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Abstract

Lactic acid fermentation is a traditional process to preserve foods and to modify their organoleptic properties. This process is generally conducted in a spontaneous way, allowing indigenous lactic acid bacteria (LAB) of the matrix and of the environment to compete and grow. The aim of this study was to better characterise LAB strains ability to modify aroma profiles in fruit and vegetable matrices, by focusing on two key enzymatic activities: β-glucosidase and alcohol dehydrogenase (ADH). Firstly, 200 LAB isolated from Cambodian and Vietnamese fermented foods were screened for their β-glucosidase activity and duplicate isolates identified through RAPD-PCR analysis were discarded. Thereby, 40 strains were found positive for β-glucosidase using *p*-nitrophenyl-β-D-glucopyranoside as substrate. Among

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

Keywords: Lactic acid bacteria; β-Glucosidases; Alcohol dehydrogenases; Volatile compounds; Fermented foods; Mashed tomatoes

I. Introduction

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

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

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

Furthermore, the genesis of flavour compounds mainly rely on the specific ability of different bacterial strains to convert precursors derived from carbohydrates, proteins, fatty acids, carotenoids, and 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). β-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 β-glucosidase activity (Michlmayr and Kneifel, 2014). This
80 phenotype is strain-specific, as reported by Renchinkhand et al. (2015) who found only six β-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,

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

II. Materials and methods

2.1. Fermented foods and bacterial strains

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

2.2. Screening for β-glucosidase activity

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

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

2.3. Evaluation of β-glucosidase activity

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

Assays were prepared in a 96-well U-bottom microplate (ThermoFisher Scientifc). Each well was filled with 40 µL of 0.2 M McIlvane buffer at pH 5.0 (0.1 M of citric acid and 0.2 M of dipotassium hydrogen phosphate), 20 µL of bacterial cell suspension, and 20 µL of *p*NPG solution (40 mM, pH 5.0). The microplate was incubated for 2 h at 37 °C. The reaction was stopped with 160 µL of Na_2CO_3 solution (0.5 M). The microplate was then centrifuged (4,000 rpm, 15 min at 4 °C) and the supernatants (200 µL) were transferred to a new 96-well flat bottom microplate. The absorbance at 400 nm (A_{400}) was measured by a multiplate spectrophotometer (FLUOstar Omega, BMG LABTECH). Reactions were carried out in triplicate. The *p*NP calibration standards were between 0.013 and 0.302 mM. One unit of enzymatic activity (UA) was defined as the amount of *p*-nitrophenol (nmol) released per minute per milligram of 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^{TM} 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^{TM} 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^{TM}. 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-µL reaction mixture contained 1 µM of primer, 200 µM of each dNTP, 0.5
167 U of Phusion^{TM} High-Fidelity DNA Polymerase (Thermo Fisher Scientific), and 6 µL 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-µL PCR mixture contained 1.5 mM of $MgCl_2$, 0.5 µM of primer,
171 200 µM of dNTP, 0.5 U of Taq polymerase (Thermo Fisher Scientific), and 6 µL 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^{+} 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 10^8 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 (10^5 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

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

2.8. Volatile compound extraction

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

2.9. Volatile compound analysis

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

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

2.10. Statistical analysis

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

III. Results

3.1. Determination of β-glucosidase activity

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

3.2. Elimination of duplicate isolates

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

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

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

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

3.4. Reduction of aldehydes and ketones into alcohols

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

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

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

3.5. Fermented tomato analysis

3.5.1. pH and bacterial enumeration

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

IV. Discussion

Besides its great potential to enhance sustainability, nutrition, and safety, fermentation can also have an impact on the sensorial properties of a product (Beena Divya et al., 2012; Leroy and De Vuyst, 2004). Although traditional spontaneous fermentation usually brings some suitable properties, the selection of starters based on specific enzymatic activities can bring a step forward in a clean label and natural oriented fermentation leading to food exhibiting specific sensorial properties. The present work follows this approach focusing on some activities able to bring important sensorial notes to tomatoes.

The first target of our work was the volatile compounds produced during plant metabolism. These volatile compounds in plant can exhibit toxicity or have a communication role and therefore, the plant itself inactivates them through glycosylation to decrease toxicity or to control communication between plant cells. Most of the volatile compounds accumulate in fruits/vegetables as non-volatile and odourless glycosides. These flavouring volatile compounds can be activated sensorially again by hydrolysing the sugar bond and releasing the volatile aglycone. This reaction can be catalysed by glucosidase enzymes (Sarry and Günata, 2004; Song et al., 2018). β-Glucosidase genes are widespread in LAB genomes. β-Glucosidase activities from LAB have been reported in many plant food matrices such as olives, soybeans, grapes, and cassava. They can hydrolyse a broad range of substrates (Michlmayr and Kneifel, 2014) but their substrate (aglycone) specificity is difficult to predict based on gene sequence. Thus, the first step in screening for specific β-glucosidases must be experimental. Aglycone specificity seems to have evolved during bacterial adaptation to the ecological niche as well as the environmental conditions for the induction of the enzymatic activity (Michlmayr and Kneifel, 2014). These assumptions suggest that the selection of LAB from various type of fermented foods would permit to get various β-glucosidase activities and therefore various aroma profiles.

366 The selected strains exhibiting β-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 β-glucosidase enzyme from almonds. In our study, terpenoids were the main volatiles
369 released after β-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 β-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 β-glucosidase activity values with ketone levels higher
373 in LP2B and LP3B than in LP4B (β-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 β-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 where 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

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

In contrast to this wide-range activity of *L. fermentum* ADH, ADHs of *L. plantarum* strains were substrate selective. *L. plantarum* C022-3B and V0023-4B2 were active only on saturated aldehydes, while C022-2B was active on unsaturated aldehydes. ADHs of *L. plantarum* strains were also active on unsaturated ketones (Table 6). LAB genomes possess several *adh* family genes which code various 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 β-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

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

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

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

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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 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 legumes	Fermented soybeans (2)	Phsar Pochentong and Phsar Depo, Phnom Penh, Cambodia
Fermented meats	Raw fermented fish (1)	Phsar Depo, Phnom Penh, Cambodia
	Raw fermented pork (4)	Chợ Hôm, Hanoi, Vietnam Dũng Nem - Đặc Sản Ước Lễ Gia Truyền, Hanoi, Vietnam
Raw material	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	*L. plantarum*	Fermented shallot roots	Cambodia
C022-3A	*L. plantarum*	Fermented shallot roots	Cambodia
C022-3B	*L. plantarum*	Fermented shallot roots	Cambodia
C022-4A	*L. plantarum*	Fermented shallot roots	Cambodia
C022-4B	*L. plantarum*	Fermented shallot roots	Cambodia
F1	*L. plantarum*	Raw fermented fish "*nem trey*"	Cambodia
F2	*L. pentosus*	Raw fermented fish "*nem trey*"	Cambodia
V0023-4B2	*L. plantarum*	Raw fermented pork "*nem chua*"	Vietnam
V053-4B	*L. plantarum*	Fermented small round aubergine	Vietnam
V073-3A1	*L. plantarum*	Fermented shallot roots	Vietnam
V073-4A	*L. plantarum*	Fermented shallot roots	Vietnam
V073-4B	*L. plantarum*	Fermented shallot roots	Vietnam
V083-4A	*L. plantarum*	Fresh banana leaf	Vietnam
V083-4B	*L. plantarum*	Fresh banana leaf	Vietnam
V013-1A	*L. fermentum*	Raw fermented pork "*nem chua*"	Vietnam
B33	*L. plantarum*	Raw fermented pork "*nem chua*"	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)	
L. plantarum C022-2B	Hexanal	0.44 ± 0.05	Hexanol	0.31 ± 0.11
	(*E*)-2-hexenal	n.d.	(*E*)-2-hexenol	n.d.
L. plantarum C022-3B	Hexanal	< 0.44	Hexanol	< 0.31
	(*E*)-2-hexenal	n.d.	(*E*)-2-hexenol	n.d.
L. plantarum V0023-4B2	Hexanal	< 0.44	Hexanol	< 0.31
	(*E*)-2-hexenal	n.d.	(*E*)-2-hexenol	n.d.
L. fermentum V013-1A	Hexanal	3.85 ± 0.18	Hexanol	3.70 ± 0.42
	(*E*)-2-hexenal	2.47 ± 0.67	(*E*)-2-hexenol	2.58 ± 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.

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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)	
L. fermentum V013-1A	(*E*)-2-heptenal	2.46 ± 0.25	(*E*)-2-heptenol	3.13 ± 0.26
	(*E*,*E*)-2,4-decadienal	3.69 ± 0.00	(*E*,*E*)-2,4-decadienol	3.22 ± 0.00
	6-Methyl-5-hepten-2-one	n.d.	6-Methyl-5-hepten-2-ol	n.d.
	6,10-Dimethyl-5,9-undecadien-2-one	1.26 ± 0.14	6,10-Dimethyl-5,9-undecadien-2-ol	0.81 ± 0.08

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.

578
579

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

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.

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

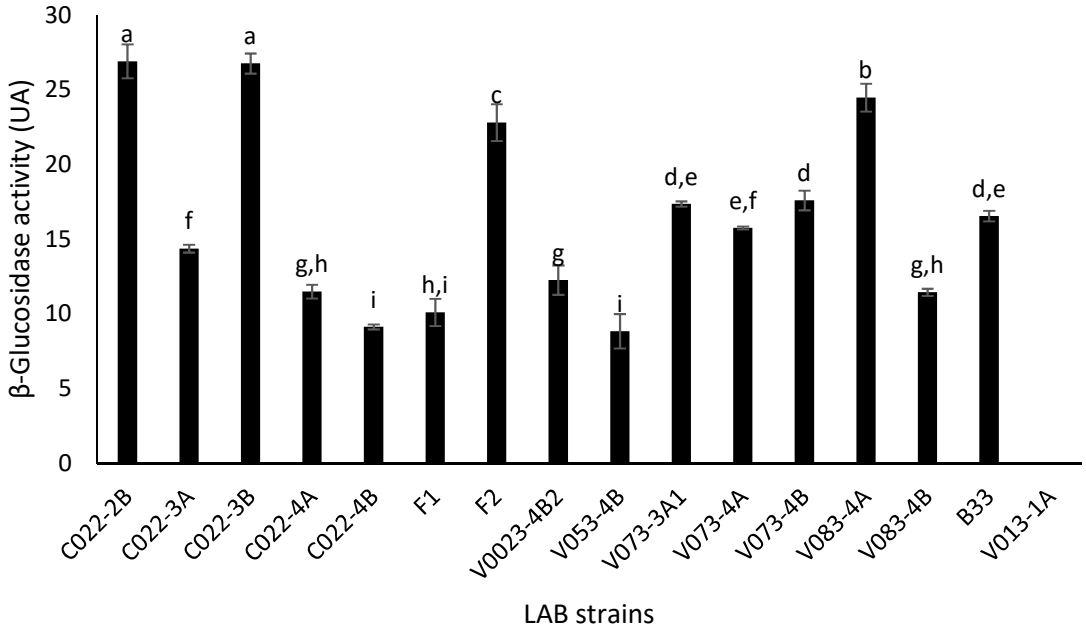
Chemical class	Treatment						
	UT	MT-LA	MT-Glu	MT-LP2B	MT-LP3B	MT-LP4B	MT-LF1A
Aldehydes							
Hexanal	135.85^{a}	92.21^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
(*E*)-2-hexenal	18.10^{a}	17.07^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
Heptanal	6.41^{a}	1.36^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
(*E*)-2-heptenal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

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

Samples were tomatoes fermented with *L. plantarum* C022-2B (sample LP2B), C022-3B (LP3B), and V0023-4B2 (LP4B) and *L. fermentum* V013-1A (LF1A) using PDMS/DVB coating with headspace-solid phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS).

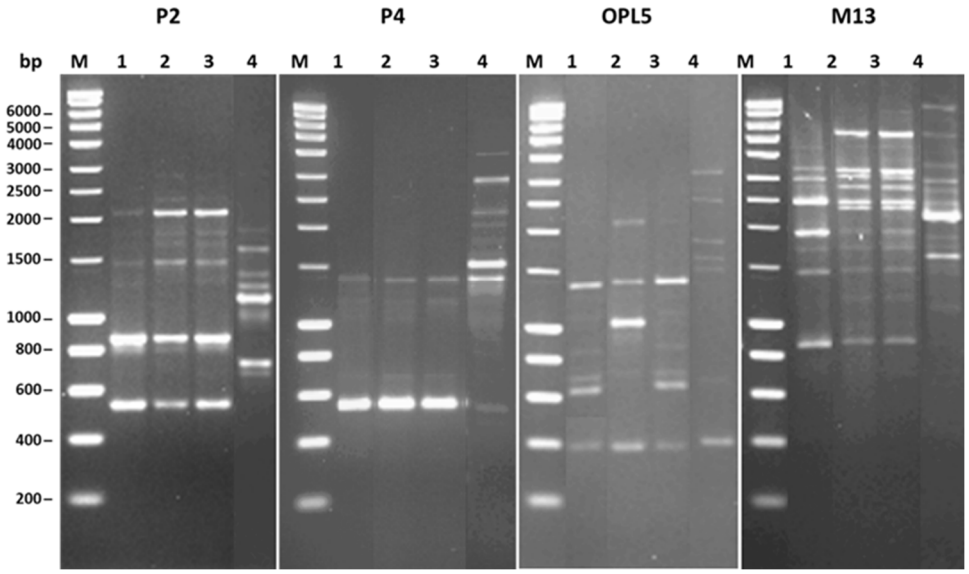
"$^{a, b, c, d}$" Values in the same line followed by different letters are significantly different ($p < 0.05$).

tr, less than 0.80 ppb; nd, not detected.



List of figures

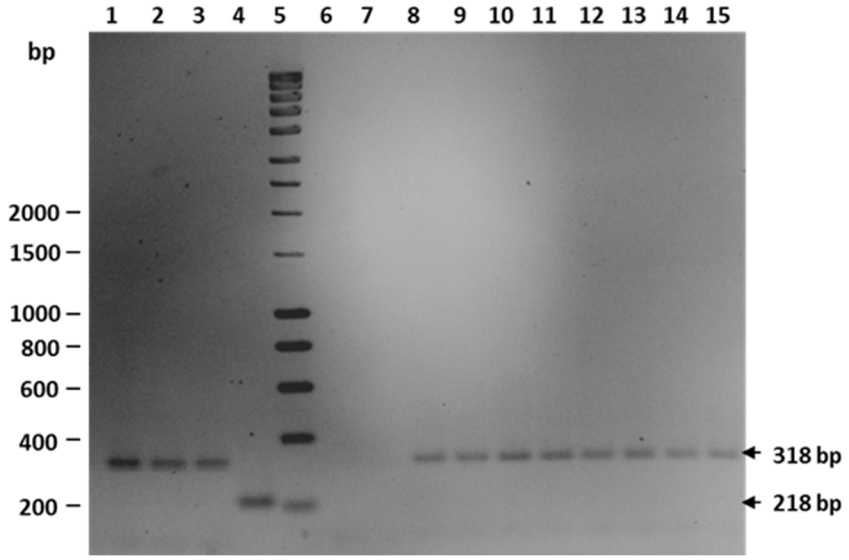
Fig. 1. β-Glucosidase activity of LAB, 15 strains and *L. plantarum* B33 grown 22 h at 25 °C. Mean values are from three



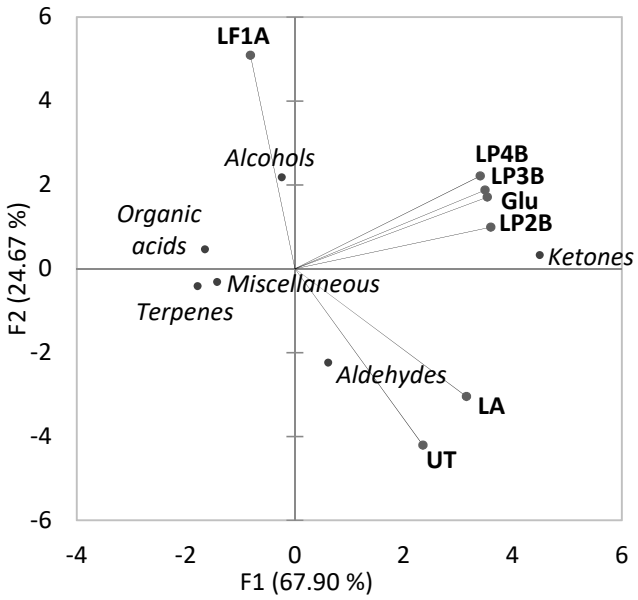
biological repeats. UA, nmol of *p*-nitrophenol per minute per milligram of cell dry weight at 37 °C. Different letters show significant differences ($p < 0.05$) among the different strains.

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**, H2O; **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.