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Linking Pélardon artisanal goat cheese microbial communities to aroma compounds during cheese-making and ripening

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21 ## Abstract

22 Pélardon is an artisanal French raw goat's milk cheese, produced using natural whey as a
23 backslopping method. The aim of this study was to identify key microbial players involved in
24 the acidification and aroma production of this Protected Designation of Origin cheese.
25 Microbial diversity of samples, collected from the raw milk to 3-months cheese ripening, was
26 determined by culture-dependent (MALDI-TOF analysis of 2877 isolates) and -independent
27 (ITS2 and 16S metabarcoding) approaches and linked to changes in biochemical profiles
28 (volatile compounds and acids). In parallel, potential dominant autochthonous microorganism
29 reservoirs were also investigated by sampling the cheese-factory environment. Complex and
30 increasing microbial diversity was observed by both approaches during ripening although
31 major discrepancies were observed regarding *Lactococcus lactis* and *Lactiplantibacillus*
32 *paracasei* fate. By correlating microbial shifts to biochemical changes, *Lactococcus lactis w*as
33 identified as the main acidifying bacterium, while *L. mesenteroides* and *Geotrichum*
34 *candidum* were prevalent and associated with amino acids catabolism after the acidification
35 step. The three species were dominant in the whey (backslop). In contrast, *L. paracasei*,
36 *Enterococcus faecalis, Penicillium commune* and *Scopulariopsis brevicaulis,* which
37 dominated during ripening, likely originated from the cheese-making environment. All these
38 four species were positively correlated to major volatile compounds responsible for goaty and
39 earthy Pélardon cheese aroma. Overall, this work highlighted the power of MALDI-TOF and
40 molecular techniques combined with volatilome analyses to dynamically follow and identify
41 microbial communities during cheese-making and successively identify the key-players
42 involved in aroma production and contributing to the typicity of Pélardon cheese.

43 **Keywords: microbial dynamics; MALDI-TOF; GC-MS; metabarcoding; backslopping**

44

45 **1. Introduction**

46 Artisanal cheeses are highly appreciated worldwide because of their typical sensory
47 attributes such as intense and complex flavors compared to industrial cheeses (Montel et al.
48 2014; Van Hoorde, Vandamme and Huys 2008). They are often made from raw milk and rely
49 on spontaneous fermentation, which implies that indigenous microorganisms (bacteria and
50 fungi) naturally present in raw materials also contribute to the fermentation in addition to
51 defined cultures sometimes added for acidification or ripening. In this context, the origin of
52 these microorganisms has been investigated and milk is considered as a main microbial
53 reservoir. However, traditional cheese-making practices such as manual salting, renneting and
54 backslopping (i.e. the use of a small portion of milk or whey from a previous successful
55 fermentation) have also been identified as microbial sources (Ercolini et al. 2008; Montel et
56 al. 2014). Furthermore, some studies highlighted the impact of the production environment on
57 the microbial communities involved in the fermentation process of some artisanal cheeses, in
58 particular the milking environment equipment such as vats and trays (Calasso et al., 2016;
59 Carpino et al., 2016), the cheese-making and ripening environment (equipments and surfaces).
60 All these potential reservoirs enrich the microbial diversity of artisanal cheeses, which
61 therefore harbor a complex microbiota (Delcenserie et al., 2014).

62 Cultural methods are classically used to characterize microbial diversity in fermented
63 foods. They rely on diverse and more or less selective media to enumerate and isolate
64 microorganisms, which constitute both the advantages and limits of the approach. While
65 enumeration and isolation are useful to understand population shifts and preserve microbial
66 diversity for further use, it only reveals the viable and cultivable fraction of the microbial
67 communities and is particularly fastidious and time-consuming. In addition, the depth of the
68 analysis depends on the number and selectivity of the media used. In the last decade, culture-
69 independent approaches, based on DNA analyses, have been widely used to describe

70 microbial communities in cheese as they have the advantage to provide an overview of the
71 microorganisms found in an ecosystem, cultivable or not. Different PCR-based techniques
72 have been applied such as PCR-DDGE (Dolci et al., 2008) or more recently high-throughput
73 sequencing techniques such as metabarcoding (De Filippis et al., 2018; Kergourlay et al.,
74 2015). Using this approach, studies were conducted to characterize the bacterial communities
75 of artisanal Irish cheeses based on their geographic production area (Quigley et al., 2012) or
76 to determine the microbiota associated with specific cheese types (Dugat-Bony et al., 2015) or
77 cheese rinds (Wolfe et al., 2014). Such methods revealed complex microbiota that were
78 previously overlooked by cultural methods. However, most studies focused on microbial
79 diversity and compared the microbial communities encountered in different cheese varieties
80 thus rendering a static view of the microbial diversity rather than investigating the changes in
81 the microbial community during cheese-making and ripening periods.
82 Cheese-making and ripening are complex phenomena during which the cheese develops
83 its aroma and other sensory characteristics. Many successive or simultaneous enzymatic
84 reactions occur throughout the process, which include lactose fermentation process,
85 proteolysis and amino acid catabolism, and lipolysis. These reactions were shown to directly
86 or indirectly involve bacteria and/or fungi depending on cheese type (Bertuzzi et al., 2018;
87 Delgado et al., 2011). As a consequence, investigating the microbial dynamics and their
88 correlation with the cheese aroma profile might help to better apprehend the key-players and
89 their respective role throughout the process.
90 Pélardon cheese is a Protected Designation of Origin (PDO) raw goat milk artisanal
91 cheese. It is mostly produced in the mountainous Cevennes and Corbières areas and in
92 Southern France and is characterized by a soft creamy texture with a thin wrinkled white and
93 blue mold rind. It is solely produced using an artisanal process by small farm-style

94 cheesemakers. Pélardon production requirements forbid the use of commercial starters but
95 authorize the use of whey from a previous batch as a backslopping inoculum.
96 The objective of this study was thus to understand the microbial dynamics associated with
97 Pélardon cheese and how they could be linked with the cheese aroma characteristics. For this
98 purpose, we first (i) described fungal and bacterial dynamics during Pélardon cheese-making
99 and ripening by both culture-dependent and -independent approaches, (ii) investigated
100 potential microbial sources in the process environment and (iii) quantified sugars, organic
101 acids and aroma compounds throughout the entire process. Finally, we assessed how
102 microbial diversity and dynamics influence the overall cheese aroma characteristics.
103

104 2. Material and methods

105 2.1. Pélardon cheese sampling strategy

106 2.1.1. Pélardon cheese-making and sample collection

107 Samples were collected from a local cheese-maker from the Southern France (Gard) in the
108 Cevennes area. Cheeses were prepared with the traditional process described by PDO
109 requirements and in the same conditions as standard manufacturing. Briefly, raw goat milk
110 from two successive milkings was supplemented with 2 % kid rennet (chymosin: 180 mg/L)
111 and 2 % whey from the previous production batch. No starter cultures were added.
112 Acidification was carried out for 18 to 24 h until the acidity reached 58 ° Dornic at 20°C with
113 controlled relative humidity (RH=80 %). Curd was manually moulded with a ladle into
114 individual jars and drained naturally at room temperature (20°C) for 24 h before salting by
115 manually sprinkling salt on the surface. After demoulding, fresh cheeses were drained for 48
116 h more prior to being placed in the drying room (T°=14°C; RH = 85 %) for 48 h. Finally,
117 ripening was carried out in a cellar, at 12°C (RH = 90 %) for a minimum period of four days
118 and up to three months.

119 Samples came from the same production batch (April 7, 2018) and sampling was done at
120 seven steps during the cheese making process: raw materials (raw milk and whey used for
121 backslopping inoculum), cheese samples after curdling (day 2), after salting and drying (day
122 8), after two weeks (day 14), two months (day 62) and three months ripening (day 90).
123 Altogether, the sampling steps provided 33 samples corresponding to the biological replicates
124 collected throughout cheese-making and ripening. Whole cheeses were used for each analysis
125 point. Samples were subjected to microbial enumeration, isolation and identification of
126 bacteria and fungi, metabarcoding analyses and biochemical analyses to quantify sugars, acids
127 and volatile compounds. General sampling strategy and experiments are summarized in Fig. 1
128 and primers used in this study in Supplementary Table S1.

129 **2.1.2.** Production environment sample collection

130 Sixteen samples were taken at day 2 from different areas in the production and ripening
131 facilities in order to track potential environmental microbial sources. Airborne
132 microorganisms from the main production, drying and ripening rooms were collected using an
133 air sampler (Sampl'air Lite, AES) operated at flow rate of 100 L/min for 10 min while ten
134 surfaces were also analysed by streaking swabs moistened with Tryptone Salt (TS) diluent
135 (sodium chloride 8.5 g/L ; tryptone 1 g/L) over a 31.5 cm² area as well as three intrants
136 (running water samples and salt) and stored at 4°C until analysis. All samples were analysed
137 within 24 h and further subjected to microbial enumeration, bacterial and fungal isolations,
138 identifications and metabarcoding analyses (except air samples).

139 **2.2.** Microbial counts during cheese-making process and in environmental samples

140 For raw materials and cheese samples, microbial populations of interest were monitored
141 at each sampling point. For cheese samples following the drying step (day 8), core and rind
142 were analyzed separately. In each case, 10 g of cheese were mixed with 90 mL of 2 %

143 trisodium citrate buffer pre-heated to 42°C and then blended with a stomacher for 3 min at
144 high speed. Serial dilutions were then prepared in TS diluent and plated on different media.
145 The following microbial populations were enumerated: mesophilic aerobic bacteria on milk
146 plate count agar (MPCA) (30°C, 72 h), mesophilic halotolerant bacterial populations on
147 MPCA supplemented with 5 % NaCl (30°C, 72 h), enterococci on Kenner fecal agar
148 supplemented with 1% (w/v) 2,3,5-triphenyltetrazolium chloride (KF) (37°C, 48 h),
149 presumptive lactococci on M17 (30°C, 48 h), presumptive anaerobic lactobacilli on de Man
150 Rogosa Sharpe (MRS) pH 5.4 (30°C, 48 h, anaerobiosis), presumptive aerobic lactobacilli on
151 MRS pH 5.4 (30°C, 48 h, aerobiosis), total fungal populations on Yeast Glucose
152 Chloramphenicol (YGC) agar (25°C, 5 days) and halotolerant fungi on YGC supplemented
153 with 5 % NaCl (25°C, 5 days). All media targeting bacterial populations were supplemented
154 with 0.1 % natamycin to inhibit fungal growth, except for KF medium.

155 For environment samples, enumerations were performed on three media: PCA (30°C, 72 h)
156 for mesophilic aerobic microorganisms, MRS (37°C, 48h, anaerobiosis) for lactic acid
157 bacteria and YGC (25°C, 5 days) for fungi after initial serial dilutions in TS diluent. Air
158 samples were directly collected by impacting 1 m^3 of air on two media: PCA (30°C, 72 h) for
159 mesophilic aerobic microorganisms and YGC (25°C, 5 days) for fungi. For those samples,
160 analyses were performed on one replicate.

161 **2.3.** Isolation and identification of microorganisms

162 **2.3.1.** Collection of microbial isolates

163 For each biological replicate of raw materials (raw milk and whey used for backslopping)
164 and cheese samples (core and rind analyzed separately from day 8 onwards), microorganisms
165 were isolated from five media: bacteria from MPCA + natamycin, M17, MRS pH 5.4
166 (anaerobiosis) and KF media and fungi from YGC medium. Twenty representative isolates (if

167 possible) were randomly selected from each medium from the Petri dish showing the highest
168 morphological diversity. Regarding environmental samples, 15 isolates (if possible) were
169 selected from PCA and YGC media.

170 **2.3.2.** Isolate dereplication using MALDI-TOF MS analysis and identification by
171 sequencing

172 A dereplication step using MALDI-TOF mass spectroscopy was performed for all
173 bacterial and fungal isolates to identify clones prior to species level molecular identifications.
174 Analyses were performed on a VITEK MS instrument (bioMérieux, Marcy l'Étoile, France)
175 equipped with the Launchpad V2.8.4 acquisition software. Bacterial and fungal isolates were
176 processed following the manufacturer's instructions. For filamentous fungi isolates, extraction
177 and plate preparation were performed as described by Quéro et al. (2019) as well as spectra
178 acquisition. Then, mass spectra of each isolate were first compared against the bioMérieux
179 bacterial or fungal industrial-clinical VITEK MS databases (V3.2.0 for bacteria and new
180 update of the VITEK MS database for fungi developed in the laboratory together with
181 bioMérieux) for presumptive identifications (internal research tool). Then, the spectra of
182 isolates identified as belonging to the same species were compared against one another to
183 build clusters based on their similarities using SARAMIS software (bioMérieux, Marcy
184 l'Etoile, France). A threshold of 65 % similarity was applied for bacteria and of 50 % for
185 fungi, as described by Lindgren et al. (2018). Representative isolates (isolates with the lowest
186 similarity within a species cluster) were then chosen within each cluster and for each
187 presumptive species. For each presumptive species, the choice of representative isolates was
188 based on the heterogeneity between their spectra and when discrepancies were observed
189 between MALDI-TOF identification and position within the cluster. An example is given for
190 *Staphycoccus* genus (Supplementary figure S1).

191 Species-level molecular identifications for filamentous fungi were done after amplification
192 and sequencing of either the internal transcribed spacer (ITS) region using ITS4/ITS5 primers
193 or the partial β-tubulin gene using Bt2a/Bt2b primers (Glass and Donaldson, 1995) depending
194 on the presumptive genus (i.e. *Penicillium, Aspergillus*) provided by MALDI-TOF MS
195 analysis. Yeasts were identified by targeting the D1-D2 domain using NL1/NL4 primers
196 (Kurtzman and Robnett, 1998). Finally, bacteria were identified by sequencing the complete
197 16S rRNA gene after amplification with fD1 and rP2 primers (Weisburg et al., 1991).
198 Sequencing was performed using the same primers as those used for PCR amplifications.
199 Sequences were assembled into contigs using Geneious software (http://www.geneious.com,
200 Kearse et al. 2012) and compared with the GenBank databases using the "Basic Local
201 Alignment Search Tool" (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST) and "SILVA
202 Incremental Aligner" (SINA) (https://www.arb-silva.de/aligner/). The obtained genus or
203 species level identifications were then confronted with MALDI-TOF identifications and
204 dendrogram analyses. When both identifications were consistent inside a given cluster, the
205 identification was expanded to all the isolates within the cluster. When the identification was
206 inconsistent, isolates were excluded.

207 **2.4.** Metabarcoding analysis

208 **2.4.1.** Raw materials, cheese and environment sample preparation

209 Metabarcoding analysis was performed on raw milk (triplicates), whey (triplicates), curd (3
210 curds), cheeses at day 8, 14, 62 and 90 and 13 environmental samples. The same raw material
211 and cheese samples (core and rind separately), diluted in citrate buffer, were used as described
212 previously for culture-dependent analyses (see 2.2). Aliquots of 1 mL were centrifuged (9000
213 *g*, 15 min, 4°C) then supernatants were removed and the cell pellets stored at -20°C until
214 DNA extraction. Regarding environmental samples, pellets were obtained from liquid

215 samples by centrifuging 1 mL (9000 *g*, 15 min, 4°C), whereas for surface samples, swabs
216 were placed in 1 mL of TS diluent prior to centrifugation (9000 *g*, 15 min, 4°C) to obtain cell
217 pellets.

218 **2.4.2.** Total DNA extraction from cheese and environment samples

219 Total DNA extractions were performed using the DNeasy Blood and tissue kit (Qiagen,
220 Germany) with a supplementary initial enzymatic lysis. First, cell pellets were thawed at room
221 temperature then resuspended in 400 µL of lysis buffer (Tris-HCl 20 mM at pH 8.0, EDTA 2
222 mM, Triton X-100 1.2 %) supplemented with lysozyme (20 mg/mL) and mutanolysin (5
223 U/µL), then Rnase (25 µg/mL; Qiagen, Germany) and lyticase (0.5 U/µL; Sigma-Aldrich,
224 Germany) were added. Samples were incubated at 37°C for 2.5 h followed by mechanical
225 lysis with 300 µL of 0.1mm zirconium beads per tube. Samples were homogenized for 2x 40 s
226 with a 30 s break and this was repeated twice with a 1 min break on ice using a Precellys
227 Evolution homogeneizer (Bertin technologies, Germany). A proteinase K (20 mg/mL)
228 treatment was finally applied for 1 h at 56°C. The remaining extraction and purification steps
229 were performed according to the manufacturer's instructions. Extracted DNA quality and
230 quantity were verified using a Nanodrop spectrophotometer and samples were stored at -20°C
231 until further analysis.

232 **2.4.3.** Quantitative PCR of *Lactiplantibacillus paracasei*

233 The number of *Lactiplantibacillus paracasei* (ex-*Lactobacillus paracasei*) in total DNA
234 extracts from day 14, 62 and 90 were determined using quantitative PCR targeting the
235 monocopy *tuf* gene, as described by Achilleos and Berthier (2013). To build the standard
236 curve, DNA was extracted from pure cultures from 1 mL of an overnight culture of *L.*
237 *paracasei* CIRM-BIA 1517. Serial dilutions of standard DNA were prepared and submitted to
238 the same amplification conditions. The curve was then created by plotting qPCR Ct values

239 against the gene copy numbers previously determined and the copy number of *L. paracasei*
240 was calculated for each cheese sample by comparing the Ct of the sample with that of the
241 standard curve.

242 **2.4.4.** Amplification and sequencing parameters

243 To study bacterial and fungal diversity in both cheese and environmental samples, PCR
244 were performed as follows. For bacteria, V3-V4 region of the 16S rRNA gene was targeted
245 using S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 primers (Klindworth et al., 2013).
246 For fungi, ITS3f/ITS4_Kyo1 primers (Toju et al., 2012) were used, targeting the ITS2 region.
247 ITS2 and V3-V4 amplifications and sequencing steps were performed in the same run at
248 Genome Quebec sequencing platform (MacGill University, Canada) using Illumina Miseq
249 PE300 technology generating 2x300 bp reads and a total of 8.2 Gb of data for both amplicon
250 types derived from the DNA extracts.

