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1 **Genetic and technological characterization of lactic acid bacteria isolated**
2 **from tropically grown fruits and vegetables**

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14

15 **Abstract**

16 Phyllosphere microorganisms are common contaminants of fruit or vegetable containing foods. The
17 aim of this study was to identify and characterize lactic acid bacteria isolated from fruits and
18 vegetables from Reunion Island, regarding possible application in food. Among 77 isolates, a large
19 diversity of species was observed, with isolates belonging to *Lactobacillus plantarum* (3 isolates),
20 other species of *Lactobacillus* (3), *Lactococcus lactis* (13), *Leuconostoc pseudomesenteroides* (25),
21 *Leuconostoc lactis* (1), *Leuconostoc mesenteroides* (7), *Leuconostoc citreum* (14), *Weissella cibaria*
22 (4), *Weissella confusa* (4), other species of *Weissella* (2) and *Fructobacillus tropaeoli* (1). Several of
23 these species, although belonging to lactic acid bacteria, are poorly characterized, because of their
24 low occurrence in dairy products. *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Weissella* isolates
25 were classified by (GTG)₅ fingerprinting in 3, 6, 21 and 10 genetic groups, respectively, suggesting a
26 large intra-species diversity. Several *Weissella* and *Lactobacillus* isolates were particularly tolerant to
27 acid and osmotic stress, whereas *Lc. pseudomesenteroides* 60 was highly tolerant to oxidative stress.
28 Isolates of *Weissella* 30, 64 and 58, *Leuconostoc* 60 and 12b, *Lactobacillus* 75 and *Fructobacillus* 77
29 present relevant characteristics for their use as starters or as preservative cultures for fruits and
30 vegetables.

31

32 **Keywords:** diversity; *Weissella*; *Leuconostoc*; starter selection; phenotype

33

34 1. Introduction

35 For food made from fruits and vegetables, raw material carries numerous microorganisms on its
36 phyllosphere, including both Gram negative and positive bacteria, yeasts and molds, which diversity
37 and number depend on agricultural practices, water quality, environmental conditions, ripening
38 stage and seasons (Leff and Fierer, 2013). Fresh fruits or vegetables may carry foodborne pathogens,
39 resulting into outbreaks (Ramos et al., 2013; Teliás et al., 2011). Yeast, molds or bacteria
40 development result in spoilage of minimally processed foods from plant origin (Francis et al., 2012).
41 In recent years, metagenomic studies investigated sources and contamination routes of fresh fruits
42 and vegetables (Alegbeleye et al., 2018; Droby and Wisniewski, 2018; Vepškaitė-Monstavičė et al.,
43 2018). It was demonstrated that raw fruits and vegetables were a considerable way of bacterial
44 contamination in canned and ready-to-eat foods (Durand et al., 2015; Guinebretiere et al., 2003;
45 Pothakos et al., 2014). Hopefully raw material contamination not only carries undesirable
46 microorganisms but also bacteria that are useful for food processing or beneficial to food quality. For
47 instance, a dominant lactic acid bacteria (LAB) isolated from tomato surface had inhibitory activities
48 against natural microbial population growth on tomato purée and could be considered as a
49 biological method to control the proliferation of contaminants (Sajur et al., 2007).

50 LAB contribute to food quality thanks to their fermentative activity, their biopreservation or their
51 probiotic properties. They are characterized by a fast growth under moderately acidic and anaerobic
52 conditions and as such are well-adapted for growth in fruit- or vegetable-based foods. Consequently,
53 LAB are frequently detected in traditional spontaneous fermented foods, including fruits- and
54 vegetables-based food (Tamang et al., 2016). During spontaneous fermentation, LAB are dominant
55 and prevent the growth of potential spoilage and pathogenic microorganisms, enhancing the safety
56 and shelf-life of food. These bacteria produce several interesting compounds, such as bacteriocins,
57 vitamins, exopolysaccharides and enzymes, which modify food composition and properties.

58 However, LAB represent a minority part of the autochthonous initial microbiota of fruit and
59 vegetable phyllosphere (Leff and Fierer, 2013). *Lactobacillus*, *Leuconostoc*, *Weissella*, *Enterococcus*
60 and *Pediococcus* are the LAB genera the most frequently isolated from raw fruits and vegetables (Di
61 Cagno et al., 2013). These genera are also the most frequent in spontaneously fermented fruits and
62 vegetables. The role and the succession stage of LAB involved in food from plant origin fermentation
63 are not clearly understood, especially for *Weissella* spp. (Fessard and Remize, 2017). Since LAB are
64 naturally present on fruit surface and produce several antimicrobial compounds, their use as
65 biological agent to control and prevent the growth of undesirable microorganisms without change of
66 sensory properties of food is also considered. A better knowledge of LAB from fruits and vegetables
67 constitutes thus an important step for the development of starter or preservative cultures for fruit-
68 and vegetable-based foods. Moreover, the phenotypic diversity of LAB species is well documented
69 for dairy applications but much less for plant origin foods or beverages. Hence, characterization of
70 the autochthonous LAB population from raw fruits and vegetables deserves a deeper investigation.

71 This study describes the identification and the characterization of LAB isolated from fruits and
72 vegetables of Reunion Island. Papaya, tomatoes and pickled cabbage were sampled. Pickled
73 cabbage, called "achards" at Reunion Island, is a mix of vegetables (white cabbage, carrots, green
74 beans, chilli pepper) with vinegar, salt, curcuma and ginger, which harbours high levels of LAB
75 (Fessard et al., 2016). Molecular biology methods such as *16S rRNA*, *pheS* and *recA* gene sequencing
76 and (GTG)₅ fingerprinting were used to identify and classify LAB. LAB from different genetic groups
77 were further characterized phenotypically, with the examination of production of EPS, the influence
78 of temperature on the growth and tolerance to acid, salt, bile salts or hydrogen peroxide in the view
79 of possible use in the food industry.

80 2. Materials and methods

81 2.1. Bacterial strains and media

82 LAB were isolated from pickled white cabbage (*Brassica oleacera* var. *capitata*), papaya (*Carica*
83 *papaya*) and tomatoes (*Lycopersicon esculantum*) grown in Reunion Island and isolates were stored
84 at -80°C. Isolation was performed as previously described (Fessard et al., 2016). Briefly, from
85 enumeration plates, different colonies were streaked out to obtain pure cultures. All isolates shared
86 the ability to grow on MRS agar with cycloheximide and were catalase negative. *Lactobacillus*
87 *plantarum* DSM 2601 and DSM20174, *Leuconostoc pseudomesenteroides* DSM 20193 and DSM5625,
88 *Weissella cibaria* DSM 14295 and DSM 15878, *Weissella confusa* DSM 20196, *Weissella koreensis*
89 DSM 15830 were used as reference strains for all experiments. Strain 1102001 (*W. confusa*), isolated
90 from green pea juice from CTCPA was added to the study.

91 2.2. Ability to produce exopolysaccharides (EPS)

92 2.2.1. EPS production from sucrose

93 Homopolysaccharide production test was performed on MRS sucrose agar (40 g.L⁻¹) as previously
94 described (Fessard et al., 2016). Experiments were performed at 25°C, 30°C and 37°C.

95 2.2.2. Sequencing of glykansucrase encoding genes

96 Primers targeting genes encoding enzymes involved in the synthesis of EPS, dextransucrase and
97 levansucrase were used in this study. The primer sets dsrk39-For/dsrk39-Rev and WconDex-
98 fw/WconDex-rev were used to detect dextransucrase from *W. cibaria* and *W. confusa* strains,
99 respectively (Bounaix et al., 2010b; Malang et al., 2015). The primer sets gtf-fw/gtf-rev and LevV-
100 fw/LevV-rev were used to detect glucansucrase and levansucrase respectively, from *Leuconostoc*
101 strains (Palomba et al., 2012). The PCR reaction was performed in a 50 µL volume with 10 µL of DNA
102 solution. PCR was performed using a Bio-rad S100 Thermal Cycler and were checked by 0.8% agarose
103 gel electrophoresis. PCR products were sequenced by Sanger method with dsrk39-For, WconDex-fw

104 and gtf-fw primers as previously described (Fessard et al., 2016). The primer set FTF2-F/FTF2-R was
105 also used to detect fructansucrase from *Weissella* and *Leuconostoc* isolates (Bounaix et al., 2009).
106 The obtained sequences were compared with NCBI Nucleotide database using BLASTN program.

107 2.3. Identification of species and typing

108 DNA extraction was performed using Instagen™ protocol (Instagen Matrix, BIORAD, Marnes la
109 Coquette, France). The supernatant containing DNA was stored at -20°C until use.

110 2.3.1. Identification

111 All isolates were identified by 16S rRNA coding region sequencing. In case of uncertainty about the
112 species, *pheS* gene sequencing was applied for *Leuconostoc*, *Lactococcus*, *Weissella* and
113 *Fructobacillus* spp. isolates whereas *recA* gene sequencing was used to identify *Lactobacillus* spp.
114 isolates, as previously described (Fessard et al., 2017). PCR products were sequenced by Sanger
115 method with FD1m, pheS-21F, PlanF and ParaF primers as previously described (Fessard et al.,
116 2016).

