



**HAL**  
open science

## Safety assessment of Gram-negative bacteria associated with traditional French cheeses

M. Imran, N. Desmasures, M. Coton, Anne Le Fleche-Mateos, F. Irlinger, C. Delbès-Paus, V. Stahl, M. Montel, Jean-Paul Vernoux

► **To cite this version:**

M. Imran, N. Desmasures, M. Coton, Anne Le Fleche-Mateos, F. Irlinger, et al.. Safety assessment of Gram-negative bacteria associated with traditional French cheeses. *Food Microbiology*, 2019, 79, pp.1-10. 10.1016/j.fm.2018.11.001 . hal-02086922

**HAL Id: hal-02086922**

**<https://normandie-univ.hal.science/hal-02086922v1>**

Submitted on 5 Jun 2020

**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.



Distributed under a Creative Commons Attribution - NoDerivatives 4.0 International License

1  
2  
3 **1 Safety assessment of Gram-negative bacteria associated with traditional French cheeses**

4  
5 **2 Imran, M<sup>1&</sup>, N. Desmasures<sup>1\*</sup>, M. Coton<sup>2§</sup>, E. Coton<sup>2§</sup>, A. Le Flèche-Matéos<sup>3</sup>, F.**

6  
7 **3 Irlinger<sup>4</sup>, C. Delbès<sup>5</sup>, V. Stahl<sup>6</sup>, M-C. Montel<sup>5</sup>, J-P. Vernoux<sup>1\*</sup>**

8  
9  
10  
11  
12 <sup>1</sup> Normandie Univ, UNICAEN, ABTE, 14000 Caen, France

13  
14 <sup>2</sup>ADRIA Normandie, Bd du 13 juin 1944, 14310 Villers-Bocage, France

15  
16 <sup>3</sup>Unité Environnement et Risques Infectieux, Cellule d'Intervention Biologique d'Urgence,  
17  
18 Institut Pasteur, F-75724 Paris Cedex 15, France

19  
20 <sup>4</sup> UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, 78850, Thiverval-Grignon,  
21  
22 France.

23  
24 <sup>5</sup>Université Clermont Auvergne, INRA, UMR 545 Fromage, Aurillac, France

25  
26 <sup>6</sup>Aérial, 250 rue Laurent Fries, F-67412 Illkirch, France

27  
28 \*Corresponding authors : [jean-paul.vernoux@unicaen.fr](mailto:jean-paul.vernoux@unicaen.fr) and [nathalie.desmasures@unicaen.fr](mailto:nathalie.desmasures@unicaen.fr)

29  
30 & Present address: Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam  
31  
32 University, Islamabad Pakistan.

33  
34 § Present address: Univ Brest, Laboratoire Universitaire de Biodiversité et Ecologie  
35  
36 Microbienne, F-29280 Plouzané, France.

37  
38  
39  
40 **18 Running title: Safety of Gram-negative dairy isolates**

60  
61  
62 **21 ABSTRACT**  
63

64 22 Twenty Gram-negative bacterial (GNB) strains were selected based on the biodiversity  
65  
66 23 previously observed in French traditional cheeses and their safety was assessed considering  
67  
68 24 various safety criteria. For the majority of tested GNB strains, only gastric stress at pH 2 (vs pH  
69  
70 25 4) resulted in low survival and no regrowth after an additional simulated gastro-intestinal stress.  
71  
72 26 Presence of milk was shown to be rarely protective. The majority of strains was resistant to  
73  
74 27 human serum and had a low level of adherence to Caco-2 cells. When tested for virulence in  
75  
76 28 *Galleria mellonella* larvae, GNB strains had LD 50 values similar to that of safe controls.  
77  
78 29 However, four strains, *Hafnia paralvei* 920, *Proteus* sp. (close to *P. hauseri*) UCMA 3780,  
79  
80 30 *Providencia heimbachae* GR4, and *Morganella morganii* 3A2A were highly toxic to the larvae,  
81  
82 31 which suggests the presence of potential virulent factors in these strains. Noteworthy, to our  
83  
84 32 knowledge, no foodborne intoxication or outbreak has been reported so far for any of the GNB  
85  
86 33 belonging to the genera/species associated with the tested strains. The role of multiple dynamic  
87  
88 34 interactions between cheese microbiota and GIT barriers could be key factors explaining safe  
89  
90 35 consumption of the corresponding cheeses.  
91  
92  
93  
94 36

95  
96 37 **Keywords:** Caco-2, *Galleria mellonella*, gastro-intestinal stress, human serum bactericidal  
97  
98 38 assay, risk factors  
99

100  
101 39  
102 40 **1. Introduction**  
103

104 41 Cheese microbiota consists of diverse microorganisms, including yeasts, moulds, Gram-  
105  
106 42 positive and -negative bacteria (Dugat-Bony et al., 2016; Gori et al., 2013; Imran et al., 2012;  
107  
108 43 Irlinger and Mounier, 2009; Larpin-Laborde et al., 2011; Martín and Coton, 2017; Mounier et  
109  
110 44 al., 2009, 2017; Wolfe et al., 2014). Microbial community diversity is important during cheese  
111  
112 45 making as it is associated with cheese sensorial quality but can also contribute to ensure  
113  
114 46 microbiological control and safety (Delbes et al., 2007; Delbès-Paus et al., 2012; Irlinger and

119  
120  
121 47 Mounier, 2009). While literature data are abundant concerning yeasts/molds and Gram-positive  
122  
123 48 bacteria (GPB) in cheese, Gram-negative bacteria (GNB) have been seldomly studied.  
124  
125 49 However, a wide diversity of GNB, found at relatively high levels in raw milk (around 3 to 4  
126  
127 50 Log CFU/mL), has been reported (Desmaures et al., 1997; Fréтин et al., 2018; Kable et al.,  
128  
129 51 2016). GNB usually represent from 18 to 60 % of the bacteria isolated from the surface of  
130  
131 52 European smear cheeses (Larpin-Laborde et al., 2011; Maoz et al., 2003; Montel et al., 2014;  
132  
133 53 Mounier et al., 2005, 2017; Wolfe et al., 2014). Most of the microorganisms found in cheese  
134  
135 54 originate from raw milk, (animal, milking machine, environment /air) (Desmaures et al., 1997;  
136  
137 55 Fréтин et al., 2018), processing steps, plant, transportation equipment, labor and cheese factory  
138  
139 56 sources (Mounier et al., 2006). GNB present on the surface of the ripened soft cheese mainly  
140  
141 57 belong to *Enterobacteriaceae*, *Moraxellaceae*, *Pseudoalteromonadaceae*, *Pseudomonadaceae*,  
142  
143 58 *Sphingobacteriaceae* and *Vibrionaceae* families (Bockelmann et al., 2005; Chaves-Lopez et  
144  
145 59 al., 2006; Maoz et al., 2003; Mounier et al., 2005; Tornadijo et al., 1993). Previous work, done  
146  
147 60 between 2008 and 2010 aimed at studying GNB associated with French milk and corresponding  
148  
149 61 cheeses. The obtained 173 isolates corresponded to at least 26 genera and 68 species, including  
150  
151 62 potential new species. *Pseudomonas*, *Chryseobacterium*, *Enterobacter*, and *Stenotrophomonas*  
152  
153 63 were the most frequent genera found in cheese core and milk samples, while *Proteus*,  
154  
155 64 *Psychrobacter*, *Halomonas* and *Pseudomonas* were the most frequent genera isolated from  
156  
157 65 cheese surface (Coton et al., 2012).

161  
162 66 Some GNB species found in cheese were reported to include some non-foodborne  
163  
164 67 strains associated with clinical cases (Delbès-Paus et al., 2012). Criteria to classify bacteria as  
165  
166 68 human pathogens or non-pathogens depends on the presence or absence of virulence factors.  
167  
168 69 Survival during gastro intestinal transit and then adhesion to enterocyte cell surfaces is often  
169  
170 70 the first step in establishing potential bacterial disease. For extracellular pathogens, adhesion is  
171  
172 71 a means to withstand mechanical cleaning. For intracellular pathogens, adhesion is often a  
173  
174  
175  
176  
177

178  
179  
180 72 prerequisite for invasion (dos Santos et al., 2015). The human adenocarcinoma cell line Caco-  
181  
182 73 2, isolated from an adult human colon, which expresses several markers characteristic of normal  
183  
184 74 small villi cells (Fogh et al., 1977; Pinto et al., 1983) has been extensively used to study bacterial  
185  
186 75 adhesion mechanisms for pathogens and probiotic strains (Greene and Klaenhammer, 1994).  
187  
188

189 76 Another barrier in the human body against invading pathogens is the action of serum  
190  
191 77 through a series of serum proteins interacting in a regulated sequential manner that eventually  
192  
193 78 leads to bacterial death due to either lysis or opsonisation (Morgan et al., 2000). Furthermore,  
194  
195 79 the safety status evaluation of a given microorganism must also be assessed by its actual  
196  
197 80 pathogenicity on an animal model. Mammals have been used for a long time to evaluate  
198  
199 81 microbial pathogen virulence but it is time consuming, labor intensive, and expensive in terms  
200  
201 82 of purchasing animals, feeding, and housing. An alternative option is the use of an insect model  
202  
203 83 (Kavanagh and Reeves, 2004) because the innate immune responses are similar. A useful model  
204  
205 84 is *Galleria mellonella* (wax moth) larvae (Ramarao et al., 2012). It has been used to evaluate  
206  
207 85 the pathogenicity of various GNB such as *Proteus mirabilis* (Morton et al., 1987), *Francisella*  
208  
209 86 *tularensis* (Aperis et al., 2007), *Yersinia pseudotuberculosis* (Champion et al., 2009),  
210  
211 87 *Stenotrophomonas maltophilia* (Nicoletti et al., 2011) and *Escherichia coli* (Walters and  
212  
213 88 Ratcliffe, 1983). For example, *Pseudomonas aeruginosa* virulence or that of *Bacillus*  
214  
215 89 *thuringiensis* and *Bacillus cereus* were correlated in *Galleria* larvae and in mice (Jander et al.,  
216  
217 90 2000; Salamitou et al., 2000). In fact, the virulence of many pathogens is similar in wax moth  
218  
219 91 larvae and mammals, including Humans (Desbois and Coote, 2012).  
220  
221

222  
223 92 The present study is a continuation of the previous work reported by Coton et al., (2012)  
224  
225 93 which focused on the diversity and potential risk factors (antibiotic resistance and biogenic  
226  
227 94 amines production) of milk and cheese GNB isolates. It aimed at further evaluating the safety  
228  
229 95 aspects of selected representative GNB strains from each genus identified in the cited study.  
230  
231 96 Growth in conditions mimicking the gastrointestinal tract (GIT) transit, Caco 2 cell adhesion,  
232  
233  
234  
235  
236

237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295

97 survival in serum conditions and pathogenicity on insect larvae, were assessed to provide a  
98 more comprehensive view about the safety these GNB isolates.

99

## 100 **2. Materials and methods**

### 101 *2.1 Strains and culture conditions*

102 Starting from an initial collection of 173 GNB strains (Coton et al., 2012), 20 strains from the  
103 raw milk or milk, cheese core or cheese surface representative of the main identified genera and  
104 of different safety status (evaluated according to low or high antibiotic resistance and biogenic  
105 amine production *in vitro*) were selected (Table 1).

106 All these strains had been previously identified by 16S rRNA or *rpoB* gene sequencing and  
107 their antibiotic resistance and biogenic amines production *in vitro* were determined (Coton et  
108 al., 2012). For initial propagation, all control and tested strains were precultured in Tryptic Soy  
109 Broth (Merck, KGaA, Darmstadt, Germany) supplemented with 2.5 g/L yeast extract (Oxoid,  
110 Basingstoke, Hampshire, England) (TSB-YE) and incubated at 37°C or 25°C (for strains unable  
111 to grow at 37°C) for 24 h under aerobic conditions.

112 For adhesion and pathogenicity assays, bacterial strains were grown in TSB-YE and  
113 incubated at 37°C or 25°C (for strains unable to grow at 37°C) for 24 h with shaking (120 rpm)  
114 using a Novotron shaker (VWR, Fontenay sous bois, France).

115

### 116 *2.2 Growth at 37°C*

117 Each precultured strain (cf. §2.1) was isolated on three plates poured with Brain Heart  
118 Infusion agar (BHA, AES, France). For each strain, three plates were inoculated  
119 simultaneously: two plates were incubated at 37°C respectively under aerobic and anaerobic  
120 (AnaeroGen Pack, Oxoid, France) conditions for 14 days and one plate was incubated at 25°C  
121 under aerobic conditions. If growth appeared as expected in aerobiosis on at least one plate and

296  
297  
298 122 for one temperature, results for growth/no growth at 37°C under anaerobic conditions were  
299  
300 123 considered.

301  
302  
303 124

### 304 125 *2.3 Gastric and gastro-intestinal media and stress simulation*

306  
307 126 For gastro-intestinal stress simulation tests, bacterial strains were first precultured in J  
308  
309 127 broth (JB) (5 g/L peptone, 15 g/L yeast extract, 2 g/L glucose, 3 g/L K<sub>2</sub>HPO<sub>4</sub>, adjusted to pH  
310  
311 128 7.2) (Clavel et al., 2004) and incubated at 25°C for 24 h with shaking to obtain optimal growth.  
312  
313 129 For all enumerations, bacteria were plated on J agar (JA) (JB supplemented with 15 g/L agar),  
314  
315 130 incubated at 25°C under aerobic conditions for 24 h.

316  
317 131 Simulated gastric medium (GM) was prepared by adding one volume of a sterile  
318  
319 132 (autoclaved 121°C, 15 min) gastric electrolyte solution (Gänzle et al., 1999) containing 4.8 g/L  
320  
321 133 NaCl, 1.56 g/L NaHCO<sub>3</sub>, 2.2 g/L KCl and 0.22 g/L CaCl<sub>2</sub> to one volume of JB (GM-JB;  
322  
323 134 autoclaved 121°C, 15 min) or milk medium (100g/L of half-skim milk powder; autoclaved  
324  
325 135 100°C, 30 min) (GM-milk (Clavel et al., 2004, 2007). After sterilization, the pH of the GM-JB  
326  
327 136 or GM-milk media were adjusted to 2 or 4 with sterile 1N HCl to simulate the acidic  
328  
329 137 environment of human gastric fluids. Finally, media were supplemented with 500U/l of a filter  
330  
331 138 sterilized (0.22µm) pepsin (gastric juice enzyme) solution prepared in water (P6887; Sigma-  
332  
333 139 Aldrich, France) just before use.

334  
335 140 For gastric stress experiments, GM-JB and GM-milk media were inoculated with each  
336  
337 141 strain at initial populations of  $N_0 = 1.10^6$  CFU/mL and incubated at 37°C with shaking (160  
338  
339 142 rpm) for 3 h to simulate human stomach conditions. Numerations were carried out during the  
340  
341 143 gastric stress simulation at 0, 1.5 and 3 h.

342  
343 144 Following the 3 h gastric stress, the inoculated GM-JB or GM-milk medium was  
344  
345 145 modified by adding one volume of intestinal medium (IM, composed of double strength  
346  
347 146 sterilized JB medium adjusted to pH 6.5 with sterile 1N HCl, (Clavel et al., 2004, 2007)), to  
348  
349  
350  
351  
352  
353  
354

355  
356  
357 147 obtain the simulated gastrointestinal medium (GIM). These two media were named GIM-JB  
358  
359 148 and GIM-milk. Bovine bile (B3883, Sigma-Aldrich, France) was added or not at 1.5 g/L.  
360  
361 149 Incubation in GIM-JB and GIM-milk was done at 37°C without shaking for 28 h to simulate  
362  
363 150 the gastro-intestinal stress. Numerations were carried out at different time intervals: 0, 1.5, 3,  
364  
365 151 5, 21 and 28 h.

366  
367 152 Bacterial survival was expressed as the log (N/N<sub>0</sub>) where N<sub>0</sub> is the initial population that was  
368  
369 153 adjusted to 10<sup>6</sup> CFU/ml and N is the number of CFU/mL obtained after 3 h (gastric stress) or  
370  
371 154 31 h (gastro-intestinal stress). The two-fold dilution, due to changing the gastric stress medium  
372  
373 155 to the gastro-intestinal stress medium, was taken into account.

374  
375 156 All numerations were done by serially diluting bacterial suspensions in Trytone Salt (TS)  
376  
377 157 diluent and plating on JA medium with a spiral system (Intersciences, France). Cell  
378  
379 158 concentrations were expressed as CFU/mL.

380  
381  
382  
383 159

#### 384 385 160 *2.4 Tissue culture and in vitro adhesion assay*

386  
387 161 The strains were tested for their adhesion ability *in vitro* on epithelial intestinal cells (Caco-2:  
388  
389 162 colon adenocarcinoma human, ATCC, USA, Lot# 4129634). The intestinal cells were routinely  
390  
391 163 cultured and used as already described (Tareb et al., 2013). Cultures were used at post-  
392  
393 164 confluence after 15 days of culture (differentiated cells). To determine the number of Caco-2  
394  
395 165 cells in a monolayer, cells were detached for 2 min with Splittix and Splitstop (Bio Media) at  
396  
397 166 ambient temperature and counted using a Thoma cell. Three non-pathogenic control strains  
398  
399 167 were used: *E. coli* Nissle 1917 (obtained from Dr. Ulrich Sonnerborn from Ardeypharm,  
400  
401 168 Germany GmbH), with a long history of safe use as a probiotic and large body of acquired  
402  
403 169 knowledge (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Wassenaar, 2016), *E. coli*  
404  
405 170 K12 (ATCC 10798) and *Lactobacillus rhamnosus* GG strain. In addition, another control was  
406  
407 171 used: *Escherichia coli* O157: H7 C267 (*stx*<sup>-</sup>, *eae*<sup>+</sup>), this strain is a shiga toxin negative mutant  
408  
409  
410  
411  
412  
413



414  
415  
416 172 (*stx1*- and *stx2*-) which has kept the gamma-intimin (adherence protein) producing gene  
417  
418 173 (*eaeA*+) (Vernozy-Rozand et al., 2000). Bacterial strains were cultured in TSB-YE incubated  
419  
420 174 at 37°C or 25°C (for strains unable to grow at 37°C) for 24 h under shaking (120 rpm) and then  
421  
422 175 washed twice with PBS. Concentrations were adjusted to  $2 \times 10^8$  cells/mL and cells were labeled  
423  
424 176 with 0.2% aqueous solution of 4', 6-diamidino-2-phenylindole (DAPI, Sigma) by incubating  
425  
426 177 for 15 min. Three washing steps were performed with PBS (0.01 M, pH 7.4) to remove excess  
427  
428 178 unbound DAPI, then cells were suspended in 0.2mL Dulbecco's Modified Eagle Medium  
429  
430 179 (DMEM) without antibiotics and put into contact with Caco-2 cells at a final concentration of  
431  
432 180  $10^7$  per  $7 \times 10^4$  Caco-2 cells for 2h under standard Caco-2 growing conditions (incubation at  
433  
434 181 37°C, 5% CO<sub>2</sub>, 95% humidity). Not adherent bacterial cells were removed by three washing  
435  
436 182 steps with PBS solution and cell fixation was performed using a 3.7% (w/v) solution of  
437  
438 183 formaldehyde in PBS.  
439

440  
441 184 Enumeration of adherent bacteria was performed using an epifluorescence microscope.  
442  
443 185 Results were determined as an average of ten observations per assay. Adhesion was calculated  
444  
445 186 by enumerating the adhered bacterial cells in 10 microscopic fields for each strain and was  
446  
447 187 expressed as the average number of adhered bacterial cells per 100 Caco-2 cells. For selected  
448  
449 188 strains, validation of bacterial adhesion was performed by using electron microscopy as  
450  
451 189 routinely done (Tareb et al., 2013).  
452  
453

454 190

#### 455 191 *2.5 Human serum bactericidal assay*

456  
457 192 To determine the sensitivity of GNB strains to human serum, human serum type male,  
458  
459 193 blood group: AB, HIV negative (Biowest, Nauville-France) was used. The bacterial strains were  
460  
461 194 cultivated overnight in TSB under shaking at 30 or 37°C, depending on the strain. GNB strains  
462  
463 195 and negative/positive control strains (see below) were added to 50% human serum solution to  
464  
465 196 obtain the initial cell density of  $10^5$  cells per mL (Jankowski et al., 1996). Each mixture was  
466  
467  
468  
469  
470  
471  
472

473  
474  
475 197 separated in two aliquots, one was plated onto Tryptic Soy Agar (TSA) in Petri plate and was  
476  
477 198 incubated at 30 or 37°C according to the tested strain. The other one was placed in a water bath  
478  
479 199 for 3 hours at 37°C then plated onto TSA and incubated at 30 or 37°C. The results were  
480  
481 200 validated by using *Hafnia alvei* 56.85 (resistant strain) as a positive control and *Hafnia alvei*  
482  
483 201 31.86 (sensitive strain) as a negative control (Jankowski et al., 1996).  
484  
485  
486 202  
487  
488 203 *2.6 Pathogenicity evaluation:*  
489

490 204 *Galleria mellonella* larvae were grown in medium containing 50.6% wheat flour, 19%  
491  
492 205 honey, 19% glycerol, 7.7% brewer's yeast and 3.7% bee hive wax. All bacterial strains were  
493  
494 206 grown as mentioned above, washed three times and cell concentrations were adjusted to  $\sim 10^{10}$   
495  
496 207 cells/mL of PBS by optical density (OD 600 nm) determination using pre-enumeration data for  
497  
498 208 each strain. The two non-pathogenic *Escherichia coli* K12 and *E. coli* Nissle 1917 strains were  
499  
500 209 used as safe controls. Additionally, as above, *E. coli* C267, O157: H7 (*stx1*-, *stx2*-, *eaeA*+) was  
501  
502 210 also used because of the presence of an adherence protein (intimin coding gene *eaeA*). *Galleria*  
503  
504 211 *mellonella* larvae, in groups of ten, were inoculated by injection into the haemocoel through the  
505  
506 212 last pro-leg by using a sterile syringe (Needle: 0.33x 12mm; 0.3mL U-100 insulin- TERUMO,  
507  
508 213 Belgium) with a needle diameter of 0.33 mm. Ten  $\mu$ l of sterile PBS (negative control) and 10  
509  
510 214  $\mu$ l of a dose series corresponding to successive 10-fold dilutions of bacterial cell suspensions  
511  
512 215 (ranging from 2 to 10 logs) were injected. After inoculation, larvae were placed in sterile Petri  
513  
514 216 dishes and incubated at 30°C for 72 h and up to the moth stage (10-15 days). Mortality rate was  
515  
516 217 assessed by the lack of movement of larvae in response to stimulation and observation of  
517  
518 218 concomitant melanization of the cuticle. LD 50 (cfu/g of larvae) was estimated at 72 h in two  
519  
520 219 independent assays as the geometric mean of the doses for which the first 100% mortality and  
521  
522 220 0% mortality were found (Lorke, 1983).  
523  
524  
525  
526 221  
527  
528  
529  
530  
531

532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590

222 2.7 Statistical analysis

223 Principal Component Analysis (PCA) using Pearson correlation (n) method to determine the  
224 correlation between different variables was carried out by using XLSTAT 2014.5.03 program.

225

226 **3. Results and Discussion**

227 3.1 Growth Assessment of Gram negative bacterial species in different conditions related to the  
228 human gastro intestinal tract (GIT) environment

229 3.1.1 Growth at 37°C

230 The ability to grow at 37°C under anaerobic conditions was first tested on Petri dishes  
231 as it is an easy way to screen bacteria in order to evaluate their potential to survive in human  
232 body temperature conditions. All strains grew at 25°C in aerobic conditions. At 37°C under  
233 aerobic conditions, only one strain *Alcaligenes faecalis* 904 did not grow. Twelve out of the 20  
234 selected isolates (60%) were able to grow at 37°C under anaerobic conditions (Table 1). As  
235 expected most of the tested *Enterobacteriaceae* (e.g. *Citrobacter*, *Klebsiella*, *Morganella*,  
236 *Proteus* or *Hafnia alvei*) were able to grow in these conditions, which is in accordance with  
237 their general physiological characteristics. Surprisingly, some *Enterobacteriaceae* (namely,  
238 *Hafnia paralvei* 920, *Proteus sp* UCMA 3779 (close to *P. hauseri*)) did not grow in these  
239 conditions. Growth of *Pseudomonas sp.* depended on the considered strain, and it was positive  
240 for *Pseudomonas putida* CV30.6 and negative for *Pseudomonas putida* VRBG37.3.  
241 Interestingly, other GNB (*i.e.* strains belonging to the *Acinetobacter*, *Alcaligenes*,  
242 *Chryseobacterium*, *Halomonas*, *Pseudomonas*, *Psychrobacter*, *Sphingobacterium* and  
243 *Stenotrophomonas* genera) showed variable growth in these conditions and were not all able to  
244 withstand anaerobic conditions. However, a limitation of this test was that it was performed in  
245 laboratory conditions with pure cultures at a relatively neutral pH without bile salts, these  
246 conditions being different from *in vivo* conditions encountered in the GIT.

591  
592  
593 247 3.1.2 Gastric and gastrointestinal stress simulation  
594

595 248 To get closer to *in vivo* conditions, survival of 20 GNB dairy strains was evaluated  
596  
597 249 successively in simulated gastric fluids and gastrointestinal stress environments over time, in  
598  
599 250 the presence or absence of milk (to mimic ingestion of a dairy product), *in vitro*. For all strains,  
600  
601 251 growth data after 3 h gastric stress and 28 h gastrointestinal stress at 37°C were plotted together  
602  
603 252 and are presented in Table 2. Additionally, changes in counts for four strains representative of  
604  
605 253 the observed behaviours during the 31h gastric and gastrointestinal stress simulation are  
606  
607 254 presented in Figures 1 A to D.  
608  
609  
610

611 255 The effects of *in vitro* simulated gastric stress were assessed over a 3 h period, for the  
612  
613 256 20 selected GNB dairy isolates, in the presence of gastric juices (containing 500 U/l pepsin),  
614  
615 257 and at pH 2 and pH 4, supplemented or not with milk. A good survival, corresponding to stable  
616  
617 258 or increased population counts, was observed for the majority of the 20 GNB isolates in gastric  
618  
619 259 juices initially adjusted to pH 4, both in the presence or absence of milk, except for *Alcaligenes*  
620  
621 260 *faecalis* 904 and to a lesser extent for *Pseudomonas putida* VRBG37.3, and in GM-milk for  
622  
623 261 *Halomonas venusta/alkaliphila/hydrothermas* 4C1A and *Sphingobacterium* sp. PCAi F2.5  
624  
625 262 (Table 2). At pH 2.0 in the absence of milk, a decrease in survival for the majority (14 strains)  
626  
627 263 of strains was reported. Six strains actually grew or presented relatively stable populations in  
628  
629 264 these conditions: *Acinetobacter* sp. PCAi E6.10, *Hafnia paralvei* 920, *Halomonas*  
630  
631 265 *venusta/alkaliphila/hydrothermas* 4C1A (Figure 1A), *Pantoea agglomerans* Q6.3, *Providencia*  
632  
633 266 *heimbachae* GR4, and *Sphingobacterium faecium* F2.5. In the presence of milk, a decrease in  
634  
635 267 survival was also mainly observed for 13 strains. In these conditions, populations remained  
636  
637 268 relatively stable for only 7 strains ( $\log(N/N_0) > -0.5$ ): *Chryseobacterium bovis* Pi18 (Figure  
638  
639 269 1B), *Hafnia paralvei* 920, *Halomonas venusta/alkaliphila/hydrothermalis* 4C1A (Figure 1A),  
640  
641 270 *Halomonas* sp. B39, *Morganella morganii* 3A2A, *Providencia heimbachae* GR4 and  
642  
643 271 *Pseudomonas putida* CV30.6 (Table 2).  
644  
645  
646  
647  
648  
649

650  
651  
652 272 These results showed that under simulated gastric stress conditions (no bile salts added),  
653  
654 273 pH 2 had a different effect on growth than pH 4; presence of milk had only a slight impact on  
655  
656 274 growth for both tested pH values.

658 275 The effects of an additional 28 h simulated gastrointestinal stress (with or without 1.5 g/L  
659  
660 276 bile salts) after 3 h gastric stress treatment, at pH 2 or pH 4, on the viability of the 20 GNB  
661  
662 277 dairy isolates was then evaluated at neutral pH (pH 6.5). In all the tested conditions, no or low  
663  
664 278 survival was observed for *Alcaligenes faecalis* 904 while two other strains exhibited good  
665  
666 279 survival in all conditions as observed by an increase in population counts or re-growth during  
667  
668 280 the simulated stress (i.e. *Halomonas* sp B39, *Panteoa agglomerans* PCA Q6.3). Surprisingly,  
669  
670 281 bile salts (studied concentration was 1.5 g/L), that are normally encountered during  
671  
672 282 gastrointestinal stress, did not modify strain survival or had only slight effects when compared  
673  
674 283 to the same conditions without bile salts (for example among others, *Chryseobacterium bovis*  
675  
676 284 Pi18 (Figure 1B) or *Pseudomonas putida* VRBG 37.3).

679 285 No or low survival of a large number of strains was observed after a pH 2.0 gastric fluids  
680  
681 286 treatment regardless of the conditions encountered in the simulated gastrointestinal tract. No or  
682  
683 287 low survival was observed for 7 strains at pH 2 , contrary to a high growth observed at pH 4 for  
684  
685 288 these strains. This was the case for *Hafnia alvei* (Biogroup 1) B16, *Proteus* sp. (close to *P.*  
686  
687 289 *hauseri*) UCMA 3779 (Figure 1C), *Pseudomonas putida* CV 30.6, *Pseudomonas stutzeri*  
688  
689 290 UCMA 3883, and *Psychrobacter celer* 91. Survival in the simulated gastrointestinal tract  
690  
691 291 containing milk was better for some strains: *Hafnia paralvei* 920, *Halomonas* sp. B39 and  
692  
693 292 *Proteus* sp. (close to *P. hauseri*) UCMA 3780, suggesting a protective effect on the overall  
694  
695 293 survival in GIM-media of these dairy isolates.

698 294 These results showed that the determinant role of initial pH persisted while bile salts  
699  
700 295 concentration had a negligible effect on growth; the addition of milk rarely showed a protective  
701  
702 296 survival effect for the tested GNB strains at both pHs after the total of 31 h of incubation.  
703  
704  
705  
706  
707  
708

709  
710  
711 297 Noteworthy, the simulated TGI stress conditions could also potentially lead to a viable but non-  
712  
713 298 culturable state for some cells and under some conditions, thus potentially underestimating  
714  
715 299 viable cell counts. This state has been previously observed for some foodborne pathogens in  
716  
717  
718 300 environmentally limiting conditions during food processing and conservation such as drastic  
719  
720 301 temperatures or the use of preservatives (Zhao et al, 2017).

721  
722 302 The protective survival effect of food in gastrointestinal simulated media has already  
723  
724 303 been observed for *Bacillus cereus* (milk media) (Clavel et al., 2007), *Bifidobacterium* (soymilk)  
725  
726 304 (Shimakawa et al., 2003) and *Lactobacillus curvatus* (meat based medium) (Gänzle et al., 1999)  
727  
728 305 and is often linked to food components such as proteins and fats. Additionally, in the intestinal  
729  
730 306 tract, bile reacts with cell membrane phospholipids and proteins and disrupts cellular  
731  
732 307 homeostasis (Begley et al., 2005). However, in this study, the presence of bile salts in the  
733  
734 308 intestinal media only had an effect on a limited number of strains and low survival was rather  
735  
736 309 due to the acidic environment encountered in the gastric fluids simulation before the  
737  
738 310 gastrointestinal stress. High variability in bile salt tolerance has also been previously observed  
739  
740 311 for GPB such as lactic acid bacteria and *Listeria monocytogenes* (Begley et al., 2005; Chateau  
741  
742 312 et al., 1994; Hyronimus et al., 2000). Finally, some species including those belonging to  
743  
744 313 *Chryseobacterium* sp., *Proteus* sp., *Halomonas* sp. and *Psychrobacter* sp., recently identified  
745  
746 314 as corresponding to the most frequent surface and/or core genera of French dairy products  
747  
748 315 (cheeses and milk, (Dugat-Bony et al., 2016)) did not survive well in the simulated gastric and  
749  
750 316 gastrointestinal media used in this study.

751  
752  
753  
754 317

### 755 318 *3.2 Adhesion analyses*

756  
757  
758 319 The observed adhesion capacities are presented in Table 3 as a mean of two biological  
759  
760 320 replicates. Results for all tested strains varied from 5 to 363 bacterial cells per 100 Caco-2 cells.  
761  
762 321 Value for attachment of *Lactobacillus rhamnosus* GG to Caco-2 cells were similar to that  
763  
764  
765  
766  
767

768  
769  
770 322 previously published (Gopal et al., 2001), thus confirming the validity of the present results.  
771  
772 323 The results for adherence of the two Gram negative species used as safe control strains were  
773  
774 324 low (<100 cells) as well for the control *E. coli* O157: H7 C267 (*stx1*-, *stx2*-, *eae*+) and twelve  
775  
776 325 other strains. The other remaining eight strains exhibited attachment > 100 cells and the most  
777  
778 326 adherent strain was *Halomonas venusta/alkaliphila/hydrothermalis* 4C1A with 363 cells per  
779  
780 327 100 Caco-2 cells, followed by 200, 152, 145, 141, 138, 114, 100 microbial units for *Alcaligenes*  
781  
782 328 *faecalis* 904, *Proteus sp* (close to *P. hauseri*) UCMA 3779, *Psychrobacter celer* 91,  
783  
784 329 *Pseudomonas group putida* VRBG 37.3, *Klebsiella oxytoca* 927, *Pseudomonas stutzeri* UCMA  
785  
786 330 3883 and *Citrobacter freundii* UCMA 4217, respectively. Adherence is a clue for probiotic  
787  
788 331 potential but is also the first step leading to potential cytotoxicity (dos Santos et al., 2015;  
789  
790 332 Pavlov et al., 2004; Pogačar et al., 2015). In the present study, adhesion capacity was overall  
791  
792 333 low for most of the tested strains and it was lower than previously tested strains of the same  
793  
794 334 species present in drinking water (Pavlov et al., 2004). The low level of adherence was also  
795  
796 335 confirmed by scanning electron microscopy (data not shown).  
797  
798  
799  
800

### 801 336 3.3 Bactericidal effect of human serum

802

803 337 The strains were described as sensitive or resistant to human serum after incubation in  
804  
805 338 the presence of 50% human serum for three hours in comparison to known resistant and  
806  
807 339 sensitive control strains of *Hafnia alvei* (e.g. in Table 4). Only 5 out of the 20 strain subsets  
808  
809 340 were sensitive, namely *Halomonas* B39, *Halomonas venusta/alkaliphila/hydrothermalis* 4C1A,  
810  
811 341 *Pseudomonas group putida* VRBG 37.3, *Psychrobacter celer* 91 and *Sphingobacterium sp.*  
812  
813 342 (close *S. faecium*) PCAi F2.5 (Table 3). Most of the studied strains showed resistance against  
814  
815 343 human serum as previously reported for GNB like *Acinetobacter sp.* (King et al., 2009). Serum  
816  
817 344 resistance is often related to capsules or lipopolysaccharides which can protect the bacteria from  
818  
819 345 entrance of the bactericidal compounds, but lipopolysaccharides are not solely responsible for  
820  
821 346 resistance (Wand et al., 2013). The complement system is a series of serum proteins interacting  
822  
823  
824  
825  
826

827  
828  
829 347 in a regulated sequence that could lead to bacterial cell death (Morgan et al., 2000). The  
830  
831 348 resistance mechanism could be due to a bacterial surface protein which binds the human factor  
832  
833 349 H (FH), and thereby inhibits complement deposition on the bacterial surface (Quin et al., 2006).  
834  
835

### 836 350 *3.4 Virulence of bacteria to Galleria mellonella*

838  
839 351 The 20 selected GNB and four control strains were tested for virulence in *G. mellonella*  
840  
841 352 (Table 3). All PBS-injected control larvae grew until the moth stage. Control strains, *E. coli*  
842  
843 353 Nissle 1917, *E. coli* K12 ATCC 10798, and *Lactobacillus rhamnosus* GG had LD 50 from  
844  
845 354 around  $10^7$  to  $>10^8$ , respectively. This range was considered as the reference non-toxic range,  
846  
847 355 since it was obtained for the three safe control strains. Thirteen GNB strains showed no  
848  
849 356 virulence score within this range. Among them *Hafnia alvei* (biogroup 1) B16 was found safe  
850  
851 357 which is reassuring as strains from this species are used as commercial ripening cultures for  
852  
853 358 many cheeses (Irlinger et al., 2012). Three strains, including *K. oxytoca* 927, *P. stutzeri* UCMA  
854  
855 359 3883, *Sphingobacterium* sp (close to *S. faecium*) PCAi F2.5 had intermediate LD 50 around  $6.5$   
856  
857 360  $10^6$  as observed for *E. coli* O157:H7 (*stx1-*, *stx2-*, *eae+*) C267 which was used as a control. Two  
858  
859 361 strains, *H. paralvei* 920 and *Proteus* sp (close to *P. hauseri*) UCMA 3780, were toxic to larvae  
860  
861 362 with a lower LD 50 around  $10^5$ . Two other strains were even more virulent, with LD50 value  
862  
863 363 lower than  $10^4$ , and corresponded to *Providencia heimbachae* GR4 and *Morganella morganii*  
864  
865 364 3A2A.  
866  
867

868 365 Assuming that *G. mellonella* larvae injection experiments are commonly used to detect  
869  
870 366 bacterial strains presenting virulence factors and that the virulence of many pathogens is similar  
871  
872 367 in wax moth larvae and mammals, including Humans (Desbois and Coote, 2012), it was  
873  
874 368 deduced that only few strains contained efficient virulent factors and could act as direct or  
875  
876 369 indirect pathogens. In the literature, a recent study showed that *Galleria mellonella* can also  
877  
878 370 provide significant insights into virulence mechanisms and that this can be applied to the study  
879  
880 371 of opportunistic human pathogens (Wand et al., 2013). Virulence clearly depended here on the  
881  
882  
883  
884  
885



886  
887  
888 372 considered strain but not on its origin as two *Proteus* sp. (close to *P. hauseri*) strains, 3779 and  
889  
890 373 3780, having the same origin, were very different in their virulence towards the wax moth  
891  
892 374 larvae. Only *Proteus* sp. (close to *P. hauseri*) UCMA 3780, *H. paralvei* 920, and even more *M.*  
893  
894 375 *morganii* 3A2A and *P. heimbachae* GR4 strains required the lowest concentrations to kill larvae  
895  
896 376 and can be considered as harbouring efficient virulence factors. Overall, the majority of the  
897  
898 377 strains tested for virulence in *Galleria mellonella* larvae were safe for this organism when  
899  
900 378 compared with the three control strains known to be safe for use in Humans through oral  
901  
902 379 absorption.  
903  
904  
905 380

### 907 381 3.5 Data analysis for safety assessment of GNB associated with traditional French cheeses

909 382 A compilation of selected results obtained in the present work (Table S1) provides a global  
910  
911 383 view about main safety characteristics of the tested GNB during simulated GIT transit after oral  
912  
913 384 ingestion of milk products and their potential virulence. In this table, the individual strains were  
914  
915 385 classified from safe to virulent based on the results obtained using the insect model *Galleria*  
916  
917 386 *mellonella* larvae. Then, *in vitro* results are presented by following the GIT transit progression  
918  
919 387 described in Figure 2 for cheese ingestion.  
920  
921

922 388 From the *in vivo* data, it was concluded that *Proteus* sp (close to *P. hauseri*) UCMA 3780, *M.*  
923  
924 389 *morganii* 3A2A, *H. paralvei* 920 and *P. heimbachae* GR4 strains presented some efficient  
925  
926 390 virulence factors. Among these four strains, *M. morganii* 3A2A and *H. paralvei* 920 survived  
927  
928 391 well in aerobic simulated gastric conditions (with milk and bile salts) while the two others  
929  
930 392 survived to a lesser extent. Only one strain, *H. paralvei* 920, did not grow at 37°C under  
931  
932 393 anaerobic conditions. PCA analysis (Figure S2) of the quantitative results given in Table S1,  
933  
934 394 for pH 2 as primary gastric stress, showed some negative correlation ( $r = -0.42$ ) between LD50  
935  
936 395 in wax moth larvae and survival in GIM-milk at pH 6.5 with bile salt (1.5g/L). So, the more  
937  
938 396 sensitive the strain to pH 2 (Figure S2A), the higher the value of LD50 (=the less toxic it was);  
939  
940  
941  
942  
943  
944

945  
946 397 at pH4 (Figure S2B), this negative correlation value was weaker ( $r = -0.29$ ) and it could be  
948  
949 398 explained by a lower number of strains sensitive to this less aggressive pH. Furthermore, at  
950  
951 399 pH4, although adherence to Caco-2 cells and survival were opposed on the F1 axis, no negative  
952  
954 400 correlation appeared ( $r = -0.21$ ) between them. Qualitative factors were not specifically  
955  
956 401 associated with any of the quantitative factors studied in Table S1.  
957  
958 402

959 403 *3.6 Safety assessment of GNB strains belonging to genera/species of strains assessed in this*  
961  
962 404 *study.*

963  
964 405 To assess the safety status of GNB strains of dairy origin studied here, a bibliographic  
965  
966 406 review on the potential human pathogenicity of strains belonging to the corresponding species,  
967  
968 407 was conducted and is listed in Table S3. Four bacteria, namely *Chryseobacterium* sp.,  
969  
970 408 *Psychrobacter celer*, *Sphingobacterium* sp., and *Stenotrophomonas maltophilia*, were so far  
971  
972 409 never reported in any clinical cases. Only individual clinical cases were reported for the GNB  
973  
974 410 species studied in Table S3 but not associated with food vectors. The only one exception was  
975  
976 411 for *Proteus* sp. that caused biogenic amine (BA) related intoxications (after eating BA  
977  
978 412 contaminated fish). BA could pose a safety risk for human health. Fortunately, the  
979  
980 413 corresponding clinical effect appeared to be very moderate except in rare cases and it could  
981  
982 414 explain that there is no regulation for BA in food, except for histamine levels in fish products.  
983  
984 415 Furthermore, a study (Delbès-Paus et al., 2012) involving eleven strains used *in vitro* in the  
985  
986 416 present study, reported that only negligible biogenic amine amounts were produced *in situ* (in  
987  
988 417 model cheeses), contrary to BA production observed *in vitro* by Coton et al., (2012).  
989  
990 418 Nevertheless, as BA production is a strain-dependant trait, strain effect and cheese type must  
991  
992 419 also be considered.  
993  
994  
995  
996 420

#### 998 421 **4. General discussion and conclusion**

999  
1000  
1001  
1002  
1003

1004  
1005  
1006 422 The safety status as well as contribution of most GNB to the cheese process is poorly  
1007  
1008 423 documented. In previous years, GNB were classically considered as indicators of hygienic  
1009  
1010 424 problems (Bockelmann et al., 2005; Tornadijo et al., 1993) and responsible for defects in cheese  
1011  
1012 425 texture and flavor due to the production of extracellular proteolytic and lipolytic enzymes  
1013  
1014 426 (Amato et al., 2012; Martins et al., 2006). For example, dairy related *Pseudomonas* spp. strains  
1015  
1016 427 have been shown to produce volatile compounds such as ethyl esters and alcohols that may  
1017  
1018 428 negatively affect cheese sensory characteristics (Arslan et al., 2011; Morales et al., 2005). The  
1019  
1020 429 idea that GNB species can be normal and interesting elements of cheese microbiota is more  
1021  
1022 430 recent (Larpin-Laborde et al., 2011). In this sense, for example, *Proteus* sp. (close to *P. hauseri*)  
1023  
1024 431 and *Psychrobacter* sp. have been shown to significantly contribute to flavor production in smear  
1025  
1026 432 cheeses (Deetae et al., 2007; Irlinger et al., 2012) raising questions about the potential beneficial  
1027  
1028 433 effects of GNB in general. Recently, it was confirmed that some GNB strains have interesting  
1029  
1030 434 specific technological properties (Schmitz-Esser et al., 2018).  
1031  
1032  
1033

1034 435 In this context, to delve deeper into the knowledge about GNB, it was necessary to  
1035  
1036 436 assess the safety status of these species. The present study was designed to evaluate the potential  
1037  
1038 437 health risks, if any, associated with the consumption of GNB present in cheeses (and coming  
1039  
1040 438 from raw milk, milk, or the milking environment), in simulated body conditions. However, risk  
1041  
1042 439 analysis is complex. You need to know, successively, the danger and its characteristics, the  
1043  
1044 440 exposure level and to set a safe threshold value, then, and only in that case, risk assessment can  
1045  
1046 441 be performed. In this study, from the present data and by using a general exposure context, only  
1047  
1048 442 part of risk analysis (i.e. the qualification of the danger) was done through an *in vivo* study of  
1049  
1050 443 pathogenicity in *Galleria mellonella* larvae and by using *in vitro* determinations of bacterial  
1051  
1052 444 growth in the simulated GIT, on Caco-2 cells and in the presence of human serum. The presence  
1053  
1054 445 of virulent factors was suggested here for some specific GNB strains but their virulence in human  
1055  
1056  
1057  
1058  
1059  
1060  
1061  
1062

1063  
1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1099  
1100  
1101  
1102  
1103  
1104  
1105  
1106  
1107  
1108  
1109  
1110  
1111  
1112  
1113  
1114  
1115  
1116  
1117  
1118  
1119  
1120  
1121

446 gut also depends on their active dose which is modulated by the effect of different gut barriers,  
447 as reported in Figure 2.

448 Data from human consumption can however be considered. In fact, the safety status of  
449 cheese in healthy consumers has been recognized for a long time, despite or thanks to microbial  
450 strain diversity and microbial interactions in the cheese microbiota that evolves during  
451 fermentation and ripening (Imran et al., 2012). Although cheeses have been implicated in 7.8%  
452 of strongly evidenced foodborne outbreaks due to zoonotic agents in the EU (European Food  
453 Safety Authority and European Centre for Disease Prevention and Control, 2016; Imran et al.,  
454 2012), none of the GNB strains studied here (nor other strains belonging to the same species  
455 than those assessed in this study) have been determined to be responsible for any known  
456 foodborne outbreaks associated to cheeses. This was also confirmed by our literature analysis  
457 described in Table S3. However, two key food risk factors (potential antibiotic resistances and  
458 biogenic amine (BA) production, see Table S1) can modulate GNB safety. Assessment of these  
459 traits has already been discussed for the 20 selected strains as well as some others (Coton et al.,  
460 2012). Particularly of interest is the variable antibiotic resistance profiles observed among  
461 certain GNB strains since their genetic material may harbour antibiotic resistance associated  
462 genes that may be potentially transferable. Additionally, the presence of potentially  
463 transferable virulence factors in cheese GNB strains must also be underlined.

464 Up until now, the absence of cheese foodborne outbreaks, might be explained by the positive  
465 bioactive impact of the cheese microbial community, as previously demonstrated in a model  
466 cheese ecosystem against *Listeria monocytogenes* (Imran et al., 2013). Additionally, it was  
467 also recently shown that *Hafnia alvei* B16 had an inhibitory effect in a model cheese  
468 ecosystem against *Escherichia coli* O26:H11 (Callon et al., 2016; Delbès-Paus et al., 2013).  
469 Multiple dynamic interactions between GIT microbiota, cheese matrix and its initial  
470 associated microbiota (detailed in Figure 2) must be taken into account. Among these barriers,

1122  
1123  
1124 471 and of special importance is the gut microbiota which was shown to play a crucial role for  
1125  
1126 472 protection against some pathogenic bacteria (Ubeda et al., 2017). This dense resident  
1127  
1128 473 microbial community, referred as the commensal microbiota, has a major function of  
1129  
1130 474 protection against colonization of pathogens and this ability to restrain pathogen growth  
1131  
1132  
1133 475 include competitive metabolic interaction, localization to intestinal niches, and induction of  
1134  
1135 476 host immune responses (Kumada et al., 2013).

1136  
1137 477 The impact of the dairy matrix on microbial interactions can also lead to changes in  
1138  
1139 478 functionality such as a means to combat against pathogens as already described (Imran et al.,  
1140  
1141 479 2012; Imran and Desmasures, 2015). In very recent scientific reports, it has been demonstrated  
1142  
1143 480 that the dairy matrix also has a significant impact on the survival and immunostimulant ability  
1144  
1145 481 (treatment of Inflammatory Bowel Diseases) of microbiota in a model gastrointestinal tract and  
1146  
1147 482 mouse model (Adouard et al., 2016; Foligné et al., 2016; Plé et al., 2015). The role of biliary  
1148  
1149 483 salts to prevent microbial adherence (Begley et al., 2005) was also recently confirmed (Sanchez  
1150  
1151 484 et al., 2016). The role of metabolites appearing during digestion must also be detailed.

1152  
1153  
1154 485 *In silico* studies could of help to complete our understanding of these interactions, but  
1155  
1156 486 it is missing for the GNB studied here. Till now only the genome sequence of several other  
1157  
1158 487 cheese-related GNB, which include the strain *P. heimbachae* GR4, was determined in a  
1159  
1160 488 subsequent study (Almeida et al., 2014). It indicated that this strain, studied in this work, is not  
1161  
1162 489 closely related to the reference strain; an adaptation to the dairy environment could be supposed.  
1163  
1164 490 For the future, *in vivo* approaches using wax worm could be further combined with *in silico*  
1165  
1166 491 approaches to seek for pathogenicity factors and their potential mobility, in the genome  
1167  
1168 492 sequence of these strains.

1169  
1170  
1171 493 To conclude, presumption of safety of most GNB strains studied here was successfully  
1172  
1173 494 established, while some could harbour virulence factors. These factors (including some  
1174  
1175 495 antibiotic resistances) could be considered as a direct or indirect risk when dissemination to

1181  
1182  
1183  
1184  
1185  
1186  
1187  
1188  
1189  
1190  
1191  
1192  
1193  
1194  
1195  
1196  
1197  
1198  
1199  
1200  
1201  
1202  
1203  
1204  
1205  
1206  
1207  
1208  
1209  
1210  
1211  
1212  
1213  
1214  
1215  
1216  
1217  
1218  
1219  
1220  
1221  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1230  
1231  
1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239

496 other bacteria of the human gut occurred. The role of multiple dynamic interactions between  
497 cheese microbiota and GIT barriers including gut microbiota could be key factors explaining  
498 safe consumption of the corresponding cheeses. However, the present study, which was partly  
499 limited to *in vitro* studies of selected individual strains, cannot replace *in vivo* studies involving  
500 actual cheese and gut microbiota ecosystem. There is always a gap between microbial behavior  
501 in *in vitro* and *in vivo* conditions, especially taking into account the complexity of the  
502 considered systems (cheese, alimentary bolus, human body). The present approach is a first step  
503 for future studies aiming at elucidating the role of each microbial component (GNB or others)  
504 within such complex microbial communities.

505 However, the present study, which was partly limited to *in vitro* studies of selected  
506 individual strains, cannot replace *in vivo* studies involving actual cheese and gut microbiota  
507 ecosystem.

508  
509 **Acknowledgements**

510 This work was funded by the French “Agence Nationale de la Recherche” in the framework of  
511 the ANR Gramme project n° ANR-07-PNRA-010. The authors would like to thank Valérie Stahl  
512 for supplying GNB strains from the Aerial collection, Dr Ulrich Sonnerborn from Ardeypharm  
513 (Germany GmbH) for supplying *E. coli* Nissle 1917, Pr Vernozy-Rozand (Biomérieux, Lyon)  
514 for supplying *E. coli*.C267 strain. Authors also acknowledged the technical support from Dr.  
515 Marion Bernardeau (Unite MILA), Dr. Didier Goux (CMABio) and Marie Joel Jacob at the  
516 University of Caen (France). M. Imran Ph D scholarship at University of Caen Normandy was  
517 funded by the Higher Education Commission of Pakistan (HEC).

518  
519 **Declarations of interest conflict:** None

520  
521 **REFERENCES**

1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1290  
1291  
1292  
1293  
1294  
1295  
1296  
1297  
1298

522 Adouard, N., Magne, L., Cattenoz, T., Guillemain, H., Foligné, B., Picque, D., Bonnarme, P.,  
523 2016. Survival of cheese-ripening microorganisms in a dynamic simulator of the  
524 gastrointestinal tract. *Food Microbiol.* 53, 30–40.  
525 <https://doi.org/10.1016/j.fm.2015.03.002>

526 Almeida, M., Hébert, A., Abraham, A.-L., Rasmussen, S., Monnet, C., Pons, N., Delbès, C.,  
527 Loux, V., Batto, J.-M., Leonard, P., Kennedy, S., Ehrlich, S., Pop, M., Montel, M.-C.,  
528 Irlinger, F., Renault, P., 2014. Construction of a dairy microbial genome catalog opens  
529 new perspectives for the metagenomic analysis of dairy fermented products. *BMC*  
530 *Genomics* 15, 1101. <https://doi.org/10.1186/1471-2164-15-1101>

531 Amato, L., Ritschard, J.S., Kurtz, O., Arias-Roth, E., Lacroix, C., Schuppler, M., Meile, L.,  
532 2012. Microbial composition of defect smear – A problem evolving during foil-  
533 prepacked storage of red-smear cheeses. *Int. Dairy J.* 27, 77–85.  
534 <https://doi.org/10.1016/j.idairyj.2012.07.012>

535 Aperis, G., Burgwynfuchs, B., Anderson, C., Warner, J., Calderwood, S., Mylonakis, E., 2007.  
536 *Galleria mellonella* as a model host to study infection by the *Francisella tularensis* live  
537 vaccine strain. *Microbes Infect.* 9, 729–734.  
538 <https://doi.org/10.1016/j.micinf.2007.02.016>

539 Arslan, S., Eyi, A., Özdemir, F., 2011. Spoilage potentials and antimicrobial resistance of  
540 *Pseudomonas* spp. isolated from cheeses. *J. Dairy Sci.* 94, 5851–5856.  
541 <https://doi.org/10.3168/jds.2011-4676>

542 Begley, M., Gahan, C.G.M., Hill, C., 2005. The interaction between bacteria and bile. *FEMS*  
543 *Microbiol. Rev.* 29, 625–651. <https://doi.org/10.1016/j.femsre.2004.09.003>

544 Bockelmann, W., Willems, K.P., Neve, H., Heller, K.H., 2005. Cultures for the ripening of  
545 smear cheeses. *Int. Dairy J.* 15, 719–732. <https://doi.org/10.1016/j.idairyj.2004.08.022>

546 Callon, C., Arliguie, C., Montel, M.C., 2016. Control of Shigatoxin-producing *Escherichia coli*  
547 in cheese by dairy bacterial strains. *Food Microbiol.* 53, 63–70.  
548 <https://doi.org/10.1016/j.fm.2015.08.009>

549 Champion, O.L., Cooper, I.A.M., James, S.L., Ford, D., Karlyshev, A., Wren, B.W., Duffield,  
550 M., Oyston, P.C.F., Titball, R.W., 2009. *Galleria mellonella* as an alternative infection  
551 model for *Yersinia pseudotuberculosis*. *Microbiology* 155, 1516–1522.  
552 <https://doi.org/10.1099/mic.0.026823-0>

553 Chateau, N., Deschamps, A.M., Sassi, A.H., 1994. Heterogeneity of bile salts resistance in the  
554 *Lactobacillus* isolates of a probiotic consortium. *Lett. Appl. Microbiol.* 18, 42–44.  
555 <https://doi.org/10.1111/j.1472-765X.1994.tb00796.x>

556 Chaves-Lopez, C., De Angelis, M., Martuscelli, M., Serio, A., Paparella, A., Suzzi, G., 2006.  
557 Characterization of the Enterobacteriaceae isolated from an artisanal Italian ewe’s  
558 cheese (Pecorino Abruzzese). *J. Appl. Microbiol.* 101, 353–360.  
559 <https://doi.org/10.1111/j.1365-2672.2006.02941.x>

560 Clavel, T., Carlin, F., Dargaignaratz, C., Lairon, D., Nguyen-The, C., Schmitt, P., 2007. Effects  
561 of porcine bile on survival of *Bacillus cereus* vegetative cells and Haemolysin BL  
562 enterotoxin production in reconstituted human small intestine media: *B. cereus* and  
563 porcine bile. *J. Appl. Microbiol.* 103, 1568–1575. <https://doi.org/10.1111/j.1365-2672.2007.03410.x>

564 Clavel, T., Carlin, F., Lairon, D., Nguyen-The, C., Schmitt, P., 2004. Survival of *Bacillus cereus*  
565 spores and vegetative cells in acid media simulating human stomach. *J. Appl. Microbiol.*  
566 97, 214–219. <https://doi.org/10.1111/j.1365-2672.2004.02292.x>

567 Coton, M., Delbès-Paus, C., Irlinger, F., Desmasures, N., Le Fleche, A., Stahl, V., Montel, M.-  
568 C., Coton, E., 2012. Diversity and assessment of potential risk factors of Gram-negative  
569 isolates associated with French cheeses. *Food Microbiol.* 29, 88–98.  
570 <https://doi.org/10.1016/j.fm.2011.08.020>

571

1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1350  
1351  
1352  
1353  
1354  
1355  
1356  
1357

572 Deetae, P., Bonnarne, P., Spinnler, H.E., Helinck, S., 2007. Production of volatile aroma  
573 compounds by bacterial strains isolated from different surface-ripened French cheeses.  
574 Appl. Microbiol. Biotechnol. 76, 1161–1171. <https://doi.org/10.1007/s00253-007-1095-5>  
575  
576 Delbes, C., Ali-Mandjee, L., Montel, M.-C., 2007. Monitoring Bacterial Communities in Raw  
577 Milk and Cheese by Culture-Dependent and -Independent 16S rRNA Gene-Based  
578 Analyses. Appl. Environ. Microbiol. 73, 1882–1891.  
579 <https://doi.org/10.1128/AEM.01716-06>  
580 Delbès-Paus, C., Miszczycha, S., Ganet, S., Helinck, S., Veisseire, P., Pochet, S., Thévenot, D.,  
581 Montel, M.-C., 2013. Behavior of *Escherichia coli* O26:H11 in the presence of *Hafnia*  
582 *alvei* in a model cheese ecosystem. Int. J. Food Microbiol. 160, 212–218.  
583 <https://doi.org/10.1016/j.ijfoodmicro.2012.10.019>  
584 Delbès-Paus, C., Pochet, S., Helinck, S., Veisseire, P., Bord, C., Lebecque, A., Coton, M.,  
585 Desmaures, N., Coton, E., Irlinger, F., Montel, M.-C., 2012. Impact of Gram-negative  
586 bacteria in interaction with a complex microbial consortium on biogenic amine content  
587 and sensory characteristics of an uncooked pressed cheese. Food Microbiol. 30, 74–82.  
588 <https://doi.org/10.1016/j.fm.2011.12.008>  
589 Desbois, A.P., Coote, P.J., 2012. Utility of Greater Wax Moth Larva (*Galleria mellonella*) for  
590 Evaluating the Toxicity and Efficacy of New Antimicrobial Agents, in: Advances in  
591 Applied Microbiology. Elsevier, pp. 25–53. <https://doi.org/10.1016/B978-0-12-394805-2.00002-6>  
592  
593 Desmaures, N., Opportune, W., Guéguen, M., 1997. *Lactococcus* spp., yeasts and  
594 *Pseudomonas* spp. on teats and udders of milking cows as potential sources of milk  
595 contamination. Int. Dairy J. 7, 643–646. [https://doi.org/10.1016/S0958-6946\(97\)00042-3](https://doi.org/10.1016/S0958-6946(97)00042-3)  
596  
597 dos Santos, P.A., Pereira, A.C.M., Ferreira, A.F., de Mattos Alves, M.A., Rosa, A.C.P., Freitas-  
598 Almeida, A.C., 2015. Adhesion, invasion, intracellular survival and cytotoxic activity  
599 of strains of *Aeromonas* spp. in HEp-2, Caco-2 and T-84 cell lines. Antonie Van  
600 Leeuwenhoek 107, 1225–1236. <https://doi.org/10.1007/s10482-015-0416-4>  
601 Dugat-Bony, E., Garnier, L., Denonfoux, J., Ferreira, S., Sarthou, A.S., Bonnarne, P., Irlinger,  
602 F., 2016. Highlighting the microbial diversity of 12 French cheese varieties. Int. J. Food  
603 Microbiol. 238, 265–273. <https://doi.org/10.1016/j.ijfoodmicro.2016.09.026>  
604 EFSA Panel on Biological Hazards (BIOHAZ), 2014. Statement on the update of the list of  
605 QPS-recommended biological agents intentionally added to food or feed as notified to  
606 EFSA 1: Suitability of taxonomic units notified to EFSA until October 2014. EFSA J.  
607 12. <https://doi.org/10.2903/j.efsa.2014.3938>  
608 European Food Safety Authority, European Centre for Disease Prevention and Control, 2016.  
609 The European Union summary report on trends and sources of zoonoses, zoonotic  
610 agents and food-borne outbreaks in 2015. EFSA J. 14.  
611 <https://doi.org/10.2903/j.efsa.2016.4634>  
612 Fogh, J., Fogh, J.M., Orfeo, T., 1977. One hundred and twenty-seven cultured human tumor  
613 cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59, 221–226.  
614 Foligné, B., Parayre, S., Cheddani, R., Famelart, M.-H., Madec, M.-N., Plé, C., Breton, J.,  
615 Dewulf, J., Jan, G., Deutsch, S.-M., 2016. Immunomodulation properties of multi-  
616 species fermented milks. Food Microbiol. 53, 60–69.  
617 <https://doi.org/10.1016/j.fm.2015.04.002>  
618 Frétin, M., Martin, B., Rifa, E., Isabelle, V.-M., Pomiès, D., Ferlay, A., Montel, M.-C., Delbès,  
619 C., 2018. Bacterial community assembly from cow teat skin to ripened cheeses is  
620 influenced by grazing systems. Sci. Rep. 8, 200. <https://doi.org/10.1038/s41598-017-18447-y>  
621



1358  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1410  
1411  
1412  
1413  
1414  
1415  
1416

622 Gänzle, M.G., Hertel, C., van der Vossen, J.M., Hammes, W.P., 1999. Effect of bacteriocin-  
623 producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic  
624 model of the stomach and the small intestine. *Int. J. Food Microbiol.* 48, 21–35.  
625 Gopal, P.K., Prasad, J., Smart, J., Gill, H.S., 2001. *In vitro* adherence properties of  
626 *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their  
627 antagonistic activity against an enterotoxigenic *Escherichia coli*. *Int. J. Food Microbiol.*  
628 67, 207–216.  
629 Gori, K., Ryssel, M., Arneborg, N., Jespersen, L., 2013. Isolation and Identification of the  
630 Microbiota of Danish Farmhouse and Industrially Produced Surface-Ripened Cheeses.  
631 *Microb. Ecol.* 65, 602–615. <https://doi.org/10.1007/s00248-012-0138-3>  
632 Greene, J.D., Klaenhammer, T.R., 1994. Factors involved in adherence of lactobacilli to human  
633 Caco-2 cells. *Appl. Environ. Microbiol.* 60, 4487–4494.  
634 Hyronimus, B., Le Marrec, C., Sassi, A.H., Deschamps, A., 2000. Acid and bile tolerance of  
635 spore-forming lactic acid bacteria. *Int. J. Food Microbiol.* 61, 193–197.  
636 Imran, M., Bré, J.-M., Guéguen, M., Vernoux, J.-P., Desmaures, N., 2013. Reduced growth of  
637 *Listeria monocytogenes* in two model cheese microcosms is not associated with  
638 individual microbial strains. *Food Microbiol.* 33, 30–39.  
639 <https://doi.org/10.1016/j.fm.2012.08.008>  
640 Imran, M., Desmaures, N., 2015. Safety and Quality Aspects of Smear Ripened Cheeses, in:  
641 Bora, N., Dodd, C., Desmaures, N. (Eds.), *Diversity, Dynamics and Functional Role*  
642 *of Actinomycetes on European Smear Ripened Cheeses*. Springer International  
643 Publishing, Cham, pp. 199–215. [https://doi.org/10.1007/978-3-319-10464-5\\_9](https://doi.org/10.1007/978-3-319-10464-5_9)  
644 Imran, M., Desmaures, N., Vernoux, J.-P., 2012. Complex Microbial Communities as Part of  
645 Fermented Food Ecosystems and Beneficial Properties, in: Mehta, B., Kamal-Eldin, A.,  
646 Iwanski, R. (Eds.), *Fermentation*. CRC Press. <https://doi.org/10.1201/b11876-3>  
647 Irlinger, F., In Yung, S.A.Y., Sarthou, A.-S., Delbès-Paus, C., Montel, M.-C., Coton, E., Coton,  
648 M., Helinck, S., 2012. Ecological and aromatic impact of two Gram-negative bacteria  
649 (*Psychrobacter celer* and *Hafnia alvei*) inoculated as part of the whole microbial  
650 community of an experimental smear soft cheese. *Int. J. Food Microbiol.* 153, 332–338.  
651 <https://doi.org/10.1016/j.ijfoodmicro.2011.11.022>  
652 Irlinger, F., Mounier, J., 2009. Microbial interactions in cheese: implications for cheese quality  
653 and safety. *Curr. Opin. Biotechnol.* 20, 142–148.  
654 <https://doi.org/10.1016/j.copbio.2009.02.016>  
655 Jander, G., Rahme, L.G., Ausubel, F.M., 2000. Positive correlation between virulence of  
656 *Pseudomonas aeruginosa* mutants in mice and insects. *J. Bacteriol.* 182, 3843–3845.  
657 Jankowski, S., Rowiński, S., Cisowska, A., Gamian, A., 1996. The sensitivity of *Hafnia alvei*  
658 strains to the bactericidal effect of serum. *FEMS Immunol. Med. Microbiol.* 13, 59–64.  
659 Kable, M.E., Srisengfa, Y., Laird, M., Zaragoza, J., McLeod, J., Heidenreich, J., Marco, M.L.,  
660 2016. The Core and Seasonal Microbiota of Raw Bovine Milk in Tanker Trucks and the  
661 Impact of Transfer to a Milk Processing Facility. *mBio* 7, e00836-16.  
662 <https://doi.org/10.1128/mBio.00836-16>  
663 Kavanagh, K., Reeves, E.P., 2004. Exploiting the potential of insects for *in vivo* pathogenicity  
664 testing of microbial pathogens. *FEMS Microbiol. Rev.* 28, 101–112.  
665 <https://doi.org/10.1016/j.femsre.2003.09.002>  
666 Kamada, N., Chen, G.Y., Inohara, N., Núñez, G., 2013. Control of Pathogens and Pathobionts  
667 by the Gut Microbiota. *Nat Immunol.* 2013 July ; 14(7): 685–690. doi:10.1038/ni.2608.  
668 King, L.B., Swiatlo, E., Swiatlo, A., McDaniel, L.S., 2009. Serum resistance and biofilm  
669 formation in clinical isolates of *Acinetobacter baumannii*. *FEMS Immunol. Med.*  
670 *Microbiol.* 55, 414–421. <https://doi.org/10.1111/j.1574-695X.2009.00538.x>

1417  
1418  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1470  
1471  
1472  
1473  
1474  
1475

- 671 Larpin-Laborde, S., Imran, M., Bonaïti, C., Bora, N., Gelsomino, R., Goerges, S., Irlinger, F.,  
672 Goodfellow, M., Ward, A.C., Vancanneyt, M., Swings, J., Scherer, S., Guéguen, M.,  
673 Desmaures, N., 2011. Surface microbial consortia from Livarot, a French smear-  
674 ripened cheese. *Can. J. Microbiol.* 57, 651–660. <https://doi.org/10.1139/w11-050>
- 675 Lorke, D., 1983. A new approach to practical acute toxicity testing. *Arch. Toxicol.* 54, 275–  
676 287.
- 677 Maoz, A., Mayr, R., Scherer, S., 2003. Temporal Stability and Biodiversity of Two Complex  
678 Antilisterial Cheese-Ripening Microbial Consortia. *Appl. Environ. Microbiol.* 69,  
679 4012–4018. <https://doi.org/10.1128/AEM.69.7.4012-4018.2003>
- 680 Martín, J.F., Coton, M., 2017. Blue Cheese, in: *Fermented Foods in Health and Disease*  
681 *Prevention*. Elsevier, pp. 275–303. <https://doi.org/10.1016/B978-0-12-802309-9.00012-1>
- 682  
683 Martins, M.L., Pinto, C.L.O., Rocha, R.B., de Araújo, E.F., Vanetti, M.C.D., 2006. Genetic  
684 diversity of Gram-negative, proteolytic, psychrotrophic bacteria isolated from  
685 refrigerated raw milk. *Int. J. Food Microbiol.* 111, 144–148.  
686 <https://doi.org/10.1016/j.ijfoodmicro.2006.06.020>
- 687 Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmaures, N.,  
688 Berthier, F., 2014. Traditional cheeses: Rich and diverse microbiota with associated  
689 benefits. *Int. J. Food Microbiol.* 177, 136–154.  
690 <https://doi.org/10.1016/j.ijfoodmicro.2014.02.019>
- 691 Morales, P., Fernández-García, E., Nuñez, M., 2005. Production of volatile compounds in  
692 cheese by *Pseudomonas fragi* strains of dairy origin. *J. Food Prot.* 68, 1399–1407.
- 693 Morgan, B.P., Daha, M., Meri, S., Nicholson-Weller, A., 2000. Into the third century of  
694 complement research. *Immunol. Today* 21, 603–605.
- 695 Morton, D.B., Dunphy, G.B., Chadwick, J.S., 1987. Reactions of hemocytes of immune and  
696 non-immune *Galleria mellonella* larvae to *Proteus mirabilis*. *Dev. Comp. Immunol.* 11,  
697 47–55.
- 698 Mounier, J., Coton, M., Irlinger, F., Landaud, S., Bonnarme, P., 2017. Smear-Ripened Cheeses,  
699 in: *Cheese*. Elsevier, pp. 955–996. <https://doi.org/10.1016/B978-0-12-417012-4.00038-7>
- 700  
701 Mounier, J., Gelsomino, R., Goerges, S., Vancanneyt, M., Vandemeulebroecke, K., Hoste, B.,  
702 Scherer, S., Swings, J., Fitzgerald, G.F., Cogan, T.M., 2005. Surface Microflora of Four  
703 Smear-Ripened Cheeses. *Appl. Environ. Microbiol.* 71, 6489–6500.  
704 <https://doi.org/10.1128/AEM.71.11.6489-6500.2005>
- 705 Mounier, J., Goerges, S., Gelsomino, R., Vancanneyt, M., Vandemeulebroecke, K., Hoste, B.,  
706 Brennan, N.M., Scherer, S., Swings, J., Fitzgerald, G.F., Cogan, T.M., 2006. Sources of  
707 the adventitious microflora of a smear-ripened cheese. *J. Appl. Microbiol.* 101, 668–  
708 681. <https://doi.org/10.1111/j.1365-2672.2006.02922.x>
- 709 Mounier, J., Monnet, C., Jacques, N., Antoinette, A., Irlinger, F., 2009. Assessment of the  
710 microbial diversity at the surface of Livarot cheese using culture-dependent and  
711 independent approaches. *Int. J. Food Microbiol.* 133, 31–37.  
712 <https://doi.org/10.1016/j.ijfoodmicro.2009.04.020>
- 713 Nicoletti, M., Iacobino, A., Prosseda, G., Fiscarelli, E., Zarrilli, R., De Carolis, E., Petrucca, A.,  
714 Nencioni, L., Colonna, B., Casalino, M., 2011. *Stenotrophomonas maltophilia* strains  
715 from cystic fibrosis patients: Genomic variability and molecular characterization of  
716 some virulence determinants. *Int. J. Med. Microbiol.* 301, 34–43.  
717 <https://doi.org/10.1016/j.ijmm.2010.07.003>
- 718 Pavlov, D., de Wet, C.M., Grabow, W.O., Ehlers, M., 2004. Potentially pathogenic features  
719 of heterotrophic plate count bacteria isolated from treated and untreated drinking water.  
720 *Int. J. Food Microbiol.* 92, 275–287. <https://doi.org/10.1016/j.ijfoodmicro.2003.08.018>

1476  
1477  
1478  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1530  
1531  
1532  
1533  
1534

- 721 Pinto, M., Robine-Leon, S., Appay, M., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B.,  
722 Simon-Assmann, P., Haffen, K., Fogh, J., Zweibaum, A., 1983. Enterocyte-like  
723 differentiation and polarization of the Human colon carcinoma cell line Caco-2 in culture  
724 47, 323–330.
- 725 Plé, C., Adouard, N., Breton, J., Dewulf, J., Pot, B., Bonnarme, P., Foligné, B., 2015. Designing  
726 specific cheese-ripening ecosystems to shape the immune effects of dairy products? J.  
727 Funct. Foods 12, 219–229. <https://doi.org/10.1016/j.jff.2014.11.021>
- 728 Pogačar, M.Š., Klančnik, A., Bucar, F., Langerholc, T., Možina, S.S., 2015. *Alpinia katsumadai*  
729 Extracts Inhibit Adhesion and Invasion of *Campylobacter jejuni* in Animal and Human  
730 Foetal Small Intestine Cell Lines: Inhibition of *Campylobacter* Adhesion in Cell Lines.  
731 Phytother. Res. 29, 1585–1589. <https://doi.org/10.1002/ptr.5396>
- 732 Quin, L.R., Onwubiko, C., Carmicle, S., McDaniel, L.S., 2006. Interaction of clinical isolates  
733 of *Streptococcus pneumoniae* with human complement factor H. FEMS Microbiol. Lett.  
734 264, 98–103. <https://doi.org/10.1111/j.1574-6968.2006.00439.x>
- 735 Ramarao, N., Nielsen-Leroux, C., Lereclus, D., 2012. The Insect *Galleria mellonella* as a  
736 Powerful Infection Model to Investigate Bacterial Pathogenesis. J. Vis. Exp.  
737 <https://doi.org/10.3791/4392>
- 738 Salamitou, S., Ramisse, F., Brehélin, M., Bourguet, D., Gilois, N., Gominet, M., Hernandez,  
739 E., Lereclus, D., 2000. The plcR regulon is involved in the opportunistic properties of  
740 *Bacillus thuringiensis* and *Bacillus cereus* in mice and insects. Microbiol. Read. Engl.  
741 146 ( Pt 11), 2825–2832. <https://doi.org/10.1099/00221287-146-11-2825>
- 742 Sanchez, L.M., Cheng, A.T., Warner, C.J.A., Townsley, L., Peach, K.C., Navarro, G., Shikuma,  
743 N.J., Bray, W.M., Riener, R.M., Yildiz, F.H., Linington, R.G., 2016. Biofilm Formation  
744 and Detachment in Gram-Negative Pathogens Is Modulated by Select Bile Acids. PLOS  
745 ONE 11, e0149603. <https://doi.org/10.1371/journal.pone.0149603>
- 746 Schmitz-Esser, S., Dzieciol, M., Nischler, E., Schornsteiner, E., Bereuter, O., Mann, E.,  
747 Wagner, M., 2018. Abundance and potential contribution of Gram-negative cheese rind  
748 bacteria from Austrian artisanal hard cheeses. Int. J. Food Microbiol. 266, 95–103.  
749 <https://doi.org/10.1016/j.ijfoodmicro.2017.11.013>
- 750 Shimakawa, Y., Matsubara, S., Yuki, N., Ikeda, M., Ishikawa, F., 2003. Evaluation of  
751 *Bifidobacterium breve* strain Yakult-fermented soymilk as a probiotic food. Int. J. Food  
752 Microbiol. 81, 131–136.
- 753 Tareb, R., Bernardeau, M., Gueguen, M., Vernoux, J.-P., 2013. *In vitro* characterization of  
754 aggregation and adhesion properties of viable and heat-killed forms of two probiotic  
755 *Lactobacillus* strains and interaction with foodborne zoonotic bacteria, especially  
756 *Campylobacter jejuni*. J. Med. Microbiol. 62, 637–649.  
757 <https://doi.org/10.1099/jmm.0.049965-0>
- 758 Tornadijo, E., Fresno, J.M., Carballo, J., Martín-Sarmiento, R., 1993. Study of  
759 Enterobacteriaceae throughout the manufacturing and ripening of hard goats' cheese. J.  
760 Appl. Bacteriol. 75, 240–246. <https://doi.org/10.1111/j.1365-2672.1993.tb02772.x>
- 761 Vernozy-Rozand, C., Feng, P., Montet, M.-P., Ray-Gueniot, S., Villard, L., Bavai, C., Meyrand,  
762 A., Mazuy, C., Atrache, V., 2000. Detection of *Escherichia coli* O157:H7 in heifers'  
763 faecal samples using an automated immunoconcentration system. Lett. Appl. Microbiol.  
764 30, 217–222. <https://doi.org/10.1046/j.1472-765x.2000.00702.x>
- 765 Walters, J.B., Ratcliffe, N.A., 1983. Studies on the *in vivo* cellular reactions of insects: Fate of  
766 pathogenic and non-pathogenic bacteria in *Galleria mellonella* nodules. J. Insect  
767 Physiol. 29, 417–424. [https://doi.org/10.1016/0022-1910\(83\)90069-0](https://doi.org/10.1016/0022-1910(83)90069-0)
- 768 Wand, M.E., McCowen, J.W.I., Nugent, P.G., Sutton, J.M., 2013. Complex interactions of  
769 *Klebsiella pneumoniae* with the host immune system in a *Galleria mellonella* infection  
770 model. J. Med. Microbiol. 62, 1790–1798. <https://doi.org/10.1099/jmm.0.063032-0>

1535  
1536  
1537  
1538  
1539  
1540  
1541  
1542  
1543  
1544  
1545  
1546  
1547  
1548  
1549  
1550  
1551  
1552  
1553  
1554  
1555  
1556  
1557  
1558  
1559  
1560  
1561  
1562  
1563  
1564  
1565  
1566  
1567  
1568  
1569  
1570  
1571  
1572  
1573  
1574  
1575  
1576  
1577  
1578  
1579  
1580  
1581  
1582  
1583  
1584  
1585  
1586  
1587  
1588  
1589  
1590  
1591  
1592  
1593

771 Wassenaar, T.M., 2016. Insights from 100 years of research with probiotic *E. coli*. Eur. J.  
772 Microbiol. Immunol. 6, 147–161. <https://doi.org/10.1556/1886.2016.00029>  
773 Wolfe, B.E., Button, J.E., Santarelli, M., Dutton, R.J., 2014. Cheese rind communities provide  
774 tractable systems for *in situ* and *in vitro* studies of microbial diversity. Cell 158, 422–  
775 433. <https://doi.org/10.1016/j.cell.2014.05.041>  
776 Ubeda, C., Djukovic, A., Isaac, S., 2017. Roles of the intestinal microbiota in pathogen  
777 protection. Clinical & Translational Immunology (2017) 6, e128;  
778 doi:10.1038/cti.2017.2  
779 Zhao, X., Zhong, J., Wei, C., Lin, C.-W., Ding T., 2017. Current Perspectives on Viable but  
780 Non-culturable State in Foodborne Pathogens. Front. Microbiol. 8:580.  
781 doi:10.3389/fmicb.2017.00580  
782

783  
784  
785

1594  
1595  
1596  
1597  
1598  
1599  
1600  
1601  
1602  
1603  
1604  
1605  
1606  
1607  
1608  
1609  
1610  
1611  
1612  
1613  
1614  
1615  
1616  
1617  
1618  
1619  
1620  
1621  
1622  
1623  
1624  
1625  
1626  
1627  
1628  
1629  
1630  
1631  
1632  
1633  
1634  
1635  
1636  
1637  
1638  
1639  
1640  
1641  
1642  
1643  
1644  
1645  
1646  
1647  
1648  
1649  
1650  
1651  
1652

786 Table 1. Identification of representative Gram-negative bacterial strains of dairy origin  
787 used in this study. Growth in anaerobic or aerobic conditions at 37°C was assessed in the  
788 present study.  
789  
790

Species	Code	Dairy source	Growth in anaerobic/aerobic condition at 37°C*
<i>Acinetobacter</i> close to <i>A. genospecies 3</i>	PCAi E6.10	Milk from Salers cow	-/+
<i>Alcaligenes faecalis</i>	904	Smear-ripened cheese	-/-
<i>Chryseobacterium</i> close to <i>C. bovis</i>	Pi18	Uncooked pressed cheese	+/+
<i>Citrobacter freundii</i>	UCMA 4217	Smear-ripened cheese	+/+
<i>Hafnia alvei</i> (biogroup 1)	B16	Smear-ripened cheese	+/+
<i>Hafnia paralvei</i>	920	Smear-ripened cheese	-/+
<i>Halomonas venusta/ alkaliphila/ hydrothermalis</i>	4C1A	Smear-ripened cheese	+/+
<i>Halomonas</i> sp. nov.	B39	Smear-ripened cheese	-/+
<i>Klebsiella oxytoca</i>	927	Smear-ripened cheese	+/+
<i>Morganella morganii</i>	3A2A	Smear-ripened cheese	+/+
<i>Pantoea agglomerans</i>	PCA Q6.3	Milk	+/+
<i>Proteus</i> sp. close to <i>P. hauseri</i>	UCMA 3779	Smear-ripened cheese	-/+
<i>Proteus</i> sp. close to <i>P. hauseri</i>	UCMA 3780	Smear-ripened cheese	+/+
<i>Providencia heimbachae</i>	GR4	Smear-ripened cheese	+/+
<i>Pseudomonas</i> group <i>putida</i>	VRBG37.3	Milk	+/+
<i>Pseudomonas</i> group <i>putida</i>	CV30.6	Milk	-/+
<i>Pseudomonas stutzeri</i>	UCMA 3883	Smear-ripened cheese	-/+
<i>Psychrobacter celer</i>	91	Mould-ripened soft cheese	-/+
<i>Sphingobacterium</i> sp. close to <i>S. faecium</i>	PCAi F2.5	Milk	+/+
<i>Stenotrophomonas maltophilia/rhizophila</i>	PCAi D6.5	Milk	+/+

791 \*all strains grew at 25°C in aerobic conditions

**Table 2. Survival of Gram-negative bacteria of dairy origin (inoculated at  $N_0=10^6$  cfu/ml) in *in vitro* simulated conditions related to gastric media (GM) and gastrointestinal (GIM) stress. Results are presented as the log (N/N<sub>0</sub>) where N is the number of CFU/ml after 3h gastric stress (GM-3) followed by 28h gastrointestinal stress simulation, with or without bile salts (GIM-28), corresponding to a total of 31 h of (GM+GIM) stress.**

Gram negative bacterial strains	Bile g/L	pH 4.0 at T <sub>0</sub>				pH 2.0 at T <sub>0</sub>			
		GM-JB		GM-milk		GM-JB		GM-milk	
		Time (h) of exposure with pH change to 6.5 at T+3h							
		3	31	3	31	3	31	3	31
<i>Acinetobacter</i> (close to <i>A. genospecies 3</i> ) PCAi E6.10	0	0.49	2.72	0.01	3.23	0.44	2.66	-5.74	-5.74
	1.5		3.01		2.62		0.73		-2.84
<i>Alcaligenes faecalis</i> 904	0	-5.20	-5.20	-4.81	-4.81	-3.30	-3.30	-5.20	-5.20
	1.5		-5.20		-4.81		-3.30		-5.20
<i>Chryseobacterium</i> (close to <i>C. bovis</i> ) Pi18	0	0.22	1.15	0.06	-1.48	-2.24	-5.84	-0.17	-3.55
	1.5		2.57		-1.47		-5.84		-5.85
<i>Citrobacter freundii</i> UCMA 4217	0	-0.11	4.04	0.85	3.25	-5.55	-2.94	-5.85	-2.85
	1.5		3.91		2.95		-2.77		-2.64
<i>Hafnia alvei</i> (biogroup 1) B16	0	0.40	3.43	0.53	3.16	-5.90	-5.90	-6.06	-2.86
	1.5		3.53		3.08		-5.90		-2.99
<i>Hafnia paralvei</i> 920	0	-0.48	3.26	0.15	3.12	-0.32	-5.99	-0.11	2.56
	1.5		3.14		3.46		-5.99		2.95
<i>Halomonas venusta/alkaliphila/hydrothermas</i> 4C1A	0	0.65	3.10	-1.28	2.03	0.92	3.55	-0.39	-5.82
	1.5		3.23		1.73		3.62		-5.82
<i>Halomonas</i> sp. nov. B39	0	-0.10	3.91	0.22	3.64	-5.66	-1.03	-0.17	3.87
	1.5		3.89		3.40		-0.88		3.84
<i>Klebsiella oxytoca</i> 927	0	-0.62	3.86	-0.26	3.59	-3.60	-3.60	-5.55	0.95
	1.5		3.64		3.93		-3.60		1.24
<i>Morganella morganii</i> 3A2A	0	-0.29	2.75	-0.34	-0.13	-0.93	-6.24	-0.06	-6.12
	1.5		2.85		-0.07		-3.64		0.20
<i>Pantoea agglomerans</i> PCA Q6.3	0	0.40	3.37	0.23	3.17	0.51	3.50	-5.41	-0.94
	1.5		3.42		3.19		3.42		-0.93
<i>Proteus</i> sp. (close <i>P. hauseri</i> ) UCMA 3779	0	-0.24	2.91	0.21	2.86	-6.00	-6.00	-6.70	-6.70
	1.5		3.47		2.15		-6.00		-6.70
<i>Proteus</i> sp. (close <i>P. hauseri</i> ) UCMA 3780	0	-0.05	3.41	-0.54	-0.74	-5.83	-5.83	-1.39	2.00
	1.5		3.90		2.90		-5.83		2.17
<i>Providencia heimbachae</i> GR4	0	0.19	2.87	-0.17	0.86	0.58	3.09	-0.46	-2.97
	1.5		2.76		0.98		2.19		-2.85
<i>Pseudomonas</i> group <i>putida</i> VRBG37.3	0	-1.04	-5.45	-1.99	-5.59	-5.61	-3.31	-4.20	-4.20
	1.5		2.56		-3.29		-2.83		-4.20
<i>Pseudomonas</i> group <i>putida</i> CV30.6	0	0.38	3.44	-0.13	2.61	-6.38	-6.38	-0.32	-6.31
	1.5		3.37		3.01		-6.38		-6.31
<i>Pseudomonas stutzeri</i> UCMA 3883	0	0.68	3.97	0.16	3.30	-5.92	-5.92	-5.75	-5.75
	1.5		3.34		4.45		-5.92		-5.75
<i>Psychrobacter celer</i> 91	0	0.47	3.26	-0.16	3.05	-6.05	-6.05	-5.68	-5.68
	1.5		3.45		3.58		-6.05		-5.68
<i>Sphingobacterium</i> sp. (close to <i>S. faecium</i> ) PCAi F2.5	0	0.20	3.56	-1.32	2.71	-0.36	3.90	-5.30	-2.15
	1.5		3.63		2.73		3.28		-1.74
<i>Stenotrophomonas maltophilia/rhizophila</i> PCAi D6.5	0	-0.23	4.29	-0.52	0.26	-4.15	-4.15	-5.14	-2.84
	1.5		3.93		-2.79		-4.15		-5.14

1712  
1713  
1714  
1715  
1716  
1717  
1718  
1719  
1720  
1721  
1722  
1723  
1724  
1725  
1726  
1727  
1728  
1729  
1730  
1731  
1732  
1733  
1734  
1735  
1736  
1737  
1738  
1739  
1740  
1741  
1742  
1743  
1744  
1745  
1746  
1747  
1748  
1749  
1750  
1751  
1752  
1753  
1754  
1755  
1756  
1757  
1758  
1759  
1760  
1761  
1762  
1763  
1764  
1765  
1766  
1767  
1768  
1769  
1770

800  
801  
802  
803

Table 3. Adhesion to Caco-2 cells, sensitivity to human serum and increasing *in vivo* pathogenicity on wax moth worm of the tested Gram-negative bacteria of dairy origin.

Identification	No. bacterial cell adhering to 100 Caco-2*	Sensitivity to human serum	50% lethal dose at 72 h (CFU/g of larva*)
<b>Control strains for safety</b>			
<i>Lactobacillus rhamnosus</i> GG (Gram +)	200	nd	7.00 10 <sup>8</sup>
<i>Escherichia coli</i> Nissle 1917	23	nd	1.70 10 <sup>7</sup>
<i>Escherichia coli</i> K12 ATCC 10798	65	nd	3.16 10 <sup>7</sup>
<b>Strain with intimin</b>			
<i>Escherichia coli</i> O157:H7 C267 ( <i>eaec</i> <sup>+</sup> , <i>stx</i> <sup>-</sup> )	35	nd	1.46 10 <sup>6</sup>
<b>Dairy strains</b>			
<i>Chryseobacterium</i> sp (close to <i>C. bovis</i> ) Pi18	73	R	3.71 10 <sup>8</sup>
<i>Psychrobacter celer</i> 91	145	S	1.41 10 <sup>8</sup>
<i>Alcaligenes faecalis</i> 904	200	R	9.83 10 <sup>7</sup>
<i>Proteus</i> sp. (close to <i>P. hauseri</i> ) UCMA 3779	58	R	9.78 10 <sup>7</sup>
<i>Acinetobacter</i> sp. (close to <i>A. genospecies</i> 3) PCAi E6.10	33	R	8.82 10 <sup>7</sup>
<i>Halomonas venusta/alkaliphila/hydrothermalis</i> 4C1A	363	S	6.06 10 <sup>7</sup>
<i>Pantoea agglomerans</i> PCA Q6.3	63	R	5.91 10 <sup>7</sup>
<i>Halomonas</i> sp. nov. B39	30	S	2.54 10 <sup>7</sup>
<i>Citrobacter freundii</i> UCMA 4217	100	R	2.18 10 <sup>7</sup>
<i>Pseudomonas</i> group <i>putida</i> VRBG 37.3	141	S	1.91 10 <sup>7</sup>
<i>Stenotrophomonas maltophilia/rhizophila</i> PCAi D6.5	70	R	1.33 10 <sup>7</sup>
<i>Pseudomonas</i> group <i>putida</i> CV30.6	5	R	1.25 10 <sup>7</sup>
<i>Hafnia alvei</i> biogroup 1 B16	43	R	9.48 10 <sup>6</sup>
<i>Pseudomonas stutzeri</i> UCMA 3883	114	R	6.57 10 <sup>6</sup>
<i>Sphingobacterium</i> sp. (close to <i>S. faecium</i> ) PCAi F2.5	45	S	6.57 10 <sup>6</sup>
<i>Klebsiella oxytoca</i> 927	138	R	6.13 10 <sup>6</sup>
<i>Hafnia paralvei</i> 920	36	R	2.80 10 <sup>5</sup>
<i>Proteus</i> sp. (close to <i>P. hauseri</i> ) UCMA 3780	152	R	9.63 10 <sup>4</sup>
<i>Providencia heimbachae</i> GR4	49	R	< 1.6 10 <sup>4</sup>
<i>Morganella morganii</i> 3A2A	54	R	< 1.16 10 <sup>4</sup>

804  
805  
806  
807

nd: not determined, R: resistant, S: sensitivite  
\*results are mean of two separate experiments

1771  
 1772  
 1773 808  
 1774 809  
 1775 810  
 1776 811  
 1777 812  
 1778  
 1779  
 1780  
 1781  
 1782  
 1783  
 1784  
 1785  
 1786  
 1787  
 1788  
 1789  
 1790  
 1791  
 1792  
 1793 813  
 1794  
 1795  
 1796  
 1797  
 1798  
 1799  
 1800  
 1801  
 1802  
 1803  
 1804  
 1805  
 1806  
 1807  
 1808  
 1809  
 1810  
 1811  
 1812  
 1813  
 1814  
 1815  
 1816  
 1817  
 1818  
 1819  
 1820  
 1821  
 1822  
 1823  
 1824  
 1825  
 1826  
 1827  
 1828  
 1829

**Table 4. Impact of human serum on Gram-negative bacteria (GNB) strains after none and 3 hours contact time: examples of results for a sensitive strain (*Pseudomonas* group *putida* VRBG 37.3) and a resistant strain (*Chryseobacterium* sp. (close to *C. bovis*) Pi18).**

Dilution	<i>Chryseobacterium</i> sp. (close to <i>C. bovis</i> ) Pi18 (number of colonies)	<i>Pseudomonas putida</i> VRBG 37.3 (number of colonies)	Control sensitive (number of colonies)	Control Resistant (number of colonies)
<b>At 0 hour</b>				
10 <sup>-1</sup>	>1000	>1000	>1000	>1000
10 <sup>-2</sup>	>1000	141	>1000	>1000
10 <sup>-3</sup>	354	6	42	152
10 <sup>-4</sup>	42	0	9	16
<b>At 3<sup>rd</sup> hour</b>				
10 <sup>-1</sup>	>1000	0	0	>1000
10 <sup>-2</sup>	>1000	0	0	>1000
10 <sup>-3</sup>	>1000	0	0	>1000
10 <sup>-4</sup>	135	0	0	426



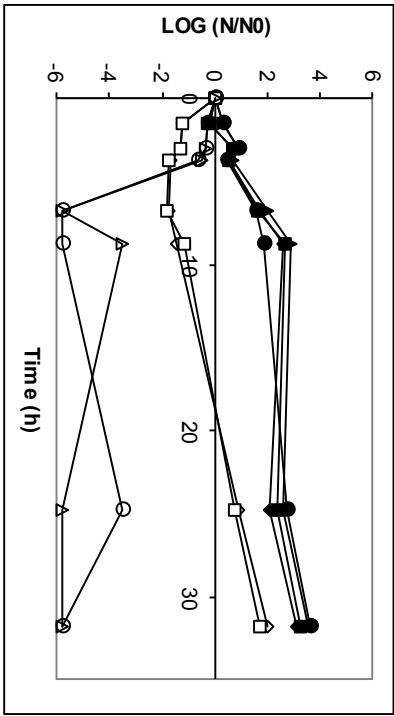
## List of Figures Captions

**Figure 1.** Changes in counts of *Halomonas venusta/alkaliphila/hydrothermalis* 4C1A (A), *Chryseobacterium* sp. close to *C. bovis* Pi18 (B), and *Protetis* sp. (close *P. hauseri*) UCMA 3779 (C), during gastric (from T0h primary adjusted to pH 2 or 4 → T3h) and gastrointestinal stress (from T3h with addition of new media initially adjusted pH 6.5 → T31h), GIM-JB (closed symbols) or GIM-milk (open symbols) media primary adjusted to pH 4.0 with 0 g/l bile salts (◆, ◇) and 1.5 g/l bile salts (■, □) or primary adjusted to pH 2.0 with 0 g/l bile salts (▲, Δ) and 1.5 g/l bile salts (●, ○). Experimental replicate for these 4 representative strains gave similar results.

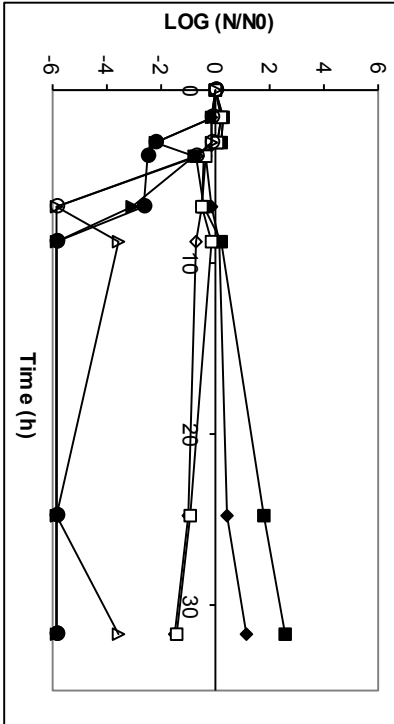
**Figure 2:** Scheme of main body barriers observed during human GIT transit of fermented cheese (1; A to F). For each barriers, beneficial (+) or adverse (-) effects involved in safety of ingested food during its GIT transit were highlighted (2; A to F).

Figure 1

A



B



C

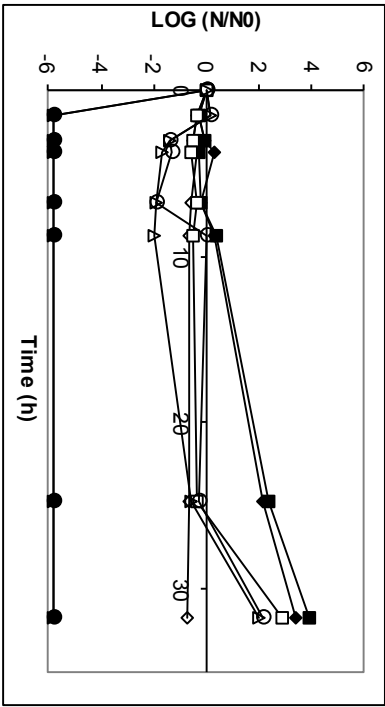
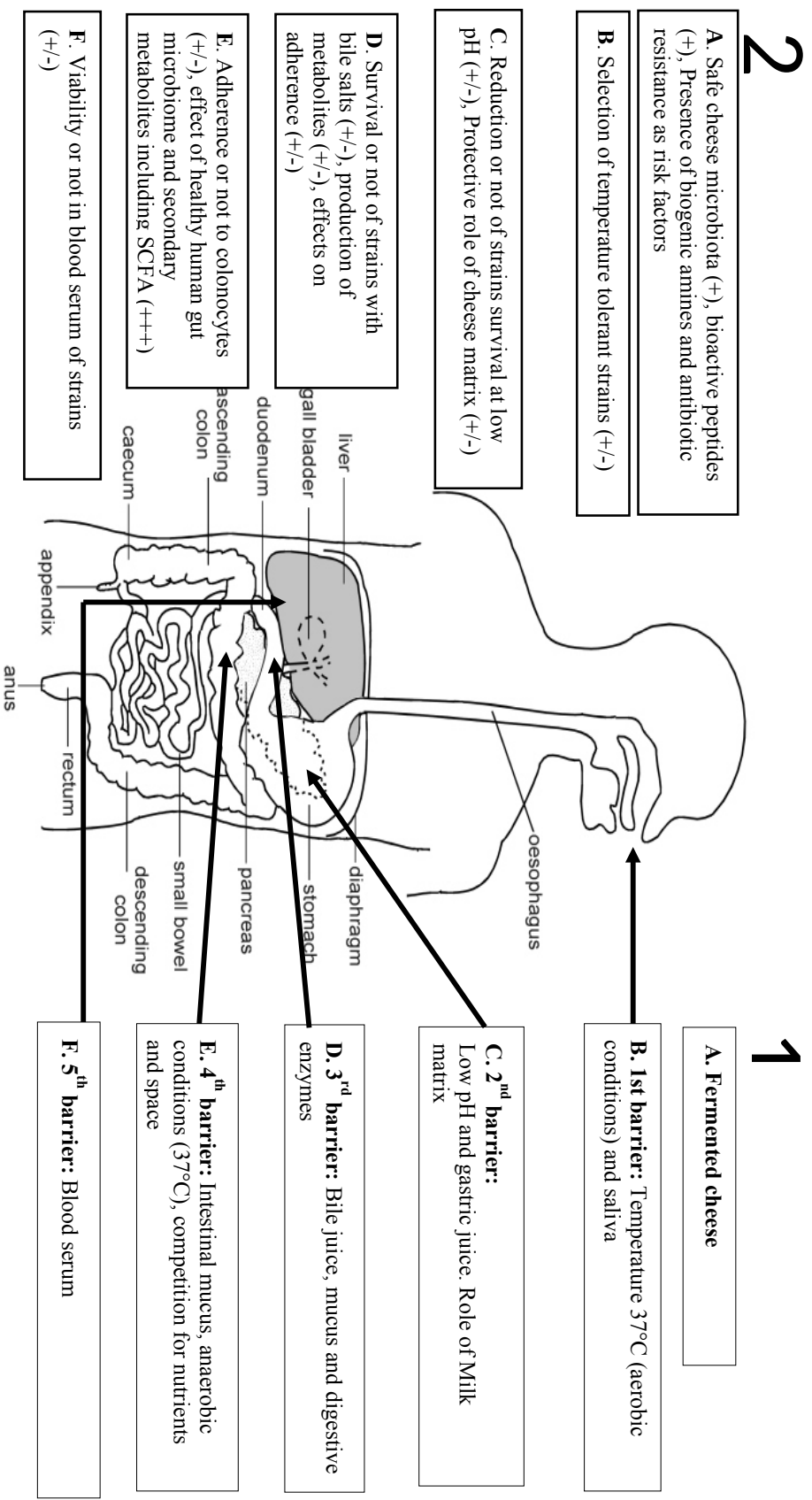


Figure 2





**Supplemental Table S1: Comprehensive view of the obtained safety data showing main characteristics of Gram negative bacteria of dairy origin *in vivo* and *in vitro* for each barrier points in simulated body GIT conditions.**

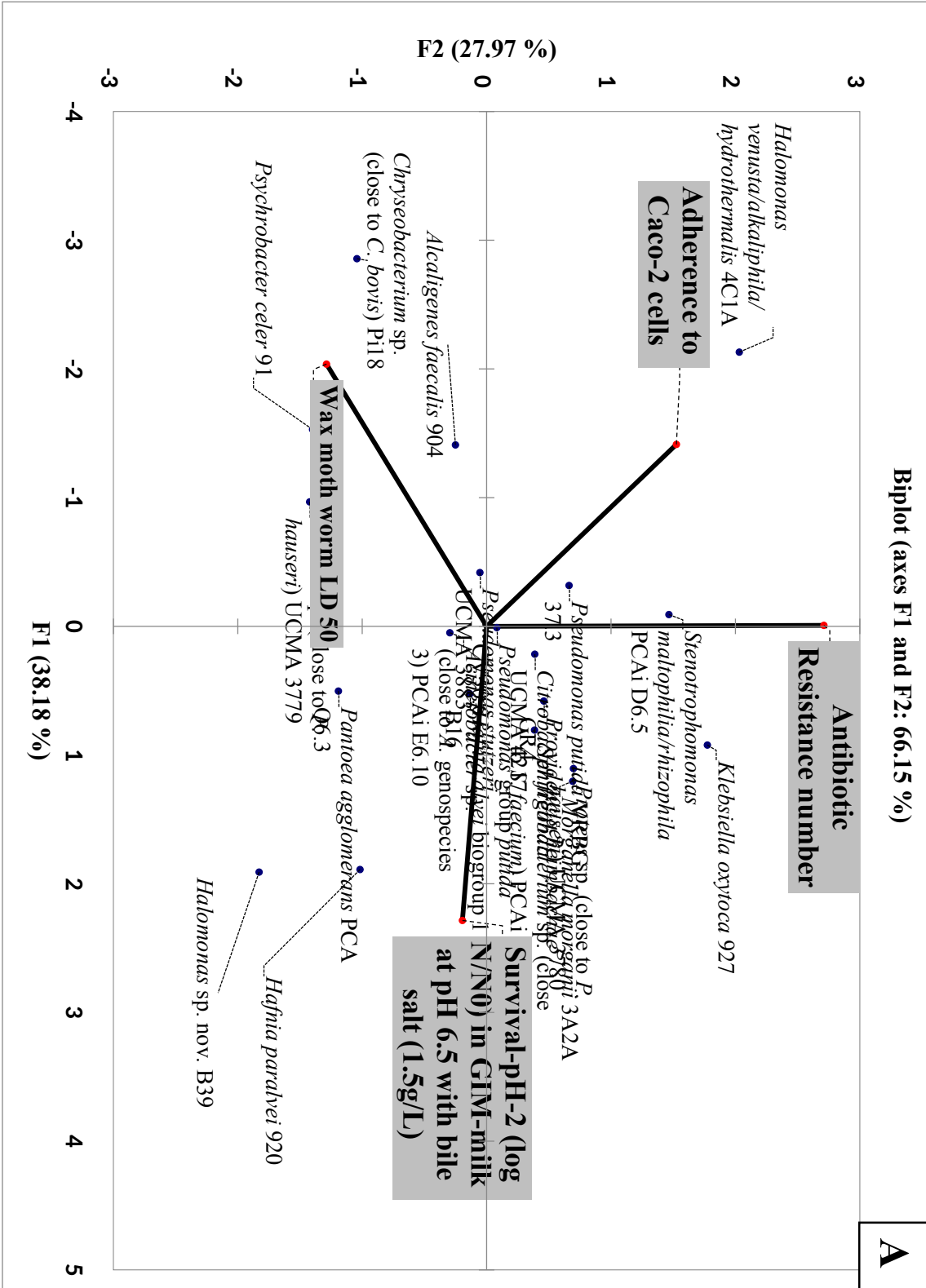
Gram negative strains	LD 50 in wax moth worm model (72h; 30°C) (this study)	Anaerobic growth at 37°C (this study)	Survival (log N/N <sub>0</sub> ) in GIM-milk at pH 6.5 with bile salt (1.5g/L) <sup>1</sup> (this study)	Adherence to Caco-2 cells (this study)	Sensitivity to human serum <sup>2</sup> (this study)	Antibiotic Resistance number (Coton et al, 2012)	Biogenic Amine production <sup>3</sup> (Coton et al, 2012)
<i>Chryseobacterium</i> sp. (close to <i>C. bovis</i> ) P1i8	3.71 10 <sup>8</sup>	+	-5.9/-1.5	73	R	15	-
<i>Psychrobacter celer</i> 91	1.41 10 <sup>8</sup>	-	-5.7/+3.6	145	S	1	+
<i>Alcaligenes faecalis</i> 904	9.83 10 <sup>7</sup>	-	-5.2/ -4.8	200	R	6	+
<i>Proteus</i> sp. (close to <i>P. hauseri</i> ) UCMA 3779	9.78 10 <sup>7</sup>	-	-6.7/+2.1	58	R	3	+
<i>Acinetobacter</i> sp. (close to <i>A. genospecies 3</i> ) PCAi E6.10	8.82 10 <sup>7</sup>	+	-2.8/+2.6	33	R	13	-
<i>Halomonas venusta/alkaliphila/hydrothermalis</i> 4C1A	6.06 10 <sup>7</sup>	+	-5.8/+1.7	363	S	15	+
<i>Pantoea agglomerans</i> PCA Q6.3	5.91 10 <sup>7</sup>	+	-0.9/+3.2	63	R	4	+
<i>Halomonas</i> sp. nov. B39	2.54 10 <sup>7</sup>	-	+3.8/+3.4	30	S	0	+
<i>Citrobacter freundii</i> UCMA 4217	2.18 10 <sup>7</sup>	+	-2.6/+2.9	100	R	13	+
<i>Pseudomonas putida</i> VRBG 37.3	1.91 10 <sup>7</sup>	+	-4.2/-3.3	141	S	13	+
<i>Stenotrophomonas maltophilia/hizophila</i> PCAi D6.5	1.33 10 <sup>7</sup>	+	-5.1/-2.8	70	R	22	+
<i>Pseudomonas</i> group <i>putida</i> CV30.6	1.25 10 <sup>7</sup>	-	-6.3/+3	5	R	14	+
<i>Hafnia alvei</i> biogroup 1 B16	9.48 10 <sup>6</sup>	+	-3/+3.1	43	R	11	+
<i>Pseudomonas stutzeri</i> UCMA 3883	6.57 10 <sup>6</sup>	-	-5.8/+4.5	114	R	8	+
<i>Sphingobacterium</i> sp. (close to <i>S.faecium</i> ) PCAi F2.5	6.57 10 <sup>6</sup>	+	-1.7/+2.7	45	S	15	+
<i>Klebsiella oxytoca</i> 927	6.13 10 <sup>6</sup>	+	+1.2/+3.9	138	R	22	+
<i>Hafnia parvhei</i> 920	2.80 10 <sup>5</sup>	-	+3/+3.5	36	R	5	+
<i>Proteus</i> sp. (close to <i>P. hauseri</i> ) UCMA 3780	9.63 10 <sup>4</sup>	+	+2.2/+2.9	152	R	13	+
<i>Providencia heimbachae</i> GR4	<1.6 10 <sup>4</sup>	+	-2.9/+1	49	R	15	+
<i>Morganella morganii</i> 3A2A	<1.16 10 <sup>4</sup>	+	+0.2/-0.1	54	R	17	+

Initial pH for GM: pH 2/pH 4

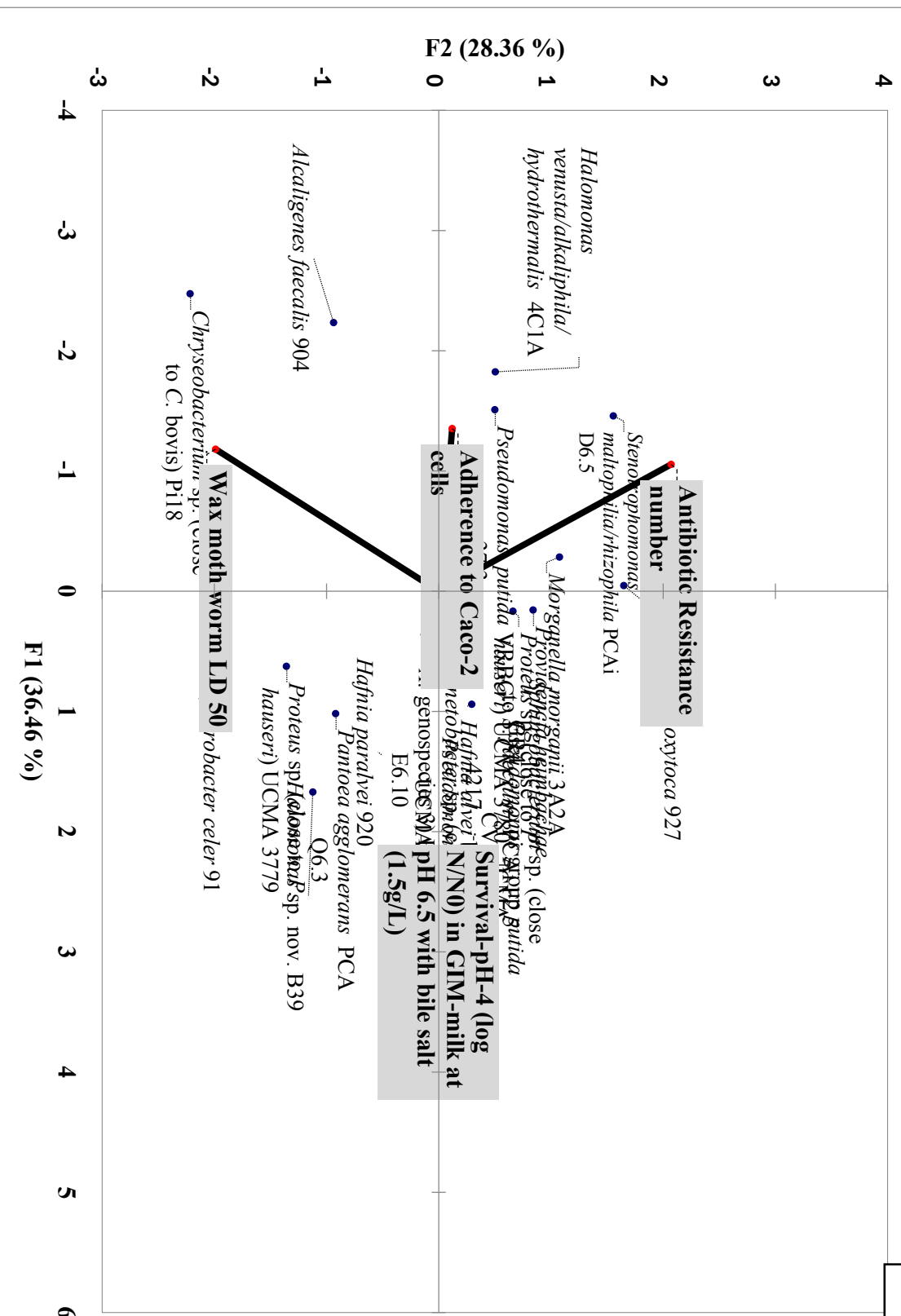
<sup>2</sup> Resistance (R) or Sensitivity (S)

<sup>3</sup> (+) : >100mg/L; (-) : <100mg/L

Supplemental Figure S2: Principal Component Analysis (PCA) of quantitative data obtained from Table 5 at initial pH 2 (A) and at initial pH 4 (B) as primary gastric stress for selected Gram-negative bacteria.



Biplot (axes F1 and F2: 64.82 %)



**Supplemental Table S3: Bibliographical assessment of pathogenicity in Human for GNB strains belonging to genera/species of strains assessed in this study.**

Species	Human clinical cases/food vector implication	References
<i>Acinetobacter</i> sp. (close to <i>A. genospecies 3</i> )	Yes/No	(Horrevorts et al., 1995) (Webster et al., 1999)
<i>Alcaligenes faecalis</i>	Yes/No	(Khokhar et al., 2002; Shimizu et al., 2002) (Bizet and Bizet, 1997; Ju et al., 2016; Khajuria et al., 2013; Pereira et al., 2000)
<i>Chryseobacterium</i> sp. (close to <i>C. bovis</i> )	No/No	(Hantisis-Zacharov et al., 2008; Iwai et al., 2012)
<i>Citrobacter freundii</i>	Yes/No	(Chen et al., 2008; Derwisoglu et al., 2008; Leski et al., 2016; Samonis et al., 2009)
<i>Hafnia alvei</i> biogroup 1	Yes/No	(Benito et al., 2008; Donato et al., 2008; Redondo et al., 2005; Rodriguez-Guardado et al., 2005)
<i>Hafnia paralvei</i>	Yes/No	(Abbott et al., 2011; Osuka et al., 2011)
<i>Halomonas venusta/alkaliphila/hydrothermalis</i>	Yes (fish bice)/No	(Berger et al., 2007; Stevens et al., 2009; von Graevenitz et al., 2000)
<i>Halomonas</i> sp.		(Berger et al., 2007; von Graevenitz et al., 2000)
<i>Klebsiella oxytoca</i>	Yes/No	(Bleich et al., 2008; Mohamed et al., 2016; Oishi et al., 2008; Shibasaki et al., 2016; Tsanaktisidis et al., 2003)
<i>Morganella morganii</i>	Yes/No	(Demiray et al., 2016; Fernandez Fuentes et al., 2014; Nitita et al., 2016; Salen and Eppes, 1997; Tsanaktisidis et al., 2003; Williams et al., 1983)
<i>Pantoea agglomerans</i>	Yes/No	(Bachmeyer et al., 2007; Cruz et al., 2007; Fernandez Fuentes et al., 2014; Uche, 2008)
<i>Proteus</i> sp. (close to <i>P. hauseri</i> )	Yes/Yes (Eating fish poisoned by biogenic amines)	(Cetre et al., 2005; Dybas et al., 2005; Fernandez-No et al., 2010; Nowakowska et al., 2004; Schaffer and Pearson, 2015)
<i>Providencia heimbachae</i>	Yes/No	(Ineid et al., 2016; Mohr O'Hara et al., 1999)
<i>Pseudomonas</i> group <i>putida</i>	Yes/No	(Carpenter et al., 2008; Lombardi et al., 2002; Miron et al., 2007; Yang et al., 1996; Zhang et al., 2012)
<i>Pseudomonas stutzeri</i>	Yes/No	(Carvalho-Assef et al., 2010; Lin et al., 2014; Miron et al., 2007; Wang et al., 2015)



<i>Psychrobacter celer</i>	No/No	(Yoon et al., 2005)
<i>Sphingobacterium</i> sp. (close to <i>S. faecium</i> )	No/No	(Mariko and Akira, 1992; Tronel et al., 2003)
<i>Stenotrophomonas maltophilia</i>	No/No	(Bin Abdulhak et al., 2009; Falagas et al., 2009; Liu et al., 2016; Spicuzza et al., 2009)

### Annexe Table S3

#### References used in supplemental Table S3

- Abbott, S.L., Moler, S., Green, N., Tran, R.K., Wainwright, K., Janda, J.M., 2011. Clinical and laboratory diagnostic characteristics and cytotoxic potential of *Hafnia alvei* and *Hafnia parva* strains. *J Clin Microbiol* 49, 3122-3126.
- Bachmeyer, C., Entressengle, H., Gibeault, M., Nedellec, G., M'Bappe, P., Delisle, F., Jacquot, F., Arlet, G., Gateau, G., Lionnet, F., 2007. Bilateral tibial chronic osteomyelitis due to *Pantoea agglomerans* in a patient with sickle cell disease. *Rheumatology (Oxford)* 46, 1247.
- Benito, M.H., Hernandez, R.S., Fernandez-Reyes, M.J., Herrando, S., 2008. Sepsis induced by *Hafnia alvei* in a kidney transplant patient. *Nefrologia* 28, 470-471.
- Berger, P., Barguelli, F., Raouf, D., Drancourt, M., 2007. An outbreak of *Halomonas phocaensis* sp. nov. bacteraemia in a neonatal intensive care unit. *J Hosp Infect* 67, 79-85.
- Bin Abdulhak, A.A., Zimmermann, V., Al Beirouti, B.T., Baddour, L.M., Tleyjeh, I.M., 2009. *Stenotrophomonas maltophilia* infections of intact skin: a systematic review of the literature. *Diagn Microbiol Infect Dis* 63, 330-333.
- Bizet, J., Bizet, C., 1997. Strains of *Alcaligenes faecalis* from clinical material. *J Infect* 35, 167-169.
- Bleich, A., Kirsch, P., Sahly, H., Fahey, J., Smoczek, A., Hedrich, H.J., Sundberg, J.P., 2008. *Klebsiella oxytoca*: opportunistic infections in laboratory rodents. *Lab Anim* 42, 369-375.
- Carpenter, R.J., Hartzell, J.D., Forsberg, J.A., Babel, B.S., Ganesan, A., 2008. *Pseudomonas putida* war wound infection in a US Marine: a case report and review of the literature. *J Infect* 56, 234-240.
- Carvalho-Assef, A.P., Gomes, M.Z., Silva, A.R., Werneck, L., Rodrigues, C.A., Souza, M.J., Asensi, M.D., 2010. IMP-16 in *Pseudomonas putida* and *Pseudomonas stutzeri*: potential reservoirs of multidrug resistance. *J Med Microbiol* 59, 1130-1131.
- Cetre, J.C., Nicolle, M.C., Salord, H., Perol, M., Tigaud, S., David, G., Bourfaul, M., Vanhems, P., 2005. Outbreaks of contaminated broncho-alveolar lavage related to intrinsically defective bronchoscopes. *J Hosp Infect* 61, 39-45.
- Chen, K.J., Sun, M.H., Hwang, Y.S., Chen, T.L., Lai, C.C., Chen, Y.P., 2008. Endophthalmitis caused by *Citrobacter* species. *Ocul Immunol Inflamm* 16, 147-153.
- Cruz, A.T., Cazacu, A.C., Allen, C.H., 2007. *Pantoea agglomerans*, a plant pathogen causing human disease. *J Clin Microbiol* 45, 1989-1992.

- Demiray, T., Aydemir, O.A., Koroglu, M., Ozbek, A., Altindis, M., 2016. A severe *Morganella morganii* endophthalmitis; followed by bacteremia. Iran J Microbiol 8, 70-72.
- Dervisoglu, E., Yumuk, Z., Yegenaga, I., 2008. *Citrobacter freundii* peritonitis and tunnel infection in a patient on continuous ambulatory peritoneal dialysis. J Med Microbiol 57, 125-127.
- Donato, K.A., Zaree, M., Jassem, A.N., Jandu, N., Alingary, N., Carusone, S.C., Johnson-Henry, K.C., Sherman, P.M., 2008. *Escherichia albertii* and *Hafnia alvei* are candidate enteric pathogens with divergent effects on intercellular tight junctions. Microb Pathog 45, 377-385.
- Dybas, I., Sidor-Wojtowicz, A., Koziol-Montewka, M., 2005. Bacterial flora and mycosis of the vagina in women with symptoms of vaginal inflammation. Ginekol Pol 76, 385-390.
- Falagas, M.E., Kastoris, A.C., Vouloumanou, E.K., Dimopoulos, G., 2009. Community-acquired *Stenotrophomonas maltophilia* infections: a systematic review. Eur J Clin Microbiol Infect Dis 28, 719-730.
- Fernandez-No, I.C., Bohme, K., Gallardo, J.M., Barros-Velazquez, J., Canas, B., Calo-Mata, P., 2010. Differential characterization of biogenic amine-producing bacteria involved in food poisoning using MALDI-TOF mass fingerprinting. Electrophoresis 31, 1116-1127.
- Fernandez Fuentes, M.A., Abriouel, H., Gadea, R., Perez Pulido, R., Galvez, A., Ortega, E., 2014. Synergistic activity of bioicides and antibiotics on resistant bacteria from organically produced foods. Microb Drug Resist 20, 383-391.
- Hantsis-Zacharov, E., Senderovich, Y., Halpern, M., 2008. *Chryseobacterium bovis* sp. nov., isolated from raw cow's milk. Int J Syst Evol Microbiol 58, 1024-1028.
- Horrevorts, A., Bergman, K., Kollee, L., Breuker, I., Tjienberg, I., Dijkshoorn, L., 1995. Clinical and epidemiological investigations of *Acinetobacter genomospecies* 3 in a neonatal intensive care unit. J Clin Microbiol 33, 1567-1572.
- Iwai, S., Fei, M., Huang, D., Fong, S., Subramanian, A., Grieco, K., Lynch, S.V., Huang, L., 2012. Oral and airway microbiota in HIV-infected pneumonia patients. J Clin Microbiol 50, 2995-3002.
- Jneid, J., Benamar, S., Pagnier, I., Levy, P.Y., Lavigne, J.P., La Scola, B., 2016. Draft Genome Sequence of *Providencia heimbachae*, Isolated from a Diabetic Foot Ulcer. Genome Announc 4.
- Ju, S., Lin, J., Zheng, J., Wang, S., Zhou, H., Sun, M., 2016. *Alcaligenes faecalis* ZD02, a Novel Nematicidal Bacterium with an Extracellular Serine Protease Virulence Factor. Appl Environ Microbiol 82, 2112-2120.
- Khajuria, A., Praharaj, A.K., Kumar, M., Grover, N., 2013. Emergence of VIM-6 metallo-beta-lactamase-producing *Alcaligenes faecalis* clinical isolates in a hospital in India. J Infect Dev Ctries 7, 494-496.
- Khokhar, D.S., Sethi, H.S., Kumar, H., Sudan, R., Sharma, N., Nayak, N., 2002. Postkeratoplasty endophthalmitis by *Alcaligenes faecalis*: a case report. Cornea 21, 232-233.
- Leski, T.A., Taitt, C.R., Bangura, U., Stockelman, M.G., Ansumana, R., Cooper, W.H., 3rd, Stenger, D.A., Vora, G.J., 2016. High prevalence of multidrug resistant *Enterobacteriaceae* isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. BMC Infect Dis 16, 167.

- Lin, K.H., Chen, C.M., Wang, J.H., Ho, M.W., 2014. *Pseudomonas stutzeri* necrotizing pneumonia in pre-existing pulmonary tuberculosis. *Intern Med* 53, 2543-2546.
- Liu, M.C., Tsai, Y.L., Huang, Y.W., Chen, H.Y., Hsueh, P.R., Lai, S.Y., Chen, L.C., Chou, Y.H., Lin, W.Y., Liaw, S.J., 2016. *Stenotrophomonas maltophilia* PhoP, a Two-Component Response Regulator, Involved in Antimicrobial Susceptibilities. *PLoS ONE* 11, e0153753.
- Lombardi, G., Luzzaro, F., Docquier, J.D., Riccio, M.L., Perilli, M., Coli, A., Amicosante, G., Rossolini, G.M., Toniolo, A., 2002. Nosocomial infections caused by multidrug-resistant isolates of *Pseudomonas putida* producing VIM-1 metallo-beta-lactamase. *J Clin Microbiol* 40, 4051-4055.
- Marko, T., Akira, Y., 1992. Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalophilum* comb. nov., and two genospecies of the genus *Sphingobacterium*, and synonymy of *Flavobacterium yabuuchiiae* and *Sphingobacterium spirivorum*. *Journal of general and applied Microbiology* 38, 465-482.
- Miron, D., Keness, Y., Bor, N., Spiegel, R., Horowitz, Y., 2007. *Pseudomonas stutzeri* knee arthritis in a child: case report and review. *J Pediatr Orthop B* 16, 419-421.
- Mohamed, A., Hall, C., Hatch, M., Ayan, M., Winn, R., 2016. Infective endocarditis caused by *Klebsiella oxytoca* in an intravenous drug user with cancer. *Proc (Bayl Univ Med Cent)* 29, 181-182.
- Mohr O'Hara, C., Steigerwalt, A.G., Green, D., McDowell, M., Hill, B.C., Brenner, D.J., Miller, J.M., 1999. Isolation of *Providencia heimbachae* from human feces. *J Clin Microbiol* 37, 3048-3050.
- Nitta, Y., Yasukata, F., Kitamoto, N., Ito, M., Sakae, M., Kikuzaki, H., Ueno, H., 2016. Inhibition of *Morganella morganii* Histidine Decarboxylase Activity and Histamine Accumulation in Mackerel Muscle Derived from Filipendula ulumaria Extracts. *J Food Prot* 79, 463-467.
- Nowakowska, M., Rogala-Zawada, D., Wiechula, B., Rudy, M., Radosz-Komoniewska, H., Zientara, M., 2004. Urinary tract infections in children--etiologic agents and susceptibility to antibiotics. *Wiad Lek* 57, 438-443.
- Oishi, H., Kagawa, Y., Mitsumizo, S., Tashiro, Y., Kobayashi, G., Udo, K., Aoki, S., Takayanagi, M., Nagasawa, Z., Araki, K., Ohza, N., Eguchi, Y., Nakashima, M., 2008. A fatal case of necrotizing fasciitis due to bacterial translocation of *Klebsiella oxytoca*. *J Infect Chemother* 14, 62-65.
- Osuka, H., Hitomi, S., Koganemaru, H., Kaneko, T., 2011. A case of bacteremia caused by *Hafnia parvhei*. *J Infect Chemother* 17, 855-857.
- Pereira, M., Perilli, M., Mantengoli, E., Luzzaro, F., Toniolo, A., Rossolini, G.M., Amicosante, G., 2000. PER-1 extended-spectrum beta-lactamase production in an *Alcaligenes faecalis* clinical isolate resistant to expanded-spectrum cephalosporins and monobactams from a hospital in Northern Italy. *Microb Drug Resist* 6, 85-90.
- Redondo, J., Maseda, E., Riquelme, A., Alday, E., Una, R., Criado, A., 2005. *Hafnia alvei* in a rare case of severe pneumonia in a postanesthesia recovery unit. *Rev Esp Anestesiol Reanim* 52, 359-362.
- Rodriguez-Guardado, A., Boga, J.A., Diego, I.D., Ordas, J., Alvarez, M.E., Perez, F., 2005. Clinical characteristics of nosocomial and community-acquired extraintestinal infections caused by *Hafnia alvei*. *Scand J Infect Dis* 37, 870-872.

- Salen, P.N., Eppes, S., 1997. *Morganella morganii*: a newly reported, rare cause of neonatal sepsis. Acad Emerg Med 4, 711-714.
- Samonis, G., Karageorgopoulos, D.E., Kofleridis, D.P., Matthaiou, D.K., Sidiropoulou, V., Maraki, S., Falagas, M.E., 2009. *Citrobacter* infections in a general hospital: characteristics and outcomes. Eur J Clin Microbiol Infect Dis 28, 61-68.
- Schaffer, J.N., Pearson, M.M., 2015. *Proteus mirabilis* and Urinary Tract Infections. Microbiol Spectr 3.
- Shibasaki, M., Komatsu, M., Sueyoshi, N., Maeda, M., Uchida, T., Yonezawa, H., Inagaki, K., Omi, A., Matsumoto, H., Murotani, M., Iwamoto, T., Kodaka, Y., Kieda, H., Tokiwa, M., Masuwa, B., Kinoshita, M., Saito, K., Katou, M., 2016. Community spread of extended-spectrum beta-lactamase-producing bacteria detected in social insurance hospitals throughout Japan. J Infect Chemother.
- Shimizu, M., Okuzumi, K., Yoneyama, A., Kunisada, T., Araake, M., Ogawa, H., Kimura, S., 2002. In vitro antibiotic susceptibility of clinical isolates from nosocomial infections. Dermatology 204 Suppl 1, 21-27.
- Spicuzza, L., Scuito, C., Vitaliti, G., Di Dio, G., Leonardi, S., La Rosa, M., 2009. Emerging pathogens in cystic fibrosis: ten years of follow-up in a cohort of patients. Eur J Clin Microbiol Infect Dis 28, 191-195.
- Stevens, D.A., Hamilton, J.R., Johnson, N., Kim, K.K., Lee, J.S., 2009. *Halomonas*, a newly recognized human pathogen causing infections and contamination in a dialysis center: three new species. Medicine (Baltimore) 88, 244-249.
- Tronel, H., Plesiat, P., Ageron, E., Grimont, P.A.D., 2003. Bacteremia caused by a novel species of *Sphingobacterium*. Clinical Microbiology and Infection 9, 1242-1244.
- Tsanaktisidis, G., Agarwal, S.A., Maloof, A.J., Chandra, J., Mitchell, P., 2003. Postoperative *Morganella morganii* endophthalmitis associated with subclinical urinary tract infection. J Cataract Refract Surg 29, 1011-1013.
- Uche, A., 2008. *Pantoea agglomerans* bacteremia in a 65-year-old man with acute myeloid leukemia: case report and review. South Med J 101, 102-103.
- von Graevenitz, A., Bowman, J., Del Notaro, C., Ritzler, M., 2000. Human infection with *Halomonas venusta* following fish bite. J Clin Microbiol 38, 3123-3124.
- Wang, L., Wang, Y., Fan, X., Tang, W., Hu, J., 2015. Prevalence of Resistant Gram-Negative Bacilli in Bloodstream Infection in Febrile Neutropenia Patients Undergoing Hematopoietic Stem Cell Transplantation: A Single Center Retrospective Cohort Study. Medicine (Baltimore) 94, e1931.
- Webster, C.A., Townner, K.J., Saunders, G.L., Crewe-Brown, H.H., Humphreys, H., 1999. Molecular and antibiogram relationships of *Acinetobacter* isolates from two contrasting hospitals in the United Kingdom and South Africa. Eur J Clin Microbiol Infect Dis 18, 595-598.
- Williams, E.W., Hawkey, P.M., Penner, J.L., Senior, B.W., Barton, L.J., 1983. Serious nosocomial infection caused by *Morganella morganii* and *Proteus mirabilis* in a cardiac surgery unit. J Clin Microbiol 18, 5-9.
- Yang, C.H., Young, T., Peng, M.Y., Weng, M.C., 1996. Clinical spectrum of *Pseudomonas putida* infection. J Formos Med Assoc 95, 754-761.
- Yoon, J.H., Lee, C.H., Kang, S.J., Oh, T.K., 2005. *Psychrobacter celer* sp. nov., isolated from sea water of the South Sea in Korea. Int J Syst Evol Microbiol 55, 1885-1890.

Zhang, W., Chen, L., Liu, D., 2012. Characterization of a marine-isolated mercury-resistant *Pseudomonas putida* strain SP1 and its potential application in marine mercury reduction. *Appl Microbiol Biotechnol* 93, 1305-1314.