and 1B1 (cactus
170 fruit), reached a higher final OD₆₀₀ in mMRS-glucose with 8% (v/v) EtOH than in mMRS-
171 glucose alone (Student t-test, $P < 0.05$) (**Table S3**).

172 None of the *L. plantarum* strains tested here were able to grow over a 48 h period when
173 incubated directly in mMRS-glucose with 12% (v/v) (260 mM) EtOH (data not shown). To
174 determine whether a more gradual exposure to high EtOH concentrations would change this
175 outcome, the strains were incubated in mMRS-glucose containing 8% (v/v) EtOH overnight
176 prior to inoculation into mMRS-glucose with 12% (v/v) EtOH. This modification resulted in
177 robust growth of 1B1 (cactus fruit) (**Fig. 2, Fig. 4, and Tables S2, S3, and S5**). Eight other
178 strains (AJ11, BGM55, BGM37, BGM40, and EL11 (fermented olives), K4 (wheat sourdough),
179 B1.1 (teff injera), and WS1.1 (fermented tomatoes)) exhibited moderate growth according to
180 AUC values as a result of the step-wise transfer to the higher (12% (v/v)) EtOH conditions (**Fig.**
181 **2, Fig. 4, and Tables S2, S3, and S5**).

182
183 **Growth in the presence of detergent (SDS) stress.** While most of the *L. plantarum* strains
184 exhibited moderate growth when SDS (0.03% (w/v) (0.10 mM)) was included in mMRS-
185 glucose, two strains BGM37 (fermented olives) and 1B1 (cactus fruit) grew robustly (**Fig. 2, Fig.**
186 **4, and Tables S2, S3, and S5**). Remarkably, the growth rate of strain B1.3 (teff injera) was
187 higher in the presence of SDS ($0.32 \pm 0.003 \text{ h}^{-1}$) (**Table S5**) as opposed to its absence ($0.20 \pm$
188 0.003 h^{-1}) (**Table S4**) and it reached a higher AUC (107 ± 0.17) (**Table S2**).

189
190 **Growth at pH 3.5 and in the presence of 4% NaCl.** Growth of *L. plantarum* was reduced in
191 mMRS-glucose adjusted to a pH of 3.5 (**Fig. 2, Fig. 4, and Table S2**). However, the strains
192 isolated from brine-based, fruit fermentations (AJ11, BGM55, BGM37, BGM40, and EL11
193 (fermented olives) and T2.5 and WS1.1 (fermented tomatoes)), grew significantly better under
194 those conditions compared to the *L. plantarum* isolated from grain fermentations (Student T-test,

195 $p < 0.05$). The strains from grain-based fermentations (K4 (wheat sourdough), 8.1 (wheat boza),
196 W1.1, B1.1, and B1.3 (teff injera)) grew poorly in the acidified mMRS (pH 3.5) (**Fig. 2, Fig. 4,**
197 **and Table S2**), yielding low growth rates ($0.06 \pm 0.01 \text{ h}^{-1}$) (**Table S5**) and final OD₆₀₀ values
198 (1.50 ± 0.19) (**Table S3**).

199 When 4% (w/v) NaCl was included in mMRS-glucose, five strains isolated from different
200 sources (BGM55, BGM37, and BGM40 (fermented olives), 8.1 (wheat sourdough), and WS1.1
201 (fermented tomatoes)) were classified as robust according to their AUC values (**Fig. 2 and Table**
202 **S2**). The growth of strain B1.3 (teff injera) was the most negatively impacted by the addition of
203 salt into the laboratory culture medium (**Fig. 4 and Tables S2, S3, and S5**).

204 All *L. plantarum* strains were inhibited in mMRS-glucose containing 4% (w/v) NaCl and
205 a starting pH of pH 3.5 (**Fig. 2, Fig. 4, and Table S2**). The final OD₆₀₀ values ranged from a low
206 of 0.23 ± 0.00 (W1.1, teff injera) to a high of 0.52 ± 0.06 (BGM37, fermented olives) (**Table**
207 **S3**). Although the AUCs of all strains were regarded to be poor, growth rates of those isolated
208 from brine-based, fruit fermentations (AJ11, BGM55, BGM37, BGM40, and EL11 (fermented
209 olives) and T2.5 and WS1.1 (fermented tomatoes)) were significantly higher than those isolated
210 from grain-based fermentations (K4 (wheat sourdough), 8.1 (wheat boza), W1.1, B1.1, and B1.3
211 (teff injera)) ($p < 0.05$, Student's T-test).

212

213 **Survival at pH 2.** Within 15 min incubation in physiological saline adjusted to pH 2, a 10^4 to
214 10^6 -fold reduction in cell viability was observed (**Fig. 5A**). After 30 min exposure to pH 2,
215 strains B1.3 (teff injera), BGM40 (fermented olives), and NCIMB8826R (saliva, reference
216 strain) were no longer detectable by colony enumeration. BGM37 (fermented olives), B1.1 (teff
217 injera), and T2.5 (fermented tomatoes) were no longer viable by 60 min (**Fig. 5A**). *L. plantarum*

218 AJ11, BGM55, and EL11 (fermented olives), 8.1 (wheat boza), and WS1.1 (fermented tomatoes)
219 exhibited the highest acid tolerance and were still viable according to colony enumerations
220 performed on cells collected after 60 min incubation. Unlike the findings for growth under acidic
221 conditions (pH 3.5) (**Fig. 4**), there were no obvious isolation-source dependent trends in *L.*
222 *plantarum* strain survival.

223
224 **Survival at 50 °C.** Survival at 50 °C spanned a 10⁶ - fold range (**Fig. 5B**). Viable B1.3 (teff
225 injera) cells were no longer detected after incubation at 50 °C for 15 min (1 x 10⁸ cells/ml present
226 in the inoculum). After 60 min, AJ11 and EL11 (fermented olives), 8.1 (wheat boza), W1.1 (teff
227 injera), 1B1 (cactus fruit), and NCIMB8826R (saliva, reference strain) were still culturable in a
228 range from 5 x 10⁴ (8.1) to 1.5 x 10² (AJ11) CFU/ml, spanning a 10³- to 10⁶-fold reduction in
229 viable cell numbers (**Fig. 5B**). Similar to survival to pH 2, no obvious isolation-source dependent
230 differences in survival were observed.

231
232 **Biofilm forming capacity.** Because biofilm formation is an indicator of bacterial capacities to
233 tolerate environmental stress (Yin *et al.*, 2019) and *L. plantarum* biofilm formation is partially
234 dependent on carbon source availability (Fernández Ramírez *et al.*, 2015), we examined the
235 capacity of *L. plantarum* to produce biofilms during growth in mMRS with glucose, fructose, or
236 sucrose. Only BGM55 and BGM37 (fermented olives), 8.1 (wheat boza), W1.1 and B1.1 (teff
237 injera), T2.5 and WS1.1 (fermented tomatoes) formed robust biofilms after growth in at least one
238 of those laboratory culture media (**Fig. 6**). Whereas injera strain W1.1 only developed a biofilm
239 when grown in mMRS-fructose, the other isolates formed robust biofilms in the presence of at
240 least two different sugars (**Fig. 6**). Both strains isolated from fermented tomatoes, T2.5 and

241 WS1.1, formed extensive biofilms when grown in the presence of either glucose or fructose.
242 Notably, biofilm formation was not associated with robust strain growth. Strain 8.1 formed a
243 biofilm in mMRS-sucrose (**Fig. 6**) despite showing poor growth (**Fig. 2**) and reaching a low final
244 OD₆₀₀ (**Table S3**) in that culture medium. Conversely, strain K4 grew well in mMRS-sucrose but
245 did not produce a biofilm.

246

247 **Antifungal activity of *L. plantarum* cell-free culture supernatant (CFCS).** Growth rates and
248 final OD₆₀₀ values of *S. cerevisiae* UCDFST 09-448 were reduced when incubated in the
249 presence of the *L. plantarum* CFCS (**Table S6**). All *L. plantarum* CFCSs inhibited *S. cerevisiae*
250 growth, however there were some strain-specific differences (**Fig. 7 and Table S6**). Collectively
251 the CFCSs from strains isolated from fermented olives (AJ11, BGM55, BGM37, BGM40, EL11)
252 and fermented tomatoes (WS1.1 and T2.5) were significantly ($p < 0.05$, Student's T-test) more
253 inhibitory than those isolated from fermented grains (K4, 8.1, W1.1, B1.1, and B1.3). Growth
254 inhibition resulting from exposure to the CFCS from olive strains ranged between $29.8\% \pm 4.87$
255 (BGM55) to $34.1\% \pm 9.4$ (BGM40). By comparison, growth inhibition with CFCS from *L.*
256 *plantarum* isolated from grain fermentations was only between $20.1\% \pm 1.06$ (B1.1) to $22.68\% \pm$
257 1.46 (8.1). Interestingly, the growth pattern of *S. cerevisiae* in the presence of teff injera strain
258 B1.3 CFCS (31.4 ± 1.27) was more similar to strains from fermented olives than grains.

259

260 **Comparisons of *L. plantarum* genomes.** Nine of the fourteen strains were selected for genome
261 sequencing (PacBio or Illumina platforms) based on the variations in their phenotypic profiles
262 (**Fig. 2**). Genome assembly for strains sequenced using PacBio resulted in fewer contigs (min of
263 3 and max of 9) and higher coverage (min of 140X and max of 148X) compared to Illumina

264 (contigs: min of 29 and max of 120; coverage (min of 27X and max of 128X) (**Table 2**).
265 Genome sizes ranged from 3.09 Mbp (B1.3 (teff injera)) to 3.51 Mbp (WS1.1 (fermented
266 tomatoes)) and total numbers of predicted coding sequences ranged from 3,088 (K4 (wheat
267 sourdough)) to 3,613 (WS1.1) (**Table 2**).

268 The core- and pan-genomes of the nine strains consisted of 2,222 and 6,277 genes,
269 respectively (**Fig. S5**), numbers consistent with previous comparisons examining larger
270 collections of *L. plantarum* strains (Siezen *et al.*, 2010; Martino *et al.*, 2016; Choi *et al.*, 2018).
271 Alignments of the predicted amino acid sequences for the genes in the core genomes indicated
272 that strains isolated from grain fermentations (K4 (wheat sourdough), 8.1 (wheat boza) and B1.1,
273 and B1.3 (teff injera)) and strain WS1.1 from fermented tomatoes are more closely related to
274 each other than isolates from olives and cactus fruit (**Fig. 1B**). B1.1 and B1.3, two strains
275 originating from the same sample of teff injera, were also shown to share similar core genomes
276 (**Fig. 1B**).

277 Just as strains 8.1 (wheat boza) and WS1.1 (fermented tomatoes) were found to have
278 similar core genomes (**Fig. 1B**), those two strains are similar according to hierarchical clustering
279 based on the numbers of genes in individual COG categories (**Fig. 8**). The three strains isolated
280 from olives formed a separate clade from those recovered from other sources and were shown to
281 have higher numbers of genes in the carbohydrate metabolism and transport (G) and
282 transcription (K) COGs. *L. plantarum* BGM37, a strain from olives that exhibited the most
283 robust growth on the different carbohydrates compared tested here (**Fig. 2**), also contained the
284 highest numbers of gene clusters annotated to the carbohydrate metabolism and transport COG
285 (256 gene clusters, **Fig 8. and Table S7**).