117 2.3.2. Genotyping

118 A rep-PCR method based on (GTG)₅ primer was used for intra-species genotypic discrimination. PCR
119 was performed according to Versalovic and Schneider (1994) as previously described (Fessard et al.,
120 2016). After electrophoresis,, agarose gels were stained with ethidium bromide and the images were
121 acquired with GelDoc (Biorad, Marnes la Coquette, France). The patterns were analyzed with CLIQS
122 1D Pro (Core Laboratory Image Quantification Software, TotalLab, Newcastle upon Tyne, England),
123 which considers both the presence/absence of bands and their relative intensity. The dendrogram
124 was generated using Pearson coefficient correlation and the arithmetic average clustering algorithm
125 (UPGMA). A coefficient of 0.15 was used to delimitate clusters. This coefficient was set up from
126 triplicate electrophoresis of three independent experiments to take into account the experimental
127 variability generated by the analyses (PCR-electrophoresis-image analysis).

128 2.4. Tolerance to acid, oxidative and osmotic stress and bile salts

129 Tolerance to stress was performed in sterile 96-well microplates. Bacterial isolates were grown for
130 48 h at 30°C in MRS broth and 20 µL of this bacterial suspension were inoculated into 180 µL of
131 corresponding broth. The control condition was MRS broth (pH 6.5). For acid stress, MRS broth
132 acidified to pH 3.0 or pH 4.5 with 2 mol.L⁻¹ HCl was used. MRS broth containing 5% or 8% NaCl was
133 used for osmotic stress. And for oxidative stress, MRS broth containing 0.025% or 0.05% or 0.075%
134 or 0.1% H₂O₂ was used. For tolerance to bile salts, MRS broth was supplemented with 0.1%, 0.2% or
135 0.3% of bile salts (Sigma Aldrich). Optical density (OD) at 660 nm was measured (Infinite M200 Pro,
136 Tecan, Lyon, France) just after inoculation (OD₀) and after 24h (OD₂₄) or 48h (OD₄₈) of incubation at
137 37°C. For acid, osmotic and oxidative stress, results were expressed as log (OD₄₈/OD₀) and indicated
138 as follows: +++ for a log (OD₄₈/OD₀) superior to 0.5 considered as a high growth yield; ++ for a log
139 (OD₄₈/OD₀) comprised between 0.3 and 0.5 considered as a moderate growth; + for a log (OD₄₈/OD₀)
140 comprised between 0.1 and 0.3 considered as a low growth; +/- for a log (OD₄₈/OD₀) comprised
141 between 0.08 and 0.1 considered as a very poor growth; - for a log (OD₄₈/OD₀) inferior to 0.08 and
142 was considered as no growth. For bile salts condition, results were expressed as a percentage of
143 control (100% corresponding to OD₂₄ in MRS broth without bile salts). Experiments were performed
144 in three independent experiments analysed in triplicate.

145 2.5. Growth parameters

146 Determination of growth parameters was performed in sterile 96-well microplates. Bacterial isolates
147 were grown for 48 h at 30°C. A volume of 20 µL of this bacterial suspension was inoculated into 180
148 µL of MRS broth. Microplates were incubated at 6 different temperatures: 12°C, 18°C, 25°C, 30°C,
149 37°C and 42°C. OD at 660 nm was measured every 2 hours (Infinite M200 Pro, Tecan, Lyon, France).
150 Experiments were performed in three independent experiments analysed in triplicates. The
151 maximum growth rate (μ_{max}) value was deduced from the curve $\ln(\text{OD } 600\text{nm}) = f(\text{time})$ using a
152 primary growth model fitting from Sym'previus software (<http://symprevius.eu/en/>). Optimum,

153 minimal and maximal growth temperature (T_{opt} , T_{min} and T_{max}) and optimum growth rate (μ_{opt}) were
154 deduced from a secondary growth model fitting also from Sym'Previus software (Rosso et al., 1993).
155 The secondary growth model is based on the gamma concept and the model was used to fit the μ_{max}
156 data as a function of temperature. The R^2 value estimates the goodness of fit of the model: the
157 closest it is to 1, the highest is the fitting of the model to experimental values. Standard deviation
158 was calculated to describe the spread of values towards the model mean. In order to confirm the
159 cardinal temperature values predicted with Sym'Previus software, growth of isolates was also
160 checked after 21 days of incubation at 2°C, 4°C, 6°C, 8°C, 10°C, 12°C, 15°C, 42°C or 45°C as previously
161 described.

162 2.6. Statistical analysis

163 The software XLSTAT (Addinsoft, Paris, France) was used for all statistical analyses. Significant
164 differences versus a control or by pairs were tested with Dunnett's or Ryan, Einot, Gabriel, Welch q
165 (REGWQ) tests respectively. A confidence interval of 95% was chosen for all statistical tests.

166

167 3. Results

168 3.1. Isolation and identification

169 A total of 77 LAB were isolated: 24 from papayas (6 different fruits), 47 from sliced cabbage (5
170 different samples) and 6 from tomatoes (1 fruit). The highest LAB population was observed for
171 pickled cabbage samples with 8.4 log CFU.g⁻¹. LAB populations of tomato and papaya ranged
172 between 2.9 and 5.1 log CFU.g⁻¹. Isolates were first identified by 16S rRNA gene sequencing but
173 distinction remained uncertain between several species. For further identification, *pheS* and *recA*
174 gene sequencing was used respectively for *Weissella* and *Leuconostoc* species and for *Lactobacillus*
175 isolates.

176 Sequencing showed the presence of 13 different species (number of isolates): *Lb. plantarum* (3), *Lb.*
177 *paraplantarum* (2), *Lb. paralimentarius/kimchii* (1), *Fructobacillus tropaeoli* (1), *Lactococcus lactis*
178 subsp. *lactis* (13), *Lc. pseudomesenteroides* (25), *Lc. citreum* (14), *Lc. mesenteroides* (7), *Lc. lactis* (1),
179 *Weissella cibaria* (4), *W. confusa* (4), *W. paramesenteroides* (1) and *W. soli* (1) (**Table 1**). Species with
180 a single isolate, *Fb. tropaeoli* 77 and *W. paramesenteroides* 37 on one side, and *Lb.*
181 *paralimentarius/kimchi* 71, *Lc. lactis* 24 and *W. soli* 58 on the other side, were isolated from papaya
182 and pickled cabbage respectively.

183 For species with multiple isolates, several isolation origins were observed. On the other side, for a
184 given sample, several isolates from the same species could be obtained. Eventually, there was no
185 correlation between a given species and an isolation material.

186 3.2. (GTG)₅-fingerprinting

187 All 77 isolates, all reference strains and strain 1102001 were subjected to rep-PCR. (GTG)₅ primer
188 generated different patterns which were used for classification into clusters (**Table 1 and Fig. 1**).
189 (GTG)₅ fingerprinting revealed a high diversity of genetic profiles since the 87 isolates or strains were
190 clustered in 48 groups, using a threshold of 0.15. This threshold was set up from triplicate
191 experiments to take into account experimental reproducibility.

192 The eight *Lactobacillus* spp. were classified into four (GTG)₅ groups. The two reference *Lb. plantarum*
193 strains DSM2601 and DSM20174 were clustered in the same (GTG)₅ group. None of our isolates
194 were classified in this group. Isolate 71 identified as *Lb. paralimentarius/kimchi* showed a specific
195 profile. *Lb. paraplantarum* isolates (73 and 74) were clustered in the same (GTG)₅ group than two *Lb.*
196 *plantarum* isolates (17a and 29a).

197 The 13 *L. lactis* subsp. *lactis* isolates were allocated to six distinct (GTG)₅ group, whereas most of
198 them were isolated from sliced cabbage. Noteworthy, some isolates (6, 14, 19, 40, 41, 53 and 65)
199 from different samples of papaya and sliced cabbage were grouped into the same cluster.

200 The 50 *Leuconostoc* spp. were allocated to 23 (GTG)₅ groups. *Lc. pseudomesenteroides* (27 strains),
201 *Lc. mesenteroides* (7 strains) and *Lc. citreum* (15 strains) were spread in 12, 6 and 4 groups,
202 respectively. None of the *Lc. pseudomesenteroides* isolates were grouped with the reference strains
203 DSM20193 and DSM5625. However, similarity was observed between *Lc. pseudomesenteroides*
204 isolates 12b, 27b and *W. koreensis* DSM15830. Two *Lc. citreum* isolates (33 and 13a) were grouped
205 with the *Lc. citreum* reference strain DSM20188. *Lc. lactis* isolate 24 showed a genetic profile distinct
206 from other *Leuconostoc* spp.

207 A high diversity was observed for the 15 *Weissella* spp. which were spread into 14 clusters. *W.*
208 *cibaria* 64, 21, 30, DSM14295 and DSM15878 patterns presented some similarity but were not
209 classified in the same cluster. (GTG)₅ profile of isolate 10b was clearly different from those of other
210 *W. cibaria* isolates. *W. confusa* 16 and 17 presented the same profile and showed some similarity
211 with *W. confusa* 59 and DSM20196 but were not classified in the same cluster. Profiles of *W. confusa*
212 1102001 and 38 were clearly different. Distinct profiles were observed also for *W. soli* 58 and *W.*
213 *paramesenteroides* 37.