251 **2.4.5.** Bioinformatic and phylogenetic analysis

252 Sequences were pre-processed for quality and length using the following parameters:
253 amplicon size between 370 and 490 bp for V3-V4 contigs and 90-500 bp for ITS2 contigs,
254 mismatch rate was set at 0.1 for both data types. Sequencing data were then analyzed using
255 the FROGS pipelines as developed by Escudié et al. (2018) under Galaxy (Afgan et al., 2018).
256 Briefly, raw paired-end reads were assembled and sequences were clustered using the Swarm
257 algorithm (Mahé et al., 2015) with an aggregation distance of 3 into Operational Taxonomic
258 Units (OTUs). Sequences underwent some filtering steps: chimeras were detected using
259 Uparse (Edgar, 2013) and 'de novo parameter' and removed, then sequences with a relative
260 abundance below $5x10^{-5}$ or present in only one sample were excluded. Finally, the affiliation
261 step was performed using SILVA database (v132 pintail 100) and UNITE (v7.1) for 16S and
262 ITS2 data, respectively. When 16S sequences were multi-affiliated by FROGS because the

263 targeted V3-V4 region was unable to discriminate species, the resulting possible species level

264 assignations were implemented into the final OTU table.

265 **2.4.6.** Biodiversity and statistical analyses

266 Processing and statistical analyses of microbial communities were performed using

267 Phyloseq package (McMurdie and Holmes, 2013) under R software. Data were normalized

268 based on the sample that had the lowest number of sequences. Alpha-diversity indexes

269 taxonomic composition and abundance distribution were then determined for each sample and

270 compared.

271 **2.5.** Biochemical analyses of cheese samples

272 **2.5.1.** pH measurement

273 pH of the whey and day 2 cheeses was measured using a WTW 3100 pH meter

274 (Weilheim, Germany) equipped with a puncture electrode (LoT406-M6-DXK GmbH, Mettler

275 Toledo, Urdorf, Switzerland) and temperature probe (WTW 325/HC) by direct insertion. For

276 cheese samples from day 8, 14, 62 and 90, pH was measured in core and rind separately.. All

277 measurements were performed on cheese triplicates.

278 **2.5.2.** Quantification of sugars and organic acids by HPLC

279 Two sugars and four organic acids were quantified in samples at six sampling points by

280 high-performance liquid chromatography (HPLC). Compound extraction was performed as

281 follows: all samples except whey were first blended in sterile distilled water (1:5) using a

282 Stomacher (Merck Eurolab, Strasbourg, France) and incubated at 40°C for 1 h. The obtained

283 dilutions were then centrifugated (3000 *g*, 30 min, 4°C) and filtered on a Whatmann 40

284 membrane. The filtrates were then diluted (3:4) with H_2SO_4 to reach a final concentration of

285 0.05 M. Samples were then frozen overnight at -20°C and, after thawing, centrifuged (8000 *g*,

286 20 min, 4°C). For each sample, supernatant was finally recovered and filtered using 0.45 µm
287 PTFE membrane into a 2 mL-vial and stocked at -20°C until analysis.
288 HPLC analyses were performed using the conditions described by Leyva Salas et al. (2019).
289 Compounds were identified and quantified by comparing retention times and peak area of
290 standard solutions injected at different concentrations (ranging from 0.05 to 1 mg/mL). All
291 samples were analyzed in the same run. When necessary, supernatants were diluted with 0.05
292 M H_2SO_4 and re-injected.

293 **2.5.3.** Volatile compounds profile analyses by headspace-GC-MS

294 Volatile profiles were obtained for six sampling points including whey and cheeses (from
295 days 2, 8, 14, 62 and 90) using headspace (HS) gas chromatography-mass spectrometry (GC-
296 MS). Compound extraction was performed using a Perkin Elmer Turbomatrix HS-40 trap
297 automatic headspace sampler with trap enrichment on 2.5 g of mixed cheese placed in 22 mL
298 vials. Analyses were performed according to methods previously described by Harlé et al.,
299 (2020). Prior to compounds identification, data were processed using PerkinElmer Turbomass
300 software, version 5.4.2.1617 and by converting the raw data to time- and mass-aligned
301 chromatographic peaks areas using the open source XCMS package implemented with the R
302 statistical language (Smith et al., 2006). Parameters were set as follows: width at half
303 maximum=5, group bandwidth=3. Volatile compound identification was achieved by
304 comparing the retention index and mass spectral values (1) from the NIST 2008 Mass
305 Spectral Library (Scientific Instrument Services, Ringoes, NJ, USA) and (2) when possible
306 with those of authentic standards (Sigma Aldrich, France) analysed in the same system and
307 (3) those reported in the literature. Finally, volatile profile changes between samples were
308 investigated by comparing relative abundance of the identified compounds using ANOVA
309 analyses with R sofware. Data were then normalized by centering and scaling the abundances.

310 Hierarchical clustering and correlation of the data was then performed using Ward's
311 minimum variance linkage and Euclidean distance method under R software.

312 **2.6.** Statistical analyses of microbial and biochemical data

313 Data from both culture-dependent and -independent analyses were implemented into a
314 Principal Component analysis (PCA). Prior to the analysis, culture-dependent identified
315 variables were reduced to one medium per species as many species were identified on more
316 than one medium. The medium with the highest specificity and abundance of each species
317 was kept and the abundance values multiplied by the corresponding microbial population
318 counts. Pearson correlation coefficients between microbial species (culture-dependent
319 approach) and biochemical compounds were then calculated and represented on a heatmap.
320 Significance levels of correlations between microbial genera and biochemical compounds
321 were then determined. All analyses were performed with R software using FactomineR,
322 Hmisc, and ggplot2 packages (Lê et al., 2008; Wickham, 2016).

323 **3. Results**

324 **3.1.** Culture-dependent analyses to determine the succession of microbial species during
325 cheese-making and ripening

326 **3.1.1.** Microbial population monitoring

327 Eight populations of interest were monitored during cheese-making and ripening.
328 Microbial counts obtained for raw materials and cheese core and rind samples are presented in
329 Fig. 2.A, 2.B and 2.C, respectively. Raw milk harbored low mesophilic bacterial populations
330 (3.4 $\log_{10}$ CFU/mL) and fungal populations (2 $\log_{10}$ CFU/mL). Higher bacterial counts by up
331 to 5 $\log_{10}$ CFU/mL were observed in the whey used for backslopping than in raw milk except
332 for enterococci and presumptive aerobic lactobacilli. Indeed, presumptive aerobic lactobacilli
333 were encountered at low levels in raw milk (around 2 $\log_{10}$ CFU/mL) and undetected in whey,

334 while enterococci counts were higher in raw milk (2.3 log_{10} CFU/mL) compared to whey (2
335 log_{10} CFU/mL). In day 2 curds, presumptive lactococci and anaerobic lactobacilli dominated
336 (up to 9.2 log_{10} CFU/g), while enterococci and presumptive aerobic lactobacilli remained
337 undetected. Fungal growth occurred during milk acidification and renneting step with
338 populations reaching 4.6 log_{10} CFU/g at day 2.
339 From the post-drying stage (day 8) onwards, differences were observed between core and rind
340 populations. Aerobic bacterial populations remained relatively stable in rinds while a decrease
341 by 1.2 log_{10} was observed in cores and up to 3 log_{10} for halotolerant bacteria. A similar trend
342 was observed for presumptive lactococci and anaerobic lactobacilli populations in cores
343 which decreased by ~1.8 log_{10} during drying (day 8) and, then remained stable (~6 log_{10}
344 CFU/g) up to 90 days. Inversely, both presumptive aerobic lactobacilli and enterococci
345 populations significantly increased during the cheese-making and ripening in both core and
346 rinds to reach 6 log_{10} CFU/g for lactobacilli and 5 log_{10} CFU/g for enterococci over the course
347 of ripening. Fungal populations were consistently 2 log_{10} higher on rinds and also increased
348 during the drying step then remained at high levels until the end of ripening (7.91 log_{10}
349 CFU/g). In contrast, they remained fairly constant in core samples, around 5.5 log_{10} CFU/g.
350 An increase in halotolerant fungal populations was also observed in rinds between day 8 and
351 day 14 while no such effect was observed in the core.

352 **3.1.2.** Identification of viable microbial communities by MALDI-TOF and sequencing

353 A total of 2001 bacterial and 563 fungal isolates were collected from the raw materials
354 and cheese-making samples and 143 bacterial and 160 fungal isolates from environmental
355 samples.
356 Isolates were first subjected to a dereplication step using MALDI-TOF analysis to select
357 representative isolates for further molecular identifications. Overall, good-quality spectra
358 were acquired for 88 % bacterial isolates (n=1876) and 99 % fungal (n= 727) isolates. These

359 spectra were thus used to build similarity dendrograms and grouped into clusters based on
360 their similarity and presumptive identifications. In total, 146 representative bacterial and 38
361 fungal isolates were identified by sequencing. Based on MALDI-TOF spectra clustering,
362 presumptive identifications and sequencing data, 1769 bacterial and 683 fungal isolates,
363 respectively representing 82 % and 93 % of the initial collection, were successfully identified
364 and included in the final analysis. Noteworthy, the presumptive identifications done by
365 MALDI-TOF analyses were confirmed by species-level molecular identification for 78 % and
366 86 % of the sequenced isolates for bacteria and fungi, respectively. Results based on all these
367 data are presented in Fig. 3.
368 Regarding raw materials, only few lactic acid bacteria species (LAB) were identified in raw
369 milk whereas high species diversity was observed for staphylococci among the isolated
370 aerobic bacteria with three species only identified in these samples namely *Staphylococcus*
371 *arlettae, Staphylococcus caprae* and *Staphylococcus epidermidis* . In contrast, in whey used
372 for backslopping and in curd (day 2), *Lactococcus lactis* was the dominant bacterial species
373 followed by *Leuconostoc mesenteroides*. Among aerobic bacteria and presumptive lactococci,
374 *L. lactis* abundance was highest at day 2 and progressively decreased throughout ripening
375 (aerobic bacteria) and was no longer detected neither in core nor rind at three months of
376 ripening. A similar trend was observed for *L. mesenteroides* as its relative abundance was
377 highest at day 8 in cores (aerobic bacteria and presumptive lactococci) and progressively
378 decreased until day 90, and was no longer detectable in rinds after day 14. These observations
379 were concomitant with the progressive growth of *Lactiplantibacillus paracasei/casei* from
380 day 8 to the end of ripening and it was the most abundant bacterial species in cores at 62 and
381 90 days of ripening (aerobic bacteria and presumptive anaerobic lactobacilli). During
382 ripening, the growth of several actinobacteria was observed such as *Glutamicibacter* spp.,
383 *Arthrobacter* spp. and *Brevibacterium* spp.; they represented up to half of the isolates among

384 aerobic bacteria in rinds. Although their populations levels were lower than the most
385 dominant bacterial groups, interesting dynamics were observed among enterococci and
386 staphylococci. Regarding enterococci, their diversity increased in rinds during ripening, with
387 only *Enterococcus durans* identified during the post-drying step (day 8) and three other
388 species namely *E. faecalis, E. faecium* and *E. hirae* identified at days 62 and 90.
389 Staphylococci species diversity also increased during ripening and numerous species were
390 identified such as *Staphylococcus xylosus, Staphylococcus equorum, Staphylococcus succinus*
391 and *Staphylococcus fleurettii*, which were not previously isolated in raw material or curds
392 (day 2).
393 Regarding fungal communities, the isolates collected from raw milk could not be identified
394 while whey was dominated by *Geotrichum candidum*. This yeast-like fungus remained
395 dominant until day 14. After curdling (day 2), despite different population levels, no striking
396 differences between core and rind composition were observed in cheese samples. Fungal
397 diversity then increased from day 14 in the rinds and from day 62 in cores. In particular,
398 *Penicillium commune* and *Scopulariopsis brevicaulis* relative abundances increased and *S.*
399 *brevicaulis* even became dominant in both core and rind at days 62 and 90. Other yeast
400 species such as *Debaryomyces hansenii* and *Yarrowia lipolytica* were also encountered in the
401 same samples but at lower abundances.

402 **3.2.** Culture-independent analyses to determine the succession of microbial species during
403 cheese-making and ripening

404 **3.2.1.** Microbial dynamics determined using 16S rRNA gene and ITS2 metabarcoding
405 analyses

406 V3-V4 16S rRNA gene sequencing analysis resulted after quality filtering in a total of 3
407 537 077 contigs with an average length of 467 nucleotides. Sequences were subsequently
408 clustered into 43 OTUs after chimera and singleton removal. Concerning metabarcoding

409 analysis of the ITS2 region, a total of 6 945 569 quality-filtered contigs (average length of 245
410 nucleotides) were obtained. After chimera and singleton removal, sequences were clustered
411 into 15 OTUs. After normalization, 67 540 and 54 622 sequences per sample were kept for
412 V3-V4 and ITS2 data analysis, respectively. Rarefaction curves are available in
413 Supplementary Fig. S2.
414 For both bacterial and fungal communities, alpha-diversity indexes were calculated from
415 OTU data to investigate the impact of three factors: cheese-making and ripening stage, sample
416 type and the two factors associated (see Supplementary Fig. S3). For bacterial communities,
417 the richness and Chao1 indexes (defined as OTU number in samples and estimated richness
418 respectively) showed respectively significant differences between sample type and stage
419 ($p<0.001$) as well as Shannon evenness index ($p<0.01$). Bacterial diversity significantly
420 increased during cheese-making and ripening, especially in rinds. Regarding fungal
421 communities, diversity increased throughout ripening. Significant differences regarding
422 richness indexes were however observed between cores and rinds and overall values were
423 lower ($p<0.001$) in cores. For both communities, low Shannon and Simpson indexes ($p>0.05$)
424 were observed compared to Chao1 index. As Shannon and Simpson indexes consider relative
425 abundances, results suggested that bacterial and fungal populations were dominated by a few
426 abundant taxa.
427 The succession of bacterial communities during Pélardon cheese-making and ripening are
428 illustrated in Fig. 4. All samples considered, a total of three phyla (Firmicutes 94 %,
429 Actinobacteria 5.6 % and Proteobacteria 0.4 % of total reads), five orders, 10 families, 12
430 genera and 21 species were identified. Overall, regardless of sample type or stage, the most
431 abundant genera were *Lactococcus* (88.9 % of all sequences) followed *Glutamicibacter* (3.8
432 %) *Leuconostoc* and *Brevibacterium* (1% each). Other genera were encountered at an
433 abundance below 1 %. Raw milk samples were characterized by a low number of reads

434 (below 200 reads per samples) and thus not formally included in this analysis. In the whey
435 used for backslopping, the most abundant genus was *Lactococcus,* with two identified species
436 (*L. lactis, L. raffinolactis*) and one OTU that could not be identified at the species-level. These
437 three OTUs represented up to 99.5 % of sequences. At day 2, after acidification, curd samples
438 were dominated by *L. lactis* and it remained dominant during the following stages in both
439 core and rind samples although its abundance progressively decreased in rinds concomitantly
440 with the appearance of other genera and species, especially belonging to staphylococci (i.e. *S.*
441 *succinus* and *S. saprophyticus*) and actinobacteria. For these groups, high variations in relative
442 abundances were observed among replicates in the late stages of ripening (days 62 and 90).
443 Indeed, *Brevibacterium* spp. abundances ranged from 2.8 to 7.6 %, *Glutamicibacter* spp. from
444 4.4 to 47 % and those of *Brachybacterium* spp. from 0.8 to 11 %.
445 Regarding fungal communities, 15 species belonging to 11 genera, nine families and two
446 phyla were identified in raw materials and cheeses (see Supplementary Fig. S4). *G. candidum*
447 was the most abundant species with a relative abundance of 99.5 % in all cases, regardless of
448 the sample type or stage. However, fungal diversity increased at the beginning of the ripening
449 period (day 14) as shown by the presence of *Penicillium* species, and in late ripening stages
450 (days 62 and 90) with the presence of *S. brevicaulis* in rinds. However, no differences in the
451 relative abundances between core and rind were observed for these species at the end of
452 ripening. Several other fungal species were punctually identified e.g. *Cladosporium* was only
453 found in whey and raw milk.

454 **3.2.2.** *Lactiplantibacillus paracasei* quantification during ripening

455 Because of some discrepancies between culture-dependent and independent analyses
456 regarding the relative abundance of *L. paracasei* at days 14, 62 and 90, its presence was
457 further investigated by qPCR, using TufLcpara specific primers. In these conditions, qPCR
458 efficiency was 94 %. Based on the standard curve, *tuf* gene copy number of *L. paracasei* per

459 gram of cheese was determined and used as a proxy of *L. paracasei* cells per gram of cheese.
460 We observed that *tuf* gene copy numbers per gram of cheese were 33-fold (cheese core) and
461 3-fold (cheese rind) higher at day 62 than at day 14, indicating that *L. paracasei* population
462 increased over ripening time (Table 1). It then remained stable until day 90. Noteworthy, at
463 day 62 and 90, the *L. paracasei* population was 10-fold higher in the cheese core than in the
464 rind.

465 **3.3.** Study of potential environmental microbial sources using culture-dependent and -
466 independent approaches

467 To investigate the potential sources of microorganisms in shaping Pélardon cheese
468 microbial communities, sampling was done inside the farmhouse. Detailed sample
469 information and viable microbial counts are shown in Table 2. All samples were analyzed
470 using culture-dependent (Fig. 5) and -independent approaches (Supplementary Fig. S5),
471 except air samples which were only assessed by the culture-dependent approach. Low
472 microbial counts for mesophilic aerobic bacteria, fungi and LAB were encountered especially
473 for surface samples (< 4 log10 CFU/cm^2 or m^3) and these results were correlated with low
474 amplification by culture-independent analysis. Concerning the main bacterial species
475 identified in cheese, *L. mesenteroides* was identified on cheese trays used in the drying room
476 (PS5) by culture-dependent analyses and surfaces from the cheese-making room (PS2, PS4
477 and PS9) by the culture-independent approaches. *L. lactis* was only isolated from a tray in the
478 drying room (PS5), while it was also identified at low relative abundance (8 %) on a clean
479 tray used for curdling (PS9) by the culture-independent approach. *Staphylococcus* species
480 were among the most frequently isolated species on trays used for ripening (PS6) and in the
481 cheese-making room (PS7) by both approaches (see Supplementary Fig. S6 for a detailed
482 comparison). The same species were also found in the air of the drying and ripening rooms.
483 Bacterial genera identified in Pélardon cheese rinds such as *Glutamicibacter* and

484 *Brevibacterium* were also isolated from surface samples collected from the cheese-making,
485 drying and ripening rooms at high abundances. Noteworthy, enterococci and lactobacilli were
486 not isolated nor detected in environmental samples. For fungi, air sample analysis showed that
487 *G. candidum* was present at high abundances in the air of the cheese-making, drying and
488 ripening rooms. *P. commune* and *S. brevicaulis* were also present but at lower abundances.
489 These three species were also identified on the surface of the trays in the different rooms
490 using culture-independent analysis.

491 **3.4.** pH, sugar and non-volatile organic acid changes

492 pH measurements were similar in the whey used for backslopping and in day 2 curd (~4.3
493 units) and continuously increased ($p<0.001$) throughout ripening until day 62 to reach 6.07
494 and 6.73 pH units in cores and in rinds, respectively. pH values were systematically and
495 significantly higher in rinds as compared to cores except on day 90 (Table 3).
496 The main sugars (lactose, galactose) and organic acids (acetic, citric, lactic acids) were
497 quantified in whey used for backslopping and during the different stages of Pélardon cheese-
498 making and ripening. Lactose, galactose as well as acetic and lactic acids showed a similar
499 trend throughout the process. Their highest concentrations were in curd after acidification
500 (day 2) and progressively decreased: lactose and acetate were detected until day 62 whereas
501 galactose and lactic acid were no longer detected as early as day 14 (early ripening). In
502 contrast, citric and 3-phenyllactic acid concentrations were below the detection level during
503 acidification (day 2) and increased during drying (for 3-phenyllactic acid) and ripening (both
504 acids).

505 **3.5.** Changes in volatile profiles during cheese-making and ripening

506 Using headspace GC-MS, 54 volatile compounds belonging to six main families were
507 detected and identified in Pélardon cheese samples during cheese-making and ripening.

508 Compound names, identification criteria and their respective abundances are listed in the
509 Supplementary Table S2.
510 To better understand these changes and the main compounds involved, a one-way ANOVA
511 analysis followed by Tukey's highest significant difference test were performed on abundance
512 data. Out of the 54 volatile compounds, 47 were detected at a significantly different
513 abundance at, at least, one stage ($p<0.05$). To confirm these trends, a correlation analysis was
514 then performed on the volatile abundances and the results are presented using a heatmap with
515 sample clustering. Overall, the identified compounds could be linked to specific cheese-
516 making or ripening stages based on their high abundances and four main groups of
517 compounds could be differentiated (Fig. 6). A first group (group I) gathered six compounds
518 present in high abundances in curd (day 2) and whey samples, i.e. acetic acid, 3-
519 hydroxybutan-2-one, 2-methylthiolan-3-one, pentane-2,3-dione, butane-2,3-dione and
520 hexanal. The post-drying (day 8) and early ripening (day 14) stages were associated with
521 compounds of group II corresponding to branched-chain and aromatic compounds (i.e. 3-
522 methylbutanoic acid, 2-phenylacetaldehyde). Finally, in the late ripening stages (day 62 and
523 90), 33 compounds were quantified at their highest abundances, mainly belonging to
524 methylketones, secondary alcohols and free fatty acids and could be separated in two groups
525 (III and IV). Group III gathered compounds identified in particularly high abundances at day
526 62 and day 90 among which were seven free fatty acids (butanoic, hexanoic, pentanoic,
527 heptanoic octanoic, nonanoic and decanoic acids), five ketones (non-8-en-2-one, octan-3-one,
528 pentan-2-one, heptan-2-one, hexan-2-one, and nonan-2-one) and one alcohol (heptan-2-ol).
529 Group IV mostly gathered compounds with high abundances in day 90 cheeses and
530 noteworthy, it included the four esters as well as ketones and secondary alcohols.

531 **3.6.** Correlation between microbial communities and changes in biochemical profiles
532 during cheese-making and ripening

533 A principal component analysis was conducted with all microbial and biochemical data to
534 correlate temporal changes in microbial communities with biochemical profiles
535 (Supplementary Fig. S7). Dimensions 1 and 3, which explained 34.2 % and 11.6 % of the
536 variance respectively, provided the best separation of samples according to the cheese-making
537 and ripening times. Day 2 and whey samples were grouped together and associated with the
538 *Lactococcus* genus, identified by both culture-dependent and -independent approaches (three
539 OTUs), and two sugars (lactose and galactose) as well as six other compounds including
540 acetic acid, lactic acid and 3-hydroxybutanone. Rind samples from day 8 and day 14 were
541 linked to 2-phenylacetaldehyde. During ripening (day 62), rinds were associated with a high
542 pH and six volatile compounds as well as three species, *S. xylosus*, *L. casei/paracasei*, and *P.*
543 *commune,* identified by culture-dependent approach. At the end of ripening (day 90), multiple
544 species identified by both culture-dependent analyses (*E. faecalis, S. equorum, S. brevicaulis*,
545 *Brevibacterium* spp.) and metabarcoding (L. lactis_62, Lactococcus sp._78, Brachybacterium
546 sp_56 and Brachybacterium sp._8) were correlated to 13 compounds mainly free fatty acids,
547 methyl ketones and esters.
548 To get a better insight into the potential links between aroma compounds and
549 microorganisms, a Pearson correlation analysis was performed based on culture-dependent
550 data, organic acid and volatile data. Pair-wise correlation coefficients and corresponding p-
551 value were thus calculated (Fig. 7). Focusing on the strongest correlations ($|r| > 0.8$ and $p <$
552 0.001), four microbial groups stood out. *L. lactis* and *G. candidum* were strongly and
553 positively correlated with lactose, acetic acid, lactic acid, and 3-hydroxybutan-2one. *L.*
554 *mesenteroides,* whose correlation profile was relatively similar to that of *L. lactis,* was
555 strongly and positively correlated to 3-methylpropanoic, 3-methylbutanoic acids and 2-
556 phenylacetaldehyde; highest abundances of these compounds being at day 8. *S. xylosus, S.*
557 *equorum* and *E. hirae* were also positively correlated with 11 compounds, mainly ketones,

558 carboxylic acids and alcohols. *P. commune*, *L. casei/paracasei*, *E. faecalis* and *S. brevicaulis*
559 were positively correlated with the same 11 compounds but also showed high correlations to
560 11 others compounds, namely octan-3-one, oct-1-en-3-ol, 2-ethylhexanoic acid, octanoic acid,
561 2-hydroxypentan-3-one, 3-methylheptan-2-one, nonanal, decan-2-one, nonanoic acid,
562 methylsulfonylmethane and ethyl butanoate. Among these species, *S. brevicaulis* showed a
563 slightly different correlation profile as strong and positive correlations were also observed for
564 ethyl hexanoate, ethyl acetate and 1-hydroxypropan-2-one. High correlations for those
565 compounds were otherwise observed only with *E. faecium*. Finally, strong positive
566 correlations were also punctually observed.

567 **4. Discussion**

568 In this study, microbial community dynamics during PDO Pélardon cheese-making and
569 ripening were unraveled for the first time by culture-dependent and -independent approaches
570 and linked to the cheese aroma profile.

571 The experimental set-up aimed at combining extensive culture-dependent and culture-
572 independent approaches to precisely and efficiently study the microbial communities
573 associated to Pélardon cheese, raw materials and the cheese-making environment. Cultural
574 methods and isolate identifications rely on tedious work and time-consuming techniques that
575 often limit the analysis scale in terms of sample number and collected isolates. In the present
576 study, we used MALDI-TOF MS as a high-throughput technique combined with molecular
577 sequencing to describe cheese microbial communities at a species-level, based on a recently
578 described microbial culturomics strategy (Lagier et al., 2015; Sarhan et al., 2019). Although
579 MALDI-TOF identifications have been recently used in food-related microbial diversity
580 studies (Nacef et al., 2017; Peruzy et al., 2019), this is the first time, to our best knowledge,
581 that this method is used to study both bacterial and fungal dynamics in a fermented product. It
582 allowed us to analyze a large collection of autochthonous isolates (n = 2877) from different

583 media targeting dominant and sub-dominant populations. Taking into account both culture-
584 dependent and independent data, 29 genera were identified during Pélardon cheese-making,
585 12 of which were identified by both approaches (Supplementary Fig. S8). Among those,
586 *Lactococcus* and *Leuconostoc* for bacteria and *Geotrichum* for fungi were identified by both
587 approaches as the most dominant during curdling while during ripening and in rinds,
588 *Brevibacterium*, *Arthrobacter, Staphylococcus, Penicillium* and *Scopulariopsis* were found
589 but in different proportion according to the method. Interestingly, enterococci were only
590 identified by the culture-dependent approach with increasing species diversity during
591 ripening; a result that was completely overlooked by the culture-independent approach.
592 Culture-independent approach however, further identified five genera, mainly highlighting the
593 subdominant halophilic bacteria (i.e. *Brachybacterium, Salinococcus, Marinhabitans)* on the
594 Pélardon cheese surface.
595 Our dynamic study of Pélardon microbial diversity revealed inconsistencies regarding
596 microbial dominances during ripening between approaches. Indeed, culture-dependent results
597 revealed that *L. lactis,* after dominating during acidification, decreased during ripening. In
598 contrast, metabarcoding results suggested that *L. lactis* remained dominant in core during the
599 entire ripening. Moreover, most surprisingly, no *Lactiplantibacillus* OTUs were detected in
600 any samples by the culture-independent approach, while it was shown to increase and
601 dominate during ripening by the culture-dependent approach. To eliminate any potential
602 discrepancies due to technical errors qPCR tests targeting *L. paracasei* were performed on
603 ripening samples to confirm culture-dependent data. *L. paracasei* was detected at high levels
604 during ripening in both cores and rinds. These results have thus led us to assume that the
605 dominance of *L. lactis* DNA from both live and dead cells following curdling masked any
606 lactobacilli DNA in the studied samples using the culture-independent approach. Indeed,
607 autolysis of starter bacteria, such as *L. lactis* in our study, during cheese ripening is a well-

608 known phenomenon although the involved mechanisms are not well established (Lazzi et al.,
609 2016; Treimo et al., 2006). It is also possible that *L. lactis* cells were in a Viable But Not
610 Cultivable state (VBNC). Bacteria in VBNC state has been discussed in the general context of
611 cheese production (Edalatian et al., 2012) and to a lesser extent for cheese starter cultures
612 (Falentin et al. 2012; Ruggirello, Dolci and Cocolin 2014), however their active metabolic
613 contribution have yet to be determined. Similar hypotheses could be done to explain the
614 differences in the proportions of *G. candidum, Penicillium* spp. and *S. brevicaulis* at each
615 ripening stage analyzed by the two approaches. Discrepancies between culture-dependent and
616 culture-independent results have been repeatedly reported regardless of the culture-
617 independent method used (Coton et al., 2017; Delbès et al., 2007) and our study led to a
618 different "picture" of the microbial communities present during ripening. Based on the
619 literature findings reported above and on our observations, the 3-step culture-dependent
620 approach we implemented in this study provided the most thorough overview of the microbial
621 community dynamics during Pélardon cheese-making although metabarcoding highlighted the
622 presence of some specific micro-organisms. Moreover, similar microbial analyses were
623 performed on another batch six months before and the same dominant species were found and
624 discrepancies between the two approaches also observed (data not shown). We thus decided
625 to mainly consider the culture-dependent results for correlation analysis.

626 As previously mentioned, Pélardon cheese is produced without any starter. Thus, the
627 plausible origin of the microorganisms identified during cheese-making and ripening was
628 investigated in the raw materials and cheese-making environment. Raw milk microbiota was
629 dominated by coagulase negative Staphylococci as previously reported by Tormo et al. (2011)
630 although most of the identified species did not persisted after acidification. However, results
631 highlighted the contribution of whey as a major source of starter LAB, mainly *L. lactis* and in
632 a lesser extent *L. mesenteroides*. This finding is consistent with other cheese technologies

633 using backslopping such as Rocamadour or Parmigiano Regiano, in which the main acidifying
634 bacteria are also brought by the whey (Coloretti et al., 2016; Demarigny et al., 2006).
635 However, in the case of Pélardon cheese, the whey also acted as a source of fungal
636 populations involved in ripening as it contained high populations of *G. candidum*. Besides, *G.*
637 *candidum* and the other most dominant fungal species were also found all over the cheese-
638 factory. In addition, some species identified on Pélardon cheeses, especially *Glutamicibacter*
639 and *Staphylococcus* species, were also found on several surfaces but not in raw materials. Our
640 results thus strongly suggested that the working environment likely contributes to the
641 enrichment of the surface microbiota of these cheeses during ripening. Overall, it would be of
642 interest in the future to investigate to what extent the microbiota revealed here is shared
643 between other Pélardon producers and if the specific microorganisms we identified as part of
644 the core microbiota can be considered as a "house microbiota" as previously described by
645 Bokulich and Mills (2013) for two artisanal washed-rinds cheeses.

646 Cheese aroma compound composition is particularly relevant for artisanal cheeses as it
647 strongly contributes to their typicity and influences the consumer's preference. In this study, a
648 correlation between the identified bacteria and fungi, the main fermentation products and
649 aroma compounds was established. Our results revealed dynamics importance of both
650 communities early-on in the cheese-making process. Indeed, whey used as an inoculum was
651 dominated by *L. lactis,* the main acidifying microorganism, along with *L. mesenteroides* and
652 none of the other species identified in raw milk persisted in curd. These two species were well
653 correlated to the major lactose fermentation products such as lactic acid and acetic acid but
654 also to hexanal and 3-hydroxybutan-2-one (acetoin) compounds associated with buttery and
655 fresh aromas. Lactic and acetic acids rapidly decreased during drying suggesting their
656 utilization, which is consistent with the significant pH increase. *L. mesenteroides* was also
657 correlated to high abundances of leucine and valine degradation products such as 3-

658 methylbutanoic and 2-methylpropanoic acid (Delgado et al., 2011), which abundances were
659 highest at day 8. This would suggest that *L. mesenteroides* is also actively involved during the
660 post-acidification stage which is often related to proteolysis activities (McSweeney and
661 Sousa, 2000). *G. candidum* was highly associated with the drying and early ripening stages
662 and is most likely responsible for the observed deacidification phenomenon. Moreover, lactic
663 acid catabolism by *G. candidum* is well-established in cheeses such as Camembert and Brie
664 (Boutrou and Guéguen, 2005). Its deamination activity has also been reported and linked to
665 the production of 2-phenylacetaldehyde and 2-phenylethnaol from phenylalanine, compounds
666 that we found to considerably increase in Pélardon cheese during drying (day 8) and early
667 ripening (day 14) stages. Interestingly, both compounds, found at high abundances at the
668 same stages, are known to bring floral and sweet aromas to the product (Majcher et al., 2014).
669 Prolonged ripening for up to three months seems to be linked to the dominance of two non-
670 starter lactic acid bacteria, *L. paracasei* and *E. faecalis,* and two filamentous fungi, *P.*
671 *commune* and *S. brevicaulis,* and associated with most of the identified volatile compounds.
672 The strongest positive correlations were observed with some ketones, which originate from
673 free fatty acid catabolism by different microorganisms in particular fungi and have low odor
674 thresholds with strong "earthy" and "cheesy" notes. Interestingly, *S. brevicaulis* was
675 previously identified in cow milk ripened cheeses (Schornsteiner et al., 2014) and natural
676 ripened cheeses as a core microorganism (Wolfe et al., 2014). This species has also been
677 associated with high proteolytic activity (Bothast et al., 1975) and considered as a cheese
678 spoiler when it predominates. Pélardon late ripening stages were also characterized by high
679 abundances of free fatty acids, which can be released by either microbial or milk lipases
680 (Collins et al., 2003). Their concentrations have similarly been shown to increase with aging
681 in other goat cheeses (Delgado et al., 2011) and hexanoic and decanoic acids, also designated
682 as caprylic and caproic acids, respectively, have previously been reported as aroma markers of

683 goat cheese. In contrast, day 14 cheeses, which corresponds to the minimal ripening time
684 required for the PDO Pélardon denomination, were not correlated with high abundances of
685 specific compounds. Thus, the typical aroma of Pélardon cheese probably results from a
686 balance between compounds, associated with fresh buttery and floral aromas, and as the
687 ripening progresses by compounds that bring increased strong, earthy and goaty aromas,
688 potentially attenuated by esters associated with fruity aromas.

689 Overall, these results highlight the dynamics of Pélardon cheese microbial community.
690 Through correlation analysis we identified the key-players of Pélardon cheese microbial
691 communities. However, more in-depth studies are necessary to first validate and secondly
692 further explore their functional role. By implementing a culture-dependent approach, we were
693 able to constitute a large collection of autochthonous isolates, hence working on individual
694 species or reconstructed consortia could help to better characterize their metabolic potential,
695 decipher interactions between the key-players and further demonstrate their contribution to
696 Pélardon cheese aroma typicity.

697

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706 **Declaration of competing interest**

707 The authors declare that they have no known competing financial interests or personal

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716 **Data and microbial resources availability**

717 Sequence data generated in this study are publicly available on the European Bioinformatics

718 Institute database (www.ebi.ac.uk) under accession numbers PRJEB37923 (bacterial 16S

719 rRNA sequencing run data) and PRJEB37922 (fungal ITS2 sequencing run

720 data).Representative isolates will be deposited in culture collections: UBOCC for fungi and

721 CIRM-BIA for bacteria and accessible on demand.

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925

926 **Table 1. *Lactiplantibacillus paracasei* quantification by qPCR during cheese ripening.** Results are expressed as number of copies per gram of
927 cheese based on mean values and standard deviation of triplicate analyses. Means with different letters in the last column are significantly
928 different (Tukey's test $p<0.05$).
929
930

Sampling time	Sample type	Ct value	*tuf* gene copy number/g of cheese
day 14	core	27.63 ± 0.71	$1.81 \times 10^6 \pm 8.07 \times 10^5$ [a]
day 62	core	22.35 ± 0.95	$6.08 \times 10^7 \pm 3.80 \times 10^7$ [b]
	rind	25.66 ± 0.77	$5.46 \times 10^6 \pm 2.07 \times 10^6$ [a]
day 90	core	21.81 ± 0.90	$7.63 \times 10^7 \pm 3.06 \times 10^7$ [b]
	rind	25.05 ± 0.54	$9.09 \times 10^6 \pm 3.04 \times 10^6$ [a]

Ct Cyle threshold

931 **Table 2. Microbial counts in environmental samples of the cheese factory**
932
933
934

Sample code	Sample type	Microbial counts		
		Mesophilic aerobic bacteria	Fungi	Presumptive LAB[a]
Surface (log_{10} CFU/cm^2)				
PS1	Cleaned plastic trays surface used for renneting A	2.95	3.62	-
PS2	Cleaned inox bench used for cheese draining	3.26	3.04	-
PS3	Cleaned plastic mould A	-	-	-
PS4	Cleaned tray stored in the cheese-making room	3.15	3.46	-
PS5	Cheese tray in the drying room	5.96	6.22	6.00
PS6	Cheese tray in the ripening room	6.48	6.23	5.26
PS7	Wall tiles in the making room	4.84	2.00	-
PS8	Cleaned plastic mould B	-	-	-
PS9	Cleaned plastic trays surface used for renneting B	3.38	3.80	2.30
PS10	Milk arrival pipe in the cheesemaking room	-	-	-
Intrants (log_{10} CFU/mL)				
PL1	Tap water arriving in the cheesemaking room	-	-	-
PL2	Tap water arriving in the cleaning area	2.77	-	-
PL3	Salt used for cheese dry salting	-	-	-
Air (log_{10} CFU/m^3)				
PAF	Main cheese-making room	>3	2.38	NP
PAS	Drying room	>3	2.34	NP
PAA	Ripening room	>3	2.45	NP

a LAB : Lactic acid bacteria ; - : Population below delection level ; NP : enumeration Not Performed

935 **Table 3. Changes in pH values, sugar and organic acid concentrations (mg/g of cheese) during cheesemaking and ripening.** Mean values
936 and standard deviations are given based on triplicate cheeses. Means with different letters within pH values or within a row for acids and sugars
937 are significantly different (Tukey's test $p<0.05$).

	pvalue	Whey	Day 2	Day 8	Day 14	Day 62	Day 90
***pH* (Units)**	**$3.42E^{-20}$**						
pH in core		4.33 ± 0.05^{a}	4.31 ± 0.07^{a}	4.62 ± 0.08^{b}	5.02 ± 0.15^{c}	6.07 ± 0.03^{e}	6.56 ± 0.02^{f}
pH in rind		/	/	4.82 ± 0.06^{bc}	5.62 ± 0.05^{d}	6.73 ± 0.09^{f}	6.69 ± 0.05^{f}
***Acids and sugars* (mg/g of cheese)**							
Lactose	**$7.3E^{-17}$**	23.61 ± 0.01^{a}	19.96 ± 0.59^{b}	10.11 ± 0.32^{c}	0.87 ± 0.73^{d}	ND	ND
Galactose	**$2.36E^{-10}$**	0.12 ± 0.03^{a}	0.14 ± 0^{b}	0.01 ± 0.01^{c}	ND	ND	ND
Lactic acid	**$6.14E^{-13}$**	5.77 ± 0^{a}	7.3 ± 0.32^{b}	4.55 ± 0.37^{c}	ND	ND	ND
Acetic acid	**$2.86E^{-10}$**	0.29 ± 0^{a}	0.47 ± 0.04^{b}	0.08 ± 0.03^{c}	0.1 ± 0.02^{c}	ND	ND
3-Phenyllactic acid	**$4.91E^{-10}$**	0.01 ± 0^{a}	0.01 ± 0^{b}	0.05 ± 0^{bc}	0.14 ± 0.01^{c}	0.07 ± 0.01^{d}	0.06 ± 0^{d}
Citric acid	**$2.13E^{-04}$**	ND	ND	ND	ND	0.61 ± 0.33^{a}	0.42 ± 0.03^{a}

ND below quantification and detection limit

938 **Figure captions**

939 **Fig. 1. Sampling strategy and experimental design used in this study for the analysis of**
940 **an artisanal goat's milk cheese: Pélardon**
941
942
943 **Fig. 2. Microbial population counts estimated by culture-dependent method during the**
944 **cheese-making and ripening of Pélardon goat cheese: (A) in raw materials and curd, (B)**
945 **in cheese core and (C) in cheese rind.** Mean values and standard deviations are given based
946 on triplicates. Cultivation conditions for each population are indicated between brackets. In
947 order to facilitate the comprehension, the curd (day 2) is represented on A, B and C.
948
949 **Fig. 3. Microbial communities diversity revealed by culture-dependent method during**
950 **Pélardon cheese-making and ripening in the core (A) and in the rind (B).** Dot plots show
951 microbial population counts as detailed on Fig. 1 (average values of 3 replicates) whereas bar
952 plots represent species composition based on isolates identification obtained via MALDI-TOF
953 analysis followed by sequencing of 16S rRNA gene (bacteria) or ITS/beta-tubulin region
954 (fungi). The isolate proportion represents the number of isolates identified as a species
955 divided by the number of isolates all three replicates. In order to facilitate the comprehension,
956 raw milk, whey used for backslopping and day 2 (curd) are represented on A and B.
957
958 **Fig. 4. Bacterial community composition during Pélardon cheese-making and ripening**
959 **determined by a culture-independent approach.** Data were obtained by metabarcoding
960 analysis targeting the V3-V4 region of the 16S rRNA gene. Results are expressed as
961 normalized counts.
962
963 **Fig. 5. Microbial diversity encountered in the environment of the cheese-factory**
964 **determined using a culture-dependent approach.** Microbial composition based on isolates
965 identification obtained via MALDI-TOF MS analysis followed by sequencing of 16S rRNA
966 gene (bacteria) or ITS/beta-tubulin region (fungi). PS refers to surface samples, PL to intrants
967 samples while PAF, PAS, PAA refer to air samples from cheese-making, drying and ripening
968 rooms respectively. For detailed information of the samples see Table 2.
969
970 **Fig. 6. Normalized heatmap showing volatile compounds changes determined by HS-**
971 **GC-MS in Pélardon cheese samples during cheese-making and ripening.** Clustering based
972 on Ward's linkage using Euclidean distance. Samples names are on the right, R1, R2 and R3
973 refer to the cheese replicates used for analysis. Identified compounds are at the bottom part of
974 the plot. Abundance data were centered and scaled prior to analysis. Color ranging from blue
975 to red correspond to normalized abundance mean levels. The different frames highlight
976 compounds with similar profile during cheese-making and ripening.
977
978 **Fig. 7. Heat-map representing Pearson correlation analysis between species identified**
979 **using culture-dependent methods and biochemical compounds.** Species are shown at the
980 bottom, compounds are displayed on the right and both were clustered using on Ward's
981 linkage using Euclidean distance. Color ranging from red to blue correspond to negative and
982 positive correlation respectively. Red square highlight the strongest positive correlations
983 ($p<0.001$) - * , compounds quantified using HPLC.

Fig. 1

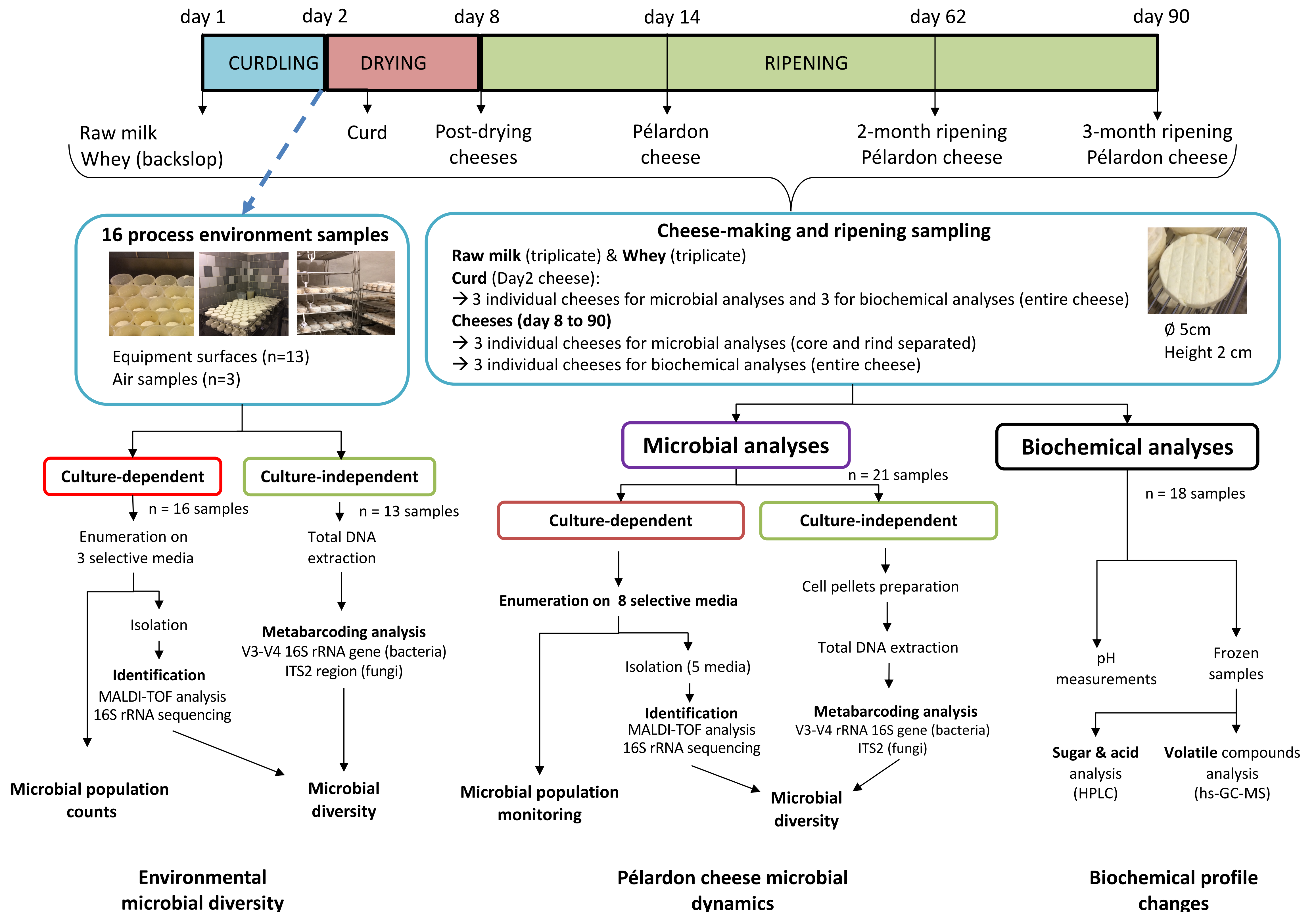
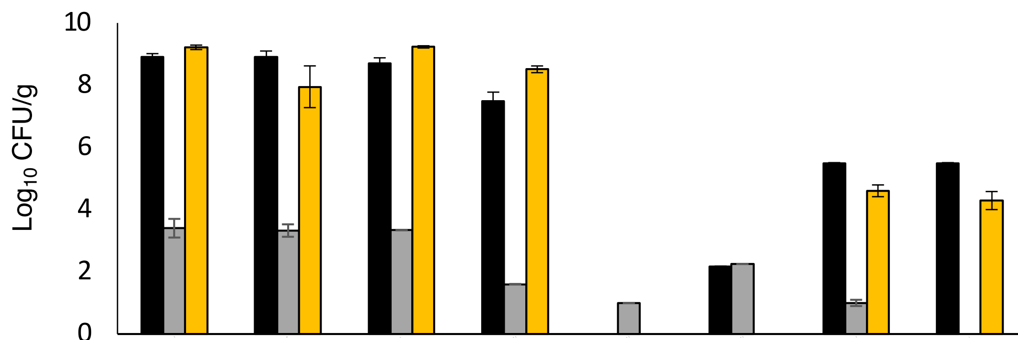
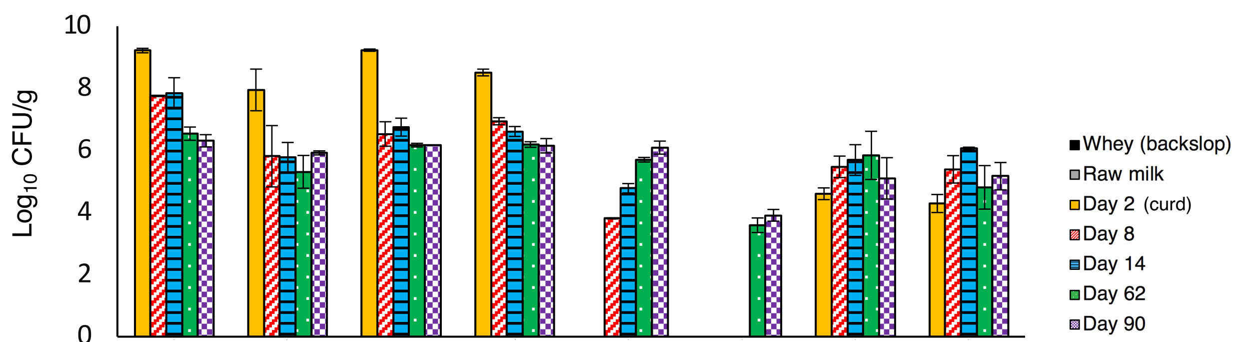


Fig. 2





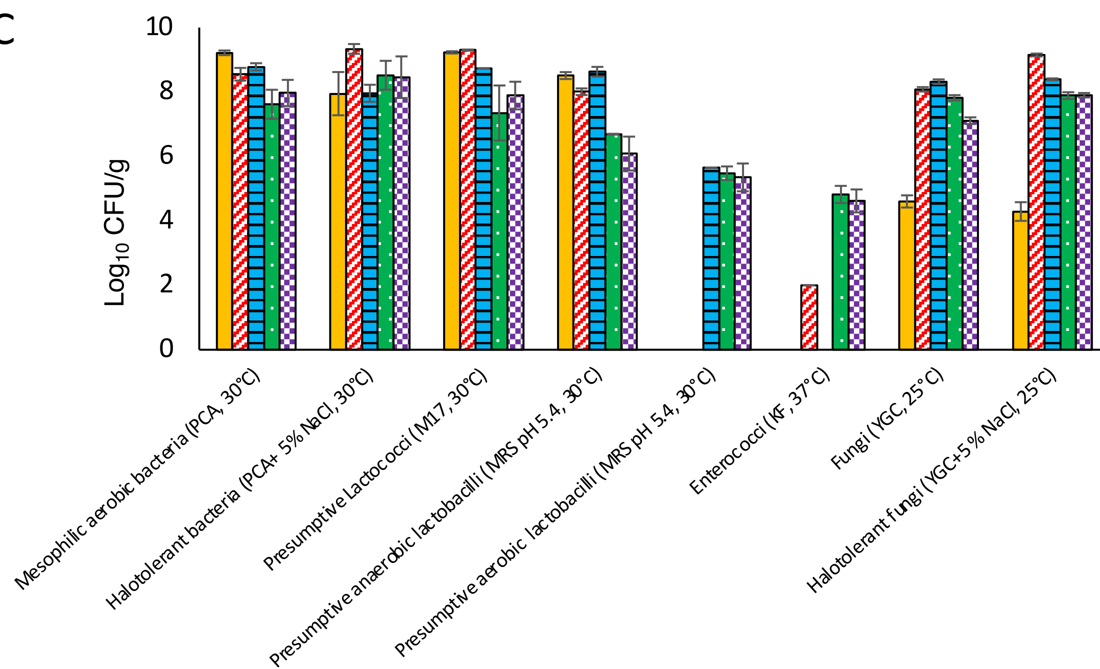
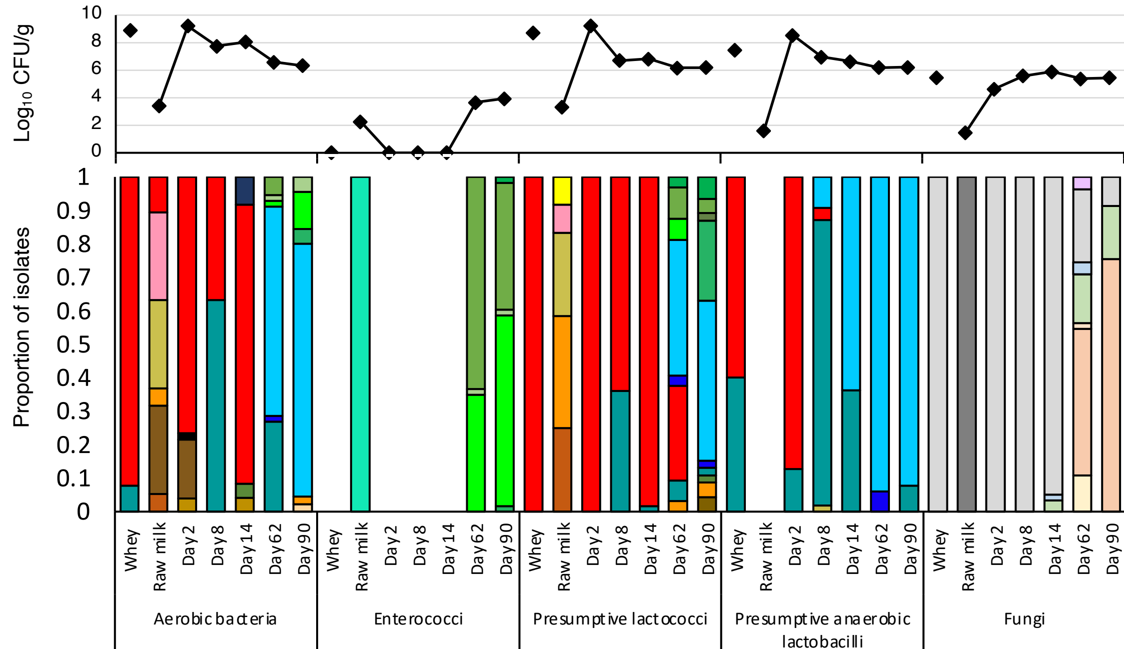
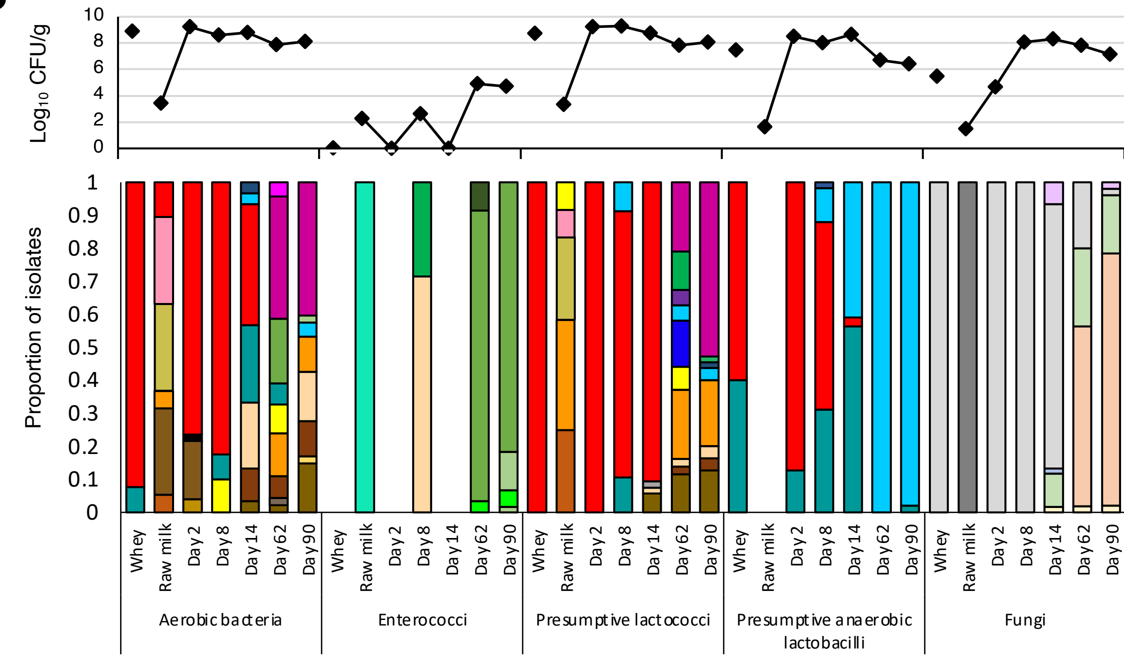


Fig 3

A



B



Bacteria

Acidovorax temperans
Arthrobacter russicus
Brevibacterium spp.
Enterococcus casseliflavus
Enterococcus faecalis
Enterococcus hirae
Enterococcus malodoratus
Glutamicibacter spp.
Lactobacillus brevis
Lactobacillus plantarum/paraplantarum
Leuconostoc mesenteroides
Micrococcus luteus
Serratia liquefaciens
Staphylococcus arlettae
Staphylococcus epidermidis
Staphylococcus fleurettii
Staphylococcus saprophyticus
Staphylococcus succinus
Staphylococcus warneri
Aerococcus viridans
Bacillus cereus/thuringiensis
Curtobacterium flacumfaciens
Enterococcus durans
Enterococcus faecium
Enterococcus italicus
Enterococcus mundtii
Kocuria spp.
Lactobacillus casei/paracasei
Lactococcus lactis
Microbacterium spp.
Rhodococcus spp.
Sphingomonas paucimobilis
Staphylococcus caprae
Staphylococcus equorum
Staphylococcus haemolyticus
Staphylococcus sciuri
Staphylococcus vitulinus/fleurettii
Staphylococcus xylosus

Fungi

Candida parapsilosis
Geotrichum candidum
Meyerozyma guilliermondii
Penicillium commune
Scopulariopsis alboflavescens
Trichosporon ovoides
Unidentified
Debaryomyces hansenii
Kluyveromyces lactis
Penicillium christenseniae
Penicillium glabrum
Scopulariopsis brevicaulis
Yarrowia lipolytica

Fig.4

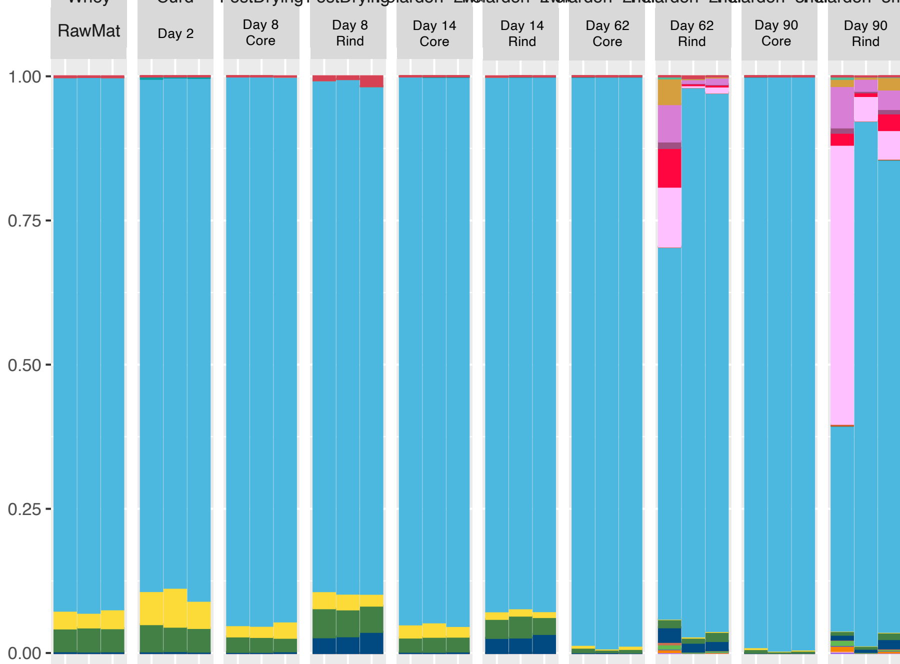


Fig. 5

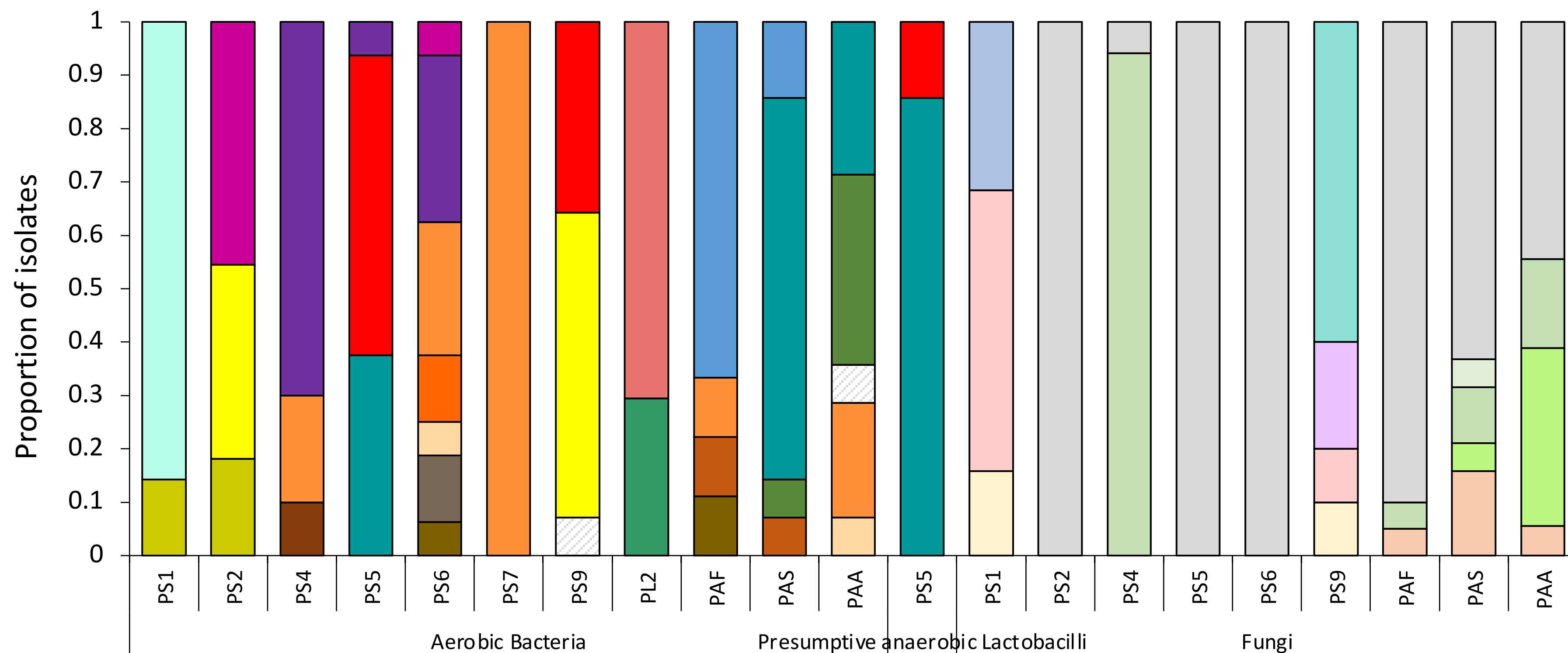


Fig. 6

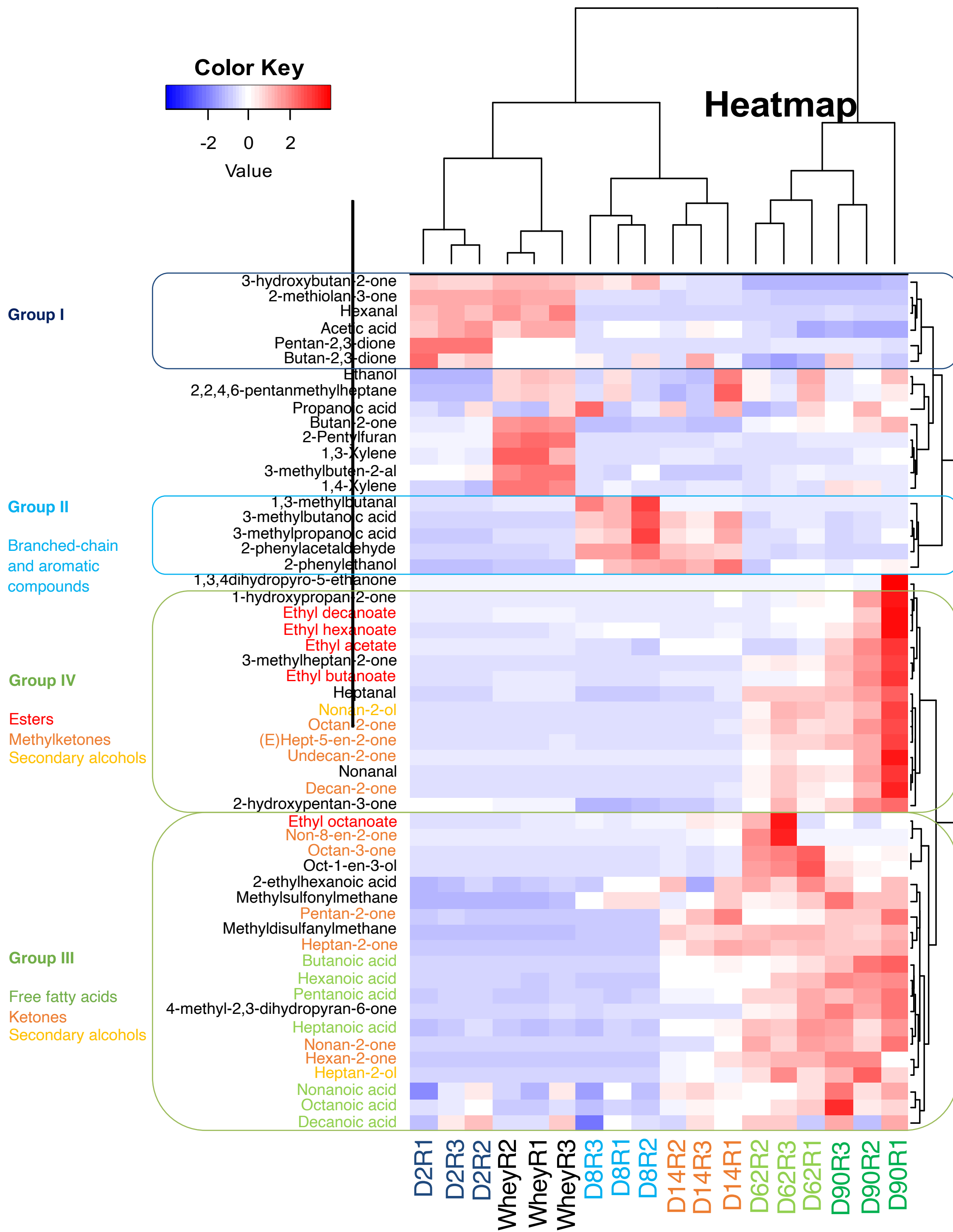
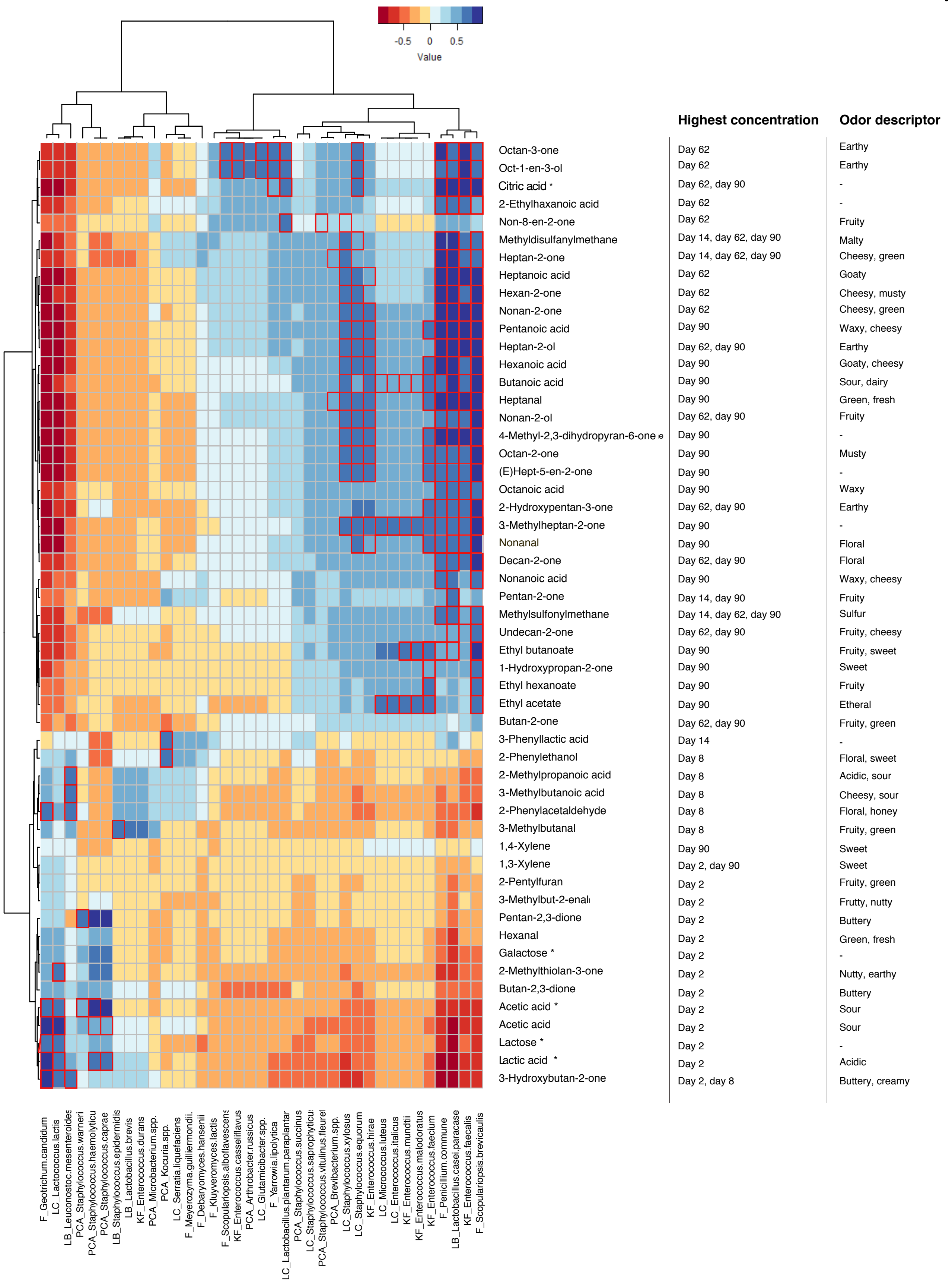


Fig. 7

Fig. 7



Supplementary material

Supplementary Fig .S1.
Example of a dendrogram of presumptive Staphylococcus species spectra obtained by MALDI-ToF analysis. Representative isolates chosen for identification by sequencing are indicated with "*" symbol. Choice was based on heterogeneity within the same cluster and discrepancies between presumptive identification by commercial database and position within the species clusters.

Supplementary Fig. S2.
Rarefaction curves of Illumina sequencing data after normalization grouped by cheese-making/ripening stage and sample type. (A) V3-V4 region of the 16S rRNA gene data (B) ITS2 sequencing data

Supplementary Fig. S3.
Alpha-diversity indexes of bacterial (A) and fungal (B) communities during cheese-making and ripening of Pélardon cheese. Samples are grouped according to the process stage and type.

Supplementary Fig. S4.
Composition of fungal communities during Pélardon cheese-making and ripening by culture-independent approach. Data were obtained by high-throughput metabarcoding analysis targeting the ITS2 region.

Supplementary Fig. S5.
Microbial community composition encountered in Pélardon cheese-making environment samples using a culture-independent approach. (A) bacterial communities (B) fungal communities using metabarcoding analyses targeting the V3-V4 region of 16S rRNA gene for bacteria and the ITS2 region for fungi. See Table 2 for legend of environmental samples

Supplementary Fig. S6.
Venn diagrams representing diversity and shared species in cheese and environmental samples using (A) culture–dependent and (B) culture–independent approaches.

Supplementary Fig. S7.
Principal component analysis biplot of microbial counts, culture-dependent (red), culture-independent (green) and biochemical data (acids and volatile compounds in blue) at the different cheese-making and ripening stages. Data are projected on dimensions 1 and 3 and only variables with a cosinus^2 superior to 0.6 are displayed.

Supplementary Fig.S8.
Venn diagrams comparing the number of genera and species identified using culture-dependent (red circles) and culture-independent (green circles) approaches for both bacteria (A) and fungi (B). Dominant genera identified by either of culture-dependent or independent approaches throughout the cheese-making or ripening are indicated in bold.

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Primers used in this study

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Volatile compounds identified and quantified (AU) during Pélardon cheese-making and ripening using HS-GSMS.

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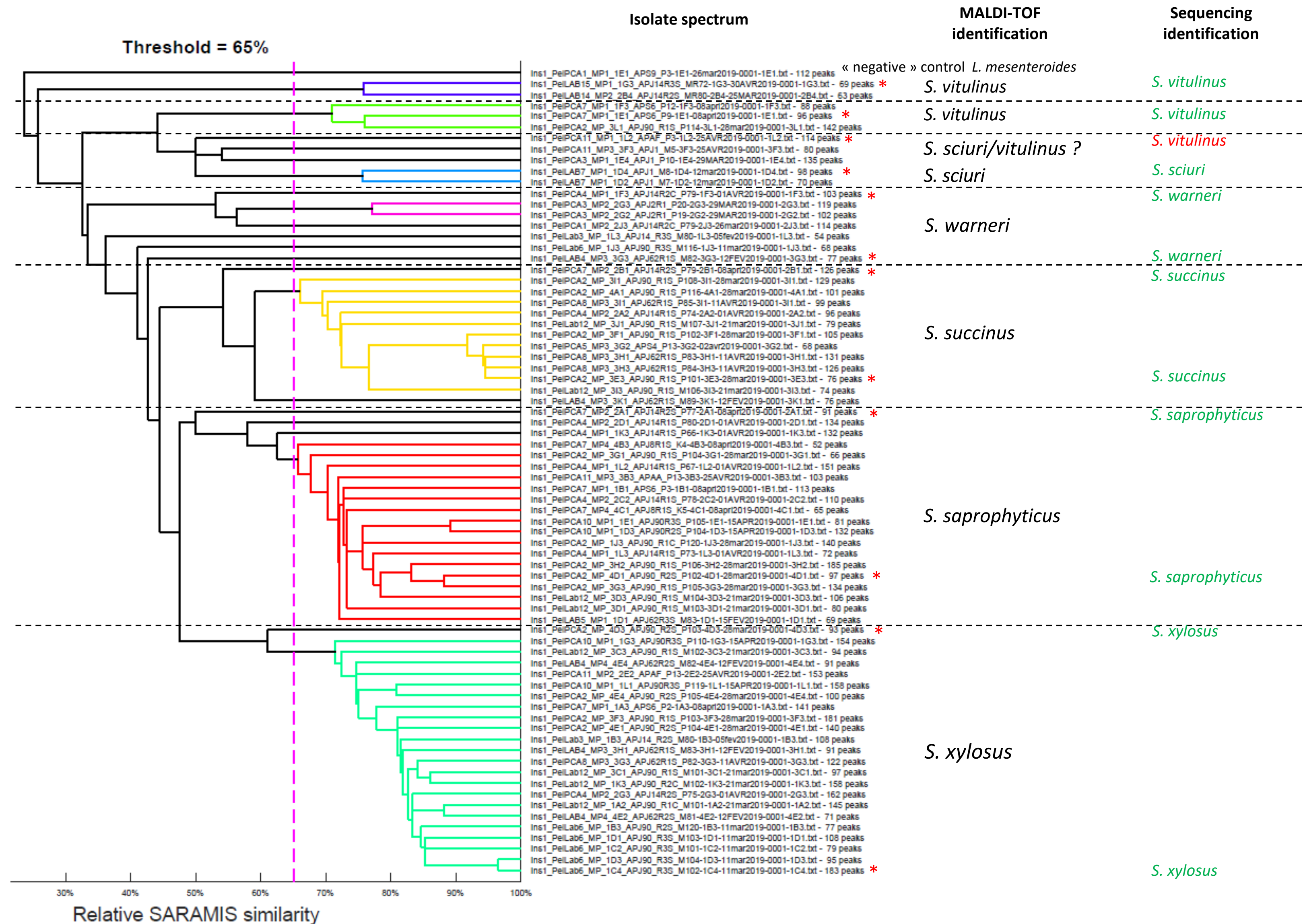

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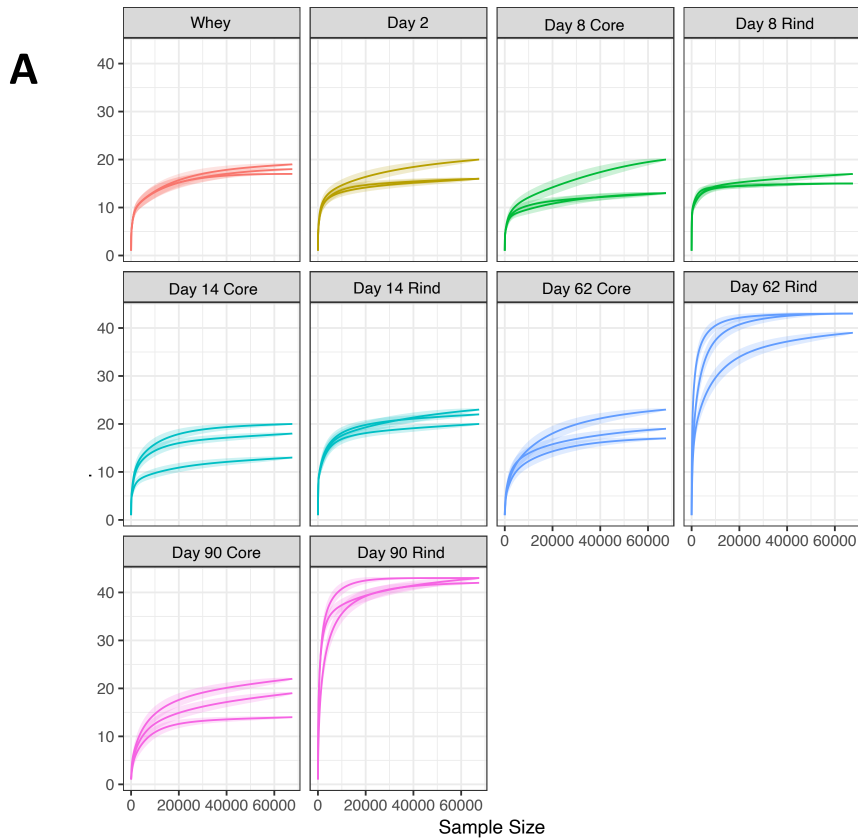

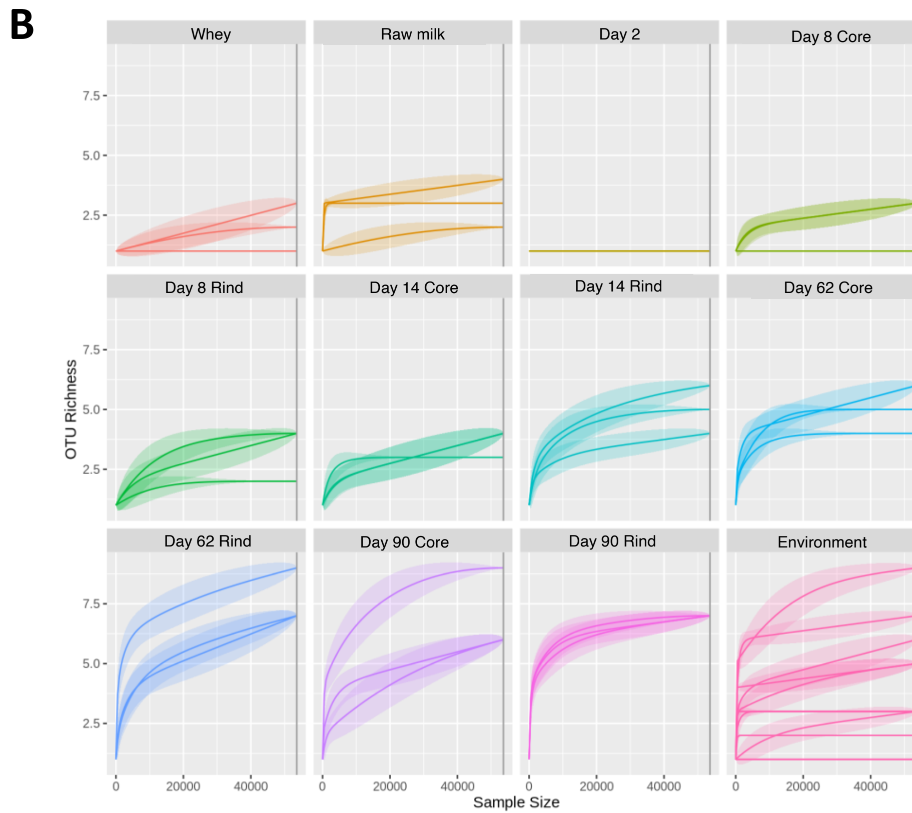

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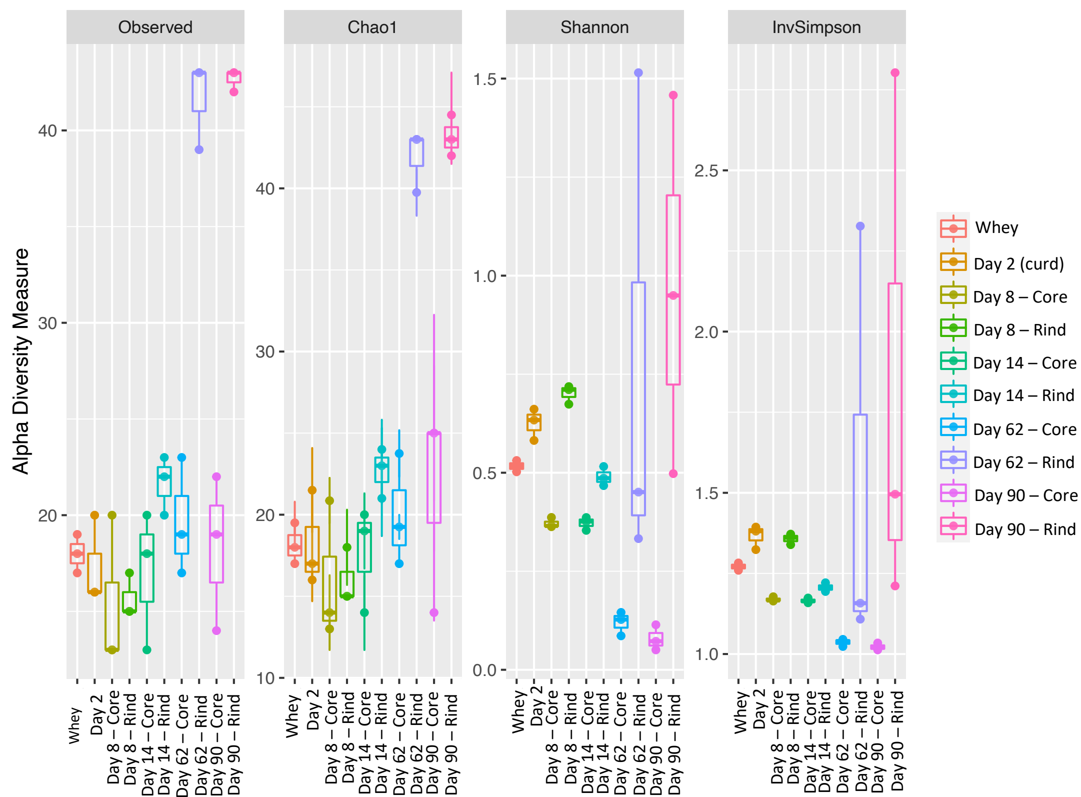

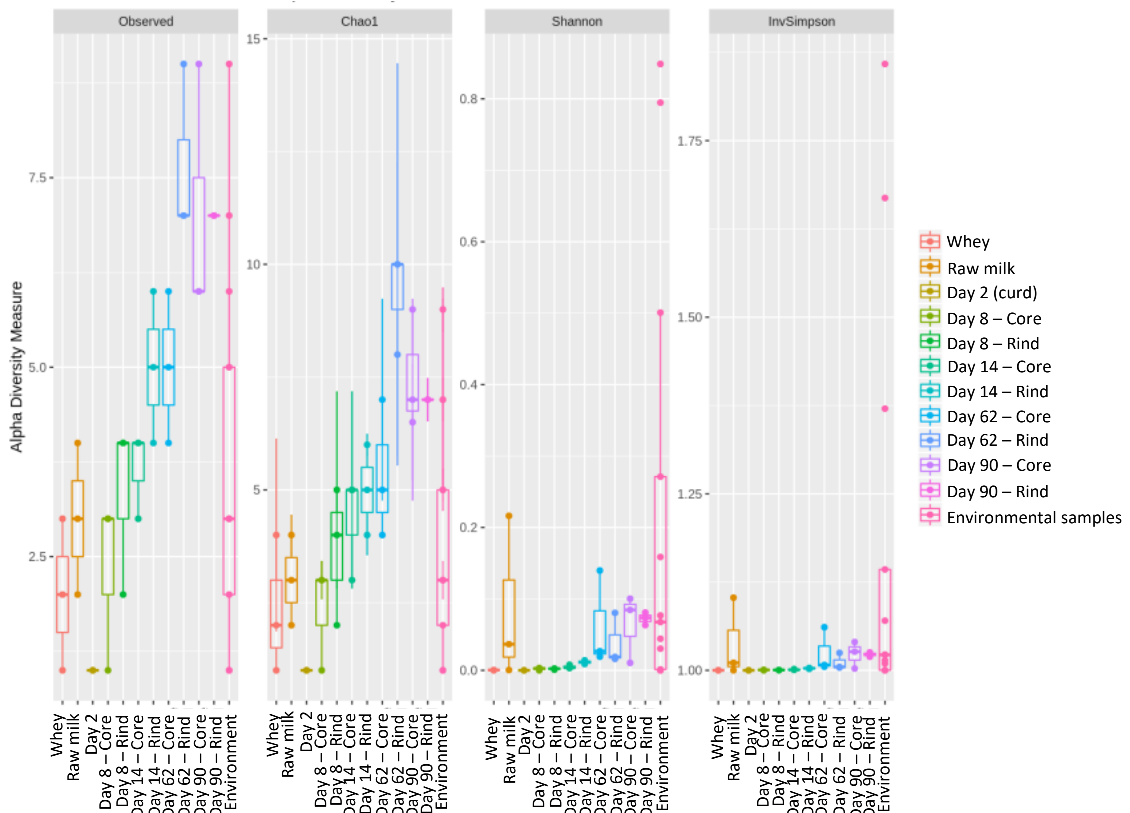

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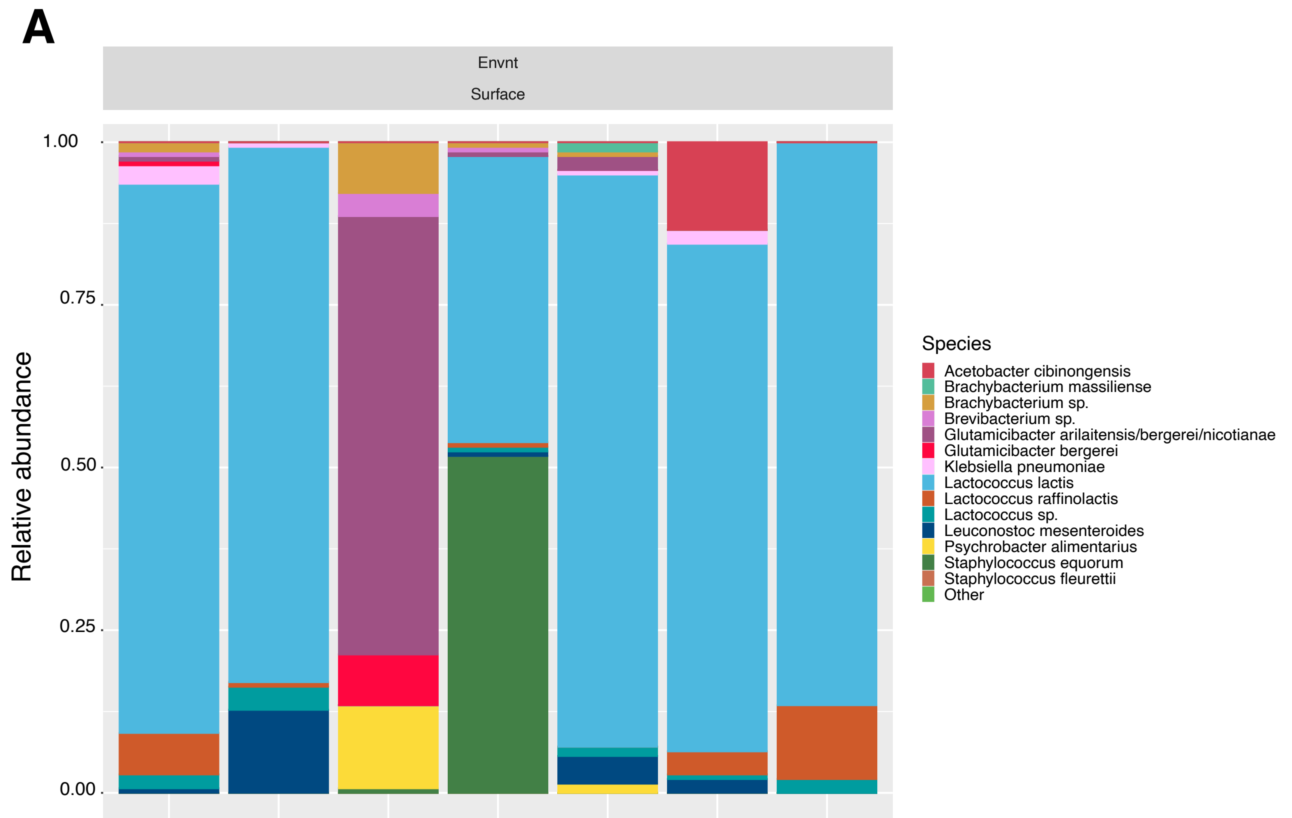

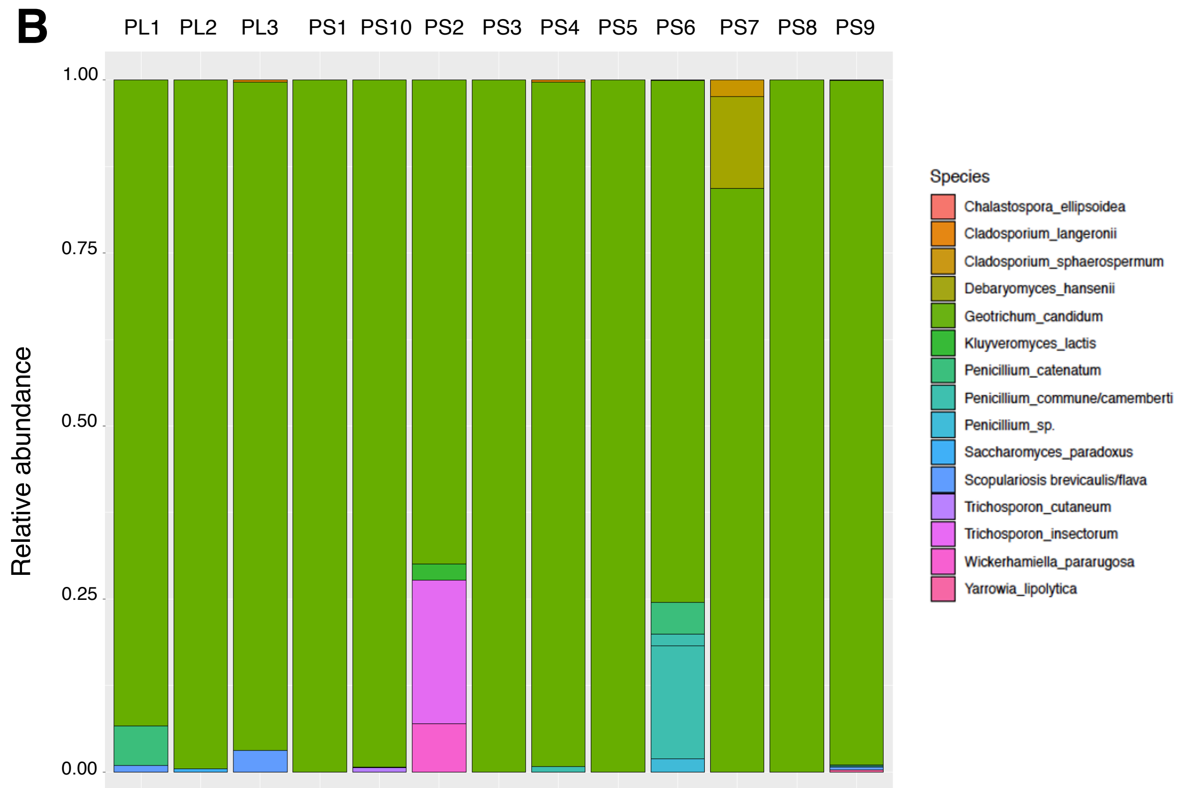

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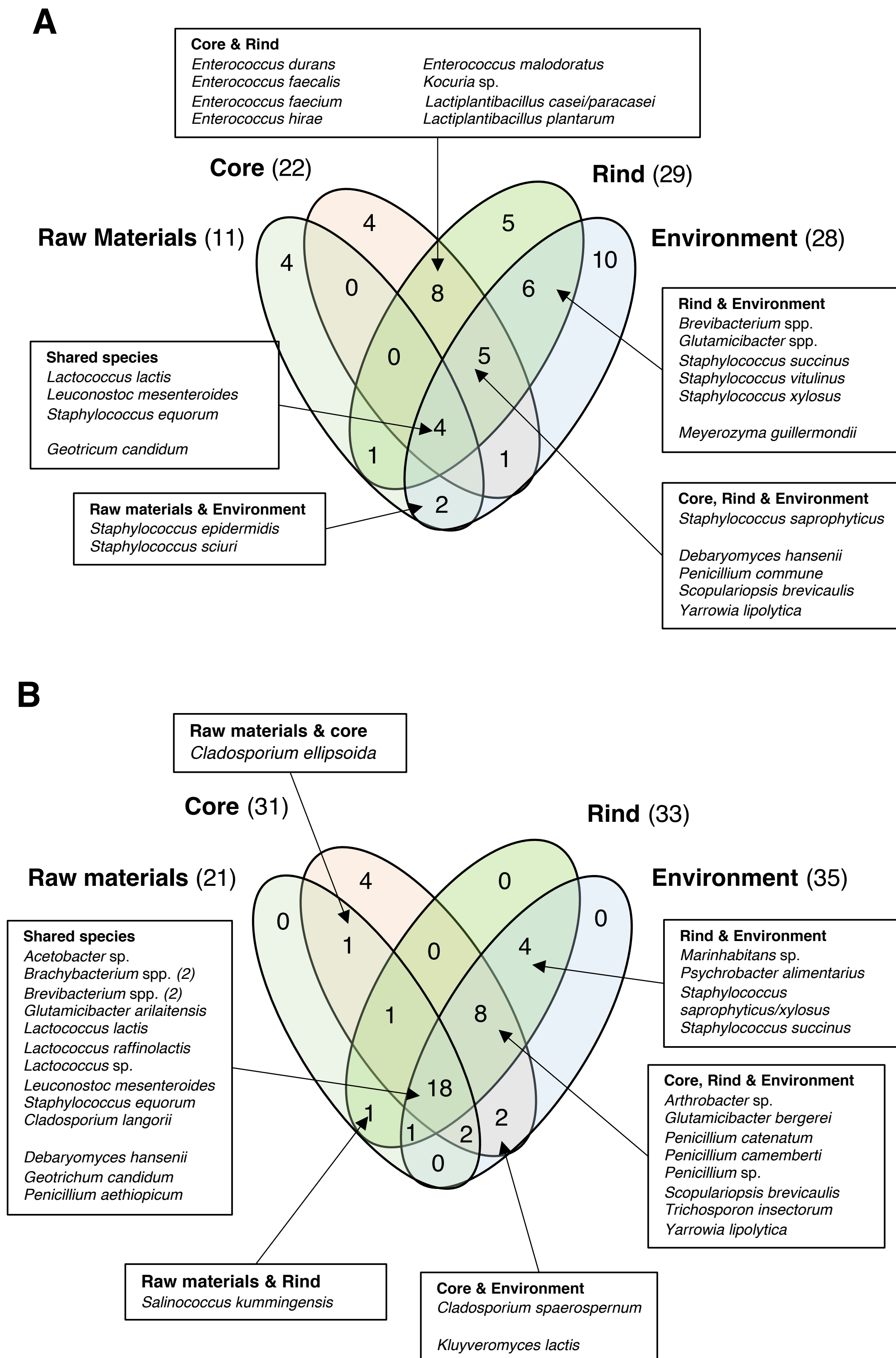

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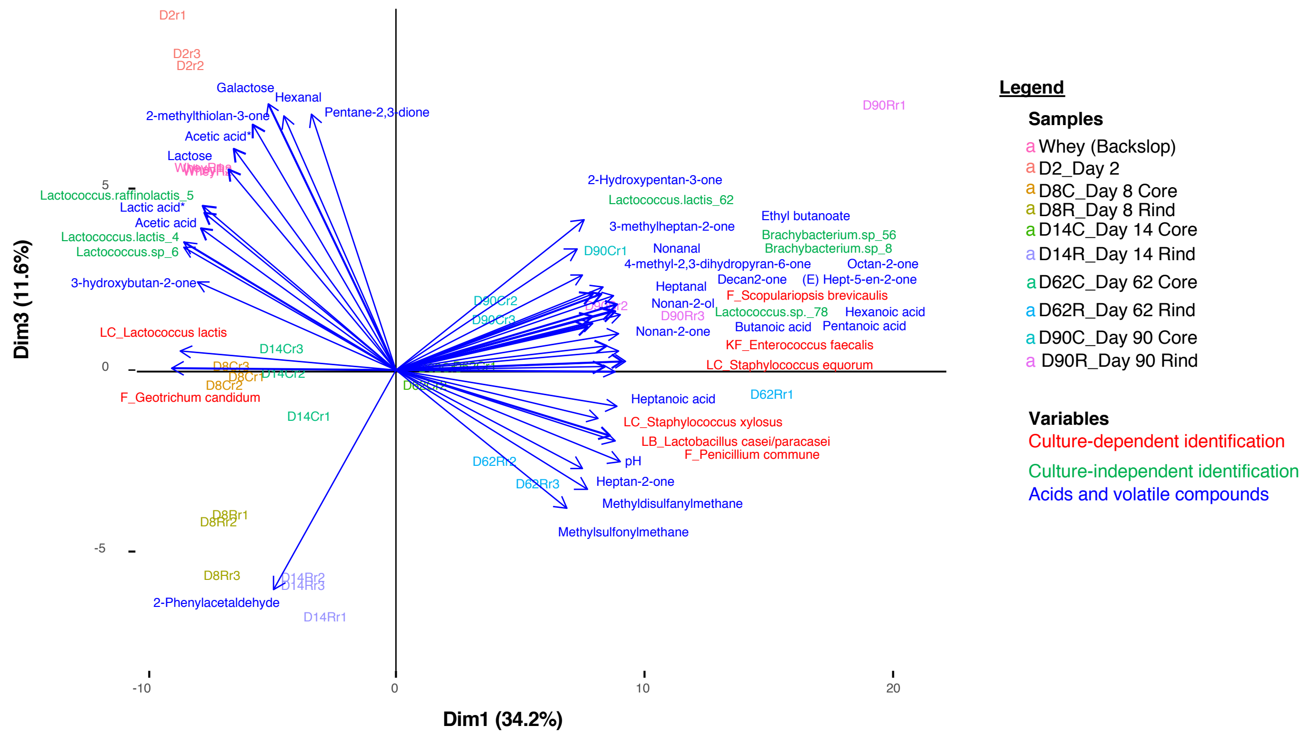

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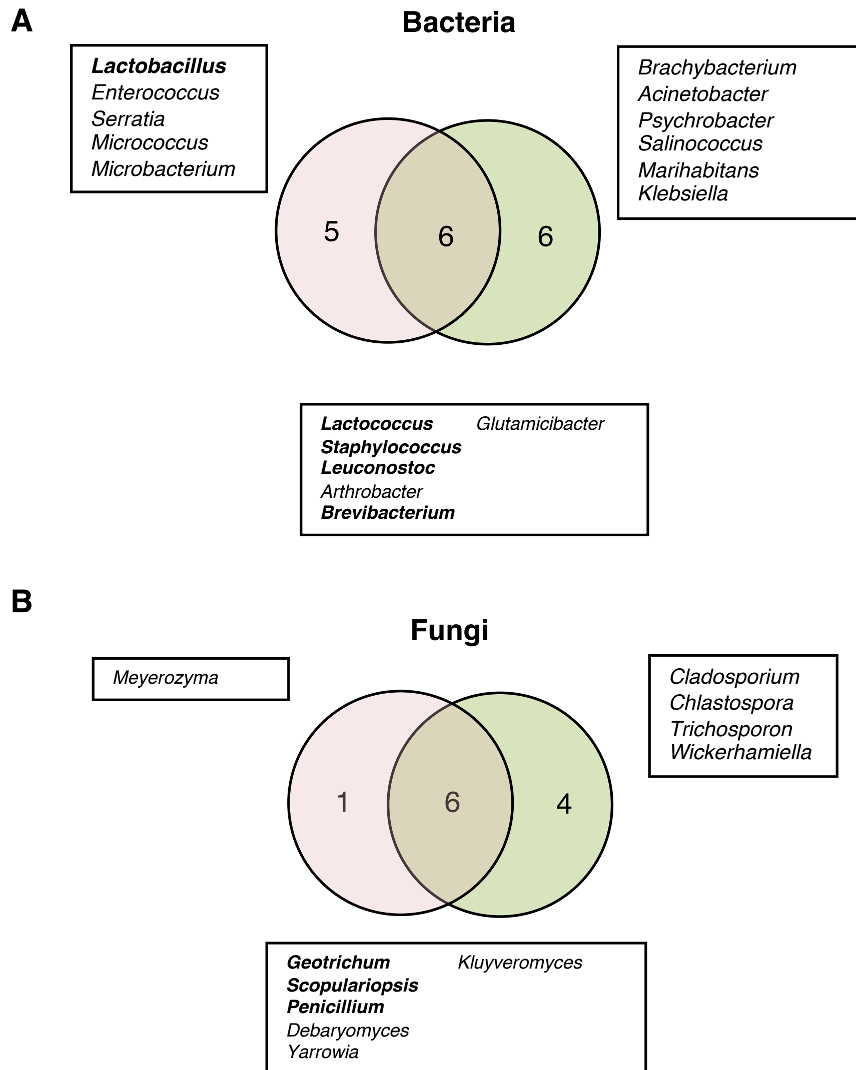

Supplementary Table S1. Primers used in this study

Primer	Sequence	Target	Reference
ITS4	5’-TCCTCCGCTTATTGATATGC-3’	ITS region	
ITS5	5’-GGAAGTAAAAGTCGTAACAAGG-3’		
Bt2a	5’-GGTAACCAAATCGGTGCTGCTTTC-3’	β-tubulin gene	Glass and Donaldson (1995)
Bt2b	5’-ACCCTCAGTGTAGTGACCCTTGGC-3’		
NL1	5’-GCATATCAATAAGCGGAGGAAAAG-3’	D1-D2 domain	Kurtzman and Robnett (1998)
NL4	5’-GGTCCGTGTTTCAAGACGG-3’		
fD1	5’-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3’	16S rRNA gene	Weisburg et al. (1991)
rP2	5’-CCCGGGATCCAAGCTTACGGCTACCTTGTTACGACTT-3’		
TuflparacF	5’-TCCGGGAACTGCTCAGC-3’	tuf gene	modified from Achilleos and Berthier (2013)
TuflparacR	5’-TGTTTCACGAACAGGTG-3’		
S-D-Bact-0341-b-S-17	5’-CCTACGGGNGGCWGCAG-3’	V3-V4 region of 16S rDNA gene	Klindworth et al. (2013)
S-D-Bact-0785-a-A-21	5’-GACTACHVGGGTATCTAATCC-3’		
ITS3f	5’-GCATCGATGAAGAACGCAGC-3’	ITS2 region	Toju et al. (2012)
ITS4_Kyo1	5’-TCCTCCGCTTWTTGWTWTGC-3’		

Supplementary Table S2. Volatile compounds identified and quantified (AU) during Pélardon cheese-making and ripening using HS-GSMS.

Compound	Pubchem #	LRI	EIC (m/z)	Identification[a]	Pvalue[b]	Abundance (AU)[b] Whey	Curd (day 2)	Post-drying (day 8)	Ripening -2weeks (day 14)	Ripening-2months (day 62)	Ripening-3months (day 90)	Odor descriptor[c]
Alcohols												
Ethanol	702	950	57	RI, DB	1.98E-01	6.8E+08 ± 4.8E+07^{a}	6.0E+07 ± 4.2E+06^{a}	3.2E+08 ± 2.2E+08^{a}	4.0E+08 ± 5.1E+08^{a}	5.1E+08 ± 2.9E+08^{a}	4.7E+08 ± 2.0E+08^{a}	Alcoholic
Heptan-2-ol	10976	1355	83	RI, DB	**2.06E-04**	5.1E+05 ± 4.9E+04^{c}	1.2E+05 ± 1.9E+04^{c}	1.0E+05 ± 4.0E+04^{c}	5.7E+07 ± 1.1E+07^{bc}	1.9E+08 ± 8.6E+07^{ba}	2.4E+08 ± 9.7E+07^{a}	Citrus, earthy, bitter
Nonan-2-ol	12367	1568	69	RI, DB	**5.83E-04**	4.1E+06 ± 1.4E+06^{bc}	1.2E+06 ± 4.3E+05^{c}	7.7E+06 ± 1.1E+07^{bc}	3.7E+07 ± 1.5E+07^{bc}	9.6E+08 ± 3.6E+08^{ba}	1.5E+09 ± 7.8E+08^{a}	Waxy, fruity, dairy, musty
Oct-1-en-3-ol	18827	1482	57	RI, DB	**2.44E-07**	2.5E+07 ± 1.3E+06^{c}	6.8E+06 ± 1.3E+06^{c}	1.2E+07 ± 3.1E+06^{c}	8.7E+07 ± 3.7E+07^{bc}	1.9E+09 ± 4.2E+08^{a}	5.9E+08 ± 2.2E+08^{b}	Funal, earthy
2-Phenylethanol	6054	NA	91	DB	**3.68E-05**	2.7E+07 ± 2.4E+06^{c}	4.5E+06 ± 3.0E+06^{c}	3.5E+08 ± 1.4E+08^{ab}	5.1E+08 ± 8.8E+07^{a}	1.4E+08 ± 8.9E+07^{bc}	1.8E+08 ± 6.4E+07^{bc}	Floral, sweet
Aldehydes												
3-Methylbut-2-enal	61020	1193	84	RI, DB	**1.60E-09**	2.9E+07 ± 7.7E+05^{a}	1.6E+07 ± 8.1E+05^{b}	1.3E+07 ± 2.2E+06^{c}	9.7E+06 ± 2.6E+05^{c}	1.3E+07 ± 1.3E+06^{c}	1.3E+07 ± 1.3E+05^{c}	Fruity, nutty, almond
3-Methylbutanal	11552	908	44	RI, DB, S	**6.30E-06**	5.2E+08 ± 6.1E+07^{b}	2.3E+08 ± 1.4E+07^{b}	8.0E+09 ± 2.6E+09^{a}	1.1E+09 ± 1.2E+08^{b}	6.8E+08 ± 2.0E+08^{b}	6.7E+08 ± 4.1E+07^{b}	Fruity, green
Hexanal	6184	1084	56	RI, DB	**8.26E-10**	1.6E+08 ± 2.5E+07^{a}	1.2E+08 ± 1.1E+07^{b}	3.3E+06 ± 5.4E+05^{c}	2.0E+06 ± 3.1E+05^{c}	4.0E+06 ± 5.9E+05^{c}	4.9E+06 ± 3.8E+06^{c}	Green, fresh
Heptanal	8130	1183	70	RI, DB, S	**1.09E-06**	4.2E+07 ± 2.7E+06^{c}	1.8E+07 ± 1.8E+06^{c}	9.9E+05 ± 4.6E+05^{c}	2.5E+07 ± 1.3E+07^{c}	1.9E+08 ±3.7E+06^{b}	2.8E+08 ± 8.2E+07^{a}	Green, fatty, fresh
Nonanal	31289	1390	70	RI, DB	**2.13E-03**	2.6E+07 ± 8.3E+05^{b}	3.8E+06 ± 2.5E+06^{b}	4.3E+06 ± 6.8E+04^{b}	1.3E+07 ± 9.7E+06^{b}	2.7E+08 ± 7.2E+07^{ba}	5.3E+08 ± 3.3E+08^{a}	Fatty, rose
2-Phenylacetaldehyde	998	1641	120	RI, DB, S	**7.43E-10**	1.9E+06 ± 6.2E+05^{c}	1.3E+06 ± 7.7E+04^{c}	5.7E+07± 8.4E+06^{a}	3.8E+06 ± 3.6E+06^{b}	3.5E+06 ± 1.5E+06^{c}	4.4E+05 ± 1.2E+05^{c}	Green, floral, honey
Fatty acids												
Acetic acid	176	1451	45	RI, DB, S	**1.62E-06**	2.2E+10 ± 3.3E+09^{a}	2.4E+10 ± 3.3E+09^{a}	1.1E+10 ± 3.4E+09^{b}	1.1E+10 ± 2.3E+09^{b}	3.7E+09 ± 3.3E+09^{bc}	4.1E+08 ± 7.0E+08^{c}	Acidic, sour
Butanoic acid	264	1630	73	RI, DB, S	**9.86E-07**	9.7E+06 ± 5.7E+06^{c}	5.3E+06 ± 3.9E+06^{c}	1.1E+07 ± 1.1E+07^{c}	2.7E+09± 4.6E+08^{bc}	3.6E+09 ± 8.5E+08^{b}	9.0E+09 ± 2.4E+09^{a}	Cheesy, sour, dairy
Propanoic acid	1032	1544	74	RI, DB, S	8.50E-01	3.9E+08 ± 2.4E+08^{a}	4.0E+08 ± 1.9E+08^{a}	5.5E+08 ± 4.2E+08^{a}	5.7E+08 ± 2.7E+08^{a}	3.7E+08 ± 2.6E+08^{a}	5.6E+08 ± 1.8E+08^{a}	Acidic, dairy, fruity
Pentanoic acid	7991	1747	73	DB, S	**9.04E-07**	8.2E+06 ± 1.5E+06^{b}	9.0E+06 ± 1.5E+06^{b}	8.2E+06 ± 1.5E+06^{b}	2.6E+07 ± 3.5E+06^{a}	5.8E+07 ± 1.1E+07^{a}	7.5E+07 ± 1.7E+07^{a}	Cheesy, acidic
Hexanoic acid	8892	NA	73	DB, S	**6.95E-09**	3.6E+08 ± 7.7E+07^{d}	5.5E+08 ± 1.1E+08^{d}	1.5E+08 ± 5.3E+07^{d}	2.0E+09 ± 3.0E+08^{c}	3.7E+09 ± 1.0E+09^{b}	6.2E+09 ± 2.2E+08^{a}	Fatty, cheesy, goaty
Heptanoic acid	8094	NA	87	DB, S	**1.46E-06**	5.8E+06 ± 9.8E+05^{c}	6.1E+06 ± 2.4E+06^{c}	5.7E+06 ± 1.6E+06^{c}	1.5E+07 ± 3.1E+05^{b}	2.5E+07 ± 5.1E+06^{a}	2.5E+07 ± 5.1E+06^{a}	Waxy, cheesy
Octanoic acid	379	NA	101	DB, S	**1.06E-02**	7.3E+07 ± 2.7E+07^{b}	1.3E+08 ± 3.4E+07^{ab}	5.4E+07 ± 2.7E+07^{b}	1.7E+08 ± 3.3E+07^{ab}	2.5E+08 ± 7.4E+07^{ab}	4.1E+08 ± 2.3E+08^{a}	Fatty, rancid, cheesy
Nonanoic acid	8158	NA	115	DB	**2.77E-02**	6.3E+06 ± 2.1E+06^{ab}	6.1E+06 ±2.7E+06^{ab}	5.4E+06 ± 2.0E+06^{b}	8.8E+06 ± 7.0E+05^{ab}	8.1E+06 ± 6.6E+05^{ab}	1.1E+07 ± 2.0E+06^{a}	Waxy, cheesy mild sweet
Decanoic acid	2969	NA	73	DB, S	6.22E-01	8.1E+07 ± 2.4E+07^{a}	8.8E+07± 3.2E+07^{a}	5.7E+07 ± 3.5E+07^{a}	8.1E+07 ± 1.8E+07^{a}	9.2E+07 ± 3.0E+07^{a}	1.0E+08 ± 4.1E+07^{a}	Rancid (-), sour, fruity
Branched-chain fatty acids												
3-Methylbutanoic acid	10430	1674	60	RI, DB, S	**1.20E-04**	1.1E+08 ± 1.3E+07^{b}	2.2E+07 ±5.9E+06^{b}	2.8E+09 ± 1.1E+09^{a}	2.0E+09 ± 6.9E+08^{b}	3.0E+08 ± 2.1E+08^{b}	3.2E+08 ± 2.1E+08^{b}	Cheesy, sour, ripe
2-Methylpropanoic acid	6590	1573	73	RI, DB, S	**2.62E-03**	9.4E+07 ± 2.9E+06^{bc}	9.7E+06 ± 2.2E+06^{c}	6.2E+08 ± 3.2E+08^{a}	4.3E+08 ± 1.5E+08^{ab}	1.3E+08 ± 5.2E+07^{bc}	1.4E+08 ± 1.1E+08^{bc}	Acidic, cheesy, sour
2-Ethylhexanoic acid	8697	NA	88	DB	**7.64E-03**	5.2E+06 ± 1.1E+06^{b}	4.7E+06 ± 2.2E+06^{b}	9.9E+06 ± 3.9E+06^{ab}	1.4E+07 ± 1.1E+07^{ab}	2.4E+07 ± 4.9E+06^{a}	1.8E+07 ± 4.8E+06^{ab}	-
Esters												
Ethyl acetate	8857	874	61	RI, DB, S	**1.14E-04**	4.1E+07 ± 6.9E+05^{b}	4.2E+07 ± 2.1E+06^{b}	1.9E+07 ± 9.5E+06^{b}	6.9E+07 ± 5.1E+06^{b}	2.5E+07 ± 1.5E+07^{b}	2.0E+08 ± 7.6E+07^{a}	Ethereal
Ethyl butanoate	7762	1032	71	RI, DB, S	**1.28E-04**	8.2E+07 ± 1.1E+07^{b}	5.0E+06 ± 5.5E+05^{b}	2.6E+07 ± 3.8E+06^{b}	1.1E+08 ± 1.6E+07^{b}	3.8E+08 ± 2.2E+08^{b}	2.6E+09 ± 1.2E+09^{a}	Fruity, fsweet, fresh
Ethyl hexanoate	31265	1233	88	RI, DB, S	**1.74E-02**	7.0E+06 ± 2.1E+06^{b}	1.3E+04 ± 1.5E+04^{b}	1.2E+08 ± 2.1E+07^{b}	5.9E+07 ± 7.9E+06^{b}	7.9E+07 ± 3.9E+07^{b}	7.7E+08 ± 6.0E+08^{a}	Fruity, etery, pineapple
Ethyl octanoate	7799	1436	88	RI, DB	1.37E-01	9.1E+05 ± 3.4E+05^{a}	2.6E+05 ± 9.1E+04^{a}	3.1E+06 ± 1.2E+06^{a}	8.3E+06 ± 4.7E+06^{a}	2.6E+07 ± 2.7E+07^{a}	4.1E+06 ± 3.5E+06^{a}	Waxy, musty, mushroom
Ethyl decanoate	8048	1639	101	RI, DB, S	6.44E-02	5.6E+06 ± 4.3E+05^{a}	1.6E+05 ± 1.2E+05^{a}	1.2E+06 ± 3.8E+05^{a}	3.7E+06 ± 3.7E+05^{a}	1.0E+07 ± 5.9E+06^{a}	8.8E+07 ± 8.6E+07^{a}	Waxy, fruity
Ketones												
Butan-2-one	6569	893	43	RI, DB, S	**1.10E-07**	2.2E+09 ± 7.7E+07^{a}	7.8E+08 ± 5.5E+07^{cd}	3.1E+08 ± 3.2E+07^{e}	3.2E+08 ± 5.5E+07de	9.9E+08 ± 1.7E+08^{bc}	1.4E+09 ± 3.7E+08^{b}	Fruity,green
Butane-2,3-dione	650	975	43	RI, DB, S	**2.68E-02**	7.7E+09 ± 1.6E+08^{ab}	1.2E+10 ± 2.8E+09^{a}	8.2E+09 ± 1.7E+09^{ab}	8.5E+09 ± 3.2E+09^{ab}	4.0E+09 ± 5.4E+08^{b}	7.4E+09 ± 2.7E+09^{ab}	Buttery, creamy
Pentan-2-one	7895	967	86	RI, DB	**2.40E-04**	3.4E+07 ± 6.3E+06^{b}	1.4E+08 ± 1.6E+08^{b}	1.7E+07 ± 3.7E+06^{b}	7.7E+09 ± 3.7E+09^{a}	3.8E+09 ± 7.3E+08^{ab}	8.7E+09 ± 3.1E+09^{a}	Fruity, fermented, sweet
Pentane-2,3-dione	11747	1066	57	RI, DB	**2.22E-15**	4.4E+07 ± 1.1E+06^{b}	1.7E+08 ± 1.3E+06^{a}	1.3E+07 ± 1.9E+06^{d}	1.4E+07 ± 2.5E+06^{cd}	2.0E+07 ± 3.1E+06^{cd}	2.2E+07 ± 6.6E+06^{c}	Buttery, toasted
Hexan-2-one	11583	1080	100	RI, DB, S	**3.29E-04**	3.8E+05 ± 1.8E+05^{b}	6.0E+05 ± 2.6E+05^{b}	1.9E+04 ± 1.4E+05^{b}	2.8E+07 ± 1.3E+07^{b}	5.4E+07 ± 6.2E+06^{a}	5.8E+07 ± 3.1E+07^{a}	Fruity, fungal, buttery
Heptan-2-one	8051	1181	43	RI, DB, S	**1.43E-07**	1.1E+08 ± 1.0E+07^{b}	6.6E+07 ± 1.2E+07^{b}	4.7E+07 ± 9.5E+06^{b}	8.7E+09 ± 2.8E+09^{a}	9.7E+09 ± 6.1E+08^{a}	9.8E+09 ± 1.2E+09^{a}	Cheesy, green, waxy
3-Methylheptan-2-one	92927	1195	72	RI, DB	**2.29E-05**	9.2E+05 ± 5.3E+04^{b}	4.4E+04 ± 3.9E+04^{b}	1.1E+05 ± 3.4E+04^{b}	2.0E+06 ± 7.2E+05^{b}	9.2E+07 ± 7.2E+06^{a}	2.8E+08 ± 1.1E+08^{a}	-
(E)-Hept-5-en-2-one	5363108	1219	112	RI, DB	**1.07E-04**	1.6E+05 ± 1.4E+05^{b}	1.0E+04 ± 7.3E+03^{b}	9.9E+03 ± 1.0E+04^{b}	9.1E+04 ± 6.9E+04^{b}	2.3E+06 ± 4.1E+05^{ba}	4.7E+06 ± 2.1E+06^{a}	-
Octan-2-one	8093	1280	58	RI, DB, S	**7.86E-05**	5.6E+06 ± 1.5E+06^{c}	4.1E+06 ± 3.7E+05^{c}	9.0E+06 ± 5.8E+05^{c}	5.0E+07 ± 2.1E+07^{bc}	4.6E+08 ± 1.2E+08^{ab}	8.6E+08 ± 3.7E+08^{a}	Musty, cheesy, parmesan
Octan-3-one	246728	1250	72	RI, DB	**3.96E-09**	3.4E+06 ± 4.1E+05^{c}	4.9E+04 ± 3.1E+04^{c}	7.0E+06 ± 9.9E+05^{c}	3.4E+07 ± 1.3E+07^{c}	4.6E+08 ± 7.8E+07^{a}	1.4E+08 ± 6.2E+06^{b}	Mushroom, cheesy, herbal
Nonan-2-one	13187	1387	43	RI, DB	**6.04E-06**	7.4E+07 ± 5.6E+06^{b}	3.9E+07 ± 1.6E+06^{b}	4.4E+07 ± 2.4E+07^{b}	2.2E+09 ± 1.3E+09^{b}	1.0E+10 ± 2.5E+09^{ab}	1.2E+10 ± 3.7E+09^{a}	Green, cheesy
Non-8-en-2-one	21108	1443	58	RI, DB	**3.66E-02**	1.3E+06 ± 4.2E+05^{a}	2.8E+05 ± 4.8E+04^{a}	4.4E+05 ± 2.6E+05^{a}	2.9E+07 ± 2.2E+07^{a}	3.3E+08 ± 2.9E+08^{a}	2.7E+07 ± 7.5E+06^{a}	Fruity, baked
Decan-2-one	12741	1492	58	RI, DB	**1.46E-02**	3.4E+06 ± 6.1E+05^{b}	6.8E+05 ± 1.5E+05^{b}	8.0E+05 ± 2.7E+05^{b}	6.5E+06 ± 3.7E+06^{b}	1.0E+08 ± 3.8E+07^{ab}	2.1E+08 ± 1.7E+08^{a}	Floral, fermented, fatty
Undecan-2-one	8163	1596	58	RI, DB	**3.07E-02**	8.4E+06 ± 9.0E+05^{ab}	3.4E+06 ± 7.5E+05^{b}	3.0E+06 ± 9.8E+05^{b}	3.2E+07 ± 2.8E+07^{ab}	7.1E+08 ± 3.8E+08^{ab}	2.1E+09 ± 1.9E+09^{a}	Fruity, floral, creamy, cheesy
3-Hydroxybutan-2-one	179	1282	43	RI, DB, S	**1.12E-09**	2.0E+10 ± 6.2E+08^{a}	1.7E+10 ± 5.7E+08^{a}	1.7E+10 ± 3.3E+09^{a}	6.1E+09 ± 4.5E+08^{b}	6.1E+08 ± 8.2E+07^{c}	9.6E+08 ± 2.8E+08^{c}	Buttery, creamy, milky
1-Hydroxypropan-2-one	8299	1301	74	RI, DB	**2.64E-02**	6.4E+06 ± 4.8E+05^{b}	4.2E+06 ± 4.8E+05^{b}	6.8E+06 ± 1.7E+06^{b}	2.1E+07 ± 1.7E+07^{ab}	4.3E+07 ± 3.6E+07^{ab}	2.8E+08 ± 2.3E+08^{a}	caramellic, sweet
2-Hydroxypentan-3-one	521790	1363	57	RI, DB	**3.94E-05**	7.7E+07 ± 4.3E+05	9.5E+07 ± 1.4E+06	1.1E+07 ± 3.7E+06	3.8E+07 ± 1.0E+07	1.2E+08 ± 4.0E+07	2.0E+08 ± 5.5E+07	Truffle, earthy, nutty
2-Methylthiolan-3-one	61664	1519	116	RI, DB	**4.63E-18**	7.4E+07 ± 1.0E+06^{a}	7.2E+07 ± 1.4E+06^{a}	1.0E+07 ± 6.0E+05^{b}	7.4E+06 ± 1.6E+06^{b}	8.6E+05 ± 1.2E+06^{c}	5.2E+05 ± 3.7E+05^{c}	Sulfurous, fruity
4-Methyl-2,3-dihydropyran-6-one	557445	NA	82	DB	**6.28E-07**	1.1E+06 ± 1.0E+06^{c}	2.7E+05 ± 1.4E+05^{c}	6.5E+05 ± 4.0E+05^{c}	2.3E+06 ± 3.0E+05^{c}	2.6E+07 ± 1.1E+07^{b}	5.0E+07 ± 9.0E+06^{a}	-
Others												
(Methyldisulfanyl)methane	12232	1067	94	RI, DB, S	**6.45E-12**	3.0E+07 ± 1.2E+06^{c}	8.5E+06 ± 1.7E+06^{c}	1.1E+08 ± 1.2E+07^{c}	8.1E+08 ± 5.3E+07^{b}	9.8E+08 ± 4.4E+07^{a}	8.9E+08 ± 9.7E+07^{b}	Sulfurous, malty
Methylsulfonylmethane	6213	NA	79	DB	**6.13E-05**	4.8E+07 ± 2.3E+07^{c}	2.3E+07 ± 1.3E+07^{c}	4.2E+08 ± 8.2E+07^{ab}	3.6E+08 ± 1.8E+08^{b}	4.5E+08 ± 8.6E+07^{ab}	6.7E+08 ± 1.6E+08^{a}	-
2-Pentylfuran	19602	1226	81	RI, DB	**2.04E-14**	4.9E+07 ± 3.0E+06^{a}	5.1E+06 ± 7.0E+05^{b}	2.3E+05± 4.4E+04^{c}	5.3E+05 ± 1.4E+05^{c}	5.0E+05 ± 1.0E+05^{c}	9.7E+05 ± 2.5E+05^{c}	Fruity, musty, green
1-(3,4-Dihydro-2H-pyrrol-5-yl)ethanone	522834	1329	111	RI, DB	2.96E-01	2.2E+05 ± 2.4E+05^{a}	6.4E+03 ± 5.6E+03^{a}	2.7E+03 ± 7.9E+02^{a}	2.5E+03 ± 2.4E+03^{a}	1.5E+05 ± 9.1E+04^{a}	1.8E+07 ± 2.6E+07^{a}	Popcorn
2,2,4,6,6-Pentamethylheptane	26058	950	99	RI, DB	3.75E-01	2.6E+07 ± 2.0E+06^{a}	1.4E+06 ± 6.5E+05^{a}	1.3E+07 ± 1.1E+07^{a}	1.8E+07 ± 2.8E+07^{a}	2.0E+07 ± 1.1E+07^{a}	1.5E+07 ± 5.1E+06^{a}	-
1,3-Xylene	7929	1125	91	RI, DB, S	**1.77E-06**	2.7E+08 ± 7.5E+07^{a}	2.1E+07 ± 1.0E+07^{b}	8.6E+05 ± 2.8E+05^{b}	6.0E+06 ± 3.7E+06^{b}	1.9E+06 ± 4.0E+05^{b}	2.7E+07 ± 2.3E+07^{b}	Sweet
1,4-Xylene	7809	1140	106	RI, DB, S	**6.01E-08**	6.2E+07 ± 0.3E+07^{a}	5.5E+06 ± 4.5E+06^{c}	1.2E+07 ± 3.0E+06^{c}	1.3E+07 ± 1.7E+06^{bc}	1.1E+07 ± 1.3E+06^{c}	2.5E+07 ± 9.7E+06^{b}	Sweet

a Peak identification method – RI comparison of retention index with literature data ; DB, tentatively identified by spectrum comparison using NIST 2008 workbook database; S comparison of spectra and retention time with those of a commercial standard analysed under the same conditions
b Anova p-value (bold value indicate p<0.05 and in red p<0.001 – different letters in the same row indicate groups with significant difference using Tukey's LSD test (p<0.05)
c odor descriptor associated with the compounds according to Thegoodscentscompany database (http://www.thegoodscentscompany.com)
NA=Not Available AU= Arbitrary units

Manuscript : Linking Pélardon artisanal goat cheese microbial communities to aroma compounds during cheese-making and ripening

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: