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## Screening of lactic acid bacteria for their potential use as aromatic starters in fermented vegetables

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26 them, 14 displayed an activity greater than 10 nmol/min/mg dry cell. Thirteen were identified as  
27 *Lactiplantibacillus (L.) plantarum* and one as *L. pentosus*. Secondly, four strains of different phenotypes  
28 for  $\beta$ -glucosidase activity were tested for ADH activity. The highest reduction ability for hexanal and (*E*)-  
29 2-hexenal was obtained for *Limosilactobacillus (L.) fermentum* V013-1A for which no  $\beta$ -glucosidase  
30 activity was detectable. The three other strains (*L. plantarum* C022-2B, C022-3B, and V0023-4B2)  
31 exhibited a lower reduction ability and only for hexanal. Thirdly, mashed tomatoes were fermented with  
32 these four strains individually to evaluate their ability to release volatile compounds from the tomato  
33 precursors. Fifty-eight volatile compounds were identified and quantified by HS-SPME/GC-MS.  
34 Untreated tomatoes were rich in aldehydes. The tomatoes fermented with *L. plantarum* strains were rich  
35 in ketones whereas those with *L. fermentum* were rich in alcohols. However, for the generation of  
36 terpenoids that provide flower and fruit flavours, our screening of  $\beta$ -glucosidase activity was not able to  
37 explain the differences among the strains. For ADH activity, *L. fermentum* exhibited a high activity in  
38 fermentation as most of the target aldehydes and ketones disappeared and were replaced by their  
39 corresponding alcohols. The *L. plantarum* strains exhibited a lower activity but with an important  
40 substrate-selectivity diversity. A better knowledge of the functionality of each LAB strain in the food  
41 matrix will permit to predict and shape the aroma profiles of fermented food.

42 *Keywords:* Lactic acid bacteria;  $\beta$ -Glucosidases; Alcohol dehydrogenases; Volatile compounds;  
43 Fermented foods; Mashed tomatoes

## 44 **I. Introduction**

45 To achieve the transition to a sustainable, affordable, trustworthy, and high-quality food system in  
46 the next decade, a WHO/FAO report recommends a minimum of 400 g of fruit and vegetable  
47 consumption per day (WHO, 2003). Lactic acid fermentation is a traditional process that could be an eco-  
48 friendly way to diversify fruit and vegetable supply and therefore increase their daily consumption.

49 Generally, five interesting features are put forward to explain the use of LAB in fermented food: increase  
50 of the shelf-life of perishable raw materials, innovation, detoxification, nutritional enrichment, and  
51 reduction of fuel consumption (Steinkraus, 1995). Whereas lactic acid fermentation is roughly anecdotic  
52 for fruits and vegetables in western countries (but including some popular examples like sauerkraut,  
53 olives, capers, turnip, and gherkins) (Di Cagno et al., 2013; Tamang et al., 2016b), it is popular in Asia.  
54 Indeed, in Asia, lactic acid fermented fruits or vegetables are served as an appetiser, a side dish or an  
55 ingredient to prepare the main course. The raw materials are very diverse, resulting in products like *sunki*  
56 (Japanese fermented red beet leaves) (Endo et al., 2008), *kimchi* (Korean fermented napa cabbage with  
57 other vegetables) (Patra et al., 2016), *sinki* (Indian fermented radish taproot) (Tamang et al., 2005), *dua*  
58 *muoi* (Vietnamese fermented mustard leave, beet, and eggplant) (Nguyen et al., 2013), and *chrouk* or  
59 *tram* (Cambodian fermented fruits or vegetables).

60 Lactic acid fermentation is considered as a simple and valuable biotechnology for preserving and/or  
61 improving the safety, nutritional, and shelf-life properties of fruits and vegetables (Fan and Hansen, 2012;  
62 Tamang et al., 2016a). It is also very important for sensory properties. Lactic acid bacteria (LAB) have  
63 been known to enhance the flavour of fermented products thanks to their metabolism. In this paper, for all  
64 LAB species, we referred to the new taxonomy (Zheng et al., 2020). Homofermentative LAB produce  
65 lactic acid as the main end-product of carbohydrate fermentation while heterofermentative LAB produce  
66 in addition other products such as acetic acid, carbon dioxide, ethanol, acetoin, and diacetyl (Gänzle,  
67 2015; Leroy and De Vuyst, 2004). The volatiles generated by heterofermentation can provide special  
68 tastes and flavours to final fermented foods. The characteristic of many fermented foods depends  
69 therefore on LAB metabolism.

70 Furthermore, the genesis of flavour compounds mainly rely on the specific ability of different  
71 bacterial strains to convert precursors derived from carbohydrates, proteins, fatty acids, carotenoids, and  
72 glycosides (Bancalari et al., 2017). The newly generated volatile compounds are added to the pool of

73 flavouring compounds that are already present in plant matrix though in plant, these latter are mostly  
74 encountered under a glycosylated form to decrease their toxicity (Song et al., 2018).  $\beta$ -Glucosidases are  
75 key enzymes for releasing aroma compounds from the glucosidic precursors found in fruits (Krisch et al.,  
76 2010). They are utilised to enhance the flavour of wine (Sestelo et al., 2004), tea (Su et al., 2010), and  
77 fruit juice (Fan et al., 2011). Some LAB such as *Lactiplantibacillus plantarum* USC1 (Sestelo et al.,  
78 2004) and *Lactobacillus* ssp. and *Pediococcus* ssp. (Grimaldi et al., 2005) isolated from wines and  
79 fermented foods are capable of exhibiting  $\beta$ -glucosidase activity (Michlmayr and Kneifel, 2014). This  
80 phenotype is strain-specific, as reported by Renchinkhand et al. (2015) who found only six  $\beta$ -glucosidase  
81 positive strains among 28 LAB strains isolated from *kimchi*. Alcohol dehydrogenase (ADH) is another  
82 family of enzymatic activities which could be important for modifying aroma profiles in fruits and  
83 vegetables. ADH catalyses the transformation of aldehydes or ketones into their corresponding alcohols,  
84 acids or esters. Despite the fact that their encoding genes are generally present in LAB genomes, ADH  
85 activities have only rarely been reported in LAB. *Lentilactobacillus kefir* DSM 20587 was able to reduce  
86 2,5-hexanedione into (2*R*,5*R*)-hexanediol (Haberland et al., 2002) in specific resting whole cells  
87 conditions. Few studies characterised purified ADHs of LAB. In cheese, Hu et al. (2019) found five  
88 ADHs of *Limosilactobacillus reuteri* DSM20016 which could reduce aldehydes into their corresponding  
89 alcohols, for instance, 3-methylbutanal to 3-methylbutanol, butyraldehyde to 2-butanol, hexanal to  
90 hexanol, phenylacetaldehyde to phenylethanol, etc.

91 To improve the aroma profiles through fermentation, fruits or vegetables have to be fermented by  
92 LAB starters possessing adequate metabolic and enzymatic activities towards plant matrix. This requires  
93 to select the starters according to their enzymatic activities in the matrix, which cannot be fully predicted  
94 in laboratory conditions. In the present study, 200 LAB isolates from Cambodian and Vietnamese  
95 spontaneously fermented foods were screened for some enzymatic activities which might be important for  
96 the development of the sensorial profile of fermented products. The first activity screened, which is,

97 according to bibliography, the less prevalent in LAB, was  $\beta$ -glucosidase. It was done using esculin iron  
98 agar and *p*-nitrophenyl- $\beta$ -D-glucoopyranoside assays. For the highest  $\beta$ -glucosidase producers, the species  
99 were identified and the strains were clustered according to RAPD profile. Four LAB isolates exhibiting  
100 distinct  $\beta$ -glucosidase activities (high, medium or negative) were then characterised for their ADH  
101 activity on several aldehyde substrates. Then, they were used as starters in mashed tomatoes. Tomato  
102 fruits were chosen for this study due to the availability of this raw material as by-products, the health  
103 benefit of this plant, and the richness of aroma precursors. The strain ability to release volatile compounds  
104 from the tomato precursors was analysed in regard to their  $\beta$ -glucosidase and ADH activities.

## 105 **II. Materials and methods**

### 106 *2.1. Fermented foods and bacterial strains*

107 Twenty-five samples were collected from different fermented foods from Cambodia or Vietnam as  
108 shown in [Table 1](#). Each sample (20 g) was suspended in 80 mL of sterile tryptone salt (8.5 g/L NaCl and  
109 1 g/L tryptone) and mixed with a Stomacher for 1 min. Serial dilutions were plated on MRS agar (VWR),  
110 and incubated for 48 h at 37 °C. Bacterial isolates of different morphology were selected and grown in  
111 MRS (pH 5.0) at 37 °C, except otherwise specified. The stationary phase of growth was reached after 20  
112 h culture. *L. plantarum* B33, previously isolated from raw Vietnamese fermented pork (*nem chua*), was  
113 used for comparison purpose as a strain which has been selected as a starter for several applications (Cao-  
114 Hoang et al., 2013).