214 3.3. EPS production and glycanucrase gene detection

215 Neither of the *Lactobacillus* spp. isolates, nor the *L. lactis* isolates, nor *Fb. tropaeoli* 77, *W.*
216 *paramesenteroides* 37 and *W. soli* 58 did produce EPS on sucrose medium (**Table 1**). On the contrary,
217 all *Leuconostoc* isolates and all the isolates of other *Weissella* species produced EPS from sucrose
218 and colony aspect was strain dependent, either liquid or creamy (**Tables 1 and 2**).

219 For these isolates, EPS production from sucrose was observed at 25°C and 30°C. At 37°C, some
220 isolates (60, 24, 1, 5, 6a, 28, 10b, 30, 38 and 1102001) did not produce EPS, whereas for some other
221 isolates (DSM20193, DSM5625, 79, 33, 2, 16, 17 and 59) a change of the EPS phenotype was noticed
222 (**Table 2**). The screening for potential glucanucrase genes from *Leuconostoc* spp. revealed 6 positive
223 strains: *Lc. mesenteroides* 1, 5, 6a, 28 and *Lc. citreum* 2, 9a. *W. cibaria* and *W. confusa* isolates gave
224 the expected fragment for the amplification of partial dextranucrase gene. Sequencing of the PCR

225 products confirmed the similarity to glucansucrase or dextransucrase genes from databases (**Table**
226 **2**). Amplification with LevV-fw/LevV-rev and with FTF2-F/FTF2-R primers was not positive suggesting
227 the absence of levansucrase and fructansucrase.

228 3.4. Growth yield in control MRS condition and tolerance to stress

229 Isolates of *Weissella*, *Leuconostoc* and *Fb. tropaeoli* from distinct (GTG)₅ group were chosen for
230 further characterization regarding their tolerance to stress, to bile salts and their growth at different
231 temperatures. Results were compared with reference strains and *Lactobacillus* isolates.

232 *Lb. plantarum* 75, *W. cibaria* 64 and 30, *W. confusa* 1102001 harboured the highest growth yields
233 over 48h compared to other isolates ($p < 0.0001$), with $\log OD_{48}/OD_0$ values of 1.16 ± 0.00 , $0.91 \pm$
234 0.08 , 0.86 ± 0.16 and 0.88 ± 0.11 , respectively. On the contrary, *W. paramesenteroides* 37 showed a
235 low growth yield in MRS pH 6.5 37°C compared to other isolates ($p < 0.0001$) (**Table 3**).

236 At a low initial pH of 4.5 (**Table 3**), $\log OD_{48}/OD_0$ values were comprised between 0.04 (*Lc.*
237 *pseudomesenteroides* 60) and 1.05 (*Lb. plantarum* 75). At a lower pH of 3, they were comprised
238 between 0.03 (*Lc. pseudomesenteroides* 56) and 0.22 (*W. confusa* 1102001). *Lb. plantarum* 17a and
239 *W. cibaria* 10b were not affected by exposure to pH 4.5, as no significant differences were detected
240 compared to control condition. Some isolates (73, 75, DSM2601, DSM20193, DSM5625, 39, 78,
241 DSM20188, 21, 30, 64 and 1102001) showed a high growth yield in MRS pH 4.5, but they were
242 clearly affected by this condition compared to control ($p < 0.0001$). At an initial pH of 3, the growth
243 yield of all isolates was reduced compared to control condition ($p < 0.0001$) and most of isolates did
244 not succeed to grow in this condition (**Table 3**). The most acid-tolerant isolates were: *Lb.*
245 *paraplantarum* 73; *Lb. plantarum* 75; *Lc. pseudomesenteroides* 12b, 27b, DSM5625, DSM20193, 39;
246 *Lc. lactis* 24; *Lc. mesenteroides* 28; *Lc. citreum* 33, 9a; *W. cibaria* 30, 64, and *W. confusa* DSM20196,
247 59 and 1102001.

248 In moderate saline stress (NaCl 5%), log OD₄₈/OD₀ values were comprised between 0.04 (*Lc.*
249 *mesenteroides* 5) and 1.16 (*Lb. plantarum* 75). *Lb. plantarum* 75, 17a, *Lb. paraplantarum* 73, *W.*
250 *cibaria* DSM15878 and *W. soli* 58 were the most tolerant to NaCl 5%, and no significant differences
251 were detected compared to control condition (**Table 3**). *Lc. citreum* 33, *W. confusa* 38 and *W.*
252 *paramesenteroides* 37 were only moderately affected by NaCl 5% ($p < 0.05$). Some isolates exhibited
253 high growth in NaCl 5% (1102001, 16, 17, 60, 64, 30 and 59), but compared to control condition,
254 growth was significantly reduced ($p < 0.0001$). When a stronger saline stress was applied (NaCl 8%),
255 log OD₄₈/OD₀ values were comprised between 0.00 (*W. cibaria* 10b) and 0.97 (*Lb. plantarum* 75). *W.*
256 *confusa* 38 seemed slightly affected by this condition but the difference with control condition was
257 not significant. *W. soli* 58 was not significantly affected by exposure to NaCl 8%. Isolates 75 and 60
258 revealed a high growth yield in the presence of 8% NaCl, however they were sensitive to this
259 condition ($p < 0.001$ and $p = 0.007$, respectively). The growth of all other isolates was severely
260 affected.

261 In a slight oxidative condition, log OD₄₈/OD₀ were comprised between 0.00 (5, DSM2601, 2, 79) and
262 1.15 (75). *Fb. tropaeoli* 77, *Lb. plantarum* 75, 17a, *Lc. pseudomesenteroides* DSM20193, DSM5625,
263 60, 39, *Lc. citreum* 9a, *W. cibaria* 21, 30, 64, DSM14295, DSM15878 and *W. confusa* 1102001 were
264 not affected by exposure to 0.025% in H₂O₂ (**Table 3**). This concentration partially inhibited the
265 growth of *W. confusa* 16 and 59 ($p < 0.05$). The growth of all other isolates was reduced in this
266 condition. With 0.05% H₂O₂, only four isolates were not affected: *Fb. tropaeoli* 77, *Lc.*
267 *pseudomesenteroides* 60 and *W. cibaria* 30 and 64. No significant differences were detected
268 between 0.075% and 0.1% H₂O₂ conditions. This concentration in H₂O₂ was very efficient to impair
269 growth of almost all LAB isolates. Only *Lc. pseudomesenteroides* 60 was not affected by 0.1% H₂O₂.
270 *Fb. tropaeoli* 77 was also able to grow in this condition but compared to control condition its growth
271 was significantly reduced ($p < 0.001$).

272 3.5. Tolerance to bile salts

273 None of the LAB strains was able to grow in 0.2% or 0.3% of bile salts after 24h (data not shown).
274 Tolerance to bile salts was thus performed with 0.1% over 24h and expressed as a percentage of OD
275 of control (MRS broth without bile salts). Four isolates showed the highest resistance to bile salts: *Lc.*
276 *pseudomesenteroides* 12b, *W. paramesenteroides* 37, *Lc. citreum* DSM20188 and *Lb. plantarum* 75,
277 with a percentage of growth comprised between 61% and 83.8% of that observed in control
278 condition (**Fig. 3**). Tolerance of strain 12b was significantly higher than that of other isolates
279 ($p < 0.001$), except compared to strains 37 and DSM20188. Other *Leuconostoc* strains showed a
280 relatively good tolerance, with a percentage of growth comprised between 35% and 55.8% of
281 control. *Weissella* isolates and *Lc. pseudomesenteroides* 60 were the less tolerant to bile salts, with a
282 percentage of growth comprised between 14.9% and 32.6% of control. The lowest tolerance was
283 observed for *Lc. pseudomesenteroides* 60 and *W. confusa* strains 38, 16, DSM20196 and 17.
284 *Leuconostoc* strains 58, 89, 27b, 2, 39, 56, 79, 33, 78 and *Weissella* strains 58 and DSM15830 were
285 not able to grow at 37°C in 24h in the presence of 0.1% bile salts: for these isolates, we cannot
286 conclude regarding their tolerance to bile salts.

287 3.6. Temperature effect on growth rate

288 Cardinal temperatures and μ_{opt} were determined using Sym'Previs software by plotting μ_{max} as a
289 function of temperature (12°C, 18°C, 25°C, 30°C, 37°C, 42°C) (**Fig. 2**). Predicted values obtained from
290 Sym'Previs model and possible growth after incubation for 21 days are indicated in **Table 4**.

291 Optimal growth temperature T_{opt} of LAB isolates ranged between 25.1°C (*Lc. citreum* 33) and 39.0°C
292 (*W. confusa* 59). The majority of *Leuconostoc* spp. harboured T_{opt} comprised between 25.0 and
293 30.0°C. *Weissella* spp. and *Lactobacillus* spp. showed T_{opt} over 30°C, above 34°C for *Weissella* isolates
294 30, 16, 17, 59 and 58. LAB isolates harboured μ_{opt} values comprised between 0.107 h⁻¹ (*Lc.*
295 *pseudomesenteroides* 27b) and 0.998 h⁻¹ (*W. confusa* 17) (**Table 4**). *W. confusa* isolates (38, 1102001,
296 16, 59, 17) and *W. cibaria* isolates (64, 30, 10b) showed the highest μ_{opt} values. Generally,
297 *Leuconostoc* isolates shown the lowest μ_{opt} values.

298 LAB isolates exhibited minimal growth temperatures T_{min} comprised between 0.6°C (*W. confusa* 59)
299 and 16.7°C (*W. cibaria* DSM15878). *Leuconostoc* spp. harboured T_{min} comprised between 1.0°C (*Lc.*
300 *mesenteroides* 5) and 11.9°C (*Lc. pseudomesenteroides* 79). *Weissella* spp. harboured T_{min} comprised
301 between 0.6°C (*W. confusa* 59) and 16.7°C (*W. cibaria* DSM15878). *Fructobacillus* and *Lactobacillus*
302 spp. harboured T_{min} comprised between 3.7°C (*Fb. tropaeoli* 77) and 14.9°C (*Lb. paraplantarum* 73).
303 Generally, *Leuconostoc* spp. have shown the lowest T_{min} values. Some Sym'previus T_{min} values were
304 not confirmed by incubation test for 21 days, as for *Lc. pseudomesenteroides* 39, 89, DSM5625 and
305 DSM20193 or *W. confusa* 16 and 17.

306 Maximal growth temperature T_{max} of LAB isolates ranged between 37.5°C (*Lc. pseudomesenteroides*
307 78) and 49.3°C (*W. confusa* 16). The majority of *Leuconostoc* spp. harboured T_{max} comprised
308 between 37.0°C and 43.0°C which was confirmed by incubation for 21 days at 42°C. Incubation for
309 21 days at 45°C confirmed that only two *Leuconostoc* isolates were able to grow between 42°C and
310 45°C, as *Lc. lactis* 24 and *Lc. pseudomesenteroides* 60. *Weissella* spp. harboured T_{max} comprised
311 between 38.1°C (*W. soli* 58) and 49.3°C (*W. confusa* 16). *Fructobacillus* and *Lactobacillus* spp.
312 harboured T_{max} comprised between 40.0°C (*Lb. plantarum* DSM2601) and 46.5°C (*Lb. plantarum* 75).
313 In most case, incubation for 21 days resulted in maximal growth temperatures which fell into the
314 confident interval given by the model, except for some strains for which the model mostly proposed
315 higher T_{max} .

316 **4. Discussion**

317 The aim of this paper was to collect and characterize LAB isolates from tropically grown fruits and
318 vegetables for possible application in food industry, especially for fruits and vegetables.

319 In this study, genetic and phenotypic characterization of 77 autochthonous LAB isolated from
320 papaya, tomato and sliced cabbage was performed. Among the species the most frequently
321 detected, *Lc. mesenteroides* was commonly isolated from fresh fruits and vegetables, as raw prickly
322 pear, sweet cherry and raw peppers (Di Cagno et al., 2016, 2011b, 2009a), *Lc. pseudomesenteroides*

323 and *Lc. citreum* have been isolated from ripe mulberries, fresh tomato, fresh coffee cherries and
324 banana fruit (Chen et al., 2017, 2010; Leong et al., 2014; Trias et al., 2008). *Lc. lactis*, hereby isolated
325 from cabbage, is mainly associated with dairy and vegetable-based fermented food, including kimchi
326 (Chen et al., 2012; Cho et al., 2006; Vos et al., 2011). To the best of our knowledge, isolation of *Lc.*
327 *lactis* from raw vegetable or ready-to-eat crude vegetable has not been reported. *W. cibaria*, *W.*
328 *confusa*, *Lb. plantarum* and *Lb. paraplantarum* were the second-most-commonly detected species.
329 These species are frequently isolated from fresh fruits and vegetables (Chen et al., 2010; Di Cagno et
330 al., 2013, 2011a, 2009b; Emerenini et al., 2013; Trias et al., 2008). *Weissella* and *Lactobacillus*
331 frequently occur in spontaneous fermentation of fruits or vegetables (Fessard and Remize, 2017),
332 highlighting their natural adaptation to fruit and vegetable environments. Our study revealed also
333 the presence of isolates of the species *W. soli* (cabbage), *W. paramesenteroides* (papaya) and *Fb.*
334 *tropaeoli* (papaya). *W. paramesenteroides* has been isolated from a variety of fermented fruit and
335 vegetable (Chen et al., 2013b, 2013a; Escalante-Minakata et al., 2008; Lan et al., 2009), and a single
336 study reported its isolation from banana fruit (Chen et al., 2017). *W. soli* has been detected in silage
337 fermentation of vegetable residues (cabbage, Chinese cabbage and lettuce) (Yang et al., 2010). *Fb.*
338 *tropaeoli* was first isolated from a flower of *Tropaeolum majus* in South Africa (Endo et al., 2011) and
339 was further isolated from spontaneous cocoa fermentation together with *W. fabalis* (Snauwaert et
340 al., 2013). Only recently, *Fb. tropaeoli* has shown fruit origins (Franquès et al., 2017; Ruiz Rodríguez
341 et al., 2017). Due to its frequent presence in raw milk, *L. lactis* has been extensively used as starter
342 culture for dairy foods, contributing to the development of texture by producing exopolysaccharides
343 (Casalta and Montel, 2008). In a recent study, two groups have been proposed for *L. lactis lactis*
344 subspecies, “domesticated” and “environmental”. The latter appears to be the main contributor to
345 genetic and phenotypic diversity within the subspecies (Laroute et al., 2017). The availability of new
346 isolates from our study would be useful to understand the relationships between origin and
347 phenotypic features. Besides, plant material is the main natural habitat of *L. lactis* and this species
348 has been detected in fresh and frozen corn, corn silks, navy beans, cabbage, lettuce or peas (Vos et

349 al., 2011), sprouted seeds and grapefruit juice (Kelly et al., 1996), by-products of pineapple and
350 cherry pulp processing (Garcia et al., 2016), ripe mulberries (Chen et al., 2010) and fresh coffee
351 cherries (Leong et al., 2014). Our results are then consistent with previous detection of these species
352 from fruit and vegetable environments. It also revealed the presence of several species, like *Lc. lactis*
353 or *W. paramesenteroides*, rarely isolated from fresh fruit and vegetables but rather from fermented
354 foods.

355 Rep-PCR revealed a high diversity of genetic profiles. The 77 LAB isolates were clustered into 41
356 genetic groups, which is a high number of groups regarding the limited number of species and
357 samples. The highest diversity was observed for *Leuconostoc* and *Weissella* isolates. Indeed, the 11
358 *Weissella* isolates were allocated to 10 genetic groups and were isolated from seven samples from
359 three different raw material. From this observed genetic diversity, we hypothesized a phenotypic
360 diversity which could be used as a stock for bacteria of technological interest.

361 Fermentation and biopreservation of minimally-processed foods from plant origin require selected
362 strains with desirable properties. The use of LAB for this purpose presents many advantages
363 regarding their history of use in foods and their ability to adapt to the specific conditions of these
364 raw materials. The advantages of autochthonous strains rely of the assumption of a higher stability
365 in their natural environment to compete microbial contaminants, and a better adaptative ability to
366 their niche (Beganović et al., 2013; Di Cagno et al., 2009b, 2008; Fessard et al., 2016; Viana de Souza
367 and Silva Dias, 2017). Although there is not a single protocol to select starter or bioprotective or
368 probiotic strains and each application requires a tailored selection, several common traits are
369 considered whatever the context (Bevilacqua et al., 2012; Kostinek et al., 2005; Leroi et al., 2015).
370 They are mainly related to adaptation of strains to technological conditions, such as acidic medium,
371 salt addition or incubation temperature. Moreover, safety of strains is of crucial importance.
372 Regarding probiotic selection, expectations of strain properties are different from those of
373 fermentation starters or biocontrol agents. A step-by-step procedure has been proposed,

374 investigating stress tolerance, adhesion ability, antipathogenic activity, safety assessment, host-
375 associated functional properties, industrial requirements and omics characterization, before clinical
376 trials (de Melo Pereira et al., 2018).

377 The behaviour of LAB at different temperatures and the determination of growth parameters are
378 thus important to consider for a rational choice of strains for a specific application. Growth of *W.*
379 *cibaria* and *W. confusa* isolates was detected between 6°C and 45°C, in accordance with those found
380 in the literature (Björkroth et al., 2002; Fusco et al., 2015). *Leuconostoc* isolates had optimum
381 growth between 25.1°C and 33.3°C, and growth up to 37°C was strain dependent, which
382 corresponds to the description of *Leuconostoc* spp. (Vos et al., 2011). *Lc. mesenteroides* 1 and 5, *Lc.*
383 *citreum* 2 and *Lc. pseudomesenteroides* 12b have shown particular ability to grow at low
384 temperatures, which it is a technological criteria of importance for their application in fruit and
385 vegetable fermentation (Fessard and Remize, 2017). In our study, μ_{max} values of *Leuconostoc* isolates
386 were lower than those observed for *Weissella* or *Lactobacillus* isolates. They were in the same range
387 than those reported by Ricciardi et al., (2009). and Drosinos et al., (2006). Maximum growth rate is
388 clearly species- and strain-dependent and several factors may affect this value such as temperature,
389 pH, oxygen or presence of toxic compounds. Regarding growth parameters, temperature control
390 appears here to be a potent lever to favour the growth of certain LAB isolates.

391 Low pH and high salt concentration are often used as selective conditions for LAB over food
392 processing steps. Exposure to low pH affected the growth of all LAB strains, but *Weissella* and
393 *Lactobacillus* spp. were more tolerant than *Leuconostoc* spp. Growth of *W. cibaria* strain in MRS
394 broth adjusted to pH 3 has been reported by Patel et al. (2012). Interestingly, *Weissella* and
395 *Lactobacillus* strains were also the most tolerant to salt stress, especially *W. soli* 58, *W. confusa* 38
396 and *Lb. plantarum* 75, which were able to grow in 8% NaCl, in accordance with Lee et al. (2012) and
397 Papamanoli et al. (2003) which reported data for strains from the same species.

398 Unexpectedly, *Fb. tropeoli* 77, *Lc. pseudomesenteroides* 60 and *W. cibaria* 21, 30 and 64 were the
399 most tolerant to hydrogen peroxide, whereas *Lb. plantarum* strains have already been shown to be
400 highly tolerant to exposure to 0.1% H₂O₂ for 30 min (Parente et al., 2010). Glutathione (GSH), a non-
401 protein thiol compound, has been described in *Lactococcus* and *Lactobacillus* spp. and may play a
402 role in the protection towards an oxidative stress (Zhang and Li, 2013). LAB are catalase negative but
403 some strains may possess a manganese-dependent form.

404 Tolerance of LAB to bile salts has been associated with their capacity to metabolize the bile salts (van
405 de Guchte et al., 2002) and constitute an important trait for the selection of cultures which can
406 survive in gut. In our study, LAB isolates were able to grow only in 0.1% of bile salts which is quite
407 low compared to the data reported in the literature. Concentrations of 0.15-0.3% of bile salts have
408 been recommended as a suitable concentration for the selection of probiotic bacteria for human use
409 (Boke et al., 2010). However, experimental time exposure to bile salts is generally limited to 6 hours,
410 which might explain the apparent discrepancy. Generally, *Leuconostoc* isolates have shown good
411 tolerance to bile salts while *W. cibaria* and *W. confusa* isolates were the most sensitive. *Lc.*
412 *pseudomesenteroides* 12b, *W. paramesenteroides* 37, and *Lb. plantarum* 75 isolates were the most
413 tolerant to bile salts. If some *Lb. plantarum* strains were shown to be resistant to 2% bile salts
414 (Papamanoli et al., 2003), tolerance to bile salts of *Lc. pseudomesenteroides* and *W.*
415 *paramesenteroides* is not described.

416 Exopolysaccharides are important in the manufacture of dairy products and have gained interest
417 recently for the manufacture of fruit or vegetable puree and smoothies (Di Cagno et al., 2011a;
418 Juvonen et al., 2015). The screening for EPS production performed on sucrose medium revealed that
419 only *Leuconostoc* spp., *W. cibaria* and *W. confusa* isolates produced EPS from sucrose. The literature
420 qualified *W. cibaria*, *W. confusa* and *Leuconostoc* spp. as high producers of EPS (Di Cagno et al.,
421 2016; Galle et al., 2010; Maina et al., 2008; Malang et al., 2015; Wolter et al., 2014), and no dextran
422 was produced from sucrose by *Fb. tropeoli* strain (Endo et al., 2011), which supports our results.

423 However, the production of EPS was also previously reported for *Lb. plantarum* strains, isolated from
424 sourdough and fish (Di Cagno et al., 2006; Hongpattarakere et al., 2012) and for *L. lactis* strain
425 isolated from raw milk (Van der Meulen et al., 2007) but our isolates of these species did not
426 produce EPS. The ability of LAB to produce EPS from sucrose is due to the action of one sucrose
427 enzyme, either glucansucrase or fructansucrase (van Hijum et al., 2006). In our study, partial
428 sequencing of glucansucrase genes was positive for *W. cibaria*, *W. confusa*, *Lc. mesenteroides* and *Lc.*
429 *citreum* isolates. Several studies already reported glucan production or glucansucrase activity from
430 *Lc. mesenteroides* and *Lc. citreum* strains (Bounaix et al., 2010a; Kang et al., 2014; Passerini et al.,
431 2015; Song et al., 2016; Zannini et al., 2016) as well as for *W. cibaria* and *W. confusa* (Amari et al.,
432 2013; Baruah et al., 2017; Bounaix et al., 2010b). Little is known about sucrose enzyme from *Lc.*
433 *pseudomesenteroides* spp. and none of our *Lc. pseudomesenteroides* isolates producing EPS were
434 positive for sucrose enzyme encoding genes. Dextran production constitutes a possible desirable
435 technological trait for our isolates. Our study revealed also that temperatures comprised between
436 25°C and 30°C were optimum for the production of EPS. It has been shown that EPS production was
437 higher at temperature comprised between 15°C and 20°C for *W. cibaria* (Hu and Gänzle, 2018). *W.*
438 *confusa* dextransucrase activity was higher between 20°C and 30°C (Amari et al., 2013). These
439 observations could explain the absence of EPS production at 37°C observed for some isolates.

440 Our study provides a stock of autochthonous LAB species from fruits and vegetables with phenotypic
441 characteristics useful for application in food. Altogether, *Weissella* strains, especially *W. cibaria* 64
442 and 30 were particularly tolerant to acidic, osmotic and oxidative conditions and produce EPS.
443 However, until now, this species is not used as commercial starter (Fessard and Remize, 2017). *W.*
444 *solis* 58 has shown high tolerance to osmotic conditions but was a relatively poor sugar fermenter in
445 MRS broth. *W. paramesenteroides* 37 was one of the isolates the most tolerant to bile salts. The
446 group of *Leuconostoc* isolates harbor a variety of diverse phenotypes. Among those, strain 12b
447 showed a high tolerance to bile salts while strain 60 was particularly tolerant to osmotic and
448 oxidative stress. *Lc. mesenteroides* 1 and 5 and *Lc. citreum* 2 were particularly tolerant to low

449 temperatures. *Lb plantarum* 75 was a high sugar fermenter, highly tolerant to low pH, salts and bile
450 salts. For all those strains, no biogenic amine production from lysine, ornithine, tyrosine and
451 histidine, and also the absence of detection of histidine, tyrosine and ornithine decarboxylase genes
452 was observed (data not shown), but the possibility of biogenic amine production via the arginine
453 deiminase pathway has to be checked.

454 *W. cibaria* 30 and 64, *Lc. pseudomesenteroides* 12b and 60 and *Lb. plantarum* 75 are powerful
455 candidates for fruits and vegetables fermentation, whereas *W. soli* 58, *Fb. tropaeoli* 77, *Lc.*
456 *mesenteroides* 1 and 5 could be investigated as preservative cultures for fruits and vegetables.

457 Despite their frequent detection in foods, genetic and phenotypic features of some LAB species
458 remain poorly documented. LAB species mostly described are *L. lactis* and *Lb. plantarum* mainly due
459 to their long and safe history of application, especially for dairy products (Leroy and De Vuyst, 2004).
460 Our study provides a useful description of several autochthonous LAB isolates from other species
461 often encountered in fruits and vegetables, including *Lactobacillus*, *Leuconostoc*, *Weissella* and
462 *Fructobacillus* spp. The comparison of the core and pan genomes towards available genomes from
463 strains of various origins, of several isolates, which exhibit a potential for their use as starter, would
464 be of particular interest to provide information on niche adaptation and go further on their possible
465 tailored-application.

466 **Conflict of interest**

467 The authors declare no conflict of interest.

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1 **FIGURE LEGENDS**

2 **Figure 1.** Dendrogram obtained from (GTG)₅-typing. The dendrogram was generated using Pearson
3 coefficient correlation and the arithmetic average clustering algorithm (UPGMA). The vertical red line
4 indicates the optimization coefficient of 0.15 used to delimitate clusters. A total of 48 genetic group
5 were generated for all isolates and strains.

6 **Figure 2.** Influence of temperature on the maximum growth rate μ_{max} (h⁻¹) for representative isolates.
7 Curve lines are predicted model calculated by Sym'previus for each isolate. Points are the μ_{max} values
8 calculated by Sym'previus from experimental growth curves.

9 **Figure 3.** Resistance of LAB isolates to 0.1% of bile salts (mean \pm standard deviation). Results are
10 expressed in percentage of OD600nm compared to control without bile salts. Different letters
11 between isolates indicate significant differences with REGWQ test ($p < 0.05$).

Figure 1.

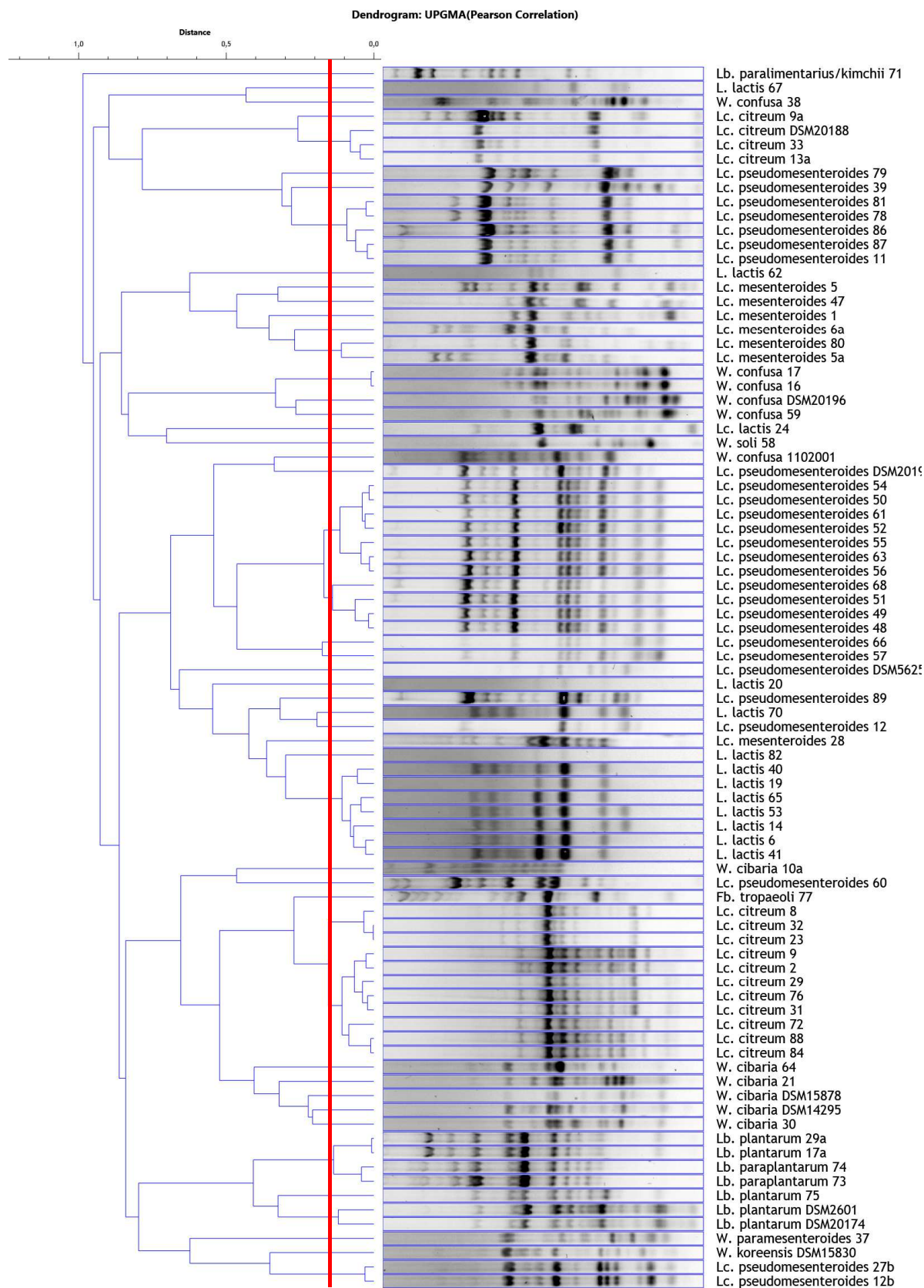


Figure 2.

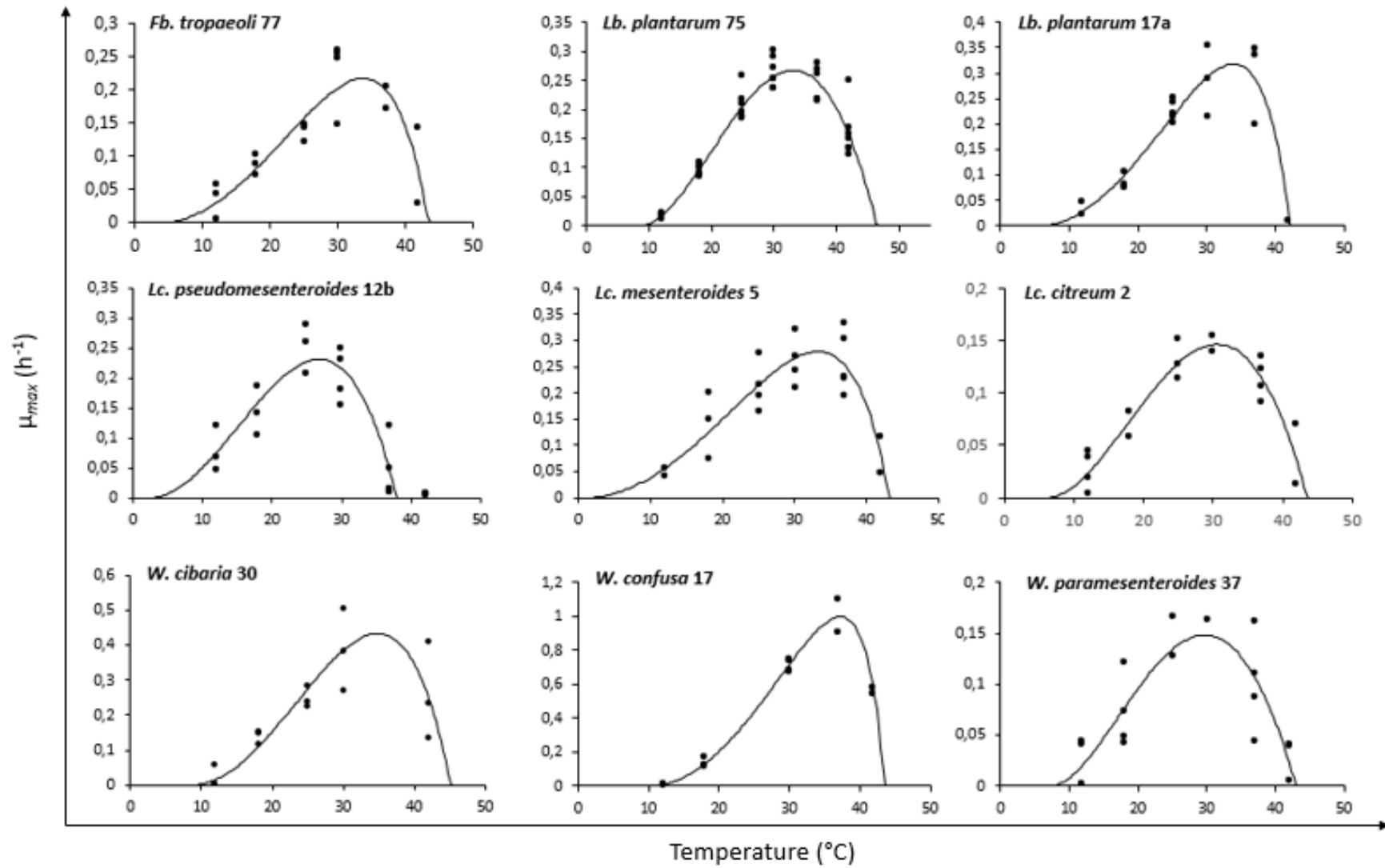


Figure 3.

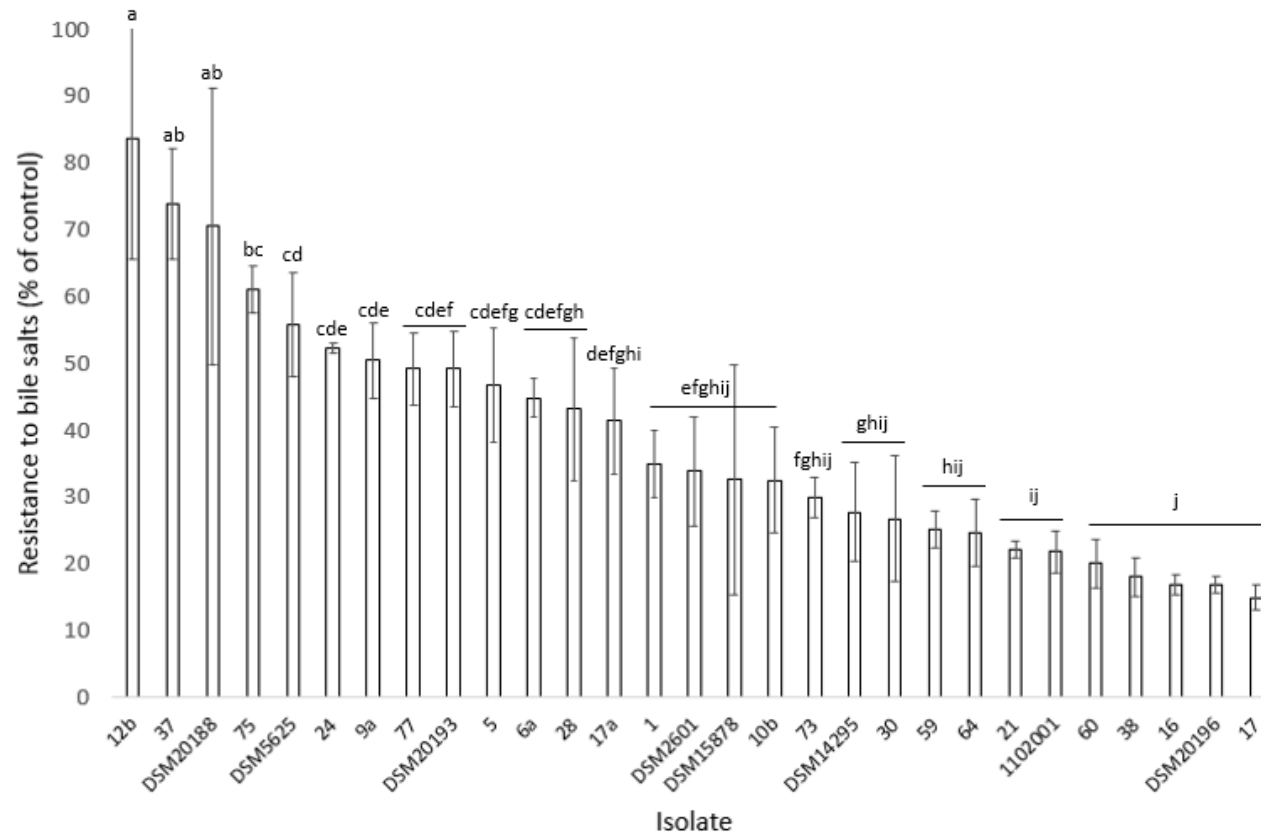


Table 1. Species, origin, EPS production and rep-PCR groups of the isolates. EPS production is indicated as follows: (+) positive phenotype and (-) no production.

Genus	(GTG) ₅ group	Species	Isolate (origin ^{1,2,3})	EPS production
Fructobacillus	Fb35	<i>Fb. tropaeoli</i>	77 (P ¹⁶)	-
Lactobacillus	Lb1	<i>Lb. paralimentarius/kimchii</i>	71 (C ²⁷)	-
	Lb43	<i>Lb. paraplantarum</i>	73, 74 (C7)	-
	Lb43	<i>Lb. plantarum</i>	17a (P), 29a (T ³)	-
	Lb44	<i>Lb. plantarum</i>	75 (C7)	-
	Lb45	<i>Lb. plantarum</i>	DSM2601, DSM20174 (pickled cabbage)	-
Lactococcus	L32	<i>L. lactis</i>	6 (C2), 14, 19 (C4), 40, 41 (P5), 53 (C6), 65 (C7)	-
	L9	<i>L. lactis</i>	62 (C7)	-
	L26	<i>L. lactis</i>	20 (C4)	-
	L2	<i>L. lactis</i>	67 (C7)	-
	L28	<i>L. lactis</i>	70 (C7)	-
	ND	<i>L. lactis</i>	42 (P5)	-
	L31	<i>L. lactis</i>	82 (P6)	-
Leuconostoc	Lc25	<i>Lc. pseudomesenteroides</i>	DSM5625 (commercial starter)	+
	Lc24	<i>Lc. pseudomesenteroides</i>	57 (C6)	+
	Lc23	<i>Lc. pseudomesenteroides</i>	66 (C6)	+
	Lc27	<i>Lc. pseudomesenteroides</i>	89 (C7)	+
	Lc29	<i>Lc. pseudomesenteroides</i>	12 (P2)	+
	Lc22	<i>Lc. pseudomesenteroides</i>	48, 49, 50, 51, 52, 54, 55, 56 (C6), 61, 63, 68 (C7)	+
	Lc21	<i>Lc. pseudomesenteroides</i>	DSM20193 (sugar cane juice)	+
	Lc48	<i>Lc. pseudomesenteroides</i>	12b (T), 27b (P)	+
	Lc34	<i>Lc. pseudomesenteroides</i>	60 (C6)	+
	Lc6	<i>Lc. pseudomesenteroides</i>	79 (P6)	+
	Lc7	<i>Lc. pseudomesenteroides</i>	39 (P5)	+
	Lc8	<i>Lc. pseudomesenteroides</i>	11 (P2), 78, 81, 86, 87 (P6)	+
	Lc18	<i>Lc. lactis</i>	24 (C5)	+
	Lc14	<i>Lc. mesenteroides</i>	5a (P), 80 (P6)	+
	Lc12	<i>Lc. mesenteroides</i>	1 (P1)	+
Lc10	<i>Lc. mesenteroides</i>	5 (C2)	+	

	Lc13	<i>Lc. mesenteroides</i>	6a (P)	+
	Lc30	<i>Lc. mesenteroides</i>	28 (C5)	+
	Lc11	<i>Lc. mesenteroides</i>	47 (C6)	+
	Lc36	<i>Lc. citreum</i>	8 (P2), 23, 32 (C5)	+
	Lc37	<i>Lc. citreum</i>	2 (C2), 9 (P2), 29, 31 (C5), 72, 76 (C7), 84, 88 (P6)	+
	Lc4	<i>Lc. citreum</i>	9a (T)	+
	Lc5	<i>Lc. citreum</i>	33 (C5), 13a (T), DSM20188 (ND ⁴)	+
Weissella	W38	<i>W. cibaria</i>	64 (C7)	+
	W39	<i>W. cibaria</i>	21 (C4)	+
	W40	<i>W. cibaria</i>	DSM15878 (chili bo)	+
	W41	<i>W. cibaria</i>	DSM14295 (kimchi)	+
	W42	<i>W. cibaria</i>	30 (C5)	+
	W33	<i>W. cibaria</i>	10b (T)	+
	W20	<i>W. confusa</i>	1102001 (green pea juice)	+
	W15	<i>W. confusa</i>	16, 17 (C4)	+
	W16	<i>W. confusa</i>	DSM20196 (cane sugar)	+
	W17	<i>W. confusa</i>	59 (C6)	+
	W3	<i>W. confusa</i>	38 (P5)	+
	W47	<i>W. koreensis</i>	DSM15830 (kimchi)	+
	W46	<i>W. paramesenteroides</i>	37 (P4)	-
	W19	<i>W. soli</i>	58 (C6)	-

¹P: papaya, ²C: cabbage, ³T: tomato, ⁴ND: not determined

Table 2. Influence of the temperature on EPS production from sucrose and glycanucrase gene detection.

Isolate	EPS production and colony aspect			Gene detection		
	25°C	30°C	37°C	Glucansucrase sequence length (pb)	% identity	Species
<i>Lc. pseudomesenteroides</i> 12b	+ (creamy)	+ (creamy)	+/-	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 27b	+ (creamy)	+ (creamy)	+/-	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 56	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 89	+ (liquid)	+ (creamy)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> DSM20193	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> DSM5625	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 60	+ (creamy)	+ (creamy)	-	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 39	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 78	+ (creamy)	+ (creamy)	+ (creamy)	ND	ND	ND
<i>Lc. pseudomesenteroides</i> 79	+ (liquid)	+ (liquid)	+ (creamy)	ND	ND	ND
<i>Lc. lactis</i> 24	+ (creamy)	+ (creamy)	-	ND	ND	ND
<i>Lc. mesenteroides</i> 1	+ (liquid)	+ (liquid)	-	609	99% AP017935.1	<i>Lc. mesenteroides</i>
<i>Lc. mesenteroides</i> 5	+ (liquid)	+ (liquid)	-	613	99% DQ249318.1	<i>Lc. mesenteroides</i>
<i>Lc. mesenteroides</i> 6a	+ (liquid)	+ (liquid)	-	526	90% JQ619633.1	<i>Lc. mesenteroides</i>
<i>Lc. mesenteroides</i> 28	+ (liquid)	+ (creamy)	-	118	92% MG869733.1	<i>Lc. mesenteroides</i>
<i>Lc. citreum</i> 33	+ (creamy)	+ (creamy)	+/- (liquid)	ND	ND	ND
<i>Lc. citreum</i> DSM20188	+ (liquid)	+ (liquid)	+/-	ND	ND	ND
<i>Lc. citreum</i> 2	+ (liquid)	+ (liquid)	+ (creamy)	615	99% DQ873511.1	<i>Lc. citreum</i>
<i>Lc. citreum</i> 9a	+ (creamy)	+ (creamy)	+/-	606	99% DQ873511.1	<i>Lc. citreum</i>
<i>W. cibaria</i> 10b	+ (creamy)	+ (creamy)	-	828	99% GU237484.3	<i>W. cibaria</i>
<i>W. cibaria</i> 21	+ (creamy)	+ (creamy)	+/-	823	99% GU237484.3	<i>W. cibaria</i>
<i>W. cibaria</i> 30	+ (creamy)	+ (liquid)	-	916	98% GU237484.3	<i>W. cibaria</i>
<i>W. cibaria</i> DSM14295	+ (creamy)	+ (creamy)	+/-	825	99% HE818409.1	<i>W. cibaria</i>
<i>W. cibaria</i> DSM15878	+ (creamy)	+ (creamy)	+ (granular)	885	99% GU237484.3	<i>W. cibaria</i>
<i>W. cibaria</i> 64	+ (creamy)	+ (creamy)	+/-	657	99% GU237484.3	<i>W. cibaria</i>
<i>W. confusa</i> 16	+ (creamy)	+ (creamy)	+ (granular)	210	98% KP729387.1	<i>W. confusa</i>
<i>W. confusa</i> 17	+ (creamy)	+ (creamy)	+ (granular)	234	98% KP729387.1	<i>W. confusa</i>
<i>W. confusa</i> DSM20196	+ (creamy)	+ (creamy)	+/-	29	100% KP729387.1	<i>W. confusa</i>

<i>W. confusa</i> 59	+ (creamy)	+ (creamy)	+ (granular)	182	99% KP729387.1	<i>W. confusa</i>
<i>W. confusa</i> 38	+ (creamy)	+ (creamy)	-	39	97% KP729387.1	<i>W. confusa</i>
<i>W. confusa</i> 1102001	+ (creamy)	+ (creamy)	-	54	91% KP729387.1	<i>W. confusa</i>

+: observed EPS production on MRS sucrose; +/- : weak EPS production, - : no EPS production; ND: no amplification

6a	++	+/-***	-***	+***	+***	-***	-***	-***
28	++	+/-***	+/-***	+***	+***	-***	-***	-***
33	+++	+***	+/-***	++*	+/-***	++***	-***	-***
DSM20188	+++	++***	-***	+***	-***	++***	-***	-***
2	++	-***	-***	-***	-***	-***	-***	-***
9a	+++	+***	+***	+***	-***	+++	+/-***	-***
Weissella								
10b	++	++	-***	+**	-***	++**	-***	-***
21	+++	++***	-***	++**	+***	+++	+++***	-***
30	+++	++***	+/-***	+++***	+***	+++	+++	+***
DSM14295	+++	+***	-***	++***	+***	+++	-***	-***
DSM15878	++	-**	*	++	*	++	-***	-**
64	+++	+++***	+/-***	+++***	+***	+++	+++	+***
16	+++	+***	-***	+++***	+/-***	+++*	-***	-***
17	+++	+***	-***	+++***	-***	+++***	-***	-***
DSM20196	+++	+***	+***	+***	+***	+++***	-***	-***
59	+++	+***	+***	+++***	+***	+++*	-***	-***
38	+++	+/-***	-***	++*	++	+***	-***	-***
DSM15830	+++	+***	-***	-***	-***	-***	-***	-***
37	+	+/-***	-***	+	+/-***	-***	-***	-***
58	++	-***	-***	++	++	-***	-***	-***
1102001	+++	+++***	+***	+++***	+***	+++	-***	-***

Table 4. Optimum growth rate (μ_{opt}) and cardinal growth temperatures (T_{opt} , T_{min} and T_{max} respectively for optimum, minimal and maximal temperatures).

Results represents the predicted values calculated by Sym'Previs software from three independent experiments. Mean \pm standard deviation (SD) is shown.

R^2 indicates the goodness of fit to the model. T_{min} and T_{max} observed in 21 days correspond to the minimal and maximal temperature, respectively, at which a significant growth was observed after 21 days of incubation.

Species	Isolate	μ_{opt} (h^{-1}) \pm SD ¹	T_{opt} ($^{\circ}C$) \pm SD	T_{min} ($^{\circ}C$) \pm SD	T_{min} observed in 21 days	T_{max} ($^{\circ}C$) \pm SD	T_{max} observed in 21 days	R^2
<i>Fb. tropeoli</i>	77	0.217 \pm 0.047	33.8 \pm 3.7	3.7 \pm 6.6	10	43.4 \pm 1.9	42	0.768
<i>Lactobacillus</i>	73	0.301 \pm 0.038	32.9 \pm 1.6	14.9 \pm 2.5	6	45.7 \pm 3.2	> 45	0.937
	75	0.268 \pm 0.016	33.0 \pm 0.8	8.3 \pm 1.1	8	46.5 \pm 1.4	42-45	0.954
	17a	0.318 \pm 0.036	33.9 \pm 1.7	5.5 \pm 3.5	8	42.1 \pm 0.2	42-45	0.92
	DSM 2601	0.298 \pm 0.156	34.0 \pm 2.4	9.8 \pm 4.5	8	40.0 \pm 12.8	42-45	0.834
<i>Leuconostoc</i>	12b	0.231 \pm 0.040	26.7 \pm 3.9	2.4 \pm 10.5	2-4	37.9 \pm 1.0	< 42	0.98
	27b	0.107 \pm 0.042	28.7 \pm 5.1	10.4 \pm 5.8	6	42.6 \pm 1.4	42	0.723
	56	0.263 \pm 0.024	28.8 \pm 1.1	10.8 \pm 0.7	8	42.5 \pm 0.2	< 42	0.998
	89	0.265 \pm 0.016	29.9 \pm 1.4	4.4 \pm 2.1	8	42.5 \pm 0.2	< 42	0.999
	DSM 20193	0.127 \pm 0.031	30.6 \pm 6.6	4.2 \pm 2.7	8	42.7 \pm 1.2	< 42	0.782
	DSM 5625	0.192 \pm 0.031	31.0 \pm 1.8	11.1 \pm 1.6	8	38.3 \pm 1.0	< 42	0.894
	60	0.186 \pm 0.024	33.3 \pm 2.4	4.3 \pm 6.5	10	50.3 \pm 7.8	42-45	0.979
	39	0.229 \pm 0.052	27.5 \pm 2.7	11.2 \pm 4.3	6	43.1 \pm 1.1	42	0.984
	78	0.205 \pm 0.107	31.8 \pm 6.4	9.0 \pm 6.4	6	37.5 \pm 1.6	42	0.787
	79	0.223 \pm 0.047	27.6 \pm 2.3	11.9 \pm 1.1	8	42.9 \pm 0.9	42	0.86
	24	0.288 \pm 0.118	29.7 \pm 5.4	10.5 \pm 4.4	8	42.7 \pm 1.7	42-45	0.895
	1	0.244 \pm 0.049	29.6 \pm 4.8	3.6 \pm 13.5	< 2	45.6 \pm 5.1	42	0.52
	5	0.279 \pm 0.035	33.2 \pm 2.5	1.0 \pm 7.4	< 2	43.1 \pm 1.0	< 42	0.787
	6a	0.202 \pm 0.007	28.6 \pm 0.6	6.6 \pm 1.1	6	43.0 \pm 0.2	< 42	0.999
28	0.322 \pm 0.062	26.1 \pm 3.9	8.1 \pm 13.2	8	42.2 \pm 0.4	< 42	0.967	

	33	0.231 ± 0.012	25.1 ± 1.5	7.1 ± ND	6	43.1 ± 0.4	< 42	0.995
	DSM 20188	0.261 ± 0.007	26.9 ± 0.5	3.5 ± 1.4	6	39.2 ± 0.3	> 42	0.999
	2	0.146 ± 0.024	30.5 ± 2.9	6.0 ± 4.7	2	43.6 ± 1.5	< 42	0.821
	9a	0.191 ± 0.047	31.8 ± 4.3	6.9 ± 5.5	8	42.9 ± 1.2	< 42	0.731
Weissella	10b	0.571 ± 0.144	30.9 ± 3.8	16.2 ± 8.0	8	44.4 ± 2.6	42-45	0.823
	21	0.209 ± 0.050	30.5 ± 4.1	8.7 ± 4.9	6	42.9 ± 1.3	42-45	0.794
	30	0.433 ± 0.120	34.8 ± 3.7	8.7 ± 4.9	6	45.1 ± 5.0	42-45	0.726
	DSM 14295	0.264 ± 0.031	29.8 ± 2.0	6.7 ± 4.8	8	43.1 ± 0.8	42-45	0.876
	DSM 15878	0.255 ± 0.052	31.0 ± 2.4	16.7 ± 2.1	6	42.9 ± 1.1	42-45	0.942
	64	0.387 ± 0.034	33.8 ± 1.3	6.7 ± 2.3	6	47.0 ± 2.7	42-45	0.981
	16	0.418 ± 0.066	32.1 ± 2.8	12.0 ± 0.8	10	49.3 ± 7.3	42-45	0.881
	17	0.998 ± 0.080	37.2 ± 0.9	10.1 ± 0.1	8	43.5 ± 0.8	42-45	0.993
	DSM 20196	0.199 ± 0.040	33.0 ± 4.5	1.8 ± 20.9	8	42.5 ± 0.9	42-45	0.886
	59	0.601 ± 0.185	39.0 ± 4.0	0.6 ± 6.5	6	43.1 ± 4.2	42-45	0.981
	38	0.336 ± 0.120	30.5 ± 4.3	8.4 ± 9.2	10	48.2 ± 8.8	42-45	0.599
	37	0.148 ± 0.035	29.7 ± 3.6	7.2 ± 6.0	8	43.0 ± 1.3	42-45	0.693
	58	0.264 ± 0.570	34.2 ± 10.8	16.2 ± 5.7	6	38.1 ± 13.7	42-45	0.749
	1102001	0.359 ± 0.087	31.0 ± 2.5	10.7 ± 2.5	10	47.8 ± 5.7	42-45	0.866

¹SD: Standard deviation.