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1 **Spatially localized expression of glutamate decarboxylase *gadB* in**  
2 ***Escherichia coli* O157:H7 microcolonies in hydrogel matrix**

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12 **Abstract:** Functional diversity within isogenic spatially organized bacterial populations has  
13 been shown to trigger emergent community properties such as stress tolerance. Taking  
14 advantage of confocal laser scanning microscopy combined with a transcriptional fluorescent  
15 fusion reporting at single cell scale the expression of the glutamic acid decarboxylase *gadB* in  
16 *E. coli* O157:H7, it was possible to visualize for the first-time spatial patterns of bacterial  
17 gene expression in microcolonies grown in a gelled matrix. The *gadB* gene is involved in *E.*  
18 *coli* tolerance to acidic conditions and its strong over-expression was observed locally on the  
19 periphery of embedded microcolonies grown in acidic hydrogels. This spatialization of *gadB*  
20 expression did not correlate with live/dead populations that appeared randomly distributed in  
21 the colonies. While the planktonic population of the pathogens was eradicated by an  
22 exposition to a pH of 2 (HCl) for 4h, mimicking a stomachal acidic stress, bacteria grown in  
23 gel-microcolonies were poorly affected by this treatment, in particular in conditions where  
24 *gadB* was spatially overexpressed. Consequences of these results for food safety are further  
25 discussed.

26

27 **Keywords:** *E. coli* O157:H7, food matrix, microcolonies, local gene expression, *gadB*,  
28 fluorescent transcriptional fusions, confocal laser scanning microscopy (CLSM), Food safety.

29 **Abbreviations:** CLSM: stands for “Confocal Laser Scanning Microscopy”; LMPA: stands  
30 for “Low Melting Point Agarose”.

31

## 32 **1) Introduction**

33 *Escherichia coli* is a commensal bacterium found in the gut of mammals that plays an integral  
34 part in the digestive process. However, some strains of *E. coli* are pathogenic and represent a  
35 public health issue when they reach the production lines of food industries. Besides obvious  
36 evisceration accidents contaminating the meat at the slaughter state, food contamination of  
37 animal products (meat and milk products), vegetable or water usually occur through direct or  
38 indirect fecal contamination<sup>1-3</sup>. Storage conditions and holding temperature are then major  
39 contributors to bacterial growth and survival in food products<sup>4</sup>. Shigatoxin (Stx) encoding  
40 *E. coli* (STEC) are the third most common foodborne zoonosis in Europe<sup>5</sup> and amongst  
41 STEC, the serotype O157:H7 is a commonly identified agent in patients. *E. coli* O157:H7 are  
42 enterohaemorrhagic *E. coli* (EHEC) responsible for bloody diarrhea when the intestinal lining  
43 is broken by the presence of Stx. A possible outcome of Stx passing in the bloodstream is  
44 damage to the kidneys that can lead to a hemolytic uremic syndrome (HUS), which itself lead  
45 to fatal outcomes in 5% of cases<sup>6</sup>. Children are especially at risk and *E. coli* O157:H7 is still  
46 the main cause of pediatric HUS<sup>7</sup>. At the level of the European union, regulations ask for the  
47 absence of this pathogen in 25g of germinated seeds (Regulation CE 209/2013, amendment  
48 2073/2055), but no equivalent exist for meat products. Precautionary measures for meats exist  
49 at a state level in the Union (France, DGAL/SSDSA/2016-353). However, the pathogen is  
50 still routinely detected at levels above 100 CFU/g in more than 1% of all red meats, the main  
51 vector of infection for this pathogen<sup>5,8</sup>.

52 Structured food matrices are a continuity of heterogeneous local microenvironments  
53 harboring multiple micro-gradients that can evolve with time and microbial activity<sup>9</sup>. This  
54 leads bacterial cells in food matrices to face different biotopes in which their growth and  
55 behavior can diverge from observations in liquid laboratory media. Therefore, environmental  
56 conditions of food matrices can prompt high phenotypic diversity in microbial populations as  
57 the cells adapt to local microenvironments<sup>10,11</sup>. In comparison with their planktonic  
58 counterparts, phenotypic diversity in structured communities can influence bacterial fitness  
59 and behavior, such as increase expression of virulence genes<sup>12</sup>, higher tolerance to  
60 antimicrobials agents and thermal stress<sup>13,14</sup>, or improved cell motility<sup>15</sup>. While several

61 studies reported emergent properties of bacterial community in food matrices at the  
62 population level <sup>13,16–19</sup>, no experimental evidence has yet been reported on the spatial  
63 heterogeneity of gene expression at the scale of single cells.

64 The stomachal phase after food ingestion exposes bacteria to strong acidic pH conditions for  
65 several hours and is credited for the highest population reduction of the bacterial load. High  
66 tolerance to acidic conditions is therefore necessary for foodborne pathogens, and involved  
67 systems that regulate intracellular pH. The glutamic acid decarboxylase (GAD) is one among  
68 various systems of acid resistance (AR) commonly found in bacteria able to survive in  
69 extreme acid conditions <sup>20–24</sup>. In *E. coli*, the GAD system is a three components system: two  
70 glutamate decarboxylases, GadA and GadB, which use cytoplasmic free protons by  
71 converting glutamate into  $\gamma$ -aminobutyrate (GABA), and the glutamate/GABA antiporter  
72 GadC. When the pH is below 5.6, cytoplasmic GadB migrates near the inner membrane to  
73 maximize collaboration with transmembrane GadC <sup>25</sup>. While *gadA* is independent in  
74 chromosomal location and *gadB* and *gadC* are organized in operon, the expression of both  
75 *gadA* and *gadBC* is transcriptionally regulated by RpoS, two AraC-like regulators GadX and  
76 GadW, and by effectors with two inhibitors, the cyclic AMP receptor protein and H-NS. H-  
77 NS and RpoS in particular determine the temporal expression, the former inhibiting *gadB*  
78 expression, whereas RpoS promotes the transcription of *gadB* once the stationary phase is  
79 reached <sup>26</sup>.

80 To decipher and model fitness and behavior of *E. coli* O157:H7, synthetic microbial ecology  
81 approaches were used in structured food matrices where the complexity of the communities  
82 and the factors of influence are reduced to their minimum, but increased in their  
83 controllability <sup>27</sup>. Such approaches have been used to describe how matrix parameters affect  
84 bacterial growth and morphodynamics of microcolonies <sup>16,28</sup>. In a recent contribution, we  
85 have shown that the volume, distribution and sphericity of microcolonies of *E. coli* O157:H7  
86 in hydrogel are dependent of the size of the inoculum, but also on the concentration of acids  
87 and NaCl, two environmental stresses frequently encountered in food products <sup>29</sup>.

88 In this study, we took advantage of a hydrogel matrix to observe the local expression of *gadB*  
89 in *E. coli* O157:H7 cells in microcolonies using confocal laser scanning microscopy (CLSM).  
90 To explore the existence of patterns of expression in microcolonies, bacterial strains with a  
91 dual transcriptional fluorescent reporter system were engineered to monitor the spatial  
92 expression of *gadB* at the single cell scale. In order to relate the impact that phenotypic  
93 heterogeneity in microcolonies can have on community function, the survival of

94 planktonically grown cells to a strongly acidic media mimicking the stomachal passage was  
95 further assessed and compared to cells grown or dispersed in hydrogels.

## 96 **2) Material and methods**

### 97 ***Bacterial strains and culture conditions***

98 From cryotubes stored at -80°C, the bacterial strain of *E. coli* (see genetic construction) was  
99 plated on Petri dishes with TSA (Tryptone Soya Agar, Oxoid, USA) and incubated overnight  
100 at 37°C. One bacterial colony was picked up and inoculated in TSB (Tryptone Soya Broth,  
101 Oxoid, England) before overnight incubation at 37°C under orbital shaking (200 rpm). When  
102 required, growth media were supplemented with chloramphenicol (Cm 25 µg/mL;  
103 EUROMEDEX, China). The strain *Lactococcus lactis* ssp. *cremoris* (Aerial N°2124) was  
104 incubated in the same conditions, but without antibiotic supplementation.

### 105 ***Genetic construction***

106 The *E. coli* O157:H7 CM454<sup>30,31</sup> is the wild type strain in this study. We used a T7  
107 polymerase (*T7pol*) amplification technique inspired from previous reports<sup>32,33</sup>, where the  
108 cassette *T7pol::Cm<sup>R</sup>* is inserted after the genetic sequence of the gene *gadB* using the  
109 Datsenko-Wanner<sup>34</sup> recombination technique (supplementary material Figure S1). Regions of  
110 identity were added at the ends of the cassette by the forward primer:  
111 5'CCGAAACTGCAGGGTATTGCCCAACAGAACAGCTTTAAACATACCTGATAACA  
112 GGAGGTAAATAATGCACACGATTAACATCGC3' and reverse primer:  
113 5'AAATTGTCCCGAAACGGGTTCGTTTCGGACACCGTTACCGTTAAACATGGAGTT  
114 CTGAGGTCATTACTG3'. The correct insertion of *T7pol::Cm<sup>R</sup>* in the construct was verified  
115 by PCR using the forward primer 5'GGAAGACTACAAAGCCTCCC3' and reverse primer  
116 5' TATTCCTGTTCGGAACCGCAC3', for sequencing (Eurofins Genomics, Germany). Based  
117 on the sequence of the pHL40 plasmid<sup>32</sup>, a new plasmid was synthesized (GeneArt,  
118 ThermoFisher Scientific, Germany) bearing the *P<sub>T7pol</sub>::GFPmut3::T<sub>p7pol</sub>* as a GFP reporter but  
119 modified by insertion of *P<sub>BBa\_J23119</sub>::mCherry2::T<sub>BBa\_B0062</sub>* (iGEM parts) for constitutive  
120 expression of a red fluorescent protein (RFP). This new plasmid, called pHL60, was  
121 transformed into competent *gadB::T7pol::Cm<sup>R</sup>* bacterial cells. This system is an indirect  
122 reporter of *gadB* transcription as the transcriptional fusion of *gadB::T7 polymerase* allows an  
123 amplified production of GFP (GFPmut3) from pHL60 and normalization of the level of  
124 expression respective to the constitutive expression of the RFP (mCherry2) from the same  
125 plasmid, to minimize variations of the fluorescence associated with variations in the number

126 of plasmids from one cell to another. To validate the genetic construction, the reporting  
127 planktonic expression of *gadB* was tested on six pH values from 4.5 to 7.0 using a microplate  
128 reader (Synergy H1, Biotek) (Supplementary material Figure S2).

### 129 ***Transparent hydrogel matrices for fluorescent imaging***

130 As previously described<sup>29</sup>, the hydrogel matrices were obtained by mixing TSB with 0.50 %  
131 low melting point agarose (LMPA) (UltraPure Agarose, Invitrogen, USA). After boiling, the  
132 liquid LMPA at neutral pH (pH=7) was cooled down to 40°C to prevent thermal stress before  
133 the bacterial inoculum was added to obtain 10<sup>4</sup> CFU/ml. When necessary, the medium was  
134 adjusted to acidic pH= 5 with HCl. After homogenizing and gentle stirring to avoid bubble  
135 formation, the inoculated gel matrix was immediately distributed in each well of a 96-well  
136 microtiter plate of microscopic grade ( $\mu$ Clear, Greiner Bio-One, France). The microtiter plates  
137 were then incubated at 20°C and observed under CLSM after 96 hours of incubation.

138 All microscopic observations were performed with a Leica HCS-SP8 confocal laser scanning  
139 microscope (CLSM) at the INRAE MIMA2 imaging platform  
140 (<https://doi.org/10.15454/1.5572348210007727E12>). The GFP (GFPmut3;  $\lambda_{ex}$ 500;  $\lambda_{em}$ 513)  
141 and RFP (mCherry2;  $\lambda_{ex}$ 589;  $\lambda_{em}$ 610) were excited respectively with laser bands 488 nm and  
142 561 nm. For Live/dead exploration, SYTO9 ( $\lambda_{ex}$ 485;  $\lambda_{em}$ 501) and IP ( $\lambda_{ex}$ 535;  $\lambda_{em}$ 617) were  
143 excited respectively with laser bands 488 nm and 561 nm. Observations were carried out with  
144 a water immersion 63x objective lens (numerical aperture of 1.20) for 184 $\mu$ m x 184 $\mu$ m fields.  
145 Bidirectional acquisition speed of 600 Hz allows a frame rate of 2.3 images per second. For  
146 3D stack analysis, a 1  $\mu$ m step between z levels was used. For each condition, a minimum of  
147 60 stacks were acquired in over a dozen independent wells. Microscopic images were treated  
148 on IMARIS v9.64 (Bitplane, Switzerland) to generate sections and projections. Kymograms  
149 reporting the spatial analysis of *gadB* expression in microcolonies were performed using  
150 BiofilmQ v0.2.2<sup>35</sup>. BiofilmQ image segmentation was performed with a threshold value set at  
151 0.1 with cubes of 1.8  $\mu$ m (vox of 10). The absence of radial fluorescence gradients in  
152 microcolonies of *E. coli* O157:H7 constitutively expressing GFP was verified prior  
153 experiments with *gadB* expression (supplementary material Figure S3).

### 154 ***Acidic digestion challenge***

155 To test the ability of *E. coli* O157:H7 population to survive the strong acidic stress during the  
156 stomachal passage, 3 ml of planktonic cells (TSB), planktonic cells grown in TSB and then  
157 encased in gel matrix (TSB-LMPA) or gel-colonies cultures (LMPA) (72h, 20°C, pH=7 or

158 pH=5) were transferred in 27 ml of NaCl 9g/L saline solution (control groups) or 27 ml of a  
159 saline solution adjusted with HCl (5 M) to a pH of 2. The cups were then incubated at 37°C  
160 for 4 hours under a 90-rpm shaking to simulate matrix digestion. All media were then  
161 homogenized to disperse bacteria (IKA Ultra-Turrax T25; Janke Kunkel) and the resulting  
162 suspensions were immediately plated on agar for enumeration and determination of the log  
163 reduction in CFU/ml before and after acidic treatment.

#### 164 *Statistics*

165 Graphics and ANOVA variance analysis were performed with Prism 9 (GraphPad; CA,  
166 USA). Differences were considered significant when  $P < 0.05$  with  $P$  being the critical  
167 probability associated with the Fisher test.

168

### 169 **3) Results**

#### 170 *Spatial patterns of gadB expression in gel-microcolonies*

171 Mean radius of microcolonies grown in neutral or acidic hydrogel showed little differences  
172 between neutral (28  $\mu\text{m}$ ) and acidic conditions (27  $\mu\text{m}$ ), the repartition of populations around  
173 these values was noted to not be statistically significant (Figure 1, obtained from  
174 measurement on 40 colonies,  $P > 0.05$ ). However, at pH 5, microcolonies appear more circular  
175 than at pH = 7 and they harbor at their edge a streamer population shedding from the colony  
176 core, forming a crown around it (Figure 2).

177 Microscopic observations of the fluorescence reporting the expression of *gadB* in cells inside  
178 microcolonies (Figure 2) show radically different patterns between the two hydrogels. In  
179 neutral pH conditions, the gene is expressed at a basal level throughout the whole  
180 microcolony with no specific spatial arrangement (Figure 2A, supplementary material Figure  
181 S4). By contrast, the expression of *gadB* is strongly overexpressed in the periphery of the  
182 colonies formed in acidic hydrogel (Figure 2B, supplementary material Figure S4). Those  
183 qualitative observations were reinforced by a quantitative exploration of the radial distribution  
184 of *gadB* expression (Figure 3). For both hydrogels, the genetic expression is monitored by the  
185 green fluorescent intensity normalized with the red constitutive fluorescent intensity. 3D  
186 kymographs integrating 40 independent microcolonies (X-axis) for each condition represent  
187 in color code *gadB* transcription from the center of the microcolony (Y-axis,  $d_{\text{CM}} = 0 \mu\text{m}$ ) to

188 the edges of the colonies and beyond. Where in neutral pH hydrogel *gadB* expression is low  
189 and almost constant over the radius of the microcolonies (Figure 3A), acidic hydrogels  
190 present a sharp band of *gadB* over-expression in between 25 and 30  $\mu\text{m}$  from the center of the  
191 microcolonies (Figure 3B). This is consistent with the observed spatial expression as the  
192 radius of the microcolonies is 27-28  $\mu\text{m}$  ( $\pm 5 \mu\text{m}$ ) in these experimental conditions.  
193 Interestingly, when microcolonies merge as they grow, they behave like a single colony in  
194 regard to the peripheral *gadB* spatial expression. Similarly, if two microcolonies are in near  
195 contact, the two sides facing each other do not present an over-expression of *gadB* or the  
196 shedding of single cells visible in other areas of the periphery (supplementary material Figure  
197 S5).

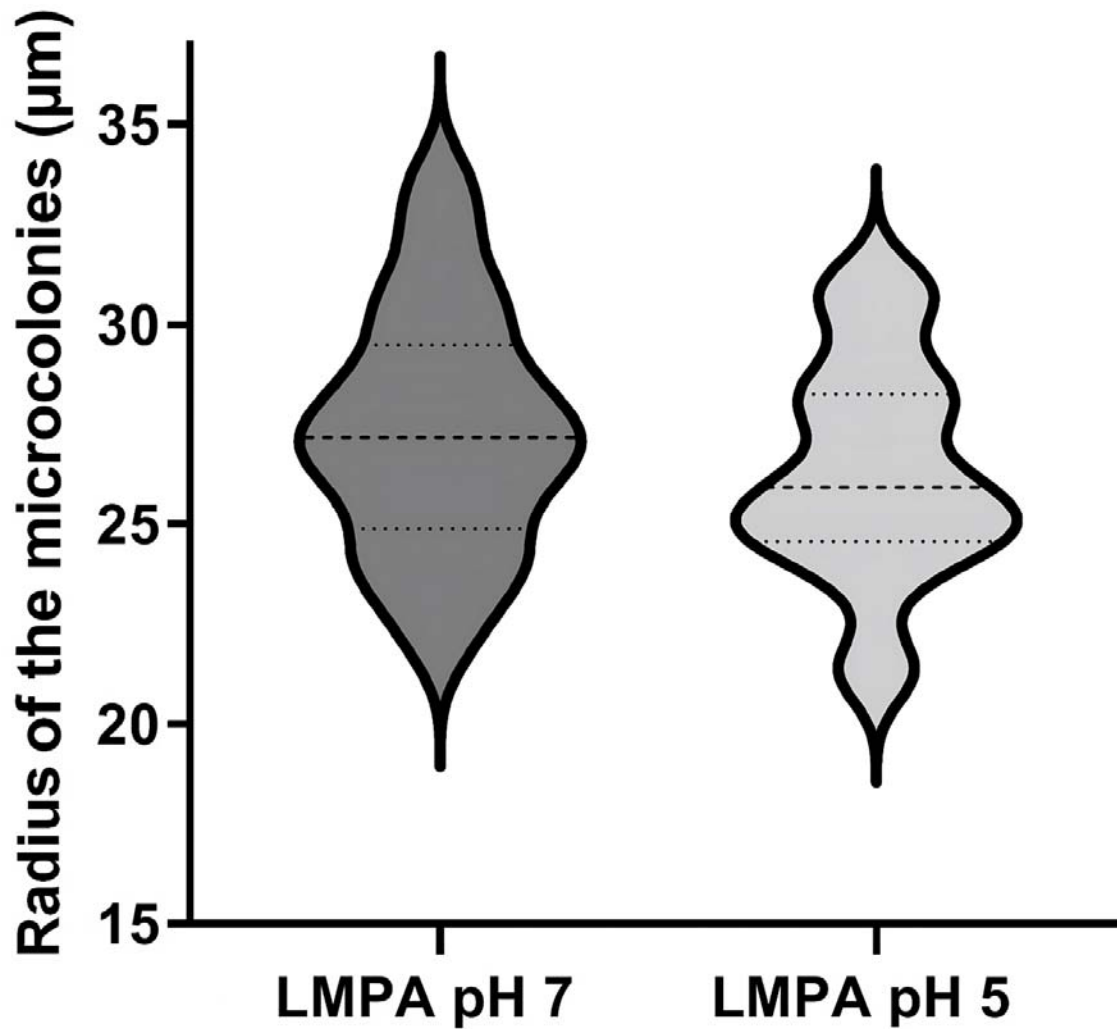
198 Control experiments performed with a constitutive expression of the green fluorescence  
199 protein did not show the spatialization of the expression as presented above (supplementary  
200 material Figure S3). A time-course microscopic analysis allows the observation of *gadB*  
201 expression spatialization in acidic hydrogels as early as microcolonies become visible under  
202 the microscope,  $\sim 48\text{h}$  after inoculation (data not shown). Finally, the spatial repartition of  
203 dead cells in gel-microcolonies as shown by live/dead fluorescent staining indicated a random  
204 distribution of the red dead cells, with no preferential localization in the microcolonies  
205 associated with cells expressing *gadB* (supplementary material Figure S6).

206 Following those results, *E. coli* O157:H7 was then cultured in the presence of *Lactococcus*  
207 *lactis* ssp. *cremoris* (Figure 4). The ability of *L. lactis* to produce *in-situ* lactic acid is used to  
208 replicate the natural acidification of food matrix containing *L. lactis* (cheese), or where a  
209 progressive accumulation of lactic acid occurs (meat). Exploration of the hydrogel was  
210 performed 72-96h after inoculation. Contrary to observations where lactic acid is added in the  
211 hydrogel preparation<sup>29</sup>, microcolonies of *E. coli* O157:H7 are present and possess the same  
212 morphology as seen in mono-cultures in the acidic condition (Figure 4A). Close up on 50  $\mu\text{m}$   
213 thick slices of microcolonies of *E. coli* O157:H7 in close proximity to microcolonies of *L.*  
214 *Lactis* clearly shows the same spatial patterns of *gadB* expression as previously encountered  
215 for the mono-cultures in acidic media (Figure 4B).

216

217

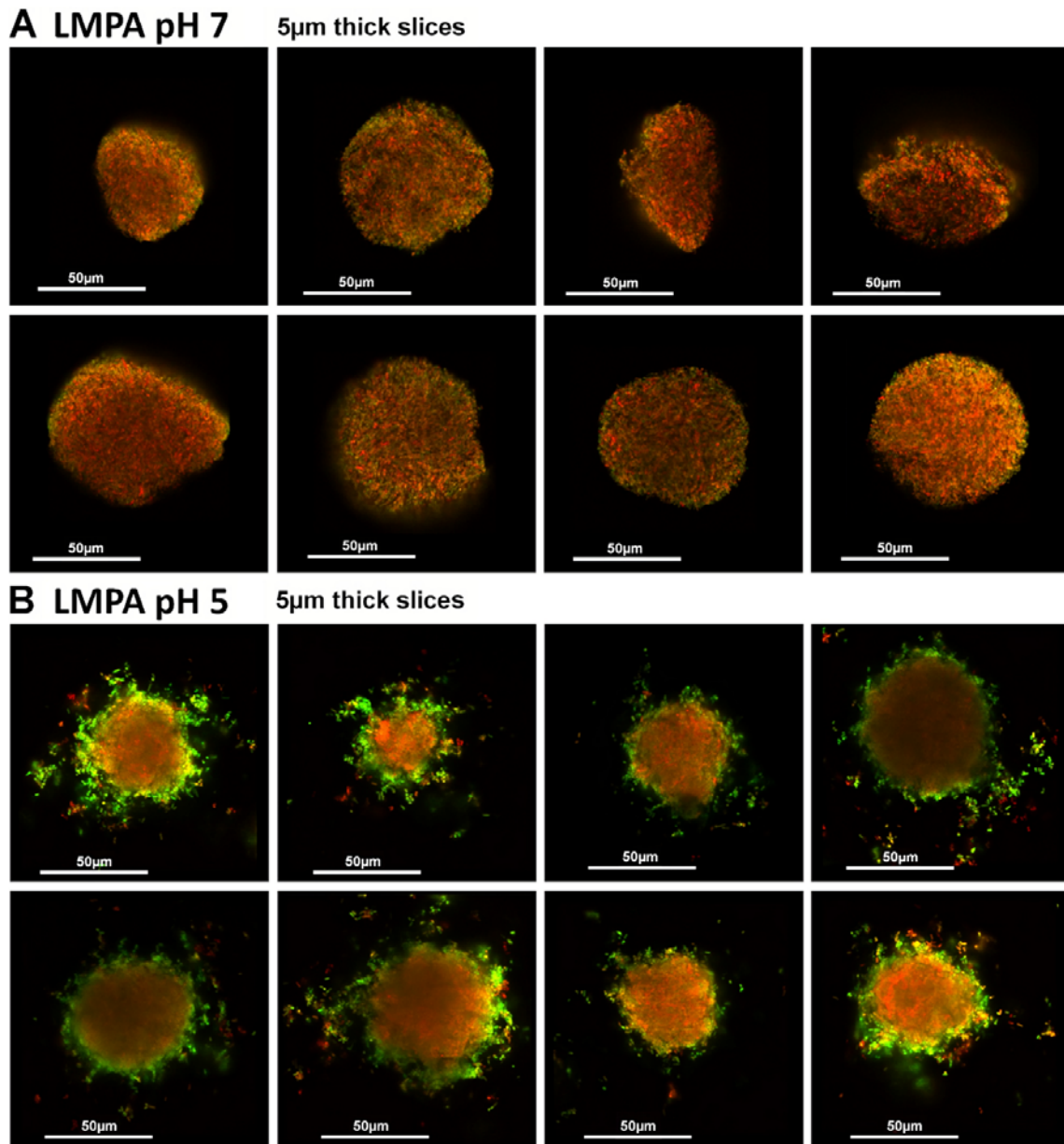




218

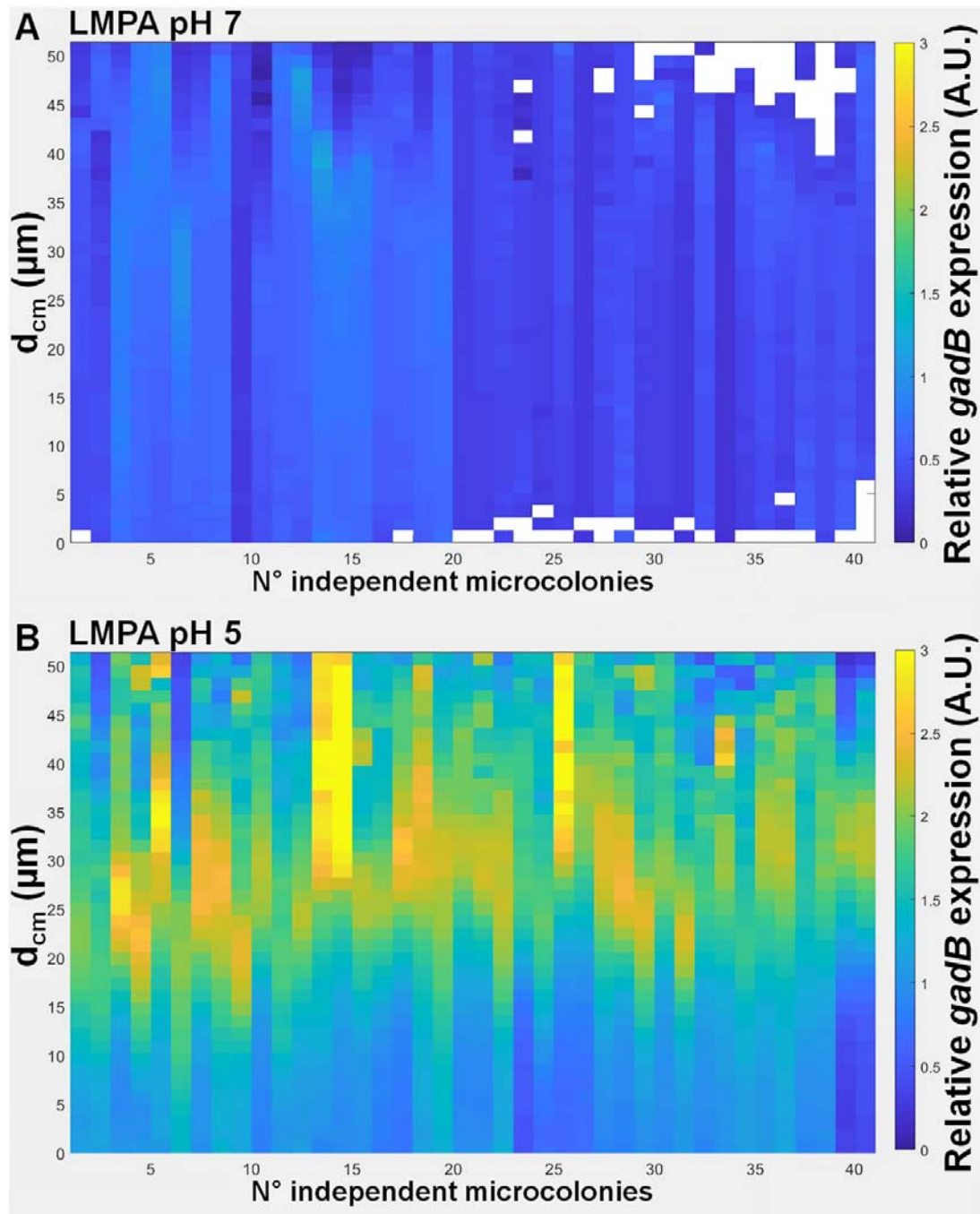
219 **Figure 1: Radius of the microcolonies.** Representation of the radius of the microcolonies in  
220  $\mu\text{m}$ , the width of each figure represents the concentration of the number of values. For each  
221 case, the slashed line is the mean value of radius, and dotted lines delimit the 75 % probability  
222 interval. Radius values were calculated from 40 independent microcolonies.

223



224

225 **Figure 2: Representative images of *gadB* spatial patterns of expression for *E. coli***  
226 **O157:H7 cultivated in neutral (pH=7) or acidic (pH=5) hydrogels.** A series of 5 $\mu$ m slices  
227 of microcolonies is presented in control LMPA pH=7 (A) and the acid matrix LMPA pH=5  
228 (B). The red fluorescence is constitutive and the green fluorescence is expressed as a function  
229 of *gadB* transcription. Supplementary material Figure S3 presents the same representation for  
230 a control constitutive GFP expression in both conditions.

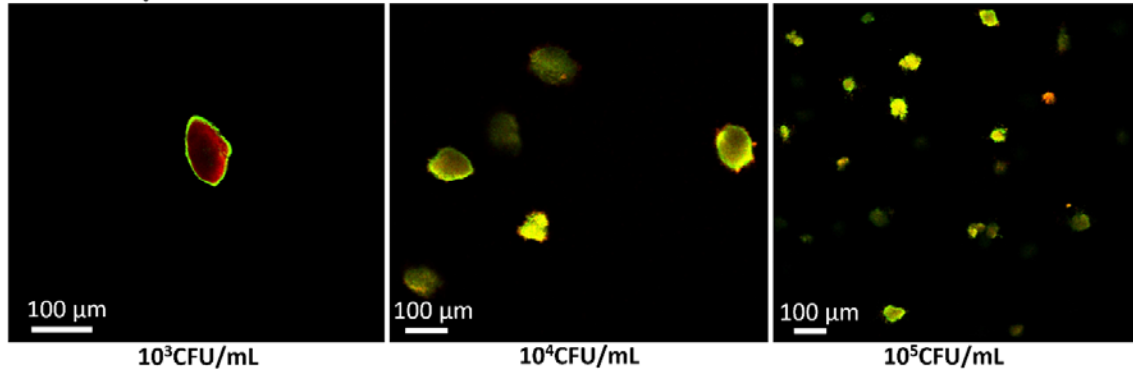


231

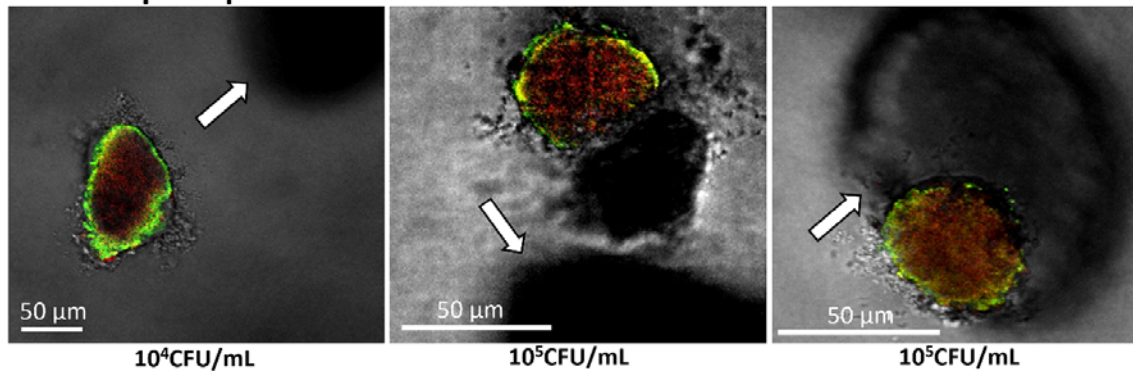
232 **Figure 3: Relative expression of *gadB* in function of the distance from the center of**  
233 **microcolony.** In this representation, the relative *gadB* expression is expressed as the ratio of  
234 green fluorescence intensity over red fluorescent intensity (GFP reporter/RFP constitutive) in  
235 function of the distance from the center of the microcolony ( $d_{cm}$ ) for forty microcolonies  
236 grown in neutral (A) or acidic hydrogels (B).

237

## A LMPA pH7



## B LMPA pH7 5μm thick slices



*E. coli* + *L. lactis*

238

239

240 **Figure 4: Spatial patterns of expression of *gadB* for *E. coli* O157:H7 in the presence of**  
241 ***Lactococcus lactis* ssp. *cremoris*, cultivated at neutral pH in hydrogels.** (A) Visualization  
242 at 96h of microcolonies of *E. coli* O157:H7 co-inoculated from 10<sup>3</sup>CFU/ml to 10<sup>5</sup>CFU/ml,  
243 with *L. lactis* inoculated at 10<sup>3</sup>CFU/ml, in a hydrogel at neutral pH. The red fluorescence is  
244 constitutive and the green fluorescence is expressed as a function of *gadB* transcription. (B)  
245 Microcolonies of *E. coli* O157:H7 close or in contact with *L. lactis* microcolonies. The two  
246 rightmost pictures were taken at 96h and the one on the left at 72h. *L. lactis* microcolonies are  
247 visualized in the bottom images thanks to the transmission detection (indicated by white  
248 arrows).

249

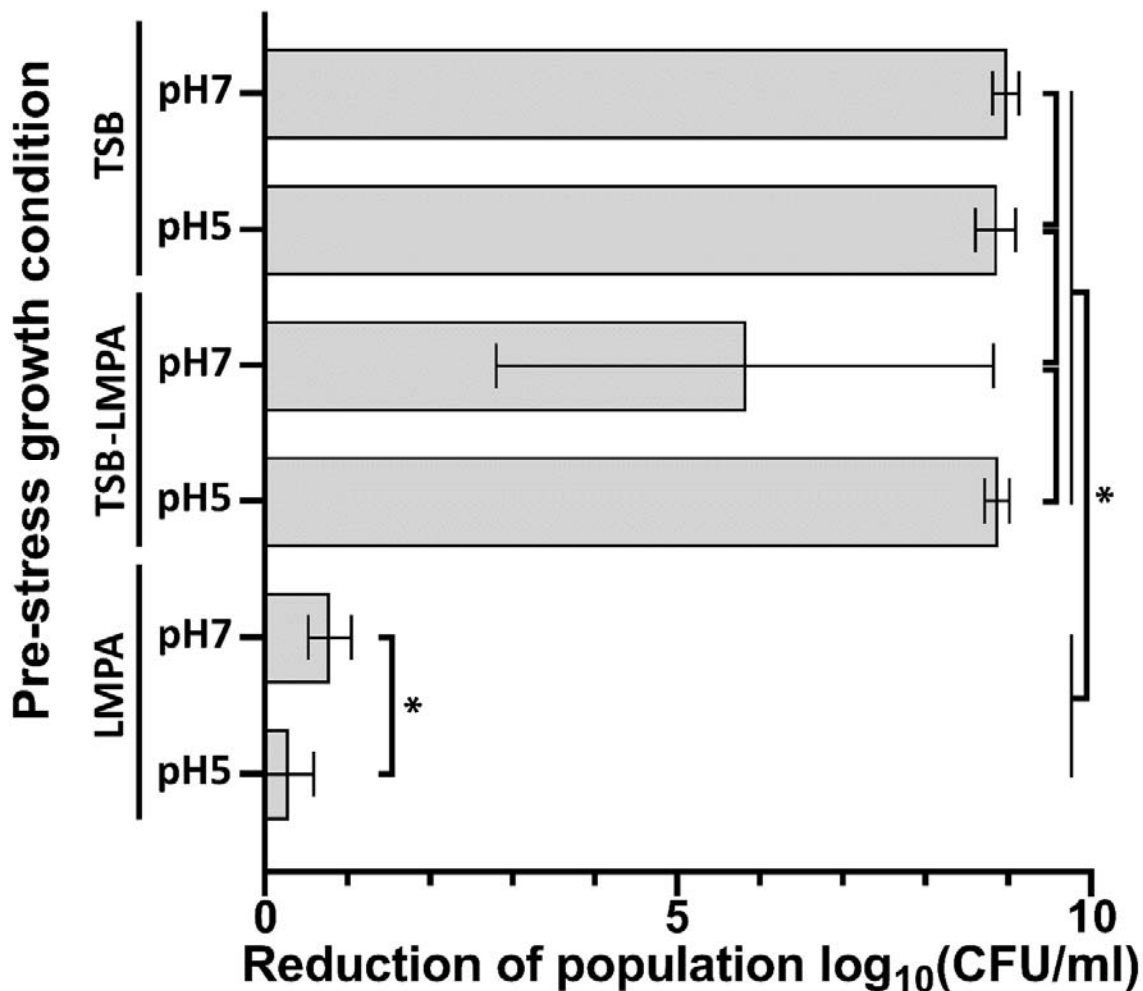
## 250 ***Increased survival to acidic stomachal stress of E. coli* grown in gel-microcolonies**

251 As the capacity of survival to stomachal acidic stress of *E. coli* O157:H7 populations is of  
252 interest for public health safety, the acid resistance of bacteria grown planktonically or in  
253 hydrogel matrices was evaluated by enumeration on agar after acid stress.

254 Cultures of *E. coli* O157:H7 adjusted to  $10^4$  CFU/ml were incubated at neutral (pH=7) or acid  
255 pH (pH=5) in either TSB or LMPA. After 96h of incubation at 20°C, the populations reached  
256 values of log CFU/ml of 9.4/9.6 in TSB at pH neutral/acid and 9.5/8.6 in LMPA at pH  
257 neutral/acid.

258 Bacteria grown in planktonic conditions (TSB) were highly sensitive to the 4-hours exposition  
259 to pH=2 with a total loss of the culturable population (9 log reduction) (Figure 5). In contrast,  
260 cells grown as spatially organized colonies in LMPA for 96h presented a statistically high  
261 tolerance to this strong acidic stress ( $P<0.05$ ). The best tolerance was observed for the  
262 microcolonies incubated at a pH of 5 with a log reduction as low as 0.29 log CFU/ml,  
263 statistically significantly lower than the reduction observed for microcolonies incubated at  
264 neutral pH, where the log reduction is 0.78 CFU/ml ( $P<0.05$ ). To test for interferences of the  
265 hydrogel to bacterial acidic stress, planktonic populations cultivated in TSB were encased in  
266 LMPA just before the survival test. Log reduction of these control planktonic populations  
267 suspended in LMPA presented significant similar sensitivity than planktonic TSB culture  
268 ( $P>0.05$ ), indicating no buffering effect of agarose to stomachal acidic stress.

## Reduction of population in acidic condition



269

270

271 **Figure 5: Population log- reduction of planktonic cultures and hydrogel microcolonies of**  
272 ***E. coli* O157:H7 upon acidic exposure.** Bacterial cells were exposed for 4 hours at a pH of 2  
273 (HCl). Representation of the mean reduction of population log between the control and  
274 survival groups. Bacteria incubated and tested in planktonic are on the top (TSB), those  
275 incubated in planktonic but encased in hydrogels before the test are on the middle (TSB-  
276 LMPA), and the results for populations incubated and tested in hydrogels are on the bottom  
277 (LMPA). A star indicates a significant difference between values ( $P < 0.05$ ). Data resulted  
278 from at least six biological replicates.

#### 279 4) Discussion

280 The behavior of microbial population in laboratory liquid growth media can strongly deviate  
281 from what is observed in real solid food matrices<sup>18,36-40</sup>. The environmental heterogeneity of  
282 structured media is listed as one of the four main causes of cellular variation, among genetic  
283 variation, aging and stochasticity of gene expression<sup>41</sup>. As such, it can trigger a large  
284 diversity of phenotypic cell expression in the same biotope, promoting the cohabitation of  
285 cells with different spectra of behaviors, such as stress response or virulence<sup>42,43</sup>. Structured  
286 food are not an optimal medium from an exploration perspective as the opacity of numerous  
287 food matrices prevents live imaging and microscopic approaches. To overcome these  
288 limitations, several studies take advantage of synthetic hydrogelled systems to simplify and  
289 control the parameters of growth of embedded bacteria. Here, low melting point agarose is  
290 used as a gelling agent in which cells can be dispersed without thermal stress and with tunable  
291 textures and media compositions. Thus, in a recent contribution, we have been able to mimic  
292 the texture of various food environments such as grounded meat or cheese<sup>29</sup>. The experiments  
293 presented in this work show that the morphology of microcolonies in the media  
294 complemented with HCl are different from the neutral pH control, in particular bacteria are  
295 shedding from the periphery of the microcolonies. This effect can be explained by a combined  
296 action of relaxed gel structures due to low pH and the higher motility of *E. coli* O157:H7  
297 when acidic conditions are encountered<sup>29,44</sup>.

298 In this study, we observed a clear overexpression of *gadB* for a subpopulation of cells  
299 localized in the periphery of microcolonies formed in acidic hydrogels. This gene is  
300 overexpressed at levels 2-3 times higher than in neutral conditions, which is in accordance  
301 with results obtained in planktonic conditions (Supplementary material Figure S2). We  
302 confirmed that this spatialization was neither associated with dead cells (supplementary  
303 material Figure S6), nor a limitation of oxygen for GFP maturation in the center of the  
304 microcolony (supplementary material Figure S3). The use of a single plasmid bearing both the  
305 genes for the constitutive and induced fluorescence means that, at the image analysis step, we  
306 prevented bias due to differences in plasmid copy numbers or differences in coloration from a  
307 mix of dye/genetic reporters<sup>45</sup>. The use of two lasers with different properties of matrix  
308 penetration could lead to a bias in the Z axis, but 3D analysis of all the cells in a microcolony  
309 reduces the bias as any offset at the bottom of the agglomerate is compensated by an opposite  
310 offset at the top.

311 Spatial patterns of genetic expression were previously reported for other genes in other  
312 bacterial species in surface biofilms either on solid or liquid, such as localized expression of  
313 *E. coli* sigma factors and type 1 pili, as well as *Pseudomonas aeruginosa*  $\beta$ -lactamase in  
314 biofilms<sup>46-48</sup>. To our knowledge, such patterns of gene expression were never reported in  
315 food or hydrogel matrices. Last experiments of co-cultures of *E. coli* O157:H7 and *L. lactis*  
316 demonstrate that the pattern of *gadB* expression could naturally occur in food matrix through  
317 a progressive accumulation and diffusion of lactic acid in the media, such as in cheese or meat  
318 products.

319 Then, we explored the consequences of growing populations in a structured media in regards  
320 of survival to an exposition to low pH media. Results showed that, regardless of the initial pH,  
321 populations incubated in a semi-solid media have a better tolerance to acid stress than those  
322 grown in liquid broths, where no surviving cells were detected. This underlines limitations in  
323 modeling food-borne pathogens behavior in food from data obtained in liquid conditions, as  
324 previously shown in other studies<sup>18,36,37,49</sup>.

325 It has been suggested from other studies that the components of the matrix could have a buffer  
326 effect that protects embedded cells by preventing the drop in pH. Our tests show that  
327 planktonic populations dispersed in hydrogel did not present the same survival fitness that  
328 those cultivated as microcolonies in the same hydrogel. The hypothesis of a buffer effect due  
329 to hydrogel interference was tested for bacteria incubated in hydrogel and it was statistically  
330 rejected. This is supported by another study in a gelified dairy matrix, where food related  
331 bacteria were dispersed without incubation in the gel before application of the acidic stress. It  
332 was reported that no protective effect existed compared to the same conditions in liquid media  
333<sup>50</sup>.

334 A parameter that could explain this difference of survival between the populations incubated  
335 or not in the hydrogels would be the spatial organization of cells. The hydrogels showed  
336 evidence of deliquescence such as unraveling of filaments and loss of stiffness but maintained  
337 enough structural integrity to ensure the microcolonies did not disperse. The ability of  
338 spatially organized populations of bacterial cells to better survive acid stress was described for  
339 pathogenic bacteria but also for auxiliary microbiota and probiotics, such as *Lactobacillus*  
340 strains<sup>51,52</sup>. The bacteria could secrete extracellular polymeric substances (EPS) when grown  
341 in communities as described in biofilms<sup>53,54</sup>. For *E. coli* O157:H7, tolerance of the bacteria to  
342 low pH could further involve the DNA binding protein Dps which is known to enhance  
343 survivability when local nutrients are exhausted<sup>55,56</sup>.



344 From this study in a hydrogel matrix, *gadB* appeared to be more expressed at the periphery of  
345 the *E. coli* O157:H7 microcolonies in acidic conditions. This correlated with an increased  
346 tolerance to the type of acid stress that can be encountered by bacterial cells after ingestion of  
347 food. These findings are of interest for public health as they underline possible differences  
348 between liquid and solid food products on the infectious dose and bacterial virulence. In this  
349 case, the tolerance to acidity could mean an increase in the bacterial load that can survive in  
350 the digestive system as well as a phenotype more likely to colonize the gut lining. In order to  
351 alleviate public health issues, differences in bacterial behavior in planktonic conditions versus  
352 microcolonies could be considered when integrating phenotypic heterogeneity in risk  
353 assessment. Modeling pathogens growth and survival should take in account the gelled  
354 environments where the spatialization of genetic expression and its resulting populational  
355 effects could deeply affect pathogens behavior and virulence after ingestion.

#### 356 **Declaration of competitive interest**

357 None

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#### 366 **Author contributions**

367 CSM, RB and MD, conceptualized the overarching aims of the research study. CSM, NC,  
368 MD, MG, AC, GJ, FDB, SL, RB and MD conceived and designed the experiments. CSM,  
369 NC, MD, MG and AC performed the experiments and data acquisition. CSM, NC, MD, AC,  
370 GJ, FDB, SL, RB and MD analyzed and interpreted the data. RB and MD had management as  
371 well as coordination responsibility for the execution of the research work. RB and MD  
372 contributed to the acquisition of the financial supports and resources leading to this  
373 publication. CSM, NC, MD, MG, AC, GJ, FDB, SL, RB and MD wrote the article, including

374 drafting and revising critically the manuscript for important intellectual content. All authors  
375 have declared no competing interests.

376 **References**

- 377 1. Fairbrother, J. M. & Nadeau, É. *Escherichia coli*: On-farm contamination of animals.  
378 *OIE Rev. Sci. Tech.* **25**, 555–569 (2006).
- 379 2. Kintz, E., Brainard, J., Hooper, L. & Hunter, P. Transmission pathways for sporadic  
380 Shiga-toxin producing *E. coli* infections: A systematic review and meta-analysis. *Int. J.*  
381 *Hyg. Environ. Health* **220**, 57–67 (2017).
- 382 3. Fremaux, B., Prigent-Combaret, C. & Vernozy-Rozand, C. Long-term survival of  
383 Shiga toxin-producing *Escherichia coli* in cattle effluents and environment: an updated  
384 review. *Vet. Microbiol.* **132**, 1–18 (2008).
- 385 4. Jackson, T. C., Hardