



HAL
open science

Temporal differences in microbial composition of Époisses cheese rinds during ripening and storage

F. Irlinger, Christophe Monnet

► To cite this version:

F. Irlinger, Christophe Monnet. Temporal differences in microbial composition of Époisses cheese rinds during ripening and storage. *Journal of Dairy Science*, American Dairy Science Association, 2021, 10.3168/jds.2021-20123 . hal-03192662v2

HAL Id: hal-03192662

<https://hal.inrae.fr/hal-03192662v2>

Submitted on 10 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 *This is the accepted version of the manuscript published in Journal of Dairy Science*
2 *(<https://doi.org/10.3168/jds.2021-20123>)*

3 *Supplemental material: <https://doi.org/10.15454/LFA0VN>*

4 **EPOISSES CHEESE RINDS**

5
6
7 **Temporal differences in microbial composition of Epoisses cheese**
8 **rinds during ripening and storage**

9
10 F. Irlinger, and C. Monnet*

11
12 Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 78850 Thiverval-Grignon,
13 France

14
15 *Corresponding author: Christophe Monnet, UMR SayFood, 1 avenue Lucien Brétignières,
16 78850 Thiverval-Grignon, France (christophe.monnet@inrae.fr; Phone: + 33 (0) 1 30 81 54
17 83)

18
19 Françoise Irlinger: ORCID ID 0000-0002-1635-9566

20 Christophe Monnet: ORCID ID 0000-0003-2647-272X

21
22 Interpretive summary:

23 Epoisses is a French Protected Designation of Origin smear-ripened cheese with an orange
24 color and a strong flavor, both generated by surface microorganisms. We evaluated the
25 microbial dynamics at the surface of Epoisses cheeses during ripening and post-
26 manufacturing storage at low temperatures. Most of the bacterial species detected by
27 amplicon-sequencing were Gram-negative species that were not deliberately inoculated
28 during cheesemaking. These microorganisms may be of interest for the development of more
29 efficient ripening cultures.

ABSTRACT

30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

Epoisses is a Protected Designation of Origin (PDO smear-ripened cheese from the Burgundy region in France. It has an orange color and a strong flavor, both of which are generated by surface microorganisms. The objective of the present study was to investigate the microbial dynamics at the surface of Epoisses cheese during ripening and post-manufacturing storage at low temperatures. Rind samples were analyzed by enumeration on agar plates and by 16S rRNA gene and Internal Transcribed Spacer amplicon sequencing. During most of the ripening process, the counts of yeasts, which corresponded to the two species *Debaryomyces hansenii* and *Geotrichum candidum*, were higher than those of the aerobic acid-sensitive bacteria. *Debaryomyces hansenii* reached a level of about 3×10^8 cfu/cm², and its viability strongly decreased at the late stage of ripening and during storage at +4°C. Two of the inoculated bacterial species, *Brevibacterium aurantiacum* and *Staphylococcus xylosus*, did not establish themselves at the cheese surface. At the end of ripening, among the 18 most abundant bacterial species detected by amplicon-sequencing, 14 were Gram-negative, mainly from genera *Psychrobacter*, *Vibrio*, *Halomonas* and *Mesonina*. It was hypothesized that the high moisture level of the Epoisses rinds, due the humid atmosphere of the ripening rooms and to the frequent washings of the curds, favored growth of these Gram-negative species. These species may be of interest for the development of efficient ripening cultures. In addition, since the orange color of Epoisses cheeses could not be attributed to the growth of *Brevibacterium*, it would be interesting to investigate the type and origin of the pigments that confer color to this cheese.

Key words: smear-ripened cheese, ripening, Epoisses, *Debaryomyces hansenii*, *Mesonina ostreae*

INTRODUCTION

57

58

59 Smear-ripened cheeses are covered by a layer of yeasts and bacteria that have a strong impact
60 on appearance and flavor (Bockelmann and John, 2011). The yeasts dominate during the early
61 stages of ripening because they are salt- and acid-tolerant, and subsequently increase the pH,
62 thereby favoring the growth of aerobic acid-sensitive bacteria. Ripening takes place at high
63 relative humidity (>95%) and the cheeses are washed several times with a dilute saline
64 solution to spread any microcolonies of yeasts or bacteria that have developed on the surface.
65 The beneficial yeasts and bacteria have to be competitive in order to prevent the growth of
66 undesired microorganisms, and most smear-ripened cheese are inoculated with defined
67 ripening cultures today (Bockelmann et al., 2005).

68

69 As for other food products, microbial analyses of smear-ripened cheeses traditionally relied
70 on culture-based techniques, but the advent of culture-independent techniques based on the
71 analysis of nucleic acids extracted from the food matrix was very useful to better characterize
72 the structure of the microbial communities (De Filippis et al., 2018; Jonnala et al., 2018).
73 However, only limited information is available about the microbial dynamics that occur
74 during the manufacturing of smear-ripened cheeses. Such information may help to better
75 understand the ecological relationships between cheese microorganisms and their adaptation
76 to the cheese habitat. For example, population dynamic studies of Gubbeen cheeses revealed a
77 progression of bacteria, with staphylococci dominating the early stages of ripening and
78 *Actinobacteria* the later stages, and the commercial strains used for smearing the cheese were
79 only present at a very low level early in ripening (Rea et al., 2007). In Tilsit cheeses,
80 biodiversity increased during ripening and *Actinobacteria* were the most prominent group in
81 the late phase.

82

83 The objective of the present study was to evaluate the microbial dynamics at the surface of
84 Epoisses, a French Protected Designation of Origin (PDO) cheese (LEGIFRANCE, 2010),
85 whose production level is approximately 1300 tons per year (CNIEL, 2020). This cheese is
86 made from whole cow's milk produced by specified dairy breeds in the Burgundy region.
87 Epoisses cheese can be manufactured from raw or from pasteurized milk and undergoes a
88 dominant lactic type coagulation, whose duration is greater than 16 hours, and draining occurs
89 spontaneously, without pressing. Salting is done using dry salt, and ripening time is at least 28

90 days, at a temperature of ~10-13°C. During ripening, the cheeses are washed one to three
91 times per week with washing solutions supplied with increasing amounts of Marc de
92 Bourgogne, a pomace brandy. Epoisses cheese has an orange color and a strong flavor, both
93 generated by surface microorganisms. A 16S rRNA amplicon-sequencing analysis revealed
94 that Epoisses rinds contain high levels of species adapted to cold aquatic and saline
95 environments (Dugat-Bony et al., 2016). In order to better understand the microbial activity
96 involved in the generation of the typical properties of Epoisses, as well as to provide
97 information about ripening cultures that are suitable for this cheese variety, it was interesting
98 to monitor the microbial composition during a typical manufacturing run and post-
99 manufacturing storage at low temperatures.

100

101

MATERIALS AND METHODS

102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133

Cheese manufacturing and sampling

All the cheeses were taken from the same batch of cheese production of an industrial manufacturing unit in 2017. The factory produced the cheeses from pasteurized milk and in compliance with the Epoisses PDO specifications. Several commercial microbial cultures were inoculated during cheesemaking. At the beginning of the manufacturing process, milk was inoculated with a mesophilic lactic starter culture, with the yeasts *Debaryomyces hansenii* and *Geotrichum candidum* and with the aerobic ripening bacteria *Brevibacterium aurantiacum*, *Staphylococcus xylosus*, and *Glutamicibacter arilaitensis*. *Brevibacterium aurantiacum* was also added to the smearing solutions used after day 6. After ripening (12°C), the cheeses were packaged and transferred to a cold room at +4°C. Three different cheeses were analyzed at eight sampling times (day 6, day 9, day 21, day 24, day 28, day 33, day 40 and day 90). The cheeses were cut perpendicular to the surface in order to produce two equivalent parts. One part was used to measure the concentrations of lactose, lactate, free amino acids and non-protein fractions. The upper and lower sections (rinds) of the other part were removed with a knife (thickness ~2-3 mm), pooled, and used for cell counts, pH measurements and DNA extraction.

Microbiological analyses

One gram of cheese rind, sampled as described above, was mixed with 9 ml of physiological saline solution (9 g/L NaCl). After dispersion with a mechanical blender (Ultra-Turrax® model T25; Ika Labortechnik, Staufen, Germany) for 1 min at 14,000 rpm, 10-fold serial dilutions were performed in physiological saline solution and plated in triplicate on agar plates. After four days of incubation at 25°C, the aerobic ripening bacteria were counted on brain heart infusion agar (Biokar Diagnostics, Beauvais, France) supplemented with 50 mg/l amphotericin (Sigma Aldrich, Saint Louis, MO, USA), which inhibits the growth of fungi. The lactic acid bacteria were counted on MRS agar (Biokar diagnostics) supplemented with 50 mg/l amphotericin after three days of incubation at 30°C under anaerobic conditions. The yeasts were counted on yeast extract-glucose-chloramphenicol agar (Biokar Diagnostics) after

134 three days of incubation at 25 °C. *Geotrichum candidum* and *D. hansenii* could be selectively
135 counted on this medium because they have distinct colony morphotypes.

136

137 **Biochemical analyses**

138

139 Biochemical analyses were performed for each of the three cheeses sampled at the eight
140 sampling times. Non-protein nitrogen content of the cheeses was measured by the Kjeldahl
141 method according to the NF EN ISO 8968 standard (Milk and milk products - Determination
142 of nitrogen content). The levels of lactate and lactose were assayed using commercially
143 available kits (Biosentec, Auzeville Tolosane, France) according to the manufacturer's
144 instructions. Free amino acids were analyzed by HPLC, as previously described (Castellote et
145 al., 2015). The pH was measured on the homogenized cheese rinds.

146

147 **DNA extraction, 16S rRNA and ITS amplicon sequencing, and data analysis**

148

149 DNA was extracted from three different cheeses at each of the eight sampling times, using the
150 bead beating-based protocol detailed in a previous study (Dugat-Bony et al., 2015). Briefly,
151 the cheese rind samples (~ 1 g) were diluted in 9 ml sodium citrate solution (20 g/l trisodium
152 citrate dihydrate) and homogenized with an Ultra Turrax® model T25 mechanical blender at
153 24,000 rpm for 2 min. A second treatment was performed after 10 min of incubation at room
154 temperature. The mixture was then centrifuged at 6,400 x g for 10 min at 4°C, and the
155 supernatant was removed. The casein pellet (containing the microbial cells) was resuspended
156 in 5 ml of a Triton X-100 aqueous solution (2.5% v/v), vigorously shaken, heated in a water
157 bath at 70°C for 10 min, centrifuged at 6,400 x g for 10 min at 4°C, and rinsed twice with 10
158 ml of a physiological saline solution. The pellet was dissolved in a mixture of 270 µL of
159 guanidium thiocyanate (4M) in Tris-HCl (pH 7.5, 0.1 M) and 30 µL of sodium lauroyl
160 sarcosinate (100 g/l), and transferred to a 2-mL tube containing 250 mg of 0.1-mm-diameter
161 zirconium beads and 250 mg of 0.5-mm-diameter zirconium beads (Biospec Products,
162 Bartlesville, OK, USA). Proteinase K treatment, bead-beating, phenol-chloroform extraction,
163 RNase treatment and ethanol precipitation were then performed as previously described
164 (Leclercq-Perlat et al., 2013), and the DNA pellet was dissolved in 120 µl of Tris EDTA
165 buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Purified DNA was quantified with Qubit
166 DNA assay kits on the Qubit 3.0 fluorometer (ThermoFischer Scientific, Villebon-sur-Yvette,
167 France). PCR amplification of the bacterial V3-V4 region (16S rRNA gene) and of the fungal

168 ribosomal Internal Transcribed Spacer 2 (ITS2) region was done with the primer pairs
169 F343/R784 and ITS3/ITS4_HYO1, as previously described (Dugat-Bony et al., 2016). The
170 amplified products were used for Illumina paired-end library preparation and cluster
171 generation, followed by 250 bp paired-end sequencing on an Illumina MiSeq instrument
172 (INRAE, GeT-PLaGE platform, Toulouse, France). Paired-end reads (~2 x 25,000 sequences)
173 were merged using Flash (Magoč and Salzberg, 2011), and the sequence data were processed
174 using the FROGS pipeline (Escudié et al., 2018), according to the standard operating
175 procedure. Briefly, operational taxonomic units (OTUs) were built using Swarm with a
176 maximum number of differences allowed to group together two amplicons (aggregation
177 distance parameter) of 3 (Mahé et al., 2014), and OTUs that accounted for <0.05% of the total
178 set of sequences were discarded. Lastly, the OTU affiliations were checked using the
179 EzBiocloud database (Kim et al., 2012) for the bacteria and the UNITE database (Nilsson et
180 al., 2019) for the fungi. Alpha-diversity metrics were determined using the Phyloseq package
181 (McMurdie and Holmes, 2013) implemented on the FROGS pipeline. Bacterial OTU-sample
182 networks were computed using Gephi software (v. 0.9.1) (Bastian et al., 2009). For a better
183 visualization, only edges corresponding to the presence of OTUs with a relative abundance >
184 0.5% in the samples were represented on the graph. Raw sequence data were deposited at the
185 Sequence Read Archive of the National Center for Biotechnology Information under the
186 accession numbers SAMN17082512 to SAMN17082535 (bioproject PRJNA685310).

187

188

RESULTS

189

190

191 In order to investigate the microbial dynamics at the surface of Epoisses cheeses, samples
192 corresponding to the same manufacturing run of a cheese factory were taken at various
193 ripening stages and during storage at +4°C. A white covering became visible at the surface of
194 the cheeses sampled at day 21, and a yellow/orange color, typical of this cheese variety,
195 appeared afterwards, its intensity increasing during storage at +4°C (Figure 1A). The pH of
196 the cheese surface increased from about 4.5 at day 6 to 6.5 at day 33, and continued to
197 increase after packaging and storage at +4°C (Figure 1B). Lactose and lactate were consumed
198 by the cheese microorganisms and were nearly exhausted in the cheeses at the end of ripening
199 (Figure 2). During ripening, the non-protein nitrogen fraction of the cheeses increased, and it
200 continued to increase during storage at +4°C. The production of non-protein nitrogen results
201 from the degradation of proteins and amino acids.

202

203 **Fungal growth at the cheese surface**

204

205 At the cheese surface, the yeast *D. hansenii* reached about 3×10^8 cfu/cm² at days 21 and 28,
206 and cell numbers strongly decreased thereafter (Figure 3). *Geotrichum candidum* was not
207 detected at days 6 and 9, but this may be due to the fact that it is not possible to distinguish *G.*
208 *candidum* colonies on the agar plates when this species represents less than about 1% of *D.*
209 *hansenii* because of the large colonies formed by the latter that rapidly cover the entire surface
210 of the agar plates. In contrast to *D. hansenii*, the level of *G. candidum* was nearly constant
211 from day 21 to day 90. The cheese rind contained lactic acid bacteria, whose level slightly
212 decreased during ripening. The level of aerobic bacteria was about 3×10^4 cfu/cm² at day 21
213 and reached 2×10^8 cfu/cm² at day 33. This growth is probably the consequence of the increase
214 in the cheese pH due to the activity of the yeasts, which favors the growth of acid-sensitive
215 surface bacteria. Despite the use of orange-pigmented *B. aurantiacum* strains as ripening
216 agents, orange colonies were not or almost never observed on the agar plates from cheeses
217 sampled from day 6 to day 40 (<1% of the colonies enumerated on brain heart infusion agar),
218 and represented only about 2% of the colonies at day 90.

219

220 ITS amplicon-sequencing analyses revealed a lower level of *G. candidum* sequences in
221 comparison to *D. hansenii* at the beginning of ripening (Figure 4A). From day 21 to day 90,

222 the changes in the proportion of the ITS sequences of the two yeasts was different than what
223 was observed by counting on agar plates (Figure 5). Indeed, after day 21, there was only a
224 slight increase in the proportion of the *G. candidum* ITS sequences, whereas a large increase
225 was observed for the proportion of *G. candidum* living cells. This may be explained by the
226 large decrease of *D. hansenii* viability that was observed after day 24. Except for *G. candidum*
227 and *D. hansenii*, no other fungi were detected in the cheeses (cutoff was set at 0.05% relative
228 abundance of the ITS sequences).

229

230 **Bacterial growth at the cheese surface**

231

232 The 16S rRNA amplicon-sequencing analyses showed the presence of two sequence clusters
233 of lactic acid bacteria (Figure 4B and Supplemental Table S1). The first corresponds to *L.*
234 *lactis* and the second (referred to as *Lactococcus* group *chungangensis*) to *L. chungangensis*
235 and *L. laudensis*. The proportion of the lactic acid bacteria 16S rRNA sequences considerably
236 decreased during ripening, especially after day 24, as the consequence of the growth of the
237 aerobic acid-sensitive bacteria. At the late stage of ripening, there was a large increase of 16S
238 rRNA sequences corresponding to *G. arilaitensis*, *Psychrobacter aquimaris*, *Psychrobacter*
239 *group nivimaris*, *Psychrobacter cibarius*, *Vibrio litoralis*, *Halomonas* group *venusta* and
240 *Mesonia ostreae*. Among these bacteria, only *G. arilaitensis* was deliberately inoculated as a
241 ripening agent. The other inoculated ripening bacteria, *B. aurantiacum* and *S. xylosus*, did not
242 establish themselves at the surface of the cheese. The 16S rRNA sequences of *S. xylosus* were
243 not detected (0.05% cutoff) and those of *B. aurantiacum* represented less than 0.5% of the
244 average relative abundance, except in one cheese sample at day 6 (1.0%) and in one cheese
245 sample at day 90 (2.2%). At day 24, *P. aquimaris* was the most abundant non-inoculated
246 species that grew at the surface of the cheeses and represented between 7 and 13% of the 16S
247 rRNA sequences. 16S rRNA sequences from this species were also abundant in one of the
248 cheeses at day 6 and one at day 21, showing that there were some differences in cheeses
249 sampled at the same ripening time. At the end of ripening at day 33, lactic acid bacteria
250 represented less than 10% of the 16S rRNA sequences, and, in addition to *P. aquimaris*, the
251 most abundant OTUs were *G. arilaitensis*, *Psychrobacter* group *nivimaris*, *V. litoralis*,
252 *Halomonas* group *venusta* and *M. ostreae*. The bacterial composition changed during storage
253 at 4°C. The proportion of *G. arilaitensis* increased, whereas there was a decrease for *P.*
254 *aquimaris*, *Psychrobacter* group *nivimaris*, *V. litoralis* and *M. ostreae*. Eight minor bacterial
255 groups ("Other" category in Figure 4B) representing an average relative abundance of

256 between 0.05 and 0.3% were detected by 16S rRNA amplicon-sequencing: *Halomonas*
257 *titanicae*, *Halomonas glaciei*, *Psychrobacter proteolyticus*, *Psychrobacter namhaensis*,
258 *Pseudoalteromonas* group *issachenkonii*, *Marinomonas polaris*, *Cobetia* group *marina* and
259 *Sphingomonas* group *insulae* (Supplemental Table S1). Shannon and Inverse Simpson alpha-
260 diversity indices showed that the highest bacterial diversity was at days 28 and 33 and
261 decreased during storage at +4°C (Supplemental Figure S1).

262

263 Since a previous study concerning the bacterial composition of Epoisses cheese rinds from
264 three different factories in 2014 was available (Dugat-Bony et al., 2016), we recovered the
265 corresponding 16S rRNA sequencing data (accession number SRP071345 of the Sequence
266 Read Archive of the National Center for Biotechnology Information) and processed them
267 using the bioinformatic pipeline described in the Materials and Methods section. The same
268 protocol for DNA extraction and amplicon sequencing was used in the two studies. An OTU-
269 sample network was built in order to visualize differences between the samples (Figure 6).
270 The cheese samples that were investigated in the present study were produced by Factory 1.
271 The absence of *Brevibacterium* and the dominance of Gram-negative species such as
272 *Psychrobacter* are observed in all of the samples. In comparison to the cheeses produced by
273 the Factory 1 in 2014, the cheeses produced in 2017 (present study) contained lower levels of
274 *P. cibarius* and *Pseudoalteromonas* group *issachenkonii*, but higher levels of *G. arilaitensis*,
275 *V. litoralis*, *M. ostreae* and *Psychrobacter* group *nivimaris*.

276

277

DISCUSSION

278

279

280 One key feature of Epoisses cheese is the type of coagulation, which is mostly of the lactic
281 type. Coagulation takes place for 16 to 24 hours, and the pH at the beginning of molding is
282 about 4.5. Mesophilic starter cultures are used for the manufacturing of this cheese variety,
283 which explains the dominance of lactococci at the beginning of ripening. Interestingly, in
284 addition to *L. lactis*, there was a high level of *L. chungangensis* or *L. laudensis*. Little is
285 known about the presence and activity of these species in cheeses, but there are at least three
286 publications in which their occurrence in cheeses has been reported (Masoud et al., 2012;
287 Fréтин et al., 2018; Park et al., 2019). It would be interesting to investigate their potential
288 functional properties relevant for cheese manufacturing.

289

290 *Debaryomyces hansenii* was the predominant yeast at the beginning of ripening. This can be
291 explained by its deliberate inoculation at the beginning of manufacturing, its acid- and salt-
292 tolerance, and its ability to grow at 5-10°C. In addition, lactose and lactate, which constitute
293 two important energy compounds for this species (Fröhlich-Wyder et al., 2019), were present
294 at the beginning of ripening. *Debaryomyces hansenii* reached about 3×10^8 cfu/cm² in the
295 Epoisses cheeses, which represents a high level, considering that in smear-ripened cheeses,
296 yeasts typically reach around 10^6 - 10^8 cfu/cm² (Cogan et al., 2014; Fröhlich-Wyder et al.,
297 2019). Smear-ripened cheeses are also referred to as "bacterial smear surface-ripened cheeses"
298 because bacteria are considered to be the dominant microorganisms in these cheeses typically
299 representing 10-100 times higher colony counts than yeasts (Corsetti et al., 2001; Cogan et al.,
300 2014). This trend was not observed for the Epoisses cheeses investigated in the present study
301 since the maximum count of surface bacteria was nearly the same as for the yeasts. One
302 possible explanation could be the low pH of the Epoisses curd at the beginning of ripening,
303 which delays the growth of the acid-sensitive aerobic bacteria. At least until day 21, the
304 increase in pH (the pH value was 5.7 at day 21) can be attributed to the activity of the yeasts
305 since the level of aerobic bacteria was very low. The yeasts contribute to the increase in the
306 pH of the cheese surface by transforming lactate to CO₂ and also by producing ammonia from
307 amino acids. Both colony counts and ITS amplicon-sequencing analyses revealed that growth
308 of *G. candidum* occurred later than that of *D. hansenii*. This is probably the consequence of
309 the high salt content of the cheese surface at the beginning of ripening due to the salting
310 procedure with dry salt. This favors the growth of *D. hansenii* since it is more tolerant to salt

311 than *G. candidum* (Boutrou and Gueguen, 2005). Interestingly, the fungal diversity in the
312 Epoisses cheeses was very low since no species other than the two inoculated fungi *D.*
313 *hansenii* and *G. candidum* could be detected. This is consistent with a previous study in which
314 Epoisses cheeses from three different producers were analyzed by amplicon high-throughput
315 sequencing (Dugat-Bony et al., 2016). Consequently, there is no evidence that adventitious
316 fungal species are common in Epoisses cheeses. Several factors may be involved in the large
317 decrease of *D. hansenii* viability that occurred during the last stage of ripening and during
318 storage at low temperatures. One of these is lactose or lactate exhaustion, which has been
319 proposed to explain *D. hansenii* lysis in experimental cheeses (Leclercq-Perlat et al., 1999).
320 Other possible factors are the lower oxygen supply after cheese packaging or the cheese
321 washing solution, which is supplemented with increasing amounts of Marc de Bourgogne
322 during the ripening process. It is possible that the decrease of *D. hansenii* viability is a key
323 feature of Epoisses cheese manufacturing since it may also correspond to proteolytic and
324 lipolytic enzymes liberated in the cheese curd by cell lysis (Kumura et al., 2002).

325

326 The present study also highlights the absence or poor growth of some ripening culture
327 components at the cheese surface, as already observed in other studies (Brennan et al., 2002;
328 Feurer et al., 2004; Mounier et al., 2005, 2006; Goerges et al., 2008; Cogan et al., 2014). The
329 inoculated *B. aurantiacum* and *S. xylosus* strains were outcompeted by adventitious strains
330 belonging to other species. It is noteworthy that among the 18 bacterial OTUs detected
331 (0.05% relative abundance cutoff), 14 corresponded to Gram-negative species. At the end of
332 ripening, Gram-negative species accounted for about 70% of the 16S rRNA sequences, with
333 the most abundant genera being *Psychrobacter*, *Halomonas*, *Mesonina*, and *Vibrio*. One
334 possible factor explaining the dominance of Gram-negative species is the high moisture level
335 of the Epoisses rinds due the humid atmosphere of the ripening rooms and to the frequent
336 washings of the curds (1 to 3 washings per week). Indeed, in a previous study of the
337 community composition of 137 cheese rinds (Wolfe et al., 2014), a positive correlation was
338 observed between rind moisture and some *Proteobacteria* genera such as *Psychrobacter* and
339 *Vibrio*, whereas there was a negative correlation for the Gram-positive genera *Brevibacterium*
340 and *Staphylococcus*, which correspond to the inoculated genera that did not establish
341 themselves at the surface of the Epoisses cheeses. One interesting feature of the cheese
342 samples investigated in the present study is the presence of *M. ostreae*. To our knowledge,
343 *Mesonina* has never been identified in cheese, except in one of the three Epoisses cheese
344 brands analyzed in 2014 (Dugat-Bony et al., 2016), and which was produced by the same

345 manufacturing unit as in the present study. The presence of *M. ostreae* is thus a specific
346 signature of the cheese manufacturing unit considered here. *Mesonia* strains have mostly been
347 isolated from marine environments, including seawater and seaweed; they are considered to
348 be salt-tolerant and some members are able to form biofilms (Lee et al., 2012; Huan et al.,
349 2019), which may constitute useful properties for the growth on the cheese surface. The
350 cheeses from the present study were manufactured from pasteurized milk, which is why the
351 presence of high levels of non-inoculated Gram-negative species probably resulted from the
352 facility-specific "house" microbiota, and especially environment microorganisms that were
353 present on processing surfaces, materials and airflows (Bokulich et al., 2016). The large
354 presence of these microorganisms in the final product also raises the question about the
355 efficiency of the inoculated ripening cultures used for manufacturing Epoisses cheeses. The
356 function of these cultures is to outcompete undesired microorganisms and to generate the
357 typical sensory properties. It may be considered that the design of more efficient cultures
358 requires the presence of Gram-negative strains belonging to the genera *Psychrobacter*,
359 *Halomonas*, *Vibrio* or *Mesonia*, which are currently not used as ripening culture components.
360 Interestingly, most Gram-negative bacteria of dairy origin investigated in a recent safety
361 assessment study were considered to be safe (Imran et al., 2019). Interestingly, *B.*
362 *aurantiacum*, the orange-pigmented bacterium that is used to give an orange color to cheeses,
363 represented only a very small minority of the bacterial population. This species is thus
364 probably not responsible for the typical color of Epoisses cheeses. In addition, HPLC profiles
365 of the carotenoids from Epoisses cheese rinds revealed the presence of yellow carotenoids
366 produced by yellow bacteria such as *Glutamicibacter arilaitensis*, but not of orange
367 carotenoids (Galaup et al., 2007). Furthermore, several studies reported the absence or
368 presence at only very low levels of *Brevibacterium* in some orange smear-ripened cheeses
369 (Brennan et al., 2002; Feurer et al., 2004; Bockelmann et al., 2005; Bockelmann and John,
370 2011; Delcenserie et al., 2014). It has also been suggested that several factors such as the
371 proteolytic activity of smear bacteria may also be important for the typical color development
372 of smear-ripened cheeses (Bockelmann and Hoppe-Seyler, 2001). Thus, it would be
373 interesting to investigate the type and origin of the pigments that confer color to Epoisses
374 cheeses.

375

376 Another noteworthy observation was that during storage at +4°C, between the packaging
377 stage and the "best-before" date (day 90), there was a considerable change in bacterial
378 composition of the Epoisses cheeses. This may also occur in other cheese varieties and it

379 would be interesting to investigate microbial growth and activity during the storage of cheeses
380 at low temperatures and its relationship to the shelf life of the product. In addition, the present
381 study also revealed some changes in the microbial composition of cheeses produced by the
382 same factory over a three year interval. Whether this is common and possibly impacts the
383 sensory properties of the final product is not known, but a previous study reported major
384 changes in the relative importance of the bacterial species present at the surface of Gubbeen
385 cheeses manufactured in the same plant (Rea et al., 2007).

386

387

388

CONCLUSIONS

389

390 As observed at the surface of other smear-ripened cheeses, the acid-tolerant yeasts grow
391 before the acid-sensitive aerobic bacteria in Epoisses cheeses. However, in this cheese variety,
392 the yeast *Debaryomyces hansenii* reaches a high level during ripening, and there is
393 subsequently a large decrease in its cell viability, which possibly impacts the organoleptic
394 properties of the final product. Most of the growth of the acid-sensitive bacteria occurs during
395 the last week of ripening, and some changes in bacterial composition also occur during post-
396 manufacturing storage at +4°C. The high moisture level probably favors Gram-negative
397 species, which are the dominant bacteria at the end of ripening. Since these species are able to
398 outcompete part of the inoculated ripening bacteria, it might be interesting to devise ripening
399 cultures containing typical Gram-negative species present in this cheese variety and with
400 previously assessed safety. The orange color of Epoisses cheeses does not seem to be due to
401 the presence of orange-pigmented bacteria, and it would thus be interesting to investigate the
402 type and origin of the pigments present in this cheese.

403

404

ACKNOWLEDGMENTS

405

406 This work has benefited from the high-throughput sequencing platform and expertise of GeT-
407 PlaGE (<http://get.genotoul.fr>). We are grateful to the INRAE MIGALE bioinformatics facility
408 (MIGALE, INRAE, 2020. Migale Bioinformatics Facility, doi:
409 10.15454/1.5572390655343293E12) and to the Genotoul Bioinformatics Platform Toulouse
410 Occitanie (Bioinfo Genotoul, doi: 10.15454/1.5572369328961167E12) for providing help,
411 computing and storage resources. We are grateful to Eric Dugat-Bony for his assistance in
412 bioinformatic analyses, and to Anne-Sophie Sarthou for her excellent technical assistance.

413

414

REFERENCES

- 415
416
417
418 Bastian, M., S. Heymann, and M. Jacomy. 2009. Gephi: An Open Source Software for
419 Exploring and Manipulating Networks. *Int. AAAI Conf. Web Soc. Media Third Int.*
420 *AAAI Conf. Weblogs Soc. Media.*
- 421 Bockelmann, W., and T. Hoppe-Seyler. 2001. The surface flora of bacterial smear-ripened
422 cheeses from cow's and goat's milk. *Int. Dairy J.* 11:307–314.
423 doi:doi.org/10.1016/S0958-6946(01)00060-7.
- 424 Bockelmann, W., and W.F. John. 2011. Cheese: smear-ripened cheeses. J.W. Fuquay, P.F.
425 Fox, and P.L.H. McSweeney, ed. Academic Press, San Diego.
- 426 Bockelmann, W., K.P. Willems, H. Neve, and K.H. Heller. 2005. Cultures for the ripening of
427 smear cheeses. *Int. Dairy J.* 15:719–732. doi:10.1016/j.idairyj.2004.08.022.
- 428 Bokulich, N.A., Z.T. Lewis, K. Boundy-Mills, and D.A. Mills. 2016. A new perspective on
429 microbial landscapes within food production. *Curr. Opin. Biotechnol.* 37:182–189.
430 doi:10.1016/j.copbio.2015.12.008.
- 431 Boutrou, R., and M. Gueguen. 2005. Interests in *Geotrichum candidum* for cheese technology.
432 *Int. J. Food Microbiol.* 102:1–20. doi:10.1016/j.ijfoodmicro.2004.12.028.
- 433 Brennan, N.M., A.C. Ward, T.P. Beresford, P.F. Fox, M. Goodfellow, and T.M. Cogan. 2002.
434 Biodiversity of the bacterial flora on the surface of a smear cheese. *Appl. Environ.*
435 *Microbiol.* 68:820–830. doi:10.1128/AEM.68.2.820-830.2002.

436 Castellote, J., S. Fraud, F. Irlinger, D. Swennen, F. Fer, P. Bonnarme, and C. Monnet. 2015.
437 Investigation of *Geotrichum candidum* gene expression during the ripening of
438 Reblochon-type cheese by reverse transcription-quantitative PCR. *Int. J. Food*
439 *Microbiol.* 194:54–61. doi:10.1016/j.ijfoodmicro.2014.11.009.

440 CNIEL. 2020. Dairy economy - edition 2020, Accessed Feb. 01, 2021 (in french).
441 [https://presse.filiere-laitiere.fr/assets/economie-laitieres-en-chiffres-edition-2020-fa05-](https://presse.filiere-laitiere.fr/assets/economie-laitieres-en-chiffres-edition-2020-fa05-ef05e.html?lang=fr)
442 [ef05e.html?lang=fr](https://presse.filiere-laitiere.fr/assets/economie-laitieres-en-chiffres-edition-2020-fa05-ef05e.html?lang=fr).

443 Cogan, T.M., S. Goerges, R. Gelsomino, S. Larpin, M. Hohenegger, N. Bora, E. Jamet, M.C.
444 Rea, J. Mounier, V. M., M. Guéguen, N. Desmasures, J. Swings, M. Goodfellow, A.C.
445 Ward, H. Sebastiani, F. Irlinger, J.F. Chamba, R. Beduhn, and S. Scherer. 2014.
446 Biodiversity of the surface microbial consortia from Limburger, Reblochon, Livarot,
447 Tilsit, and Gubbeen cheeses. C.W. Donnelly, ed. ASM Press, Washington, DC.

448 Corsetti, A., J. Rossi, and M. Gobbetti. 2001. Interactions between yeasts and bacteria in the
449 smear surface-ripened cheeses. *Int. J. Food Microbiol.* 69:1–10. doi:10.1016/S0168-
450 1605(01)00567-0.

451 De Filippis, F., E. Parente, and D. Ercolini. 2018. Recent Past, Present, and Future of the
452 Food Microbiome. *Annu. Rev. Food Sci. Technol.* doi:10.1146/annurev-food-
453 030117-012312.

454 Delcenserie, V., B. Taminiau, L. Delhalle, C. Nezer, P. Doyen, S. Crevecoeur, D. Roussey, N.
455 Korsak, and G. Daube. 2014. Microbiota characterization of a Belgian protected
456 designation of origin cheese, Herve cheese, using metagenomic analysis. *J. Dairy Sci.*
457 97:6046–6056. doi:10/ggrh6z.

458 Dugat-Bony, E., L. Garnier, J. Denonfoux, S. Ferreira, A.-S. Sarthou, P. Bonnarme, and F.
459 Irlinger. 2016. Highlighting the microbial diversity of 12 French cheese varieties. *Int.*
460 *J. Food Microbiol.* 238:265–273. doi:10.1016/j.ijfoodmicro.2016.09.026.

461 Dugat-Bony, E., C. Straub, A. Teissandier, D. Onésime, V. Loux, C. Monnet, F. Irlinger, S.
462 Landaud, M.-N. Leclercq-Perlat, P. Bento, S. Fraud, J.-F. Gibrat, J. Aubert, F. Fer, E.
463 Guédon, N. Pons, S. Kennedy, J.-M. Beckerich, D. Swennen, and P. Bonnarme. 2015.
464 Overview of a surface-ripened cheese community functioning by meta-omics
465 analyses. *PLoS ONE* 10. doi:10.1371/journal.pone.0124360.

466 Escudié, F., L. Auer, M. Bernard, M. Mariadassou, L. Cauquil, K. Vidal, S. Maman, G.
467 Hernandez-Raquet, S. Combes, and G. Pascal. 2018. FROGS: Find, Rapidly, OTUs
468 with Galaxy Solution. *Bioinformatics* 34:1287–1294.
469 doi:10.1093/bioinformatics/btx791.

470 Feurer, C., T. Vallaey, G. Corrieu, and F. Irlinger. 2004. Does smearing inoculum reflect the
471 bacterial composition of the smear at the end of the ripening of a french soft, red-
472 smear cheese?. *J. Dairy Sci.* 87:3189–3197. doi:10.3168/jds.S0022-0302(04)73454-2.

473 Fréтин, M., B. Martin, E. Rifa, V.-M. Isabelle, D. Pomiès, A. Ferlay, M.-C. Montel, and C.
474 Delbès. 2018. Bacterial community assembly from cow teat skin to ripened cheeses is
475 influenced by grazing systems. *Sci. Rep.* 8:1–11. doi:10/gcvd5v.

476 Fröhlich-Wyder, M.-T., E. Arias-Roth, and E. Jakob. 2019. Cheese yeasts. *Yeast* 36:129–141.
477 doi:10/gfqr3.

478 Galaup, P., A. Gautier, Y. Piriou, A. De Villeblanche, A. Valla, and L. Dufossé. 2007. First
479 pigment fingerprints from the rind of French PDO red-smear ripened soft cheeses

480 Epoisses, Mont d'Or and Maroilles. *Innov. Food Sci. Emerg. Technol.* 8:373–378.
481 doi:10.1016/j.ifset.2007.03.017.

482 Goerges, S., J. Mounier, M.C. Rea, R. Gelsomino, V. Heise, R. Beduhn, T.M. Cogan, M.
483 Vancanneyt, and S. Scherer. 2008. Commercial ripening starter microorganisms
484 inoculated into cheese milk do not successfully establish themselves in the resident
485 microbial ripening consortia of a south German red smear cheese. *Appl. Environ.*
486 *Microbiol.* 74:2210–2217. doi:10.1128/aem.01663-07.

487 Huan, R., J. Huang, D. Liu, M. Wang, C. Liu, Y. Zhang, C. Yi, D. Xiao, and H. He. 2019.
488 Genome Sequencing of *Mesonia* algae K4-1 Reveals Its Adaptation to the Arctic
489 Ocean. *Front. Microbiol.* 10. doi:10/ggqdtb.

490 Imran, M., N. Desmasures, M. Coton, E. Coton, A. Le Flèche-Matéos, F. Irlinger, C. Delbès-
491 Paus, V. Stahl, M.-C. Montel, and J.-P. Vernoux. 2019. Safety assessment of Gram-
492 negative bacteria associated with traditional French cheeses. *Food Microbiol.* 79:1–10.
493 doi:10/gf2fvm.

494 Jonnala, Y., B. R. P.L.H. McSweeney, J.J. Sheehan, and P.D. Cotter. 2018. Sequencing of the
495 Cheese Microbiome and Its Relevance to Industry. *Front. Microbiol.* 9.
496 doi:10.3389/fmicb.2018.01020.

497 Kim, O.-S., Y.-J. Cho, K. Lee, S.-H. Yoon, M. Kim, H. Na, S.-C. Park, Y.S. Jeon, J.-H. Lee,
498 H. Yi, S. Won, and J. Chun. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA
499 gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst.*
500 *Evol. Microbiol.* 62:716–721. doi:10.1099/ijs.0.038075-0.

501 Kumura, H., K. Takagaki, T. Sone, M. Tsukahara, T. Tanaka, and K. Shimazaki. 2002. Casein
502 Digestion by *Debaryomyces hansenii* Isolated from Cheese. *Biosci. Biotechnol.*
503 *Biochem.* 66:1370–1373. doi:10.1271/bbb.66.1370.