### 115 *2.2. Screening for $\beta$ -glucosidase activity*

116  $\beta$ -glucosidase activity assays were conducted as described by Renchinkhand et al. (2015) with some  
117 modifications. Esculin (coumarin glucoside) was used to visualise the  $\beta$ -glucosidase activity that  
118 hydrolyses esculin to release  $\beta$ -D-glucose and esculetin. Esculetin reacts thereafter with iron salts, which

119 form a brown or black complex. Therefore, a colony corresponding to an isolate positive for  $\beta$ -  
120 glucosidase activity turns brown or black. The esculin iron agar (EIA) at pH 6.60 was composed of  
121 esculin 1 g/L (Acros Organics), iron ammonium citrate 0.5 g/L, meat extract 3 g/L, peptone 5 g/L, and  
122 agar 16 g/L. Bacterial isolates were grown for 24 h at 37 °C, plated on EIA, and incubated for 72 h at the  
123 same temperature.

### 124 2.3. Evaluation of $\beta$ -glucosidase activity

125  $\beta$ -Glucosidase activity was evaluated by the release of *p*-nitrophenol (*p*NP, Sigma-Aldrich) from *p*-  
126 nitrophenyl- $\beta$ -D-glucopyranoside (*p*NPG, Sigma-Aldrich N7006). The  $\beta$ -glucosidase activity was  
127 determined according to the method described by Grimaldi et al. (2005) with some adjustments. Bacterial  
128 cell suspension from 22 h culture at 25 °C was harvested by centrifugation (4,000 rpm, 7 min at 4 °C),  
129 washed twice with physiological water (0.9% NaCl, w/v), and normalised to a final optical density at 600  
130 nm (OD<sub>600</sub>) of 0.8.

131 Assays were prepared in a 96-well U-bottom microplate (ThermoFisher Scientific). Each well was  
132 filled with 40  $\mu$ L of 0.2 M McIlvane buffer at pH 5.0 (0.1 M of citric acid and 0.2 M of dipotassium  
133 hydrogen phosphate), 20  $\mu$ L of bacterial cell suspension, and 20  $\mu$ L of *p*NPG solution (40 mM, pH 5.0).  
134 The microplate was incubated for 2 h at 37 °C. The reaction was stopped with 160  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> solution  
135 (0.5 M). The microplate was then centrifuged (4,000 rpm, 15 min at 4 °C) and the supernatants (200  $\mu$ L)  
136 were transferred to a new 96-well flat bottom microplate. The absorbance at 400 nm (A<sub>400</sub>) was measured  
137 by a multiplate spectrophotometer (FLUOstar Omega, BMG LABTECH). Reactions were carried out in  
138 triplicate. The *p*NP calibration standards were between 0.013 and 0.302 mM. One unit of enzymatic  
139 activity (UA) was defined as the amount of *p*-nitrophenol (nmol) released per minute per milligram of  
140 cell dry weight. Each strain was grown in three independent cultures to determine the biological variation.

141 *2.4. Genotypic identification of LAB*

142 ***DNA extraction and molecular identification by 16S rRNA gene sequencing***

143 Genomic DNA was extracted using the InstaGene™ Matrix (Bio-Rad) with modifications. Each  
144 isolate (1 mL) grown 20 h at 37 °C was pelleted by centrifugation , washed twice with ultra-pure water,  
145 resuspended in 200 µL of InstaGene™ Matrix, and incubated at 56 °C for 30 min. After vortex mixing at  
146 high speed for 10 s, the tubes were placed in a boiling water bath for 10 min. The supernatant containing  
147 the genomic DNA was recovered by centrifugation at 10,000 g for 3 min and stored at -20°C until use.  
148 DNA were quantified by NanoDrop™. The V1-V9 regions of 16S rRNA gene were amplified using  
149 primers 16S-27 (5'-AGAGTTTGATCMTGGCTCAG-3') and 16S-1492R (5'-  
150 TACGGYTACCTTGTTACGACTT-3'). DNA sequencing reactions were performed by Eurofins  
151 Genomics (Germany) using both forward and reverse primers (16S-SeqF, 5'-  
152 AGTAGGGAATCTTCCACA-3' and 16S-SeqR, 5'-CTTGCCACCTACGTATTA-3'). Taxonomic  
153 identification was determined by comparing the sequence of each isolate with those reported in the basic  
154 BLAST database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

155 ***Multiplex PCR assay***

156 Multiplex PCR assays were realised as described by Torriani et al. (2001). The PCR mixture was  
157 composed of four primers: 0.25 µM paraF (5'-GTCACAGGCATTACGAAAAC-3'), 0.25 µM pentF (5'-  
158 CAGTGGCGCGGTTGATATC-3'), 0.12 µM planF (5'-CCGTTTATGCGGAACACCTA-3'), and 0.25  
159 µM pREV (5'-TCGGGATTACCAAACATCAC-3'). The difference in size of the amplicons allows to  
160 discriminate *L. plantarum*, *L. paraplantarum*, and *L. pentosus* species.

161 ***Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis***

162 Differential identification of the isolates was carried out through RAPD analysis. Four primers  
163 (Eurogentec) with arbitrarily chosen sequences (OPL5, 5'-ACGCAGGCAC-3'; P2, 5'-ATGTAACGCC-



164 3'; P4, 5'-CCGCAGCGTT-3'; M13, 5'- GAGGGTGGCGGTTCT-3') were tested singly in four series of  
165 amplification (Corsetti et al., 2003; Di Cagno et al., 2009a; Lucena-Padrós et al., 2014). In the case of  
166 using the M13 primer, each 25- $\mu$ L reaction mixture contained 1  $\mu$ M of primer, 200  $\mu$ M of each dNTP, 0.5  
167 U of Phusion<sup>TM</sup> High-Fidelity DNA Polymerase (Thermo Fisher Scientific), and 6  $\mu$ L of DNA extract.  
168 The PCR program was an initial denaturation at 98 °C for 30 s, 35 cycles of 98 °C for 10 s, 46 °C for 60  
169 s, 72 °C for 120 s, and a final extension at 72 °C for 5 min.

170 For OPL5, P2 or P4 primer, the 25- $\mu$ L PCR mixture contained 1.5 mM of MgCl<sub>2</sub>, 0.5  $\mu$ M of primer,  
171 200  $\mu$ M of dNTP, 0.5 U of Taq polymerase (Thermo Fisher Scientific), and 6  $\mu$ L of DNA extract. The  
172 PCR program was: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 30 °C for 60 s, 72°C for 120 s, and 72 °C  
173 for 5 min.

174 The total PCR products were analysed by electrophoresis at 80 V for 4 h on 1.5% (w/v) agarose gel  
175 in 1X TAE buffer and the DNA was detected by UV transillumination after staining with ethidium  
176 bromide (0.5 mg/L). The molecular sizes of the amplified DNA fragments were estimated by comparison  
177 with Smartladder (Eurogentec). Randomly amplified polymorphic DNA-PCR (RAPD-PCR) analysis  
178 profiles were acquired by using the Gel Doc XR<sup>+</sup> system (Bio-Rad) and were compared using Image Lab  
179 software (Bio-Rad).

## 180 *2.5. Reduction of aldehydes and ketones into alcohols by LAB resting cells*

181 The ADH activity of LAB resting cells was evaluated through the reduction of aldehydes or ketones  
182 into their corresponding alcohols. Substrates for the reduction reaction included hexanal (98%), (*E*)-2-  
183 hexenal (95%), (*E*)-2-heptenal (95%), (*E,E*)-2,4-decadienal (89%), 6-methyl-5-hepten-2-one (98%), and  
184 6,10-dimethyl-5,9-undecadien-2-one (97%). They were purchased from Sigma-Aldrich. 4-Nonanol  
185 (96.5%) from Merck Millipore, was used as the internal standard. Stock solutions (1 g/L) were prepared  
186 in absolute ethanol. Working solutions (160 mg/L) were obtained by diluting the stock solutions with

187 distilled water. The bacterial cell suspension from a-20-h-culture was obtained by centrifugation (4,000  
188 rpm, 7 min at 4 °C), washed twice with physiological water, and diluted to 10<sup>8</sup> CFU/mL according to the  
189 CFU/OD correspondance obtained previously.

190 Bacterial ADH assays were carried out in 4 mL glass vials. Each vial contained 0.5 mL of substrate  
191 (20 mg/kg), 0.5 mL of glucose solution (3 g/kg), 0.5 mL of physiological water, and 17 µL of bacterial  
192 suspension to initiate the reduction reaction. The reduction reaction was conducted in triplicate at 37 °C  
193 for 1 h under agitation. Volatile compounds were quantified by gas chromatography coupled with flame  
194 ionisation detector (GC/FID, PerkinElmer, Clarus® 500) after extraction with diethyl ether according to  
195 Try et al. (2018) using 4-nonanol (35 µL, 1.83 g/kg) as the internal standard. The oven temperature  
196 programme was modified to have an increase from 48 °C (hold for 2 min) to 110 °C at 2 °C/min, then  
197 from 110 °C to 120 °C at 10 °C/min and finally from 110 °C to 250 °C (hold for 2 min) at 30 °C/min.  
198 One unit of enzymatic activity (UA) was defined as the amount of a substrate (nmol) reduced into a  
199 product per minute per CFU.

## 200 *2.6. Mashed tomato treatment*

201 Peeled frozen tomatoes (Picard Surgelés, France) were thawed and blended with a conventional  
202 blender (Philips HR2056/00, 350 W) for 1 min to obtain mashed tomatoes (MT). The MT (60 g) were  
203 fermented in a 100 mL Duran glass jar with a screw-cap. Stationary phase cultures were used to inoculate  
204 MT (10<sup>5</sup> CFU/g of MT) into hermetic jars. MT were mixed and the jars were then kept in a static  
205 incubator for 24 h at 37 °C. The control “acid hydrolysis sample” was MT acidified to pH 3.7 with lactic  
206 acid. The “enzymatic hydrolysis sample” corresponded to MT treated with 1 U of almond β-glucosidases  
207 (Sigma-Aldrich, G0395-2.5KU) per g of tomato as control of the enzymatic activity. These conditions  
208 were noted LA and Glu, respectively. The control samples were also incubated for 24 h at 37 °C.  
209 Analyses were made extemporaneously. The MT were analysed immediately after blending as a reference

210 without any treatment, and this condition was called “untreated tomatoes” (UT). The entire experiment  
211 was carried out in triplicate.

## 212 *2.8. Volatile compound extraction*

213 Volatile compounds were extracted by headspace-solid phase microextraction (HS-SPME). Three  
214 fibre coatings were tested on the MT to select the most suitable fibre coating for the tomato volatile  
215 compounds. 100 µm polydimethylsiloxane (PDMS), 65 µm PDMS/divinylbenzene (PDMS/DVB), and 75  
216 µm carboxen/PDMS (CAR/PDMS) were purchased from Sigma-Aldrich. The extraction was carried out  
217 in 20 mL headspace glass vial sealed tightly with crimp cap and PTFE/silicone septum. The MT (5 g) and  
218 10 µL of 4-nonanol (70.77 mg/kg) as internal standard were placed in the vial. Before inserting the fibre,  
219 the vial was previously conditioned by stirring for 20 min at 40 °C. The fibre was then exposed to the  
220 headspace for 40 min at the same temperature (Di Cagno et al., 2009b). The volatile compounds adsorbed  
221 by SPME fibre were desorbed by splitless injection at 250 °C for 5 min in the GC injector port.

## 222 *2.9. Volatile compound analysis*

223 GC/FID was used to select the fibre coatings and gas chromatography (PerkinElmer, Clarus 580)  
224 coupled with mass spectrometer (GC/MS, PerkinElmer, Clarus SQ 8S) was utilised to identify and  
225 quantify the volatile compounds. GC/FID was equipped with UBWax capillary column (30 m by 320 µm  
226 by 0.25 µm) and GC/MS was equipped with Elite-5MS capillary column (30 m by 250 µm by 0.25 µm).  
227 Nitrogen (GC/FID) and helium (GC/MS) were the carrier gas at a linear flow rate 4.3 mL/min and 1.0  
228 mL/min, respectively. The oven temperature was programmed to increase from 48 °C (hold for 2 min) to  
229 110 °C at 2 °C/min, then from 110 to 250 °C at 10 °C/min (hold for 5 min). Flame ionisation temperature  
230 was 300 °C and interface temperature was 240 °C. Mass spectra were obtained using an ionisation source  
231 with an electronic impact of 70 eV. Mass scans of the sample quadrupole type monitoring ranged from  
232 40-350 m/z. The identification of volatile compounds was mainly achieved by comparing their mass

233 spectra with those of known compounds held in the NIST mass spectral database as well as laboratory  
234 databases.

### 235 2.10. Statistical analysis

236 All experiments were carried out at least twice independently. The data were analysed using  
237 analysis of variance (one-way Anova) and Tukey's Honestly Significant Difference test (HSD) (the level  
238 of significance was 5%) by the XLSTAT statistical software (version 16.0). In this study, principal  
239 component analysis (PCA) was used to detect the correlation between variables, the volatile compounds  
240 in fermented tomatoes and their treatments.

## 241 III. Results

### 242 3.1. Determination of $\beta$ -glucosidase activity

243 Two hundred isolates were selected from 25 fermented foods and screened on EIA for  $\beta$ -  
244 glucosidase activity. Eighty isolates were positive for  $\beta$ -glucosidase according to the brown or black  
245 colour of their colony. The  $\beta$ -glucosidase activity of these 80 isolates was evaluated at 37 °C (from 22 h  
246 culture). Fourteen isolates had an activity greater than 10 UA (Fig. 1), while the other 66 had a  $\beta$ -  
247 glucosidase activity lower than 10 UA (data not shown). The highest activity detected was 27 UA for  
248 isolates C022-2B and C022-3B. Other isolates exhibited about half this activity like V0023-4B2 (12 UA).  
249 For comparison, *L. plantarum* B33 presented a  $\beta$ -glucosidase activity of 17 UA. The quantification of  
250 activity was also checked for V013-1A, which was negative for  $\beta$ -glucosidase activity on EIA plate, and  
251 no activity was detected in the *p*NPG assay, confirming its phenotype.

### 252 3.2. Elimination of duplicate isolates

253 To discard duplicate isolates among the  $\beta$ -glucosidase-positive isolates, the RAPD-PCR profiles  
254 from 55 isolates (the 14 isolates with the highest activities and 41 isolates randomly selected) were

255 analysed. As an example, the different banding patterns obtained with four isolates are shown in Fig. 2. In  
256 the case of the two isolates C022-3B and V0023-4B2, the PCR-amplified DNA fragments with the  
257 primers P2, P4, and M13 were identical. On the contrary, the primers OPL5 generated two different  
258 profiles which enabled us to distinguish these isolates (Fig. 2). The analysis of the amplification profiles  
259 of the 55 isolates with the primers P2, P4, OPL5, and M13 released 30, 33, 33, and 36 different RAPD-  
260 PCR profiles, respectively. Finally, the comparison of the combined RAPD-PCR fingerprinting profiles  
261 provided 40 different strains. It revealed redundancy among the 41 isolates with  $\beta$ -glucosidase activity  
262 lower than 10 UA but not among the 14 isolates with an activity greater than 10 UA.

### 263 3.3. Identification of bacterial species by 16S rRNA and multiplex PCR

264 All the 14  $\beta$ -glucosidase-positive strains, belonged to *Lactiplantibacillus* group (*L. plantarum*, *L.*  
265 *paraplantarum*, and *L. pentosus*) and the  $\beta$ -glucosidase-negative (V013-1A) was *Limosilactobacillus*  
266 *fermentum* according to the 16S rRNA gene sequencing, with homology threshold values higher than  
267 99% (Table 2). As 16S rRNA gene sequencing cannot discriminate between the three species, multiplex  
268 PCR assays were carried out on the 14  $\beta$ -glucosidase-positive strains. Thirteen strains had fragments of  
269 the same size of 318 bp corresponding to *L. plantarum*. Only strain F2 gave a fragment of 218 bp  
270 corresponding to *L. pentosus* (Fig. 3).

### 271 3.4. Reduction of aldehydes and ketones into alcohols

272 Four strains selected for their diverse  $\beta$ -glucosidase activity were tested for their potential to reduce  
273 aldehydes into their corresponding alcohols: *L. plantarum* C022-2B and C022-3B, the two highest  
274 producer strains, *L. plantarum* V0023-4B2, an intermediate producer, and *L. fermentum* V013-1A,  
275 exhibiting no detectable  $\beta$ -glucosidase activity. Hexanal and (*E*)-2-hexenal were used as test substrates as  
276 they are short-chain saturated and unsaturated aldehydes present in mashed tomatoes and important  
277 contributors to the tomato aroma (Wang et al., 2016). *L. fermentum* V013-1A was able to reduce hexanal

278  $(3.85 \times 10^{-8}$  UA) and (*E*)-2-hexenal ( $2.47 \times 10^{-8}$  UA) into hexanol and (*E*)-2-hexenol, respectively (Table  
279 3). No alcohol was detected in the *L. plantarum* C022-2B, C022-3B, and V0023-4B2 vials under the  
280 above conditions. Therefore, an adjustment was made by concentrating bacterial cells ten times. Indeed,  
281 *L. plantarum* C022-2B, C022-3B, and V0023-4B2 could only reduce hexanal with a far lower reduction  
282 rate than *L. fermentum* V013-1A. For *L. plantarum* strains, no reduction of (*E*)-2-hexenal was observed.

283 *L. fermentum* V013-1A seemed to have the greatest potential for aldehyde reduction ability.  
284 Consequently, more aldehyde and ketone substrates were tested. As shown in Table 4, *L. fermentum*  
285 V013-1A was capable of converting (*E*)-2-heptenal ( $2.46 \times 10^{-8}$  UA) and (*E,E*)-2,4-decadienal ( $3.69 \times$   
286  $10^{-8}$  UA) to (*E*)-2-heptenol and (*E,E*)-2,4-decadienol, respectively. This strain could also convert 6,10-  
287 dimethyl-5,9-undecadien-2-one ( $1.26 \times 10^{-8}$  UA) to 6,10-dimethyl-5,9-undecadien-2-ol but not 6-methyl-  
288 5-hepten-2-one. Therefore, *L. fermentum* V013-1A was able to reduce aldehydes and ketones to their  
289 corresponding alcohols with a reduction ability greater for aldehydes than for ketones.

### 290 3.5. Fermented tomato analysis

#### 291 3.5.1. pH and bacterial enumeration

292 The four strains exhibiting various enzymatic properties were used individually as starters for  
293 tomato fermentation.. For the four strains, during fermentation of MT (initial pH 4.3) the pH value  
294 dropped to 3.5-3.8 and the total number of lactobacilli increased from  $1.0 \times 10^5$  CFU/g (initial  
295 inoculation) to  $1.2 \times 10^8$  -  $1.5 \times 10^9$  CFU/g, demonstrating that the four strains were able to grow and  
296 ferment MT individually. For LA and Glu, lactobacilli counts were  $1.1 \times 10^7$  and  $5.4 \times 10^7$  CFU/g after  
297 incubation, showing that a spontaneous fermentation took place reaching lower bacterial counts than with  
298 starters (Table 5).

299 3.5.2. Volatile compound analysis

300 The volatile compounds in UT, LA, Glu, and fermented tomatoes were extracted by HS-SPME.  
301 Among the three fibre coatings tested, PDMS/DVB was the preferred coating material as it gave results  
302 with all the families of compounds. The volatile compounds were identified and quantified by GC/MS.  
303 Fifty-eight volatile compounds were grouped according to the chemical classes: aldehydes, alcohols,  
304 ketones, terpenes, organic acids, and miscellaneous (Table 6).

305 As shown in Table 6, most of the aldehydes found in untreated tomato (UT) had their concentration  
306 decreasing, at least three times, and sometimes even reached zero during fermentation and  $\beta$ -glucosidase  
307 treatment. They also decreased, but slightly (about 1.4 folds), during the lactic acid treatment (LA).  
308 Among fermented tomatoes, LF1A (tomatoes fermented with *L. fermentum* V013-1A) contained the  
309 lowest amount of aldehydes with a 36-fold decrease compared to UT. Only  $\beta$ -cyclocitral,  $\beta$ -citral, and  $\alpha$ -  
310 citral remained in all fermented tomatoes. The levels of aldehydes derived from the LOX pathway such as  
311 hexanal, (*E*)-2-hexenal, (*E*)-2-octenal, and (*E*)-2-nonenal were similar in UT and LA whereas they were  
312 not detectable or very low in the other treatments.

313 Unlike aldehydes, most alcohols detected in our study were not found in UT and LA (Table 6).  
314 Inversely correlated with the degradation of aldehydes, the concentration of alcohols significantly  
315 increased during fermentation and  $\beta$ -glucosidase treatment. The level of alcohol was the highest in LF1A,  
316 where it was about twice as high as in other fermented tomatoes. The alcohols present in high  
317 concentrations in all fermented tomatoes were hexanol and (*E*)-2-octenol (both derived from the LOX  
318 pathway), and 6-methyl-5-hepten-2-ol and 6,10-dimethyl-5,9-undecadien-2-ol. Low concentrations of  
319 phenolic volatiles, including 2-phenylethanol and 4-ethylphenol, appeared during fermentation and  
320 treatment. 2,3-Butanediol only increased significantly in LP2B (tomatoes fermented with *L. plantarum*  
321 C022-2B).

322 The concentration of ketones increased moderately in all fermented tomatoes and in Glu and LA,  
323 except in LF1A where the concentration decreased drastically (Table 6). Ketone levels were similar in  
324 LP2B and LP3B (tomatoes fermented with *L. plantarum* C022-2B and C022-3B, respectively). These  
325 levels were both higher than those in LP4B (tomatoes fermented with *L. plantarum* V0023-4B2), Glu and  
326 LA. 6-Methyl-5-hepten-2-one (MHO) and geranylacetone were the most abundant ketones in UT, Glu,  
327 LA, and fermented tomatoes, but they decreased significantly in LF1A. Although the total concentration  
328 of terpenes did not change considerably during fermentation, several terpenes important for aroma  
329 profiles appeared, including (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, and D-limonene (Table  
330 6).

331 Acetic acid was undetected in UT but it was found in LP2B and LF1A.

332 Principal component analysis (PCA) was used to identify patterns and detect correlations between  
333 volatile compounds and tomato treatments. The first and second components represented 67.90% and  
334 24.67% of the total variance, respectively (Fig. 4). LA was close to UT, which indicates that they were  
335 significantly positively correlated. Acid hydrolysis therefore was unlikely to modify the aroma profiles of  
336 tomatoes. In contrast, LP2B, LP3B, LP4B, and Glu were almost orthogonal with UT, which signifies that  
337 they were not correlated with UT. LF1A was nearly on the opposite side of the UT, which suggests that  
338 they were significantly negatively correlated. Consequently,  $\beta$ -glucosidase activity and LAB had a  
339 considerable effect on the volatile compounds of tomatoes. The score plot (Fig. 4) clearly shows the  
340 effects of the treatments on volatile compounds of tomatoes. UT and LA were rich in aldehydes. LP2B,  
341 LP3B, LP4B, and Glu were likely abundant in ketones. LF1A differed considerably from other fermented  
342 tomatoes with the abundant presence of alcohols and acetic acid. These results highlight the effect of  
343 LAB on volatile compounds of fermented tomatoes.



#### 344 **IV. Discussion**

345 Besides its great potential to enhance sustainability, nutrition, and safety, fermentation can also  
346 have an impact on the sensorial properties of a product (Beena Divya et al., 2012; Leroy and De Vuyst,  
347 2004). Although traditional spontaneous fermentation usually brings some suitable properties, the  
348 selection of starters based on specific enzymatic activities can bring a step forward in a clean label and  
349 natural oriented fermentation leading to food exhibiting specific sensorial properties. The present work  
350 follows this approach focusing on some activities able to bring important sensorial notes to tomatoes.  
351 The first target of our work was the volatile compounds produced during plant metabolism. These volatile  
352 compounds in plant can exhibit toxicity or have a communication role and therefore, the plant itself  
353 inactivates them through glycosylation to decrease toxicity or to control communication between plant  
354 cells. Most of the volatile compounds accumulate in fruits/vegetables as non-volatile and odourless  
355 glycosides. These flavouring volatile compounds can be activated sensorially again by hydrolysing the  
356 sugar bond and releasing the volatile aglycone. This reaction can be catalysed by glucosidase enzymes  
357 (Sarry and Günata, 2004; Song et al., 2018).  $\beta$ -Glucosidase genes are widespread in LAB genomes.  $\beta$ -  
358 Glucosidase activities from LAB have been reported in many plant food matrices such as olives,  
359 soybeans, grapes, and cassava. They can hydrolyse a broad range of substrates (Michlmayr and Kneifel,  
360 2014) but their substrate (aglycone) specificity is difficult to predict based on gene sequence. Thus, the  
361 first step in screening for specific  $\beta$ -glucosidases must be experimental. Aglycone specificity seems to  
362 have evolved during bacterial adaptation to the ecological niche as well as the environmental conditions  
363 for the induction of the enzymatic activity (Michlmayr and Kneifel, 2014). These assumptions suggest  
364 that the selection of LAB from various type of fermented foods would permit to get various  $\beta$ -glucosidase  
365 activities and therefore various aroma profiles.

366 The selected strains exhibiting  $\beta$ -glucosidase activity have been evaluated for their capability to  
367 release volatile compounds in comparison with the effect of lactic acid to stimulate hydrolysis and with  
368 the addition of a  $\beta$ -glucosidase enzyme from almonds. In our study, terpenoids were the main volatiles  
369 released after  $\beta$ -glucosidase treatment. A slight increase in the concentration of terpene ketones was  
370 observed not only in Glu but also in LA (Table 6), confirming that, besides  $\beta$ -glucosidase activity, acid  
371 hydrolysis had also an impact on ketone generation. For the glucosidase-exhibiting strains, the result of  
372 volatile compounds generation correlates with the  $\beta$ -glucosidase activity values with ketone levels higher  
373 in LP2B and LP3B than in LP4B ( $\beta$ -glucosidase activity: 27 UA for *L. plantarum* C022-2B and C022-3B  
374 and 12 UA for *L. plantarum* V0023-4B2). MHO and geranylacetone were the most abundant ketones in  
375 all treatments except in LF1A (Table 6). These compounds give fruity or floral notes in tomato fruits  
376 (Wang et al., 2016) and they are initially derived from lycopene (Vogel et al., 2010).

377 Other important terpenes such as (*Z*)-geraniol, 6,7-dihydrogeraniol, melonol, linalool, and D-  
378 limonene were also detected in fermented tomatoes (Table 6). They can theoretically be released from  
379 fruit glycosides by  $\beta$ -glucosidase or acid hydrolysis (Fenoll et al., 2009; Hellín et al., 2010; Ortiz-Serrano  
380 and Gil, 2010). However, the results in LA and Glu were not significantly different from the ones in UT  
381 whereas several important increases in concentration were observed after fermentation although this was  
382 strain- and compound-specific. For instance, geraniol appeared in all fermentations while limonene  
383 increased significantly in three media but not in LP2B (Table 6). This latter medium was the one  
384 containing the highest concentration of melonol and linalool. All these results suggest that the release of  
385 terpenoids could either come from glycosides after the action of specific glucosidases or from other  
386 metabolic activities present differently in the lactic acid bacteria. Our results show that the production of  
387 volatile terpenes is a characteristic showing differences at the strain level in LAB.

388 A family of flavouring compounds exhibiting a huge importance in many plant products is the  
389 green note family. These compounds, volatile aldehydes and alcohols, are generated from the

390 lipoxygenase (LOX) pathway and are responsible for the fresh and green sensorial notes in fruits and  
391 vegetables. The LOX pathway is a multi-enzymatic system in which polyunsaturated fatty acids are  
392 converted into aldehydes and alcohols by the sequential action of lipoxygenase, hydroperoxide lyase  
393 (HPL) and alcohol dehydrogenase (ADH) (Akacha and Gargouri, 2015; Christensen et al., 2007). This  
394 pathway generally occurs in fruits during ripening or upon tissue disruption (maceration or blending).  
395 Therefore, most of the aldehydes detected in UT and LA were likely released from fatty acids via the  
396 LOX pathway. The LOX-pathway-derived aldehydes include hexanal, (*E*)-2-hexenal, heptanal, (*Z*)-2-  
397 heptenal, (*E*)-2-octenal, nonanal, and (*E*)-2-nonenal (Table 6). The LOX-alcohols homologues of  
398 aldehydes were not detected in UT, showing that the final reduction of aldehydes by alcohol  
399 dehydrogenase was not active. However, this reduction of green notes is important for the sensorial  
400 properties as alcohols exhibit either the same sensorial properties as aldehydes but with higher detection  
401 thresholds or exhibit notes less green and fruitier (Hu et al., 2019; Wang et al., 2016). Green notes are  
402 typical of tomato flavour but their transformation to fruity notes can bring interesting sensorial properties  
403 to the fermented product. When we investigated the ADH activity of the various strains in resting cells,  
404 we noticed that *L. fermentum* exhibited considerably higher activities than *L. plantarum* strains on most  
405 aldehydes and ketones present in the medium (Table 3 and 4). Our test with resting cells presented a  
406 slight difference in ketone reduction compared to what occurred in tomatoes. For example, 6-methyl-5-  
407 hepten-2-one (MHO) was not reduced with resting cells in buffer (Table 4) but its corresponding alcohol  
408 6-methyl-5-hepten-2-ol was found in the complex LF1A (Table 6).

409 In contrast to this wide-range activity of *L. fermentum* ADH, ADHs of *L. plantarum* strains were  
410 substrate selective. *L. plantarum* C022-3B and V0023-4B2 were active only on saturated aldehydes,  
411 while C022-2B was active on unsaturated aldehydes. ADHs of *L. plantarum* strains were also active on  
412 unsaturated ketones (Table 6). LAB genomes possess several *adh* family genes which code various  
413 functional domains (Haberland et al., 2002; Hu et al., 2019). For instance, *L. plantarum* WCFS1

414 possessed nine *adh* genes and *L. fermentum* IFO 3956 ten *adh* genes among which only four are *L.*  
415 *plantarum* homologs (Bioinformatic analysis using Biocyc database) (Karp et al., 2017). The differences  
416 in substrate specificity observed between ADH activities of the two species could be due to the presence  
417 of specific ADH enzymes. This point of characterisation of the diversity of *adh* genes in relation with the  
418 activity will be our interest for the next study.

419 Eventually, besides the strain potential to release terpenoids or to transform aldehydes and ketones  
420 into alcohols, we also found a sign of heterolactic fermentation in fermented products. Indeed, acetic acid  
421 was encountered in fermented matrices but not in UT (Table 6). Unsurprisingly, the medium containing  
422 the highest concentration was LF1A. This is consistent with the fact that *L. fermentum* V013-1A is  
423 obligately heterofermentative (Ibrahim, 2016). Acetic acid together with 2,3-butanediol were also  
424 identified in LP2B (Table 6), showing that *L. plantarum* C022-2B was probably heterofermentative  
425 during tomato fermentation (Ibrahim, 2016; Renchinkhand et al., 2015)

426 Similar research by Di Cagno et al. (2009b) on tomato juices fermented with allochthonous *L.*  
427 *plantarum* LP54 was discriminated from autochthonous strains, especially by high levels of esters,  
428 alcohols and sulphur compounds and a few furans.

## 429 **V. Conclusions**

430 Our results confirm that fermentation has a significant impact on the presence of volatile  
431 compounds in tomatoes. The strategy focusing on strains exhibiting various levels of  $\beta$ -glucosidase and  
432 alcohol dehydrogenase activities is of interest as it leads to various levels of volatile compounds.  
433 However, for the generation of terpenoids that provide flower and fruit flavours, our screening procedure  
434 was not able to explain the differences among the strains.

435 For ADH activity, *L. fermentum* exhibited a high activity, which confirms in fermentation as most  
436 of the target aldehydes and ketones disappeared and were replaced by their corresponding alcohols. The

437 *L. plantarum* strains exhibited a lower activity but with an important substrate-selectivity diversity. The  
438 differences in substrate specificity observed between ADH activities of the two species could be due to  
439 the presence of specific ADH enzymes. This point of characterisation of the diversity of *adh* genes in  
440 relation with the activity will be our interest for the next study.

441 The presence of products of the heterolactic pathway also depended on the strains with the  
442 obligately heterofermentive *L. fermentum* producing mainly acetic acid whereas butanediol was also  
443 detected in *L. plantarum* C022-2B.

444 Together, these results bring about data confirming the metabolic diversity at the strain level and  
445 they can be utilised for the building of starters able to formulate specific flavours.

446

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560

561 **List of tables**562 **Table 1.** Fermented foods collected for this study and their origin.

Matrix	Sample	Origin
Fermented vegetables	Fermented gherkins (2)	Phsar Pochentong, Phnom Penh, Cambodia
	Fermented cucumber pieces (1)	Phsar Depo, Phnom Penh, Cambodia
	Fermented young melons (1)	Phsar Pochentong, Phnom Penh, Cambodia
	Fermented mustard cabbages (5)	Phsar Pochentong and Phsar Depo, Phnom Penh, Cambodia
		Chợ Châu Long, Hanoi, Vietnam
	Fermented shredded wild mustard leaves (2)	Phsar Depo, Phnom Penh, Cambodia
		Chợ Chính Kinh, Hanoi, Vietnam
	Fermented shredded white cabbages and carrots (1)	Chợ Châu Long, Hanoi, Vietnam
	Fermented Thai small round white aubergines (1)	Chợ Chính Kinh, Hanoi, Vietnam
	Fermented Thai big round white aubergines (1)	Chợ Chính Kinh, Hanoi, Vietnam
Fermented legumes	Fermented shallot roots (2)	Phsar Depo, Phnom Penh, Cambodia
		Chợ Chính Kinh, Hanoi, Vietnam
	Fermented chili pepper puree (1)	Mường Khương, Lao Cai district, Vietnam
Fermented meats	Fermented soybeans (2)	Phsar Pochentong and Phsar Depo, Phnom Penh, Cambodia
	Raw fermented fish (1)	Phsar Depo, Phnom Penh, Cambodia
Raw material	Raw fermented pork (4)	Chợ Hâm, Hanoi, Vietnam
	Fresh banana leaf (1)	Dũng Nem - Đặc Sản Ước Lễ Gia Truyền, Hanoi, Vietnam

563

564 **Table 2.** Lactic acid bacteria species identification by molecular approaches and their fermented foods origin

Strain code	Species	Fermented food	Country
C022-2B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-3A	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-3B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-4A	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
C022-4B	<i>L. plantarum</i>	Fermented shallot roots	Cambodia
F1	<i>L. plantarum</i>	Raw fermented fish “ <i>nem trey</i> ”	Cambodia
F2	<i>L. pentosus</i>	Raw fermented fish “ <i>nem trey</i> ”	Cambodia
V0023-4B2	<i>L. plantarum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam
V053-4B	<i>L. plantarum</i>	Fermented small round aubergine	Vietnam
V073-3A1	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V073-4A	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V073-4B	<i>L. plantarum</i>	Fermented shallot roots	Vietnam
V083-4A	<i>L. plantarum</i>	Fresh banana leaf	Vietnam
V083-4B	<i>L. plantarum</i>	Fresh banana leaf	Vietnam
V013-1A	<i>L. fermentum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam
B33	<i>L. plantarum</i>	Raw fermented pork “ <i>nem chua</i> ”	Vietnam

565 **Table 3.** Reduction of aldehydes into alcohols by ADH activity of *L. plantarum* C022-2B, C022-3B, and V0023-4B2 and *L.*  
566 *fermentum* V013-1A.

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)		Product appearance rate ( $\times 10^{-8}$ UA)	
	<i>L. plantarum</i> C022-2B	Hexanal	$0.44 \pm 0.05$	Hexanol
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> C022-3B	Hexanal	< 0.44	Hexanol	< 0.31
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. plantarum</i> V0023-4B2	Hexanal	< 0.44	Hexanol	< 0.31
	( <i>E</i> )-2-hexenal	n.d.	( <i>E</i> )-2-hexenol	n.d.
<i>L. fermentum</i> V013-1A	Hexanal	$3.85 \pm 0.18$	Hexanol	$3.70 \pm 0.42$
	( <i>E</i> )-2-hexenal	$2.47 \pm 0.67$	( <i>E</i> )-2-hexenol	$2.58 \pm 0.26$

567 n.d. = not detectable

568 Values are expressed as means of triplicate experiments. One unit of enzymatic activity (UA) was defined as the amount of a  
569 substrate (nmol) reduced into a product per minute per CFU.

570

571

572 **Table 4.** Reduction of aldehydes and ketones into alcohols by ADH activity of *L. fermentum* V013-1A.

Strain	Substrate disappearance rate ( $\times 10^{-8}$ UA)	Product appearance rate ( $\times 10^{-8}$ UA)
<i>L. fermentum</i> V013-1A	( <i>E</i> )-2-heptenal	2.46 $\pm$ 0.25
	( <i>E,E</i> )-2,4-decadienal	3.69 $\pm$ 0.00
	6-Methyl-5-hepten-2-one	n.d.
	6,10-Dimethyl-5,9-undecadien-2-one	1.26 $\pm$ 0.14

573 n.d. = not detectable

574 Values are expressed as means of triplicate experiments. One unit of enzymatic activity (UA) was defined as the amount of a  
575 substrate (nmol) reduced into a product per minute per CFU.

576 **Table 5.** Total lactobacilli count on MRS agar plates (CFU/g) and pH of tomatoes fermented with the selected LAB strains and  
577 controls.

Treatment	pH	Total lactobacilli (CFU/g)
Untreated tomatoes (UT)	4.3	8.3 $\times 10^2$
Lactic-acid-treated tomatoes (LA)	3.7	1.1 $\times 10^7$
Glucosidase-treated tomatoes (Glu)	4.1	5.4 $\times 10^7$
Fermented tomatoes with <i>L. plantarum</i> C022-2B (LP2B)	3.7	7.5 $\times 10^8$
Fermented tomatoes with <i>L. plantarum</i> C022-3B (LP3B)	3.7	7.0 $\times 10^8$
Fermented tomatoes with <i>L. plantarum</i> V0023-4B2 (LP4B)	3.8	1.5 $\times 10^9$
Fermented tomatoes with <i>L. fermentum</i> V013-1A (LF1A)	3.5	1.2 $\times 10^8$

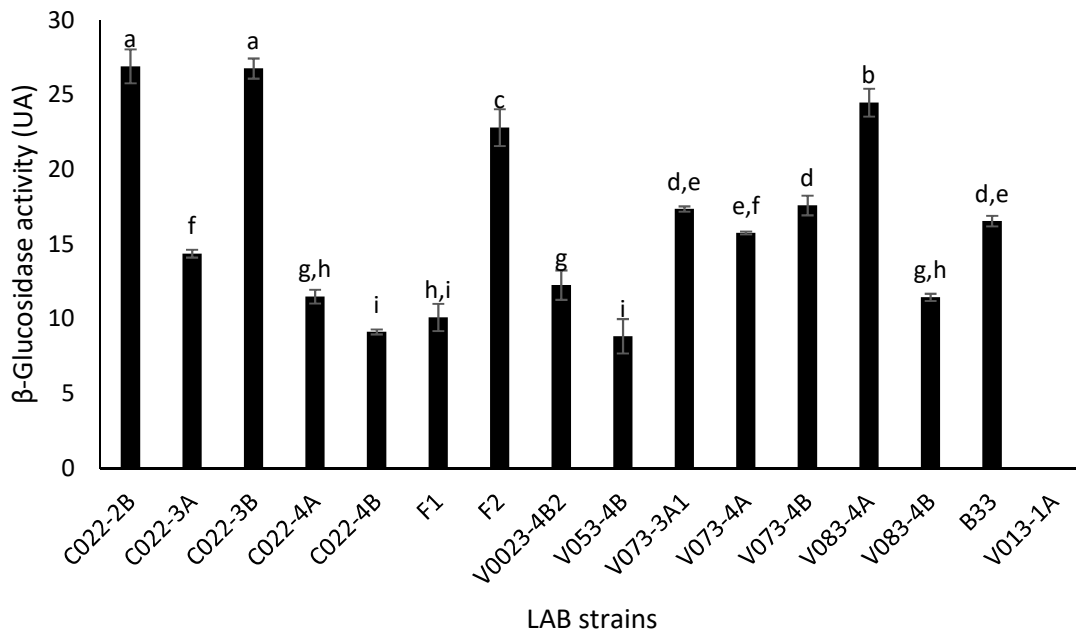
584 For all conditions, tomatoes were incubated for 24 h at 37 °C and analysed immediately, except for UT for which tomatoes  
585 were not incubated. Data are means of three independent experiments.

**Table 6.** Volatile compounds (ppb) found in tomato after treatment.

Chemical class	Treatment						
	UT	MT-LA	MT-Glu	MT-LP2B	MT-LP3B	MT-LP4B	MT-LF1A
<b>Aldehydes</b>							
Hexanal	135.85 <sup>a</sup>	92.21 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E</i> )-2-hexenal	18.10 <sup>a</sup>	17.07 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Heptanal	6.41 <sup>a</sup>	1.36 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E</i> )-2-heptenal	15.65 <sup>a</sup>	14.81 <sup>a</sup>	nd <sup>a</sup>	7.77 <sup>a</sup>	tr <sup>a</sup>	3.56 <sup>a</sup>	nd <sup>a</sup>
( <i>E</i> )-2-octenal	60.19 <sup>a</sup>	58.46 <sup>a</sup>	1.78 <sup>b</sup>	49.63 <sup>ab</sup>	11.92 <sup>ab</sup>	13.60 <sup>ab</sup>	nd <sup>b</sup>
Nonanal	16.77 <sup>a</sup>	6.7 <sup>b</sup>	nd <sup>c</sup>	tr <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
( <i>E</i> )-2-nonenal	9.38 <sup>a</sup>	9.70 <sup>a</sup>	nd <sup>b</sup>	3.29 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Decanal	9.97 <sup>a</sup>	5.99 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
( <i>E,E</i> )-2,4-decadienal	31.68 <sup>ab</sup>	18.17 <sup>bc</sup>	nd <sup>c</sup>	42.98 <sup>a</sup>	32.97 <sup>ab</sup>	nd <sup>c</sup>	nd <sup>c</sup>
Benzaldehyde	nd <sup>b</sup>	nd <sup>b</sup>	13.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
β-Cyclocitral	4.76 <sup>a</sup>	5.85 <sup>a</sup>	3.54 <sup>a</sup>	3.74 <sup>a</sup>	4.44 <sup>a</sup>	4.06 <sup>a</sup>	4.09 <sup>a</sup>
β-Citral	11.62 <sup>a</sup>	9.51 <sup>a</sup>	10.44 <sup>a</sup>	8.11 <sup>a</sup>	5.95 <sup>a</sup>	6.79 <sup>a</sup>	1.88 <sup>a</sup>
α-Citral	31.12 <sup>a</sup>	15.26 <sup>b</sup>	16.25 <sup>b</sup>	14.36 <sup>b</sup>	8.02 <sup>b</sup>	8.48 <sup>b</sup>	4.28 <sup>b</sup>
4-Methyl-3-cyclohexene-1-carboxaldehyde	tr <sup>a</sup>	1.60 <sup>a</sup>	2.97 <sup>a</sup>	tr <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>
3-Methyl-3-(4-methyl-3-pentenyl)-oxiranecarboxaldehyde	6.48 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>358.80</b>	<b>256.69</b>	<b>48.37</b>	<b>131.48</b>	<b>64.10</b>	<b>36.49</b>	<b>10.25</b>
<b>Alcohols</b>							
2,3-Butanediol	nd <sup>b</sup>	nd <sup>b</sup>	2.46 <sup>b</sup>	12.27 <sup>a</sup>	tr <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
Hexanol	nd <sup>c</sup>	6.25 <sup>c</sup>	32.10 <sup>bc</sup>	27.93 <sup>bc</sup>	51.31 <sup>ab</sup>	50.45 <sup>ab</sup>	74.05 <sup>a</sup>
1-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	3.86 <sup>a</sup>	nd <sup>b</sup>
2-Heptanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.29 <sup>a</sup>
( <i>E</i> )-2-heptenol	nd <sup>b</sup>	nd <sup>b</sup>	7.98 <sup>a</sup>	7.37 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.06 <sup>a</sup>
1-Octanol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	4.43 <sup>b</sup>	nd <sup>c</sup>	15.07 <sup>a</sup>	nd <sup>c</sup>
1-Octen-3-ol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	1.50 <sup>a</sup>
( <i>E</i> )-2-octenol	nd <sup>d</sup>	nd <sup>d</sup>	22.20 <sup>a</sup>	15.42 <sup>b</sup>	6.60 <sup>c</sup>	5.75 <sup>c</sup>	20.47 <sup>a</sup>
1-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.37 <sup>a</sup>	3.41 <sup>a</sup>	tr <sup>b</sup>	2.10 <sup>a</sup>
2-Nonanol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	2.70 <sup>a</sup>
( <i>Z</i> )-3-nonenol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	7.64 <sup>a</sup>
( <i>E</i> )-2-nonenol	nd <sup>b</sup>	nd <sup>b</sup>	1.34 <sup>b</sup>	6.39 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	5.65 <sup>a</sup>
( <i>E,E</i> )-2,4-decadienol	nd <sup>b</sup>	nd <sup>b</sup>	2.74 <sup>ab</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	4.96 <sup>a</sup>
6-Methyl-5-hepten-2-ol	nd <sup>d</sup>	nd <sup>d</sup>	9.84 <sup>d</sup>	33.70 <sup>b</sup>	35.38 <sup>b</sup>	21.74 <sup>c</sup>	163.19 <sup>a</sup>
6,10-Dimethyl-5,9-undecadien-2-ol	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	10.49 <sup>b</sup>	10.33 <sup>b</sup>	12.74 <sup>b</sup>	25.75 <sup>a</sup>
2-Methoxy phenol	5.86 <sup>a</sup>	5.97 <sup>a</sup>	5.21 <sup>a</sup>	3.55 <sup>a</sup>	10.39 <sup>a</sup>	5.41 <sup>a</sup>	5.93 <sup>a</sup>
2-Phenylethanol	nd <sup>d</sup>	1.74 <sup>d</sup>	21.17 <sup>a</sup>	9.70 <sup>bc</sup>	16.32 <sup>ab</sup>	12.28 <sup>bc</sup>	5.20 <sup>cd</sup>
4-Ethylphenol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	15.50 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>5.86</b>	<b>13.96</b>	<b>105.44</b>	<b>134.62</b>	<b>150.05</b>	<b>128.09</b>	<b>330.47</b>
<b>Ketones</b>							
1-Octen-3-one	3.24 <sup>a</sup>	0.84 <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>

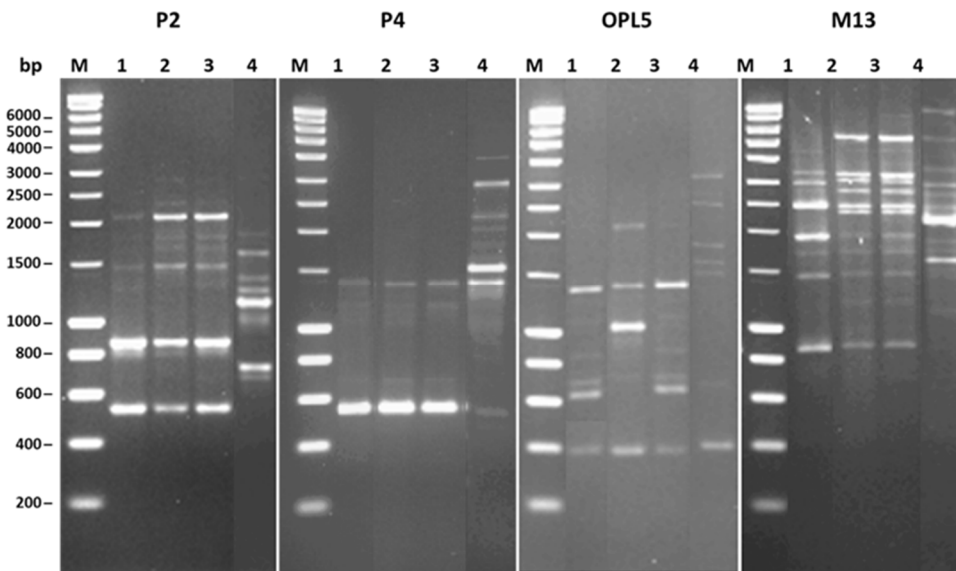
6-Methyl-5-hepten-2-one	139.57 <sup>a</sup>	184.69 <sup>a</sup>	219.43 <sup>a</sup>	246.69 <sup>a</sup>	269.49 <sup>a</sup>	207.21 <sup>a</sup>	4.31 <sup>b</sup>
6,10-Dimethyl-5,9-undecadien-2-one	78.88 <sup>a</sup>	84.91 <sup>a</sup>	81.83 <sup>a</sup>	78.46 <sup>a</sup>	62.47 <sup>ab</sup>	51.51 <sup>ab</sup>	4.23 <sup>b</sup>
2-Undecanone	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	28.83 <sup>a</sup>	8.85 <sup>b</sup>
α-Ionone	nd <sup>b</sup>	2.10 <sup>a</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	tr <sup>ab</sup>	nd <sup>b</sup>
1-(2-Methyl-cyclopenten-1-yl)-ethanone	tr <sup>b</sup>	3.20 <sup>ab</sup>	tr <sup>b</sup>	5.54 <sup>a</sup>	4.74 <sup>a</sup>	tr <sup>b</sup>	3.25 <sup>ab</sup>
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	12.23 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>
5-Ethyl-2,4-dimethyl-4-hepten-3-one	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	6.05 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
<b>Total</b>	<b>222.49</b>	<b>275.75</b>	<b>302.86</b>	<b>349.78</b>	<b>337.49</b>	<b>289.15</b>	<b>21.43</b>
<b>Terpenes</b>							
(Z)-geraniol	nd <sup>b</sup>	nd <sup>b</sup>	1.73 <sup>b</sup>	9.86 <sup>a</sup>	6.06 <sup>a</sup>	6.94 <sup>a</sup>	7.73 <sup>a</sup>
6,7-Dihydrogeraniol	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	tr <sup>b</sup>	3.12 <sup>a</sup>	4.21 <sup>a</sup>
Melonol	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	5.16 <sup>a</sup>	tr <sup>c</sup>	2.69 <sup>b</sup>	tr <sup>c</sup>
Linalool	nd <sup>b</sup>	1.52 <sup>ab</sup>	tr <sup>ab</sup>	5.35 <sup>a</sup>	3.84 <sup>ab</sup>	tr <sup>ab</sup>	2.58 <sup>ab</sup>
D-limonene	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	9.38 <sup>a</sup>	8.15 <sup>a</sup>	5.43 <sup>a</sup>
(Z)-2,6-dimethyl-2,6-octadiene	8.82 <sup>a</sup>	7.90 <sup>a</sup>	5.77 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>	nd <sup>b</sup>
(3Z,5Z)-2,7-dimethyl-3,5-octadiene	6.98 <sup>a</sup>	1.75 <sup>b</sup>	4.23 <sup>b</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>
<b>Total</b>	<b>15.80</b>	<b>11.17</b>	<b>12.52</b>	<b>20.37</b>	<b>20.88</b>	<b>21.69</b>	<b>20.75</b>
<b>Organic acid and their derivatives</b>							
Acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	26.69 <sup>c</sup>	93.56 <sup>b</sup>	tr <sup>c</sup>	7.36 <sup>c</sup>	148.58 <sup>a</sup>
Hexyl ester acetic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	tr <sup>c</sup>	5.45 <sup>a</sup>	nd <sup>c</sup>	3.08 <sup>b</sup>
Octanoic acid	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	nd <sup>c</sup>	5.74 <sup>a</sup>	4.27 <sup>b</sup>
Methyl ester octanoic acid	5.94 <sup>a</sup>	3.25 <sup>b</sup>	tr <sup>b</sup>	3.13 <sup>b</sup>	tr <sup>b</sup>	tr <sup>b</sup>	2.36 <sup>b</sup>
<b>Total</b>	<b>5.94</b>	<b>3.25</b>	<b>27.49</b>	<b>97.49</b>	<b>7.05</b>	<b>13.90</b>	<b>158.28</b>
<b>Miscellaneous</b>							
Methoxy-phenyl-oxime	1.89 <sup>a</sup>	2.82 <sup>a</sup>	2.18 <sup>a</sup>	nd <sup>a</sup>	tr <sup>a</sup>	3.90 <sup>a</sup>	6.56 <sup>a</sup>
2,7-Dimethyl-oxepine	nd <sup>a</sup>	7.29 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	8.73 <sup>a</sup>	8.65 <sup>a</sup>	8.26 <sup>a</sup>
2-Isobutylthiazole	14.89 <sup>a</sup>	tr <sup>c</sup>	nd <sup>c</sup>	18.26 <sup>a</sup>	12.37 <sup>ab</sup>	13.66 <sup>ab</sup>	4.52 <sup>bc</sup>
3-(4-Methyl-3-pentenyl)-furan	7.79 <sup>ab</sup>	7.30 <sup>b</sup>	8.32 <sup>ab</sup>	14.01 <sup>ab</sup>	14.46 <sup>ab</sup>	13.93 <sup>ab</sup>	16.88 <sup>a</sup>
1-Isocyano-3-methyl benzene	nd <sup>d</sup>	nd <sup>d</sup>	nd <sup>d</sup>	6.17 <sup>a</sup>	tr <sup>c</sup>	3.39 <sup>b</sup>	3.66 <sup>b</sup>
Methyl salicylate	11.66 <sup>a</sup>	9.91 <sup>a</sup>	nd <sup>a</sup>	5.74 <sup>a</sup>	7.22 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>
<b>Total</b>	<b>36.23</b>	<b>28.12</b>	<b>10.50</b>	<b>44.18</b>	<b>44.54</b>	<b>43.54</b>	<b>158.28</b>

- 587 Samples were tomatoes fermented with *L. plantarum* C022-2B (sample LP2B), C022-3B (LP3B), and V0023-4B2 (LP4B) and
- 588 *L. fermentum* V013-1A (LF1A) using PDMS/DVB coating with headspace-solid phase microextraction/gas chromatography-
- 589 mass spectrometry (HS-SPME/GC-MS).
- 590 “a, b, c, d” Values in the same line followed by different letters are significantly different ( $p < 0.05$ ).
- 591 tr, less than 0.80 ppb; nd, not detected.



592 **List of figures**

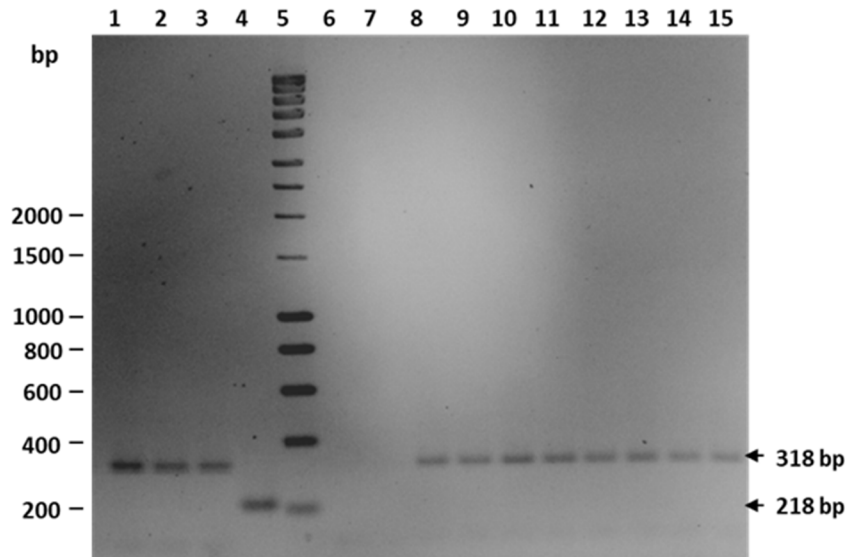
593 **Fig. 1.**  $\beta$ -Glucosidase activity of LAB, 15 strains and *L. plantarum* B33 grown 22 h at 25 °C. Mean values are from three



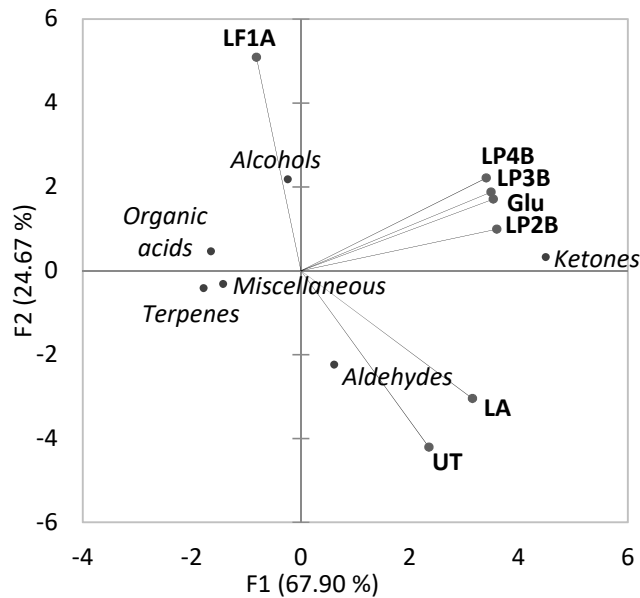
594 biological repeats. UA, nmol of *p*-nitrophenol per minute per milligram of cell dry weight at 37 °C. Different letters show  
 595 significant differences ( $p < 0.05$ ) among the different strains.

596 **Fig. 2.** RAPD-PCR profiles of LAB isolates from fermented foods using primers P2, P4, OPL5, and M13. Lanes: **1**, C022-2B;  
597 **2**, C022-3B; **3**, V0023-4B2; **4**, F2; **M**, Molecular weight markers (SmartLadder; Eurogentec).





598 **Fig. 3.** PCR multiplex analysis of the *Lactiplantibacillus* group. Lane: 1, V0023-4B2; 2, V053-4B; 3, F1; 4, F2; 5, Molecular  
 599 weight markers (SmartLadder; Eurogentec); 6, H<sub>2</sub>O; 7, V013-1A; 8, C022-2B; 9, C022-3A; 10, C022-3B; 11, C022-4A; 12,



600 C022-4B; 13, V073-3A1; 14, V073-4A; 15, V073-4B. DNA molecular weight markers (bp) are shown on the left.

601 **Fig. 4.** Score plot of the first and second principal component (PC) after PC analysis based on volatile compounds  
 602 concentration found in tomato after treatments (Table 6). Abbreviations: LP2B, tomatoes fermented with *L. plantarum* C022-  
 603 2B; LP3B, with *L. plantarum* C022-3B; LP4B, with *L. plantarum* V0023-4B2; LF1A, with *L. fermentum* V013-1A; UT,  
 604 untreated tomatoes; LA, lactic-acid-treated tomatoes; Glu, glucosidase-treated tomatoes.