286 Strain B1.3 was found to contain the lowest number of gene clusters in the carbohydrate
287 metabolism and transport COG (206 gene clusters, **Fig. 8 and Table S7**) and is specifically
288 lacking in several genes required for sugar metabolism and sugar-importing phosphotransferase
289 (PTS) systems (data not shown). Conversely, the genome of B1.3 harbors at least two-fold
290 higher numbers of genes and genetic elements in the mobilome (X) COG compared to the other
291 strains examined (352 gene clusters, **Table S7**). These genomic features include prophages,
292 insertion sequence elements, and transposases that are interspersed throughout the genome and
293 frequently located between genes with known function. For example, a transposon (3.8 kb) is
294 located between the glucose-6-phosphate isomerase (lp_2502) and glucose/ribose porter family
295 sugar transporter (lp_2503) genes that are annotated to be associated with glucose metabolism.
296 Other genes were not present in the B1.3 genome such as the sucrose-associated PTS (lp_3819;
297 *pts24BCA*), possibly indicating why this strain exhibited poor growth in mMRS-sucrose. Strain
298 8.1, the only other *L. plantarum* strain tested here that grew to a limited extent on sucrose (**Fig.**
299 **2**), lacks the first 650 bp of *pts1BCA* (lp_0185), a gene in the sucrose phosphoenolpyruvate
300 (PEP)- dependent phosphotransferase system (PTS) (Saulnier *et al.*, 2007; Yin *et al.*, 2018).

301 The number of gene clusters in the other COG categories was largely conserved between
302 strains (**Fig. 8 and Table S7**). These COG categories encode pathways required energy
303 metabolism (glycolysis), synthesis of macromolecules (proteins, nucleotides, and lipids), and
304 stress response. The genomes of all nine strains contain genes encoding chaperones (DnaJK,
305 GroEL, GroES, GrpE, ClpB, ClpL), proteases (ClpX, ClpP, ClpE), DNA repair proteins (RecA,
306 UvrABC), and transcriptional regulators (HrcA, CtsR) critical for *L. plantarum* tolerance to
307 numerous environmental stresses (Papadimitriou *et al.*, 2016). Although genes required for
308 citrate metabolism (*citCDEF*) were previously found to be associated with EtOH tolerance (Veen

309 *et al.*, 2011) and that locus was flanked by mobile elements in several of the *L. plantarum* strains
310 examined here, the presence of those mobile elements was not correlated with EtOH sensitivity.

311

312 **Discussion**

313 This study investigated the phenotypic and genetic properties of *L. plantarum* strains
314 from (fermented) plant sources. The findings broadly show that strains obtained from the same
315 or similar plant environments tend to be more genetically related and share similar carbohydrate
316 utilization and stress tolerance capacities. However, there were still significant differences
317 between all strains, irrespective of their source, a result which suggests that *L. plantarum* has
318 adapted for growth in specific habitats (e.g., olive fermentations) but that intraspecific variation
319 of this generalist species may afford the opportunity for *L. plantarum* strain coexistence by niche
320 differentiation.

321 Our use of growth curve AUC rankings and the monitoring of growth rates and final
322 OD₆₀₀ values provided a detailed view of *L. plantarum* carbohydrate utilization capacities. The
323 majority of strains exhibited robust growth on glucose, maltose, sucrose, and galactose, moderate
324 growth on raffinose and fructose, and only limited to no growth on ribose, arabinose, and xylose.
325 The moderate or poor growth observed for a few strains when incubated the presence galactose
326 or fructose, was likely not due to carbon catabolite repression (Görke and Stülke, 2008;
327 Kremling *et al.*, 2015), but rather a lack of enzymatic capacity to utilize those sugars. These
328 conserved carbohydrate consumption patterns are consistent with prior reports on *L. plantarum*
329 isolated from plants and other host-associated sources (Westby *et al.*, 1993; Saulnier *et al.*, 2007;
330 Siezen *et al.*, 2010; Filannino *et al.*, 2014; Siragusa *et al.*, 2014). The strains tested here were
331 also able to grow in the presence of 0.03% (w/v) SDS and were severely impaired when

332 incubated in mMRS at pH 3.5 with 4% (w/v) NaCl or inoculated directly into mMRS with 12%
333 (v/v) EtOH.

334 Other findings were strain specific and similarly consistent with reported phenotypic
335 (Parente *et al.*, 2010; Siezen *et al.*, 2010; Guidone *et al.*, 2014; Ferrando *et al.*, 2015, 2016;
336 Ghezzi *et al.*, 2019; Prete *et al.*, 2020) and genomic variations (Molenaar *et al.*, 2005; Siezen *et*
337 *al.*, 2010; Siezen and van Hylckama Vlieg, 2011; Martino *et al.*, 2016; Choi *et al.*, 2018)
338 observed for the *L. plantarum* species. We found that *L. plantarum* growth was highly variable
339 following the sequential incubation in 8% (v/v) and then 12% (v/v) EtOH. Strain growth rates in
340 mMRS with 8% (v/v) EtOH were correlated with those observed for mMRS containing 0.03%
341 SDS ($r = 0.561$, $p < 0.05$), thereby indicating overlapping mechanisms in *L. plantarum* strain
342 tolerance to membrane-disruptive compounds (Seddon *et al.*, 2004; Bravo-Ferrada *et al.*, 2015;
343 Mukhopadhyay, 2015). High temperature tolerance also differed between the *L. plantarum*
344 isolates, such that incubation at 50 °C for 60 min resulted in over a 10⁵ - fold range in strain
345 survival. Survival at pH 2 followed a similar trend, such that some strains were no longer
346 culturable after 15 min, while other strains still formed colonies after prolonged (60 min)
347 incubation. Notably, only two strains from olive fermentations (AJ11 and EL11) and 8.1 from
348 boza survived well under both high temperature and low pH conditions. Although, the genomes
349 were found contain chaperones and proteases known to be involved in *L. plantarum* heat and
350 acid shock responses (Corcoran *et al.*, 2008; Mills *et al.*, 2011), the unique proteins or pathways
351 expressed by those strains which confer heightened stress tolerance remain to be determined.

352 Despite the conserved and variable aspects of *L. plantarum* carbohydrate utilization and
353 environment stress tolerance phenotypes, there were other remarkable trends associated with
354 strain isolation source. For example, the isolates from acidic, brine-containing ferments (olives

355 and tomatoes) were more resistant to acidic pH (pH 3.5) and high NaCl (4% w/v) concentrations
356 than those recovered from grain fermentations (wheat boza, wheat sourdough, and teff injera).
357 Genome comparisons using concatenated core gene amino acids showed that strains isolated
358 from grain fermentations are more related to each other than those from other sources. Genetic
359 conservation between olive fermentation-associated strains was observed by MLST and COG
360 gene numbers.

361 The strains from fermented olives also showed the greatest capacity to consume raffinose
362 (a tri-saccharide composed of galactose, glucose, and fructose). It is also notable that two of
363 those isolates (BGM37 and BGM55) grew equally well in mMRS-galactose as in mMRS-
364 glucose. These results are consistent with the findings that olives leaves and roots contain both
365 raffinose ($2.7 \pm 0.1 \mu\text{mol}$) and galactose ($4.8 \pm 0.3 \mu\text{mol}$) (Cataldi *et al.*, 2000) and that the fruits
366 contain galactose along with higher concentrations of glucose, mannitol, and fructose (Gómez-
367 González *et al.*, 2010). All strains from olive fermentations also exhibited at least moderate or
368 robust growth in mMRS in the presence of 8% (v/v) EtOH, and the CFCSs from those strains
369 resulted in greater inhibition of *S. cerevisiae* UCDFST-09-448 compared to the CFCSs from *L.*
370 *plantarum* isolated from other environments. Because yeast are normal members of olive
371 fermentation microbiota, the inhibitory capacity may indicate the presence of shared mechanisms
372 required to prevent yeast overgrowth.

373 Several strains also showed unique properties illustrative of the phenotypic range of the
374 *L. plantarum* species. Among those strains was BGM37 isolated from the brine of fermented
375 olives. This strain exhibited the most robust growth on the carbohydrates tested here, showed the
376 highest tolerance to 8% EtOH and 0.03% SDS, and was able to form biofilms in the presence of
377 glucose, fructose, and sucrose. Compared to the other strains for which genome sequences were

378 obtained, BGM37 was found to have the second largest genome size (3.46 Mbp) after WS1.1
379 (3.51 Mbp), a magnitude comparable to the other *L. plantarum* strains with large (complete)
380 genomes published at NCBI (maximum of 3.70 Mbp as of Jan 2021).

381 *L. plantarum* 1B1, a strain isolated from ripe cactus fruit, is notable because of its robust
382 growth in the presence of either SDS or EtOH. Although other studies reported growth of *L.*
383 *plantarum* in the presence of EtOH (Veen *et al.*, 2011, Brizuela *et al.*, 2019, Duley, 2004), the
384 capacity to grow well at 12% EtOH is an unusual trait even among oenological-associated *L.*
385 *plantarum* (Succi *et al.*, 2017). Thus, the unique properties of this single isolate from a fresh fruit
386 source may indicate the presence of a broader diversity of LAB present in the carposphere (Yu *et*
387 *al.*, 2020).

388 Lastly, strain B1.3 from teff injera exhibited the most restrictive carbon utilization
389 capacities and the lowest levels of environmental stress tolerance among all isolates tested. B1.3
390 grew poorly on glucose and most other carbohydrates, whereas the other strains from teff injera
391 B1.1 and W1.1 exhibited robust growth on a variety of sugars. Limitations in the ability of B1.3
392 to consume different sugars was also shown by the lower numbers of gene clusters in the B1.3
393 genome that are responsible for carbohydrate transport and metabolism. The overall smaller
394 genome size of this strain (3.09 Mbp) and high numbers of genes in the mobilome COG
395 potentially indicates that this strain is undergoing genome reduction for habitat specialization as
396 found for other LAB (e.g., *Lactobacillus bulgaricus* (yogurt) (van de Guchte *et al.*, 2006),
397 *Lactobacillus iners* (vagina) (France *et al.*, 2016), *Apilactobacillus apinorum* (honeybee) (Endo
398 *et al.*, 2018)). Remarkably, the higher growth rate of B1.3 in mMRS-fructose and in the presence
399 of SDS indicates it may be fructophilic and capable of withstanding the presence of membrane
400 disrupting compounds in teff flour. The finding that the CFCS from B1.3 inhibited *S. cerevisiae*

401 UCDFST 09-448 growth also suggests that B1.3 may be adapted to compete with yeast in teff
402 injera. This result is consistent with the proximity of B1.3 to the olive-associated strains in the
403 MLST phylogenetic tree. However, it is also noteworthy that B1.3 shares genetic similarity with
404 the teff injera isolate (B1.1) and other grain-associated *L. plantarum* according to core genome
405 comparisons.

406 Although disruptions in sucrose PTS systems may indicate why neither strain B1.3 nor
407 8.1 was able to grow in the presence of sucrose, the specific genes and pathways conferring the
408 phenotypic variations observed in this study remain to be determined. To this regard,
409 identification of the genome composition alone is insufficient to understand the full metabolic
410 and functional potential of this species. For example, there still remains a lack of resolution in
411 some PTS and other carbohydrate transport and metabolic pathways among lactobacilli (Gänzle
412 and Follador, 2012; Zheng *et al.*, 2015) and stress response mechanisms frequently involve
413 numerous pathways with overlapping cell functions (e.g., membrane synthesis, protein turnover,
414 and energy metabolism pathways) (Papadimitriou *et al.*, 2016).

415 The genetic and phenotypic variation observed for the *L. plantarum* isolates indicate this
416 species has evolved towards specialization in different plant-associated habitats (e.g., fruit vs
417 cereal grains), but at the same time is under selective pressure for sustaining intraspecific
418 diversity within those habitats, possibly as a mechanism promoting *L. plantarum* species stability
419 through co-occurrence in those ecosystems (Maynard *et al.*, 2019). Investigating this diversity
420 and the importance of conserved and variable *L. plantarum* traits on plants and fermented plant
421 foods is expected to be useful for understanding bacterial interactions and habitat partitioning in
422 other complex host-associated (e.g., Lloyd-Price *et al.*, 2017; Truong *et al.*, 2017; Ma *et al.*,
423 2020; Bongrand and Ruby, 2019) and environmental (e.g., Ellegaard *et al.*, 2015; Props and

424 Deneff, 2020; Koch *et al.*, 2020) sites wherein significant intraspecies diversity has been found
425 but not yet understood. These findings may also be used to guide the selection of robust, multi-
426 strain starter cultures that are suited to inter- and intra-species selection pressures in fruit and
427 vegetable fermentations to result in optimal sensory and safety characteristics.

428

429 **Experimental Procedures**

430 **Bacterial strains and growth conditions.** *L. plantarum* strains used in this study are shown in
431 **Table 1**. The isolates from olive fermentations and cactus fruit were described previously
432 (Golomb *et al.*, 2013; Tyler *et al.*, 2016) and NCIMB8826R, a rifampicin-resistant variant (Yin
433 *et al.*, 2018) of strain NCIMB8826 (Hayward and Davis, 1956), was used as a reference. For *L.*
434 *plantarum* isolation from injera batter, the batter was mixed with phosphate buffered saline
435 (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄-7H₂O, 1.4 mM KH₂PO₄) (pH 7.2) at a
436 ratio of 1:10. For isolation from boza and sourdough, the batter was mixed with physiological
437 saline (145 mM NaCl) (pH 7.0) at a ratio of 1:10. For isolation from fermented tomatoes, three
438 tomatoes were placed in sterile bags containing mesh filters (Nasco, Modesto, CA) with 1 ml of
439 PBS (pH 7.2) and macerated by hand. Serial dilutions of the injera, boza, sourdough and tomato
440 suspensions were then plated on de Man, Rogosa, and Sharpe (MRS) agar from a commercial
441 source (BD, Franklin Lakes, NJ) (cMRS). Natamycin (25 µg/mL) (Dairy Connection,
442 Wisconsin, WI) was included in the cMRS agar to inhibit fungal growth. The cMRS agar plates
443 were incubated at 30 °C under aerobic or anaerobic conditions (BD BBL GasPak system (BD,
444 Franklin Lakes, NJ) for 48 h. Single colony isolates were repeatedly streaked for isolation on
445 cMRS prior to characterization. For phenotypic and genotypic analysis, the *L. plantarum* strains
446 were routinely grown in cMRS without aeration at 30 °C.

447

448 **Strain identification and typing.** *L. plantarum* 16S rRNA genes were amplified from individual
449 colonies using the 27F and 1492R primers (Lane *et al.* 1991) (**Table S8**) with *ExTaq* DNA
450 polymerase (TaKaRa, Shiga, Japan). Thermal cycling conditions were as follows: 95 °C for 3
451 min, 30 cycles of 94 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 90 sec, and a final elongation
452 step of 72 °C for 5 min. The PCR products were purified (Wizard SV Gel and PCR Clean-Up
453 System (Promega, Madison, WI)) and sequenced at the UC Davis DNA Sequencing Facility
454 <http://dnaseq.ucdavis.edu/>. The DNA sequences were compared against the National Center for
455 Biotechnology Information (NCBI) database using the nucleotide Basic Local Alignment Search
456 Tool (BLASTn) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Ribosomal Database Project
457 (RDP) (<http://rdp.cme.msu.edu/>). Multiplex PCR targeting the *recA* gene was also used to
458 confirm *L. plantarum* at the species level according to methods described by (Torriani *et al.*,
459 2001) (**Table S8**). The 16S rRNA sequencing data for the strains in this study can be found
460 National Center for Biotechnology Information (BankIt) under accession numbers MT937284-
461 MT937296.

462 For multilocus sequence typing (MLST), genomic DNA was isolated with the Qiagen
463 DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's
464 instructions. PCR was then performed using primers targeting the variable regions of *L.*
465 *plantarum* *pheS*, *pyrG*, *uvrC*, *recA*, *clpX*, *murC*, *groEL*, and *murE* (**Table S8**) (Xu *et al.*, 2015).
466 PCR amplification was performed using *ExTaq* DNA polymerase (TaKaRa, Shiga, Japan) as
467 previously described (Xu *et al.*, 2015). The PCR products were sequenced in both directions
468 using the forward and reverse primers at the UC Davis DNA Sequencing Facility
469 (<http://dnaseq.ucdavis.edu/>) and Genewiz (South Plainfield, NJ). DNA sequences were aligned,

470 trimmed, and analyzed using the MEGA 7.0 software package (Kumar *et al.*, 2016). Based on
471 the findings, unique nucleotide sequences for a gene were defined as an allele and unique allelic
472 profiles were defined as a sequence type. The concatenate sequences in the order of *pheS*, *pyrG*,
473 *uvrC*, *recA*, *clpX*, *murC*, *groEL*, and *murE* was used for phylogenetic tree analysis with
474 maximum likelihood supported with a multilocus bootstrap approach using MEGA 7.0 (Kumar
475 *et al.*, 2016). For comparisons to other strains of *L. plantarum*, the sequences of 264 strains of *L.*
476 *plantarum* were downloaded from the National Center for Biotechnology Information (NCBI)
477 database (<https://www.ncbi.nlm.nih.gov/>), and a minimum spanning tree of the 278 strains was
478 made using PHYLOVIZ Online (Ribeiro-Gonçalves *et al.*, 2016). The MLST DNA sequences
479 can be found in the National Center for Biotechnology Information (BankIt) under gene
480 accession numbers MT864201-MT864291 and MT880889-MT880901,

481
482 **Genome sequencing, assembly, annotation, and analysis.** Nine strains were selected for
483 genome sequencing by either Illumina MiSeq (Illumina, San Diego, CA) (B1.1, WS1.1, 1B1,
484 AJ11, BGM37, EL11) or Pacific Biosciences (PacBio, Menlo Park, CA) (B1.3, 8.1, K4) DNA
485 sequencing methods. For the Illumina MiSeq, approximately 3×10^9 cells were suspended in lysis
486 buffer containing 200 mM NaCl, 20 mM EDTA, 500 μ l of 793 mM SDS and 300 mg of
487 zirconium beads (0.1 mm, BioSpec Products, Bartlesville, OK). The cells were then
488 mechanically lysed by bead-beating at 6.5m/s for 1 min with a FastPrep-24 (MP Biomedical,
489 Santa Ana, CA). To obtain larger DNA fragments appropriate for PacBio DNA sequencing, total
490 genomic DNA was extracted from each strain by incubating approximately 3×10^9 cells in the
491 presence of 20 mg/ml lysozyme (Sigma-Aldrich, St. Louis, MO) at 37 °C for 60 min. After

492 extraction by either mechanical or enzymatic lysis, DNA was purified using phenol-chloroform
493 and EtOH precipitation methods (Sambrook and Russell, 2006).

494 Illumina libraries were prepared for paired-end 250-bp sequencing (2 X 250 bp) using the
495 Nextera DNA Flex Library kit (Illumina, San Diego, CA). The libraries were sequenced at the
496 UC Davis Genome Center (Davis, CA) (<https://genomecenter.ucdavis.edu/>) on an Illumina
497 MiSeq V2 according to the manufacturer's protocol. Genomes were assembled with Spades
498 (v3.12.0, using k-mers 31, 51, 71), and QUAST (v 4.6.3) was used to confirm assembly quality.
499 The assembled genome sequences were then annotated with RASTtk and PATRIC (Wattam *et*
500 *al.*, 2017). PATRIC comprehensive genome analysis was run using default auto parameters. This
501 program encompasses BayesHammer for read error correction, Velvet, IDBA, and Spades for
502 assembly, and ARAST to verify assembly quality (Wattam *et al.*, 2017).

503 PacBio libraries were prepared and sequenced at the UC Davis Genome Center (Davis, CA)
504 (<https://genomecenter.ucdavis.edu/>) on a Pacific Biosciences RSII instrument using P6-C4
505 sequencing chemistry. Sequence SMRTcell files were imported into the PacBio SMRT portal
506 graphical interface unit (<https://www.pacb.com/>) for de novo assembly using the hierarchical
507 genome-assembly process (HGAP) protocol (Chin *et al.*, 2013) and RS HGAP Assembly 2 in
508 Smart analysis version 2.3 software. The resulting assemblies were used for subsequent
509 annotation with RASTtk (<https://rast.nmpdr.org/>) and PATRIC (Wattam *et al.*, 2017). The whole
510 genome sequencing data for this study can be found in the National Center for Biotechnology
511 Information under the BioProject PRJNA598971.

512 EDGAR 2.0 was used to evaluate the size of the pangenome and identify the number of
513 genes shared between all nine sequenced strains as well as to identify the phylogenetic
514 relationships between the different strains (Blom *et al.*, 2016). The pan and core genomes were

515 identified, and the results were presented as ortholog sets. To evaluate phylogenetic
516 relationships, concatenate core amino acid sequences were aligned using MUSCLE (Edgar,
517 2004). The resulting alignment was used to construct a phylogenetic tree using a maximum
518 likelihood method with bootstrapping in MEGA 7.0 (Kumar *et al.*, 2016). Anvi'o (v6.1) was
519 used to group orthologous protein sequences into gene clusters for Cluster of Orthologues Group
520 (COG) functional assignments using the program 'anvi-pan-genome' (Eren *et al.*, 2015; Delmont
521 and Eren, 2018) with the flags '-use-ncbi-blast' (Altschul *et al.*, 1990) and parameters '-minibit
522 0.5' (Benedict *et al.*, 2014) and 'mcl-inflation 10'. COG frequency heat map with hierarchical
523 clustering was generated using RStudio with the package 'pheatmap'
524 (<https://www.rstudio.com/>). To confirm the truncation of *ptsIBC*A in *L. plantarum* 8.1, the
525 *ptsIBC*A gene was amplified from genomic DNA from strains B1.3, K4, 8.1, and NCIMB8826R
526 using the *ptsIBC*A_trunF (5'- TCGTCACCGAGTGTTTCGTTT) and *ptsIBC*A_trunR (5'-
527 AGTTGCTGGCCACTGTTCAT) primers (Table S8) and *ExTaq* DNA polymerase (TaKaRa,
528 Shiga, Japan). Thermal cycling conditions were as follows: 95 °C for 3 min, 30 cycles of 94 °C
529 for 30 sec, 50 °C for 30 sec, and 72 °C for 90 sec, and a final elongation step of 72 °C for 5 min.
530 PCR products were visualized on a 1% agarose gel.

531
532 **Carbohydrate utilization.** *L. plantarum* strains were first incubated in cMRS for 24 h at 30 °C.
533 The cells were then collected by centrifugation at 5,000 x g for 5 min, washed twice in PBS to
534 remove residual nutrients (pH 7.2), and then suspended in a modified MRS (mMRS) without
535 beef extract or dextrose (pH 6.5) (De MAN *et al.*, 1960). The cell suspensions were then
536 distributed into 96-well microtiter plates (Thermo Fisher Scientific, Waltham, MA) at an optical
537 density (OD) at 600 nm (OD₆₀₀) of 0.2. To test the capacity to grow on different sugars, mMRS

538 was amended to contain 2% (w/v) of D-glucose (111 mM) (Fisher Scientific, Fair Lawn, NJ), D-
539 maltose monohydrate (55 mM) (Amresco, Solon, OH), sucrose (58 mM) (Sigma, St. Louis,
540 MO), D-galactose (111 mM) (Fisher Scientific, Fair Lawn, NJ), D-raffinose pentahydrate (40
541 mM) (VWR International, Solon, OH), D-fructose (55 mM) (Fisher Scientific, Fair Lawn, NJ),
542 D-xylose (133 mM) (Acros Organics, Morris Plains, NJ), D-ribose (133 mM) (Acros Organics,
543 Morris Plains, NJ), or L-arabinose (133 mM) (Acros Organics, Morris Plains, NJ). The OD₆₀₀
544 values were measured hourly for 48 h in a Synergy 2 microplate reader (Biotek, Winooski, VT)
545 set at 30 °C without aeration.

546

547 **Growth during exposure to EtOH, SDS, NaCl, and pH 3.5.** *L. plantarum* was incubated in
548 cMRS for 24 h at 30 °C. The cells were then collected by centrifugation at 5,000 x g for 5 min,
549 washed twice in PBS (pH 7.2), and then suspended in mMRS-glucose (2% (w/v) (111 mM) D-
550 glucose) (pH 6.5). The cell suspensions were then distributed into 96-well microtiter plates
551 containing mMRS-glucose amended to contain EtOH (8% (v/v) (174 mM) or 12% (v/v) (260
552 mM)), SDS (0.03% (w/v) (0.10 mM)), or NaCl (4% (w/v) (68 mM)). For measuring the effects
553 of low pH, mMRS-glucose was adjusted to pH 3.5 with 1 M HCl. For measuring the effect of
554 both low pH and high NaCl concentration, mMRS-glucose (pH 3.5) was supplemented with 4%
555 (w/v) (68 mM) NaCl. Each strain was also incubated in mMRS diluted with water between (4 -
556 12% (v/v)) to control for dilution of mMRS due to amendment addition. The OD₆₀₀ was used to
557 monitor growth during incubation at 30 °C for 48 h without aeration using a Synergy 2
558 microplate reader (Biotek, Winooski, VT).

559

560 **Survival at pH 2 or 50 °C.** For assessing acid tolerance, *L. plantarum* was incubated in cMRS
561 for 24 h at 30 °C prior to collection by centrifugation at 5,000 x g for 5 min and washing twice in
562 physiological saline (145 mM NaCl) (pH 7.0). *L. plantarum* was then inoculated at a
563 concentration of 1×10^8 cells/ml in physiological saline adjusted to pH 2 with 5 M HCl in 1.5mL
564 tubes. Survival was measured after 0, 15, 30, and 60 min incubation at 30 °C. At each time point,
565 three tubes were retrieved per stain for centrifugation at 10,000 x g for 1 min. The supernatant
566 was discarded, and the resulting cell pellet was suspended in 1mL physiological saline (pH 7.0).
567 Serial dilutions were then plated on cMRS agar and incubated at 30 °C for 48 h prior to colony
568 enumeration.

569

570 **Survival at 50 °C.** To measure thermal tolerance, *L. plantarum* was incubated in cMRS for 24 h
571 at 30 °C prior to collection by centrifugation at 5,000 x g for 5 min and washing twice in PBS
572 (pH 7.2). The suspensions were then distributed into 0.2 mL tubes at approximately 1×10^8
573 CFU/ml and incubated in a C1000 Thermal Cycler (Bio-Rad Laboratories, Foster City, CA) at 50
574 °C for 0, 15, 30, and 60 min. At each time point, three tubes were retrieved per strain. Serial
575 dilutions of the cell suspensions were plated onto cMRS agar and incubated at 30 °C for 48 h
576 prior to colony enumeration.

577

578 **Biofilm formation assay.** The potential for *L. plantarum* to form biofilms was assessed by
579 measuring adherence to polystyrene according to previously described methods (Kopit *et al.*,
580 2014) with several modifications. Briefly, 96-well polystyrene plates (Thermo Fisher Scientific,
581 Waltham, MA) containing either mMRS-glucose, mMRS-fructose, or mMRS-sucrose were
582 inoculated with *L. plantarum* to a starting OD₆₀₀ of 0.2 and the plates were incubated at 30 °C for

583 48 h. The wells were then rinsed with PBS (pH 7.2), stained with 0.05% (w/v) crystal violet
584 (CV), dried in an inverted position for 30 min, and then rinsed again three times with PBS (pH
585 7.2). Absorbance at OD₅₉₅ was measured with a Synergy 2 microplate reader (Biotek, Winooski,
586 VT) to determine adherence. Wells containing mMRS with the corresponding sugar without *L.*
587 *plantarum* inoculum were included as controls.

588

589 **Yeast inhibition assay.** *L. plantarum* cell-free culture supernatants (CFCS) were prepared from
590 the spent media collected after *L. plantarum* incubation in cMRS for 24 h at 30 °C. CFCS was
591 collected by centrifugation at 4,000 x g for 10 min at 4 °C followed by filtration of the
592 supernatant through a 0.45 µm polyethersulfone (PES) filter (Genesee Scientific, San Diego,
593 CA). To eliminate the effects of differences of pH on yeast inhibition, the CFCS was adjusted
594 with lactic acid (1.3 M) to pH 3.8, the lowest pH reached by *L. plantarum* after incubation in
595 cMRS (data not shown). *S. cerevisiae* UCDFST 09-448 (Golomb *et al.*, 2013), a strain shown to
596 cause olive tissue damage and spoilage during olive fermentations, was grown in Yeast Mold
597 (YM) broth (BD, Franklin Lakes, NJ) for 24 h at 30 °C with aeration at 250 rpm. Cells were
598 collected by centrifugation at 20,000 x g for 5 min at 4 °C and then washed twice with PBS. *S.*
599 *cerevisiae* UCDFST 09-448 was then inoculated into 96-well microtiter plates containing 1:1
600 ratio of 2X YM and CFCS at a starting OD₆₀₀ of 0.05. OD₆₀₀ was measured in a Synergy 2
601 microplate reader (Biotek, Winooski, VT) set at 30 °C for 24 h aerated every hour by shaking for
602 10 sec before each read. Controls included *S. cerevisiae* UCDFST 09-448 incubated in YM and
603 YM supplemented with cMRS (pH 3.8).

604

605 **Statistical analysis.** Area under the curve (AUC) was used to examine the growth and survival
606 of *L. plantarum* under different conditions (Sprouffske and Wagner, 2016). The AUC was
607 calculated with GraphPad Prism 8 (Graph Pad Software, San Diego, CA). Hierarchical clustering
608 was generated using RStudio with the package ‘pheatmap’ based on AUC values
609 (<https://www.rstudio.com/>). Unpaired, two-tailed Student t-tests were used to compare between
610 the different *L. plantarum* groups (e.g., brine- and grain-based fermentations). P values of <0.05
611 were considered significant.

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618 619 **Conflict of Interest**

620 The authors declare that the research was conducted in the absence of any commercial or
621 financial relationships that could be construed as a potential conflict of interest.

623 624 **References**

625 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local
626 alignment search tool. *J Mol Biol* **215**: 403–410.

627
628 Aquilanti, L., Santarelli, S., Silvestri, G., Osimani, A., Petruzzelli, A., and Clementi, F. (2007)
629 The microbial ecology of a typical Italian salami during its natural fermentation. *Int J of Food*
630 *Microbiol* **120**: 136–145.

631
632 Barache, N., Ladjouzi, R., Belguesmia, Y., Bendali, F., and Drider, D. (2020) Abundance of
633 *Lactobacillus plantarum* strains with beneficial attributes in blackberries (*Rubus sp.*), fresh figs

- 634 (*Ficus carica*), and prickly pears (*Opuntia ficus-indica*) grown and harvested in Algeria.
635 *Probiotics & Antimicro Prot.*
- 636
- 637 Benedict, M.N., Henriksen, J.R., Metcalf, W.W., Whitaker, R.J., and Price, N.D. (2014) ITEP:
638 An integrated toolkit for exploration of microbial pan-genomes. *BMC Genomics* **15**: 8.
639
- 640 Blom, J., Kreis, J., Spänig, S., Juhre, T., Bertelli, C., Ernst, C., and Goesmann, A. (2016)
641 EDGAR 2.0: an enhanced software platform for comparative gene content analyses. *Nucleic*
642 *Acids Res* **44**: W22–W28.
643
- 644 Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., et al. (2011)
645 Why intraspecific trait variation matters in community ecology. *Trends Eco Evol* **26**: 183–192.
646
- 647 Bongrand, C. and Ruby, E.G. (2019) Achieving a multi-strain symbiosis: strain behavior and
648 infection dynamics. *ISME J* **13**: 698–706.
649
- 650 Bravo-Ferrada, B.M., Gonçalves, S., Semorile, L., Santos, N.C., Tymczyszyn, E.E., and
651 Hollmann, A. (2015) Study of surface damage on cell envelope assessed by AFM and flow
652 cytometry of *Lactobacillus plantarum* exposed to ethanol and dehydration. *J Appl Microbio* **118**:
653 1409–1417.
654
- 655 Brizuela, N., Tymczyszyn, E.E., Semorile, L.C., Valdes La Hens, D., Delfederico, L., Hollmann,
656 A., and Bravo-Ferrada, B. (2019) *Lactobacillus plantarum* as a malolactic starter culture in
657 winemaking: A new (old) player? *Electron J Biotech* **38**: 10–18.
658
- 659 Cataldi, T.R.I., Margiotta, G., Iasi, L., Di Chio, B., Xiloyannis, C., and Bufo, S.A. (2000)
660 Determination of sugar compounds in olive plant extracts by anion-exchange chromatography
661 with pulsed amperometric detection. *Anal Chem* **72**: 3902–3907.
662
- 663 Chin, C.-S., Alexander, D.H., Marks, P., Klammer, A.A., Drake, J., Heiner, C., et al. (2013)
664 Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat*
665 *Methods* **10**: 563–569.
666
- 667 Choi, S., Jin, G.-D., Park, J., You, I., and Kim, E.B. (2018) Pan-genomics of *Lactobacillus*
668 *plantarum* revealed group-specific genomic profiles without habitat association. *J Microbiol*
669 *Biotechnol* **28**: 1352–1359.
670
- 671 Ciocia, F., McSweeney, P.L.H., Piraino, P., and Parente, E. (2013) Use of dairy and non-dairy
672 *Lactobacillus plantarum*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus* strains as
673 adjuncts in cheddar cheese. *Dairy Sci & Technol* **93**: 623–640.
674
- 675 Corcoran, B.M., Stanton, C., Fitzgerald, G., and Ross, R.P. (2008) Life under stress: the
676 probiotic stress response and how it may be manipulated. *Curr Pharm Des* **14**: 1382–1399.
677

- 678 Crakes, K.R., Rocha, C.S., Grishina, I., Hirao, L.A., Napoli, E., Gaulke, C.A., et al. (2019)
679 PPAR α -targeted mitochondrial bioenergetics mediate repair of intestinal barriers at the host-
680 microbe intersection during SIV infection. *PNAS* **116**: 24819–24829.
681
- 682 De MAN, J.C., Rogosa, M., and Sharpe, M.E. (1960) A medium for the cultivation of
683 *Lactobacilli*. *Journal of Applied Bacteriology* **23**: 130–135.
684
- 685 Delgado, S., Flórez, A.B., and Mayo, B. (2005) Antibiotic Susceptibility of *Lactobacillus* and
686 *Bifidobacterium* species from the human gastrointestinal tract. *Curr Microbiol* **50**: 202–207.
687
- 688 Delmont, T.O. and Eren, A.M. (2018) Linking pangenomes and metagenomes: the
689 *Prochlorococcus* metapangenome. *PeerJ* **6**: e4320.
690
- 691 Di Cagno, R., Surico, R.F., Siragusa, S., De Angelis, M., Paradiso, A., Minervini, F., et al.
692 (2008) Selection and use of autochthonous mixed starter for lactic acid fermentation of carrots,
693 French beans or marrows. *Int J Food Microbiol* **127**: 220–228.
694
- 695 Duar, R.M., Lin, X.B., Zheng, J., Martino, M.E., Grenier, T., Pérez-Muñoz, M.E., et al. (2017)
696 Lifestyles in transition: evolution and natural history of the genus *Lactobacillus*. *FEMS*
697 *Microbiol Rev* **41**: S27–S48.
698
- 699 Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high
700 throughput. *Nucleic Acids Res* **32**: 1792–1797.
701
- 702 Ehlers, B.K., Damgaard, C.F., and Laroche, F. (2016) Intraspecific genetic variation and species
703 coexistence in plant communities. *Biol Lett* **12**: 20150853.
704
- 705 Ellegaard, K.M., Tamarit, D., Javelind, E., Olofsson, T.C., Andersson, S.G., and Vásquez, A.
706 (2015) Extensive intra-phylo-type diversity in lactobacilli and bifidobacteria from the honeybee
707 gut. *BMC Genomics* **16**: 284.
708
- 709 Endo, A., Maeno, S., Tanizawa, Y., Kneifel, W., Arita, M., Dicks, L., and Salminen, S. (2018)
710 Fructophilic lactic acid bacteria, a unique group of fructose-fermenting microbes. *Appl Environ*
711 *Microbiol* **84**: e01290-18.
712
- 713 Ercolini, D. (2017) Exciting strain-level resolution studies of the food microbiome. *Microb*
714 *Biotechnol* **10**: 54–56.
715
- 716 Eren, A.M., Esen, Ö.C., Quince, C., Vineis, J.H., Morrison, H.G., Sogin, M.L., and Delmont,
717 T.O. (2015) Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* **3**:
718 e1319.
719
- 720 Fernández Ramírez, M.D., Smid, E.J., Abee, T., and Nierop Groot, M.N. (2015) Characterization
721 of biofilms formed by *Lactobacillus plantarum* WCFS1 and food spoilage isolates. *Int J Food*
722 *Microbio* **207**: 23–29.
723

- 724 Ferrando, V., Quiberoni, A., Reinheimer, J., and Suárez, V. (2016) Functional properties of
725 *Lactobacillus plantarum* strains: A study in vitro of heat stress influence. *Food Microbiol* **54**:
726 154–161.
727
- 728 Ferrando, V., Quiberoni, A., Reinheimer, J., and Suárez, V. (2015) Resistance of functional
729 *Lactobacillus plantarum* strains against food stress conditions. *Food Microbiol* **48**: 63–71.
730
- 731 Filannino, P., Cardinali, G., Rizzello, C.G., Buchin, S., Angelis, M.D., Gobbetti, M., and Cagno,
732 R.D. (2014) Metabolic responses of *Lactobacillus plantarum* strains during fermentation and
733 storage of vegetable and fruit juices. *Appl Environ Microbiol* **80**: 2206–2215.
734
- 735 France, M.T., Mendes-Soares, H., and Forney, L.J. (2016) Genomic comparisons of
736 *Lactobacillus crispatus* and *Lactobacillus iners* reveal potential ecological drivers of community
737 composition in the vagina. *Appl Environ Microbiol* **82**: 7063–7073.
738
- 739 Gänzle, M.G. and Follador, R. (2012) Metabolism of oligosaccharides and starch in Lactobacilli:
740 A Review. *Front Microbiol* **3**: 340.
741
- 742 Gheziel, C., Russo, P., Arena, M.P., Spano, G., Ouzari, H.-I., Kheroua, O., et al. (2019)
743 Evaluating the probiotic potential of *Lactobacillus plantarum* strains from Algerian infant feces:
744 towards the design of probiotic starter cultures tailored for developing countries. *Probiotics &*
745 *Antimicro Prot* **11**: 113–123.
746
- 747 Golomb, B.L., Morales, V., Jung, A., Yau, B., Boundy-Mills, K.L., and Marco, M.L. (2013)
748 Effects of pectinolytic yeast on the microbial composition and spoilage of olive fermentations.
749 *Food Microbiol* **33**: 97–106.
750
- 751 Gómez-González, S., Ruiz-Jiménez, J., Priego-Capote, F., and Luque de Castro, M.D. (2010)
752 Qualitative and quantitative sugar profiling in olive fruits, leaves, and stems by gas
753 chromatography–tandem mass spectrometry (GC-MS/MS) after ultrasound-assisted leaching. *J*
754 *Agric Food Chem* **58**: 12292–12299.
755
- 756 Görke, B. and Stülke, J. (2008) Carbon catabolite repression in bacteria: many ways to make the
757 most out of nutrients. *Nat Rev Microbiol* **6**: 613–624.
758
- 759 van de Guchte, M., Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., et al. (2006) The
760 complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive
761 evolution. *Proc Natl Acad Sci U S A* **103**: 9274–9279.
762
- 763 Guidone, A., Zotta, T., Ross, R.P., Stanton, C., Rea, M.C., Parente, E., and Ricciardi, A. (2014)
764 Functional properties of *Lactobacillus plantarum* strains: A multivariate screening study. *LWT -*
765 *Food Sci and Technol* **56**: 69–76.
766
- 767 Hayward, A.C. and Davis, G.H.G. (1956) The isolation and classification of *Lactobacillus*
768 strains from Italian saliva samples. *Br Dent J* **101**: 2733–2741.
769

- 770 Hurtado, A., Reguant, C., Bordons, A., and Rozès, N. (2012) Lactic acid bacteria from fermented
771 table olives. *Food Microbiol* **31**: 1–8.
772
- 773 Jose, N.M., Bunt, C.R., and Hussain, M.A. (2015) Comparison of microbiological and probiotic
774 characteristics of Lactobacilli isolates from dairy food products and animal rumen contents.
775 *Microorganisms* **3**: 198–212.
776
- 777 Koch, H., Germscheid, N., Freese, H.M., Noriega-Ortega, B., Lücking, D., Berger, M., et al.
778 (2020) Genomic, metabolic and phenotypic variability shapes ecological differentiation and
779 intraspecies interactions of *Alteromonas macleodii*. *Sci Rep* **10**: 809.
780
- 781 Kopit, L.M., Kim, E.B., Siezen, R.J., Harris, L.J., and Marco, M.L. (2014) Safety of the
782 surrogate microorganism *Enterococcus faecium* NRRL B-2354 for use in thermal process
783 validation. *Appl Environ Microbiol* **80**: 1899–1909.
784
- 785 Kremling, A., Geiselman, J., Ropers, D., and de Jong, H. (2015) Understanding carbon
786 catabolite repression in *Escherichia coli* using quantitative models. *Trends in Microbiol* **23**: 99–
787 109.
788
- 789 Kumar, S., Stecher, G., and Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics
790 Analysis Version 7.0 for bigger datasets. *Mol Biol Evol* **33**: 1870–1874.
791
- 792 Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A.B., et al. (2017)
793 Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* **550**: 61–
794 66.
795
- 796 Ma, B., France, M.T., Crabtree, J., Holm, J.B., Humphrys, M.S., Brotman, R.M., and Ravel, J.
797 (2020) A comprehensive non-redundant gene catalog reveals extensive within-community
798 intraspecies diversity in the human vagina. *Nature Commun* **11**: 1–13.
799
- 800 Marco, M. (2010) *Lactobacillus plantarum* in foods. In *Encyclopedia of Biotechnology in*
801 *Agriculture and Food*. CRC Press, pp. 360–362.
802
- 803 Martino, M.E., Bayjanov, J.R., Caffrey, B.E., Wels, M., Joncour, P., Hughes, S., et al. (2016)
804 Nomadic lifestyle of *Lactobacillus plantarum* revealed by comparative genomics of 54 strains
805 isolated from different habitats. *Environ Microbiol* **18**: 4974–4989.
806
- 807 Maynard, D.S., Serván, C.A., Capitán, J.A., and Allesina, S. (2019) Phenotypic variability
808 promotes diversity and stability in competitive communities. *Ecology Letters* **22**: 1776–1786.
809
- 810 Miller, E.R., Kearns, P.J., Niccum, B.A., O'Mara Schwartz, J., Ornstein, A., and Wolfe, B.E.
811 (2019) Establishment Limitation constrains the abundance of lactic acid bacteria in the Napa
812 cabbage phyllosphere. *Appl Environ Microbiol* **85**: e00269-19.
813
- 814 Mills, S., Stanton, C., Fitzgerald, G.F., and Ross, R.P. (2011) Enhancing the stress responses of
815 probiotics for a lifestyle from gut to product and back again. *Microb Cell Fact* **10**: S19.

816
817 Molenaar, D., Bringel, F., Schuren, F.H., Vos, W.M. de, Siezen, R.J., and Kleerebezem, M.
818 (2005) Exploring *Lactobacillus plantarum* genome diversity by using microarrays. *J Bacteriol*
819 **187**: 6119–6127.
820
821 Mukhopadhyay, A. (2015) Tolerance engineering in bacteria for the production of advanced
822 biofuels and chemicals. *Trends in Microbiol* **23**: 498–508.
823
824 Papadimitriou, K., Alegría, Á., Bron, P.A., Angelis, M. de, Gobbetti, M., Kleerebezem, M., et al.
825 (2016) Stress physiology of lactic acid bacteria. *Microbiol Mol Biol Rev* **80**: 837–890.
826
827 Parente, E., Ciocia, F., Ricciardi, A., Zotta, T., Felis, G.E., and Torriani, S. (2010) Diversity of
828 stress tolerance in *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus*
829 *paraplantarum*: A multivariate screening study. *Int J Food Microbiol* **144**: 270–279.
830
831 Parichehreh, S., Tahmasbi, G., Sarafrazi, A., Imani, S., and Tajabadi, N. (2018) Isolation and
832 identification of *Lactobacillus* bacteria found in the gastrointestinal tract of the dwarf honeybee,
833 *Apis florea* Fabricius, 1973 (Hymenoptera: Apidae). *Apidologie* **49**: 430–438.
834
835 Prete, R., Long, S.L., Joyce, S.A., and Corsetti, A. (2020) Genotypic and phenotypic
836 characterization of food-associated *Lactobacillus plantarum* isolates for potential probiotic
837 activities. *FEMS Microbiol Lett*.
838
839 Props, R. and Deneff, V.J. (2020) Temperature and nutrient levels correspond with lineage-
840 specific microdiversification in the ubiquitous and abundant freshwater genus *Limnohabitans*.
841 *Appl Environ Microbiol* **86**: e00140–20.
842
843 Ribeiro-Gonçalves, B., Francisco, A.P., Vaz, C., Ramirez, M., and Carriço, J.A. (2016)
844 PHYLOViZ Online: web-based tool for visualization, phylogenetic inference, analysis and
845 sharing of minimum spanning trees. *Nucleic Acids Res* **44**: W246–W251.
846
847 Salvetti, E., Harris, H.M.B., Felis, G.E., and O’Toole, P.W. (2018) Comparative genomics of the
848 genus *Lactobacillus* reveals robust phylogroups that provide the basis for reclassification. *Appl*
849 *Environ Microbiol* **84**: e00993-18.
850
851 Sambrook, J. and Russell, D.W. (2006) Purification of Nucleic Acids by Extraction with
852 Phenol:Chloroform. *Cold Spring Harb Protoc* **2006**: pdb.prot4455.
853
854 Saulnier, D.M.A., Molenaar, D., Vos, W.M. de, Gibson, G.R., and Kolida, S. (2007)
855 Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum* WCFS1
856 through microarrays. *Appl Environ Microbiol* **73**: 1753–1765.
857
858 Seddik, H.A., Bendali, F., Gancel, F., Fliss, I., Spano, G., and Drider, D. (2017) *Lactobacillus*
859 *plantarum* and its probiotic and food potentialities. *Probiotics & Antimicro Prot* **9**: 111–122.
860

- 861 Seddon, A.M., Curnow, P., and Booth, P.J. (2004) Membrane proteins, lipids and detergents: not
862 just a soap opera. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1666**: 105–117.
863
- 864 Siezen, R.J. and van Hylekama Vlieg, J.E. (2011) Genomic diversity and versatility of
865 *Lactobacillus plantarum*, a natural metabolic engineer. *Microb Cell Fact* **10**: S3.
866
- 867 Siezen, R.J., Tzeneva, V.A., Castioni, A., Wels, M., Phan, H.T.K., Rademaker, J.L.W., et al.
868 (2010) Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from
869 various environmental niches. *Environ Microbiol* **12**: 758–773.
870
- 871 Siragusa, S., De Angelis, M., Calasso, M., Campanella, D., Minervini, F., Di Cagno, R., and
872 Gobbetti, M. (2014) Fermentation and proteome profiles of *Lactobacillus plantarum* strains
873 during growth under food-like conditions. *J Proteom* **96**: 366–380.
874
- 875 Sprouffske, K. and Wagner, A. (2016) Growthcurver: an R package for obtaining interpretable
876 metrics from microbial growth curves. *BMC Bioinformatics* **17**: 172.
877
- 878 Succi, M., Pannella, G., Tremonte, P., Tipaldi, L., Coppola, R., Iorizzo, M., et al. (2017) Sub-
879 optimal pH preadaptation improves the survival of *Lactobacillus plantarum* strains and the malic
880 acid consumption in wine-like medium. *Front Microbiol* **8**: 470.
881
- 882 Sun, Z., Harris, H.M.B., McCann, A., Guo, C., Argimón, S., Zhang, W., et al. (2015) Expanding
883 the biotechnology potential of lactobacilli through comparative genomics of 213 strains and
884 associated genera. *Nat Commun* **6**: 8322.
885
- 886 Torriani, S., Felis, G.E., and Dellaglio, F. (2001) Differentiation of *Lactobacillus plantarum*, *L.*
887 *pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with
888 *recA* gene-derived primers. *Appl Environ Microbiol* **67**: 3450–3454.
889
- 890 Truong, D.T., Tett, A., Pasolli, E., Huttenhower, C., and Segata, N. (2017) Microbial strain-level
891 population structure and genetic diversity from metagenomes. *Genome Res* **27**: 626–638.
892
- 893 Tyler, C. a., Kopit, L., Doyle, C., Yu, A. o., Hugenholtz, J., and Marco, M. l. (2016) Polyol
894 production during heterofermentative growth of the plant isolate *Lactobacillus florum* 2F. *J Appl*
895 *Microbiol* **120**: 1336–1345.
896
- 897 Veen, H. van B. de, Abee, T., Tempelaars, M., Bron, P.A., Kleerebezem, M., and Marco, M.L.
898 (2011) Short- and long-term adaptation to ethanol stress and its cross-protective consequences in
899 *Lactobacillus plantarum*. *Appl Environ Microbiol* **77**: 5247–5256.
900
- 901 Wattam, A.R., Davis, J.J., Assaf, R., Boisvert, S., Brettin, T., Bun, C., et al. (2017)
902 Improvements to PATRIC, the all-bacterial bioinformatics database and analysis Resource
903 Center. *Nucleic Acids Res* **45**: D535–D542.
904

- 905 Westby, A., Nuraida, L., Owens, J.D., and Gibbs, P.A. (1993) Inability of *Lactobacillus*
906 *plantarum* and other lactic acid bacteria to grow on D-ribose as sole source of fermentable
907 carbohydrate. *J Appl Bacteriol* **75**: 168–175.
908
- 909 Xu, H., Sun, Z., Liu, W., Yu, J., Song, Y., Lv, Q., et al. (2014) Multilocus sequence typing of
910 *Lactococcus lactis* from naturally fermented milk foods in ethnic minority areas of China. *J*
911 *Dairy Sci* **97**: 2633–2645.
912
- 913 Yang, J., Cao, Y., Cai, Y., and Terada, F. (2010) Natural populations of lactic acid bacteria
914 isolated from vegetable residues and silage fermentation. *J Dairy Sci* **93**: 3136–3145.
915
- 916 Yin, W., Wang, Y., Liu, L., and He, J. (2019) Biofilms: The microbial “protective clothing” in
917 extreme environments. *Int J Mol Sci* **20**: 3423.
918
- 919 Yin, X., Heeney, D.D., Srisengfa, Y.T., Chen, S.-Y., Slupsky, C.M., and Marco, M.L. (2018)
920 Sucrose metabolism alters *Lactobacillus plantarum* survival and interactions with the microbiota
921 in the digestive tract. *FEMS Microbiol Ecol* **94**: fiy084.
922
- 923 Yu, A.O., Leveau, J.H.J., and Marco, M.L. (2020) Abundance, diversity and plant-specific
924 adaptations of plant-associated lactic acid bacteria. *Environ Microbiol Rep* **12**: 16–29.
925
- 926 Zago, M., Scaltriti, E., Bonvini, B., Fornasari, M.E., Penna, G., Massimiliano, L., et al. (2017)
927 Genomic diversity and immunomodulatory activity of *Lactobacillus plantarum* isolated from
928 dairy products. *Benef Microbes* **8**: 597–604.
929
- 930 Zaragoza, J., Bendiks, Z., Tyler, C., Kable, M.E., Williams, T.R., Luchkovska, Y., et al. (2017)
931 Effects of exogenous yeast and bacteria on the microbial population dynamics and outcomes of
932 olive fermentations. *mSphere* **2**: e00315-16.
933
- 934 Zheng, J., Ruan, L., Sun, M., and Gänzle, M. (2015) A genomic view of *Lactobacilli* and
935 *Pediococci* demonstrates that phylogeny matches ecology and physiology. *Appl Environ*
936 *Microbiol* **81**: 7233–7243.
937
- 938 Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., et al. (2020)
939 A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended
940 description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and
941 *Leuconostocaceae*. *Int J Syst Evol Microbiol* **70**: 2782–2858.
942

Table 1. *L. plantarum* strains used in this study.

Strain name	Isolation source	Isolation date ^a	References
AJ11	Fermented olives; commercial fermentation	12/02/2010	Golomb <i>et al.</i> 2013
BGM55	Fermented olives; pilot- scale fermentation inoculated with <i>S.</i> <i>cerevisiae</i> 09-448	03/07/2011	Golomb <i>et al.</i> 2013
BGM37	Olive fermentation brine; commercial fermentation	01/04/2011	Golomb <i>et al.</i> 2013
BGM40	Fermented olives; commercial fermentation	01/26/2011	Golomb <i>et al.</i> 2013
EL11	Fermented olives; commercial fermentation	12/04/2009	Golomb <i>et al.</i> 2013
K4	Wheat sourdough starter	09/15/2014	This study
8.1	Wheat boza	09/15/2014	This study
W1.1	White flour teff injera	04/04/2015	This study
B1.1	Brown flour teff injera	04/04/2015	This study
B1.3	Brown flour teff injera	04/04/2015	This study
T2.5	Fermented tomatoes	08/20/2015	This study
WS1.1	Fermented tomatoes (spoiled)	08/20/2015	This study
1B1	Ripe cactus fruit (<i>Opuntia</i> <i>ficus-indicia</i>)	10/25/2011	Tyler <i>et al.</i> 2016
NCIMB8826R	Human saliva	N/A	Yin <i>et al.</i> 2018

^a Month/Day/Year. N/A, not available

Table 2. *L. plantarum* genome coverage and assembly statistics.

Strain ^a	Accession No.	Genome Size (Mb)	# of Contigs	Coverage	N50	L50	% GC Content	# of CDS
AJ11	WWDD00000000	3.27	29	27X	252487	6	44.54	3,214
BGM37	WWDC00000000	3.46	46	66X	155998	7	44.15	3,467
EL11	WWDB00000000	3.28	29	128X	1944449	5	44.31	3,231
K4	WWDF00000000	3.16	3	148X	3157988	1	44.60	3,088
8.1	WWDE00000000	3.37	9	140X	3066287	1	44.40	3,366
B1.1	WWCZ00000000	3.17	120	76X	59472	19	44.55	3,242
B1.3	WWCY00000000	3.09	5	145X	2939357	1	44.50	3,157
WS1.1	WWDA00000000	3.51	99	30X	78600	12	44.11	3,613
1B1	WWDG00000000	3.34	60	28X	109565	11	44.34	3,371

^a The genomes of AJ11, EL11, BGM37, WS1.1, and 1B1 were sequenced by Illumina MiSeq V2 (2 X 250). The genomes of strains

K4, 8.1, and B1.3 were sequenced by PacBio RSII (P6-C4 sequencing chemistry).

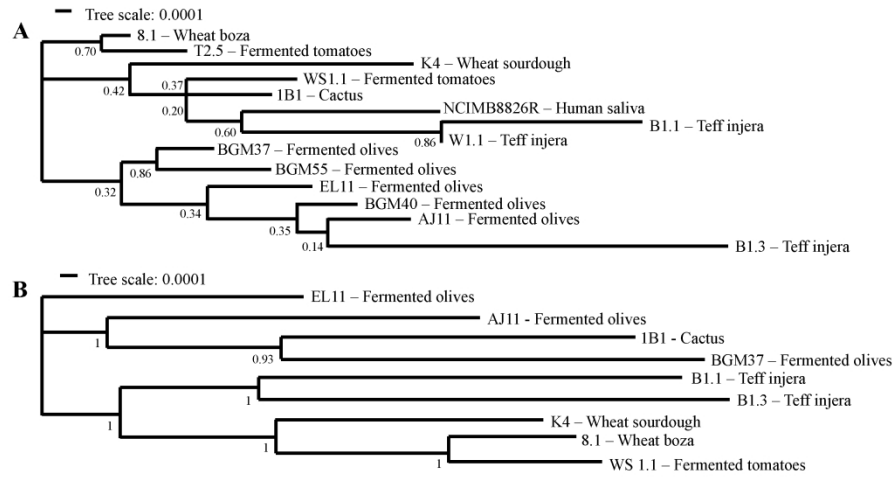


Fig. 1. Phylogenetic relationships between *L. plantarum* strains. (A) Phylogenetic relationships of 14 strains of *L. plantarum* based on MLST profiles with *pheS*, *pyrG*, *uvrC*, *recA*, *clpX*, *murC*, *groEL*, and *murE* (Table S8) and (B) nine *L. plantarum* strains based on concatenated core protein sequences using the maximum likelihood method with bootstrap values calculated from 500 replicates using MEGA (7.0) (Kumar et al., 2016).

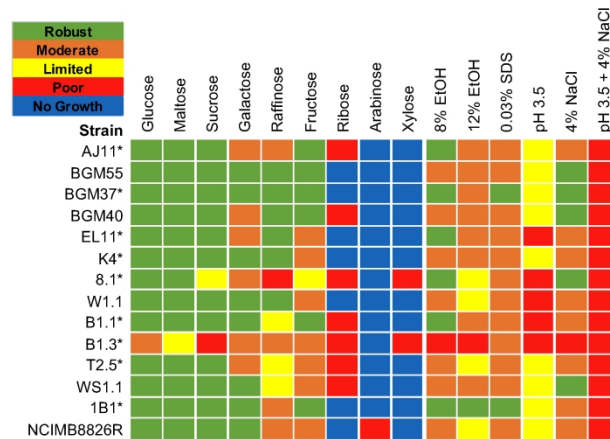


Fig. 2. *L. plantarum* phenotype profiles. Area under the curve (AUC) values were used to illustrate *L. plantarum* capacities to grow in mMRS containing different sugars and in mMRS-glucose in the presence of 8% (v/v) EtOH, 8% (v/v) EtOH and then 12% (v/v) EtOH (12% EtOH), 0.03% (w/v) SDS, 4% (w/v) NaCl or set at pH 3.5 without or with 4% (w/v) NaCl. AUC values for the growth curves were ranked as "robust" (AUC between 150 and 115), "moderate" (AUC between 114 and 80), "limited" (AUC between 79 and 45), "poor" (AUC < 45), or "no growth" (AUC was equivalent to the strain growth in mMRS lacking a carbohydrate source). *L. plantarum* growth in mMRS-glucose supplemented with an equal volume of water instead of EtOH, NaCl, or SDS was not significantly different compared to growth in mMRS-glucose ($p > 0.05$). * indicates strains examined by whole genome sequencing.

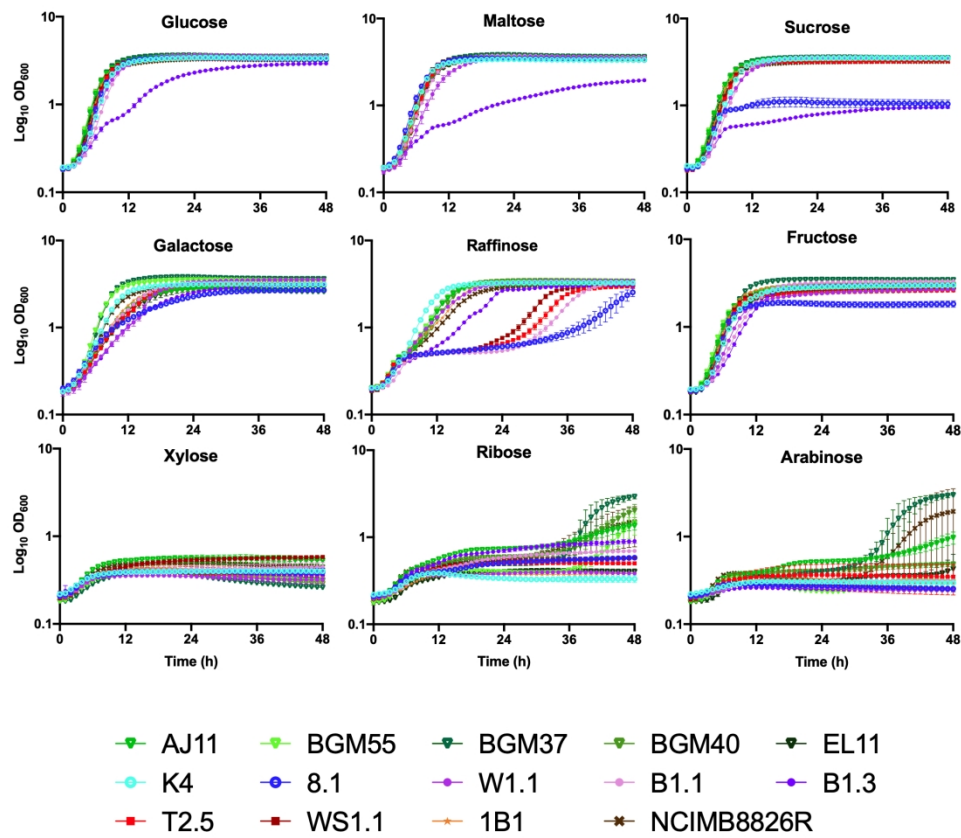


Fig. 3. Growth of *L. plantarum* in mMRS containing different mono-, di-, and tri-saccharides. *L. plantarum* was incubated in mMRS containing 2% (w/v) of each sugar at 30 °C for 48 h. The avg \pm stdev OD600 of three replicates for each strain are shown.

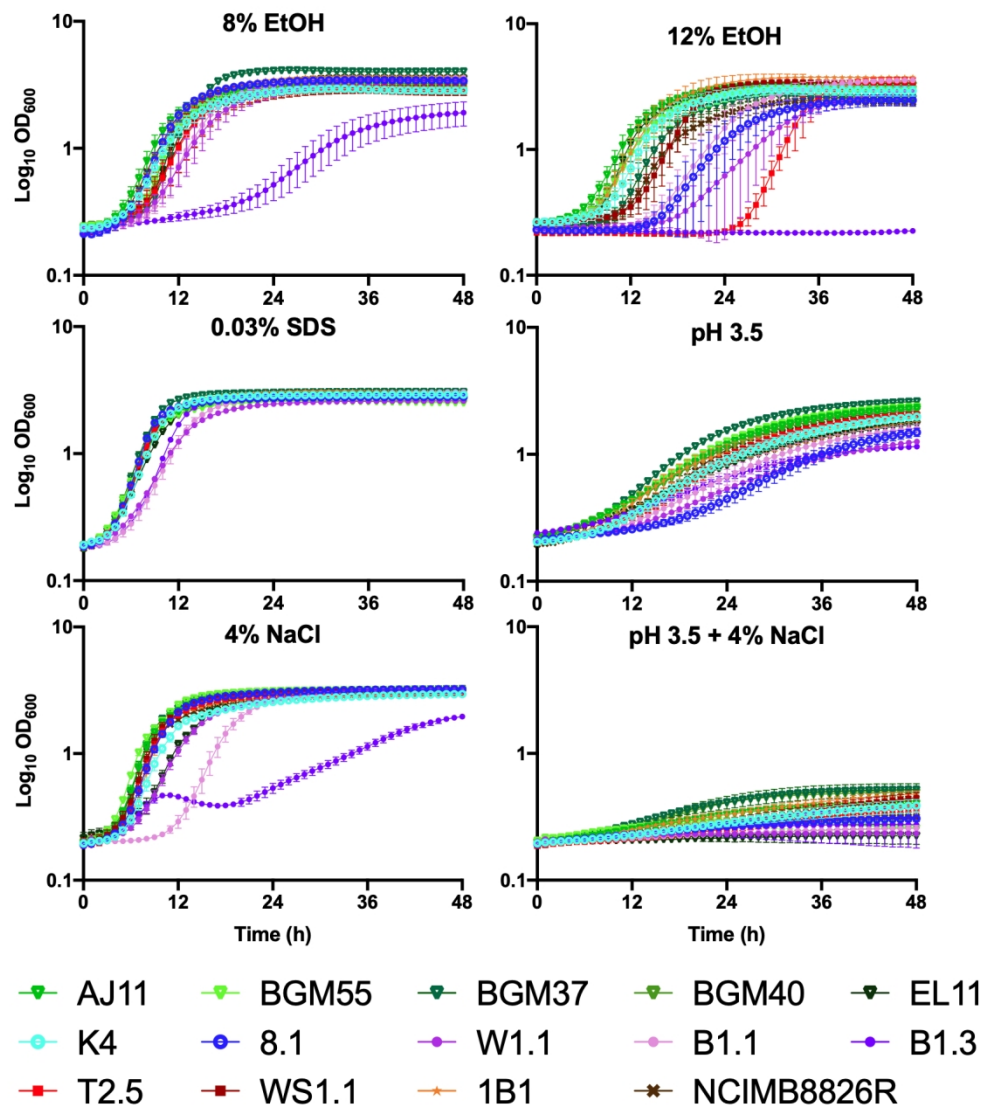


Fig 4. Growth of *L. plantarum* in mMRS-glucose exposed to different environmental stressors. *L. plantarum* was incubated in mMRS-glucose containing 8% (v/v) EtOH, 12% (v/v) EtOH, 0.03% (w/v) SDS, or 4% (w/v) NaCl with or without adjustment to pH 3.5 and incubated at 30 °C for 48 h. The avg ± stdev OD600 of three replicates for each strain are shown.

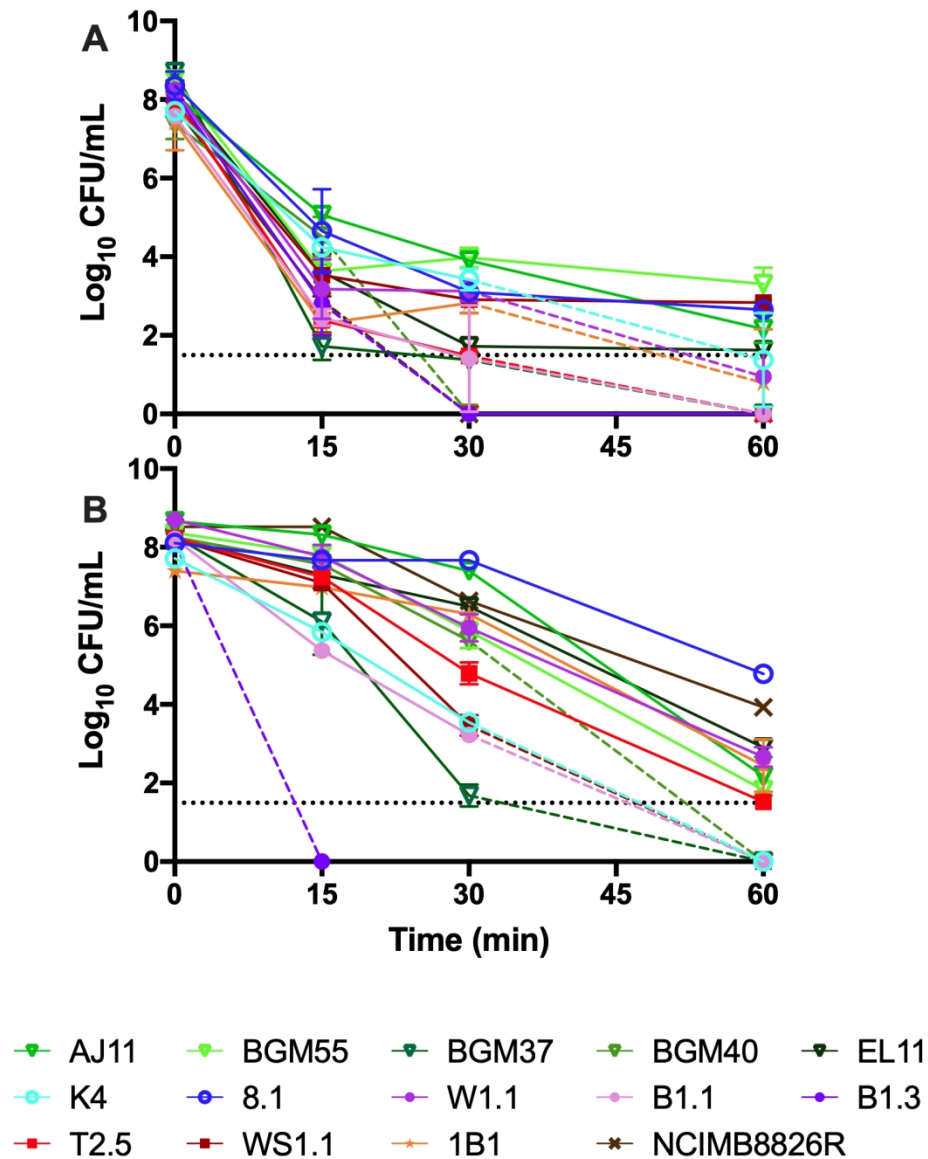


Fig 5. Survival of *L. plantarum* at (A) pH 2 and at (B) 50 °C. (A) Viable cells were enumerated after 0, 15, 30, and 60 min of incubation in physiological saline at pH 2 or (B) in PBS at 50 °C. The dashed lines indicate when the number of viable cells were below the detection limit (34 CFU/mL). The avg \pm stdev CFU/mL values of three replicates for each strain are shown.

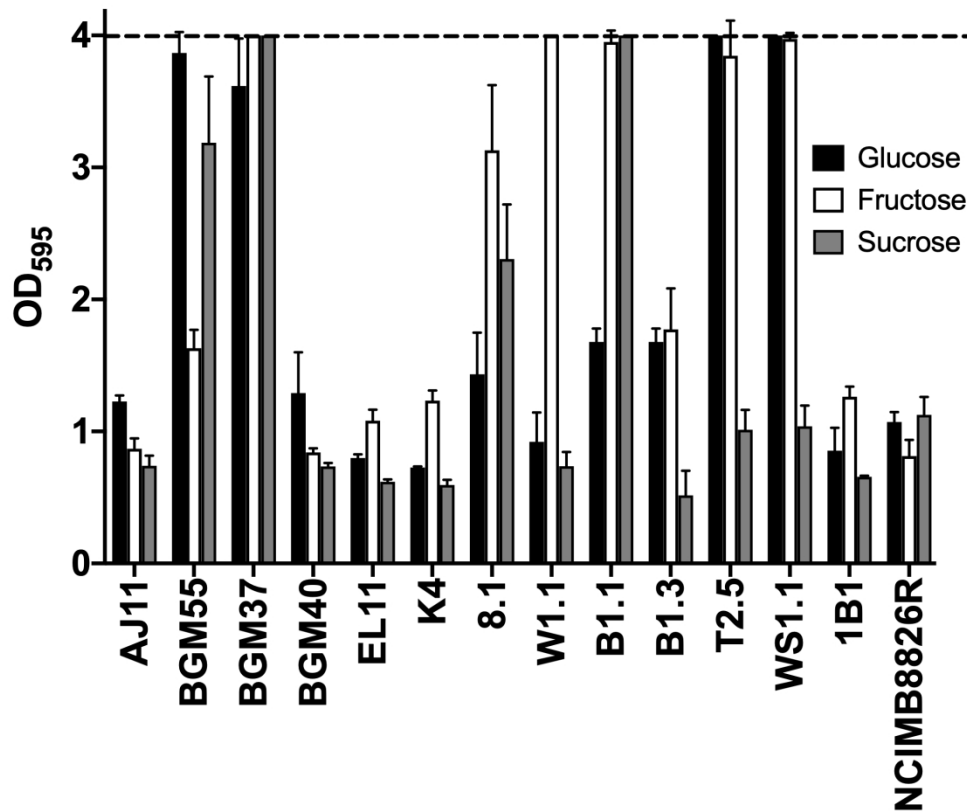


Fig 6. *L. plantarum* biofilm formation during growth in mMRS with glucose, fructose, or sucrose. *L. plantarum* was incubated in mMRS-glucose, mMRS-fructose, and mMRS-sucrose in 96-well, polystyrene microtiter plates at 30 °C for 48 h. The non-adherent cells were removed by washing with PBS. The remaining cells were stained with 0.05% crystal violet (CV). OD₅₉₅ values of wells without cells did not exceed 0.22. The upper detection limit as indicated by the stippled line was an OD₅₉₅ of 4.0. The avg ± stdev OD₅₉₅ of three replicate wells after CV staining are shown.

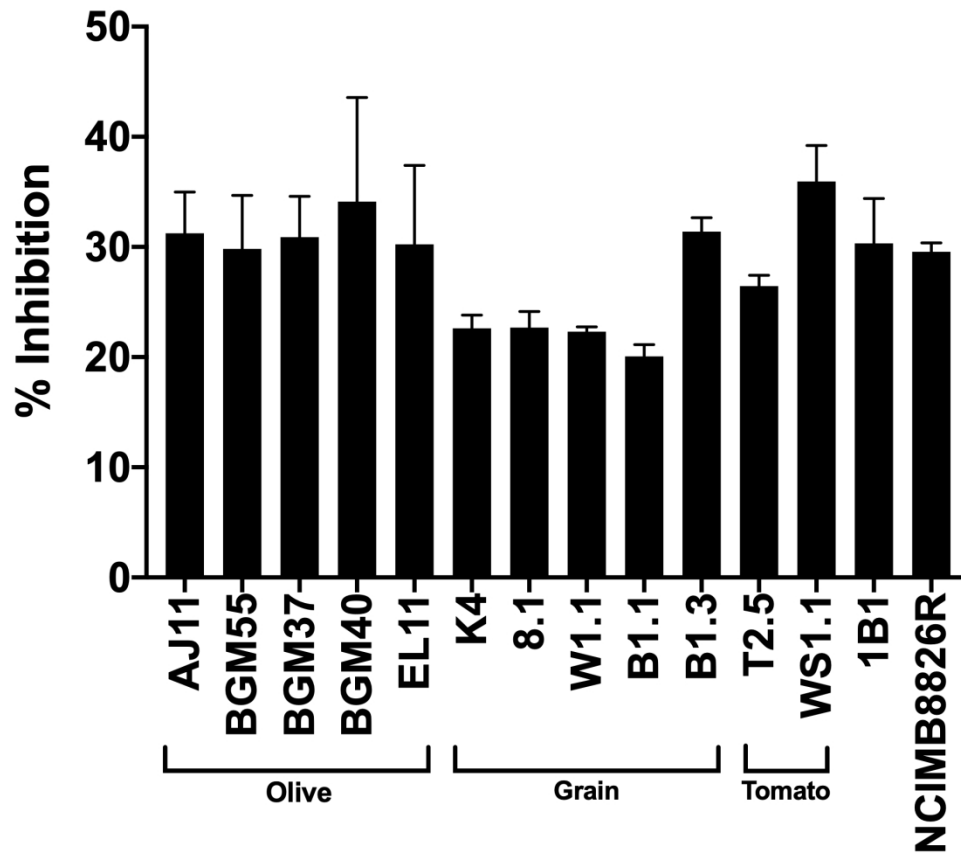


Fig 7. *S. cerevisiae* growth inhibition in the presence of *L. plantarum* CFCS. *S. cerevisiae* UCDFST-09-448 was incubated in a 1:1 ratio of 2X YM and pH adjusted (pH 3.8) *L. plantarum* CFCS from cMRS. Growth was measured by monitoring the change in OD₆₀₀ over 24 h. Percent inhibition was determined by comparing the final OD₆₀₀ of *S. cerevisiae* grown in the presence of CFCS to growth in a 1:1 ratio of 2X YM and pH adjusted (pH 3.8) cMRS.

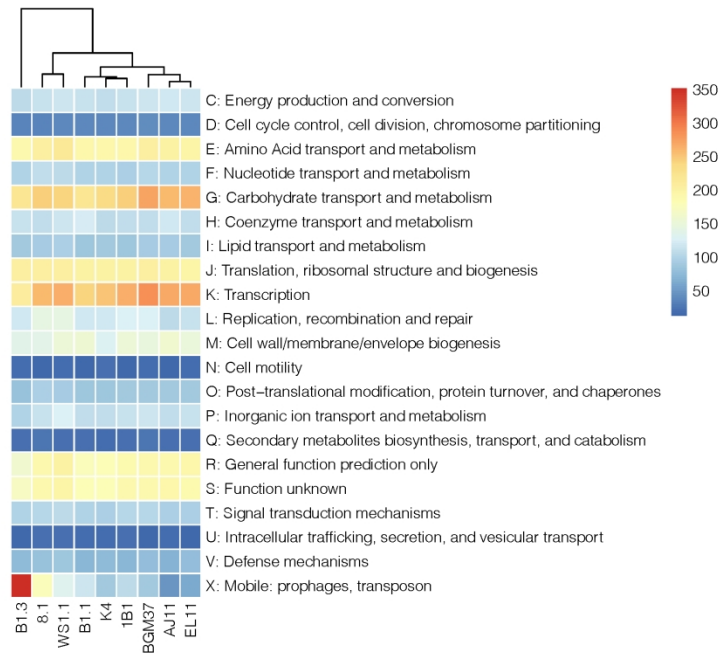


Fig 8. Distribution of COG Categories across *L. plantarum* genomes. Hierarchical clustering of *L. plantarum* based on the number of gene clusters assigned to each functional COG category. Number of gene clusters present in each strain was denoted by the color gradient.