504 Leclercq-Perlat, M.N., A. Oumer, J.L. Bergere, H.E. Spinnler, and G. Corrieu. 1999. Growth
505 of *Debaryomyces hansenii* on a bacterial surface-ripened soft cheese. *J. Dairy Res.*
506 66:271–281. doi:10.1017/S0022029999003362.

507 Leclercq-Perlat, M.N., D. Picque, S.T. Martin del Campo Barba, and C. Monnet. 2013.
508 Dynamics of *Penicillium camemberti* growth quantified by real-time PCR on
509 Camembert-type cheeses at different conditions of temperature and relative humidity.
510 *J. Dairy Sci.* 96:4031–4040. doi:10.3168/jds.2012-6372.

511 Lee, S.-Y., M.-H. Lee, and J.-H. Yoon. 2012. *Mesonium ostreae* sp. nov., isolated from
512 seawater of an oyster farm, and emended description of the genus *Mesonium*. *Int. J.*
513 *Syst. Evol. Microbiol.* 62:1804–1808. doi:10/cg6hg3.

514 LEGIFRANCE. 2010. Decree n°2010-1092 relating to the Protected Designation of Origin
515 (PDO) “Epoisses”, Accessed Feb. 01, 2021 (in french).
516 <https://www.legifrance.gouv.fr/loda/id/JORFTEXT000022825025/2021-02-01/>.

517 Magoč, T., and S.L. Salzberg. 2011. FLASH: Fast Length Adjustment of Short Reads to
518 Improve Genome Assemblies. *Bioinformatics* btr507.
519 doi:10.1093/bioinformatics/btr507.

520 Mahé, F., T. Rognes, C. Quince, C. de Vargas, and M. Dunthorn. 2014. Swarm: robust and
521 fast clustering method for amplicon-based studies. *PeerJ* 2:e593. doi:10/gfzhq3.

522 Masoud, W., F.K. Vogensen, S. Lillevang, W. Abu Al-Soud, S.J. Sørensen, and M.
523 Jakobsen. 2012. The fate of indigenous microbiota, starter cultures, *Escherichia coli*,
524 *Listeria innocua* and *Staphylococcus aureus* in Danish raw milk and cheeses
525 determined by pyrosequencing and quantitative real time (qRT)-PCR. *Int. J. Food*
526 *Microbiol.*

527 McMurdie, P.J., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive
528 analysis and graphics of microbiome census data. *PloS One* 8:e61217. doi:10/gdq975.

529 Mounier, J., R. Gelsomino, S. Goerges, M. Vancanneyt, K. Vandemeulebroecke, B. Hoste, S.
530 Scherer, J. Swings, G.F. Fitzgerald, and T.M. Cogan. 2005. Surface microflora of four
531 smear-ripened cheeses. *Appl. Environ. Microbiol.* 71:6489–6500.
532 doi:10.1128/AEM.71.11.6489-6500.2005.

533 Mounier, J., S. Goerges, R. Gelsomino, M. Vancanneyt, K. Vandemeulebroecke, B. Hoste,
534 N.M. Brennan, S. Scherer, J. Swings, G.F. Fitzgerald, and T.M. Cogan. 2006. Sources
535 of the adventitious microflora of a smear-ripened cheese. *J. Appl. Microbiol.* 101:668–
536 681. doi:10.1111/j.1365-2672.2006.02922.x.

537 Nilsson, R.H., K.-H. Larsson, A.F.S. Taylor, J. Bengtsson-Palme, T.S. Jeppesen, D. Schigel,
538 P. Kennedy, K. Picard, F.O. Glöckner, L. Tedersoo, I. Saar, U. Kõljalg, and K.
539 Abarenkov. 2019. The UNITE database for molecular identification of fungi: handling
540 dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47:D259–D264.
541 doi:10/gfx5s6.

542 Park, W., J. Yoo, S. Oh, J. Ham, S. Jeong, and Y. Kim. 2019. Microbiological Characteristics
543 of Gouda Cheese Manufactured with Pasteurized and Raw Milk during Ripening

544 Using Next Generation Sequencing. *Food Sci. Anim. Resour.* 39:585–600.
545 doi:10/ghwkgc.

546 Rea, M.C., S. Gorges, R. Gelsomino, N.M. Brennan, J. Mounier, M. Vancanneyt, S. Scherer,
547 J. Swings, and T.M. Cogan. 2007. Stability of the biodiversity of the surface consortia
548 of Gubbeen, a red-smear cheese. *J Dairy Sci* 90:2200–2210. doi:10.3168/jds.2006-
549 377.

550 Wolfe, B.E., J.E. Button, M. Santarelli, and R.J. Dutton. 2014. Cheese rind communities
551 provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell*
552 158:422–433. doi:10.1016/j.cell.2014.05.041.

553
554
555

556

FIGURES

557

558

559

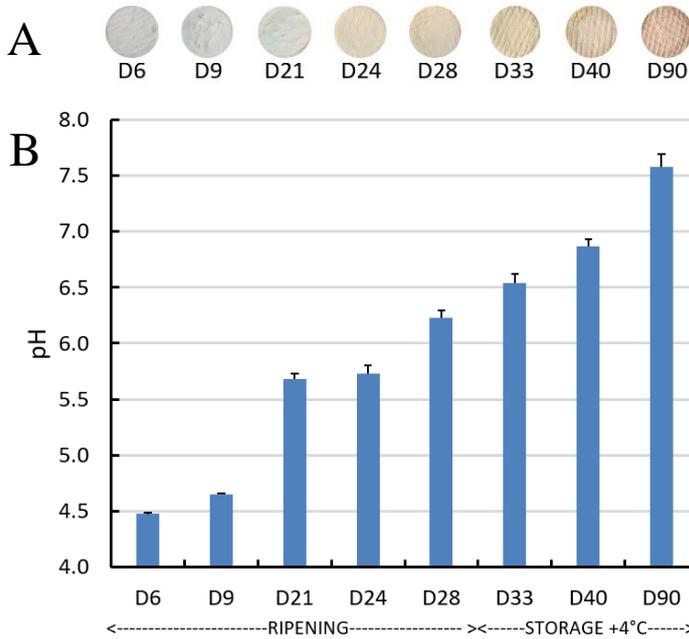


Figure: 8.9-cm wide

560

561

562

563

564

565

566

567

568

569

570

571 **Figure 1.** (A) Appearance of the Epoisses cheeses during their manufacturing and storage.

572 (B) Changes in the pH values at the surface of the cheeses. Bars show mean \pm sd of three

573 replicates. The samples D6, D9, D21, D24, D28, D33, D40 and D90 correspond to cheeses

574 sampled from 6 to 90 days after the beginning of manufacturing.

575

576

577

578

579

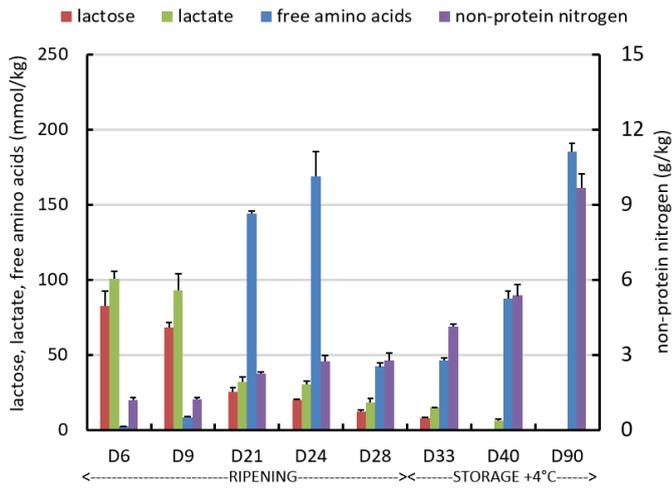


Figure: 8.9 cm wide

581

582

583 **Figure 2.** Concentration of lactose, lactate, free amino acids and non-protein nitrogen
 584 fractions during the manufacturing and storage of Epoisses cheeses. Bars show mean \pm sd of
 585 three replicates. The samples D6, D9, D21, D24, D28, D33, D40 and D90 correspond to
 586 cheeses sampled from 6 to 90 days after the beginning of manufacturing.

587

588

589

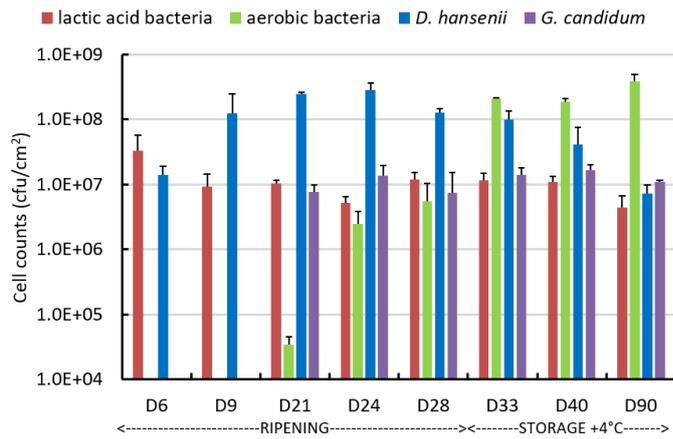
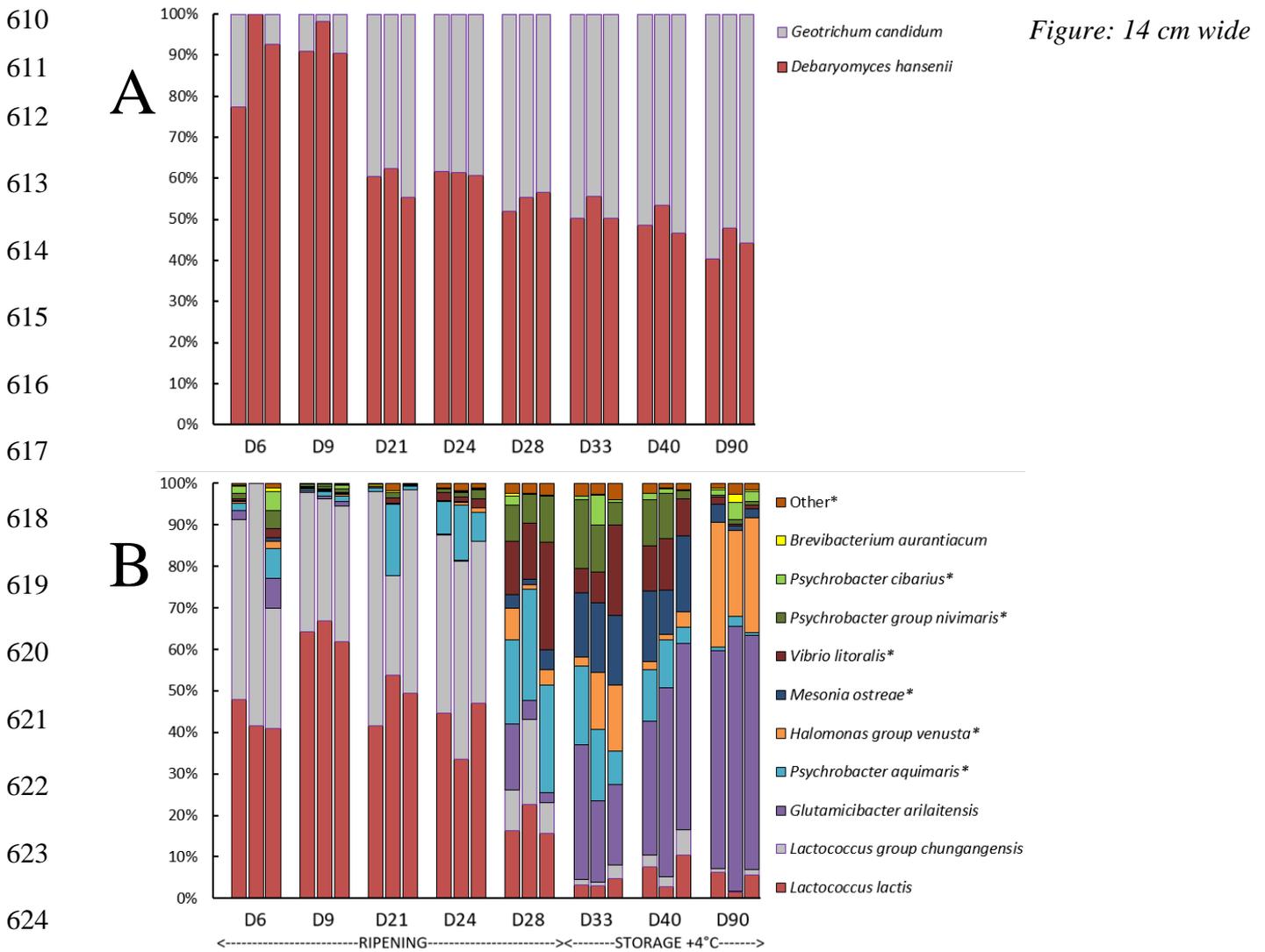


Figure: 8.9 cm wide

590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609

Figure 3. Bacterial and yeast counts at the surface of the Epoisses cheeses during their manufacturing and storage. Bars show mean \pm sd of three replicates. The samples D6, D9, D21, D24, D28, D33, D40 and D90 correspond to cheeses sampled from 6 to 90 days after the beginning of manufacturing.



625

626 **Figure 4.** Relative abundances of the fungal (A) and bacterial (B) species at the surface of the
627 Epoisses cheeses during their manufacturing and storage. Abundances were estimated by 16S
628 rRNA and ITS marker gene analysis. Microorganisms that were not inoculated during
629 cheesemaking are indicated by an asterisk. The "Other" category corresponds to the
630 subdominant species *Halomonas titanicae*, *Halomonas glaciei*, *Psychrobacter proteolyticus*,
631 *Psychrobacter namhaensis*, *Pseudoalteromonas group issachenkonii*, *Marinomonas polaris*,
632 *Cobetia group marina* and *Sphingomonas group insulae*. Three separate cheeses were
633 analyzed at each sampling time. The samples D6, D9, D21, D24, D28, D33, D40 and D90
634 correspond to cheeses sampled from 6 to 90 days after the beginning of manufacturing.

635

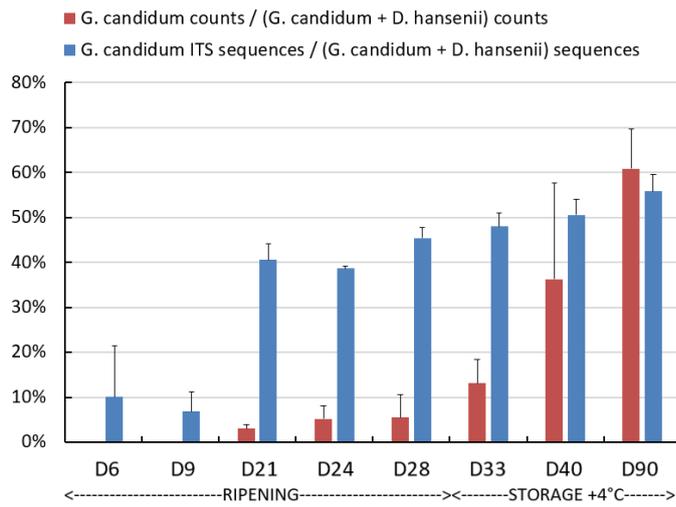


Figure: 8.9 cm wide

636

637

638

639 **Figure 5.** Proportion of *G. candidum* among the yeasts at the surface of the Epoisses cheeses

640 during their manufacturing and storage. Abundances were estimated by colony counting and

641 by ITS marker gene analysis. The samples D6, D9, D21, D24, D28, D33, D40 and D90

642 correspond to cheeses sampled from 6 to 90 days after the beginning of manufacturing.

643

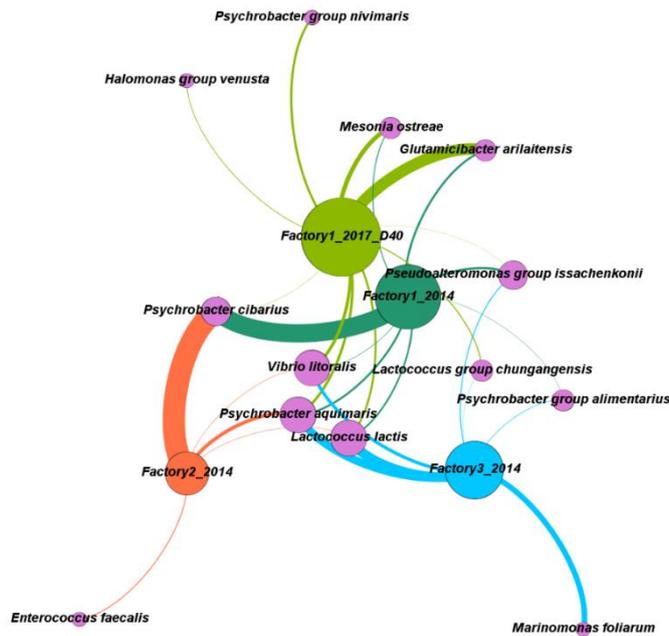


Figure: 8.9 cm wide

645

646 **Figure 6.** OTU-sample network summarizing the relationships between bacterial species of
 647 the cheese rinds from the present study at day 40 (sampled in 2017) and of cheeses sampled in
 648 2014 and that were manufactured by the same factory (Factory 1) or by two other Epoisses
 649 factories (Factories 2 and 3). Nodes represent OTUs and cheeses. Connection lines indicate
 650 the detection of OTUs in the cheeses. Only lines corresponding to OTUs detected at >0.5%
 651 relative abundance in the cheese rinds (mean of three samples) are shown. For the cheese
 652 nodes, different colors (green, dark green, orange and blue) are used to differentiate cheese
 653 factories, and sizes are proportional to bacterial richness (i.e., number of connected OTU
 654 nodes per sample). For OTU nodes, sizes are proportional to the number of occurrences in the
 655 cheese rinds. The thickness of the connecting edges is proportional to the relative abundance
 656 of the OTU in the considered cheese.

657

658

659

SUPPLEMENTARY MATERIAL

Supplemental Table S1 Proportion of bacterial or fungal operational taxonomic units (OTUs) in the samples (%)

	D6a	D6b	D6c	D9a	D9b	D9c	D21a	D21b	D21c	D24a	D24b	D24c	D28a	D28b	D28c	D33a	D33b	D33c	D40a	D40b	D40c	D90a	D90b	D90c	Mean
bacterial OTUs																									
Lactococcus lactis	47,877	41,522	40,862	64,167	66,859	61,761	41,494	53,752	49,335	44,684	33,590	46,964	16,265	22,653	15,644	3,159	2,963	4,793	7,673	2,910	10,520	6,251	1,545	5,668	28,871
Lactococcus group	43,383	58,404	29,107	33,667	29,416	32,743	56,576	23,981	48,034	42,969	47,629	39,024	9,949	20,509	7,331	1,311	0,915	3,314	2,816	2,301	5,938	0,958	0,233	1,365	22,620
chungangensis																									
Glutamicibacter ariflakensis	2,251	0,000	7,080	0,491	0,625	1,228	0,004	0,100	0,022	0,051	0,357	0,015	15,728	4,461	2,577	32,447	19,712	19,382	32,158	45,543	44,966	52,428	63,823	56,264	16,738
Psychrobacter aquimaris	1,646	0,005	7,309	0,387	1,089	1,275	0,901	17,065	0,908	7,857	13,269	6,979	20,360	26,845	25,908	19,089	17,153	8,092	12,346	11,475	3,815	0,927	2,420	0,842	8,665
Psychrobacter venusta	0,386	0,000	1,760	0,117	0,267	0,281	0,030	0,172	0,027	0,287	0,632	1,118	7,604	1,221	3,537	2,223	13,589	15,883	2,031	1,337	3,774	30,068	20,572	27,627	5,606
Mesonnia ostreae	0,323	0,000	0,902	0,061	0,125	0,233	0,004	0,023	0,000	0,051	0,065	0,030	3,305	1,167	4,811	15,440	16,785	16,800	17,090	10,672	18,250	4,396	1,138	2,225	4,746
Vibrio littoralis	0,435	0,005	2,115	0,135	0,369	0,343	0,354	1,434	0,135	1,810	1,119	2,192	12,745	13,590	26,108	5,806	7,603	21,779	10,839	12,412	8,956	1,736	0,556	6,696	5,553
Psychrobacter group	1,285	0,000	4,328	0,357	0,486	0,790	0,060	1,321	0,153	0,917	1,101	2,251	8,734	6,827	10,929	16,646	11,258	5,433	11,104	10,930	2,020	0,358	1,090	1,051	4,143
nivimaris																									
Psychrobacter cibarius	1,688	0,005	4,528	0,291	0,461	0,980	0,009	0,032	0,004	0,009	0,194	0,005	2,305	0,151	0,135	0,768	7,113	0,601	1,507	1,164	0,186	1,363	3,933	2,188	1,234
Brevibacterium aurantiacum	0,373	0,034	0,988	0,200	0,157	0,214	0,286	0,335	0,112	0,194	0,301	0,236	0,689	0,281	0,117	0,039	0,327	0,092	0,121	0,054	0,073	0,436	2,160	0,528	0,348
Pseudoalteromonas group	0,066	0,000	0,188	0,013	0,012	0,010	0,004	0,344	0,013	0,079	0,047	0,044	0,350	0,708	0,674	0,421	0,290	0,301	0,892	0,431	0,432	0,187	1,515	0,550	0,316
Issachenkonii																									
Marinomonas polaris	0,025	0,000	0,078	0,017	0,007	0,019	0,000	0,005	0,000	0,000	0,000	0,005	0,249	0,124	0,400	1,820	0,649	1,164	0,866	0,286	0,708	0,345	0,143	0,378	0,304
Cobetia group marina	0,037	0,000	0,147	0,013	0,025	0,019	0,043	0,742	0,072	0,556	0,697	0,552	0,294	0,383	0,426	0,113	0,154	0,370	0,031	0,016	0,104	0,000	0,000	0,005	0,200
Halonomas titanicae	0,000	0,000	0,016	0,004	0,007	0,005	0,004	0,036	0,000	0,030	0,108	0,094	0,260	0,065	0,400	0,080	0,450	1,310	0,067	0,027	0,076	0,317	0,446	0,150	0,165
Psychrobacter namaensis	0,017	0,000	0,114	0,004	0,022	0,014	0,009	0,194	0,036	0,282	0,404	0,079	0,424	0,605	0,591	0,231	0,279	0,085	0,166	0,102	0,041	0,025	0,018	0,023	0,157
Sphingomonas group insulae	0,021	0,020	0,045	0,039	0,025	0,019	0,218	0,425	0,148	0,218	0,400	0,291	0,350	0,383	0,222	0,055	0,082	0,108	0,081	0,043	0,062	0,028	0,023	0,045	0,140
Halonomas glaciei	0,087	0,000	0,147	0,013	0,030	0,014	0,004	0,032	0,000	0,007	0,069	0,123	0,203	0,022	0,174	0,132	0,338	0,432	0,152	0,172	0,073	0,137	0,170	0,341	0,120
Psychrobacter proteolyticus	0,100	0,005	0,286	0,022	0,017	0,052	0,000	0,009	0,000	0,000	0,017	0,000	0,186	0,005	0,017	0,220	0,330	0,062	0,058	0,124	0,007	0,040	0,215	0,055	0,076
fungal OTUs																									
Debaryomyces hansenii	77,428	99,887	92,624	90,852	98,276	90,489	60,520	62,480	55,353	61,711	61,524	60,679	51,859	55,442	56,476	50,204	55,634	50,271	48,493	53,323	46,538	40,436	47,793	44,199	63,020
Geotrichum candidum	22,572	0,113	7,376	9,148	1,724	9,511	39,480	37,520	44,647	38,289	38,476	39,321	48,141	44,558	43,524	49,796	44,366	49,729	51,507	46,677	53,462	59,564	52,207	55,801	36,980

693 **Supplemental Figure S1** Alpha-diversity indices of bacterial communities during cheese-
694 making and storage

695

696

697

698

699

700

701

702

703

704

705

706

707

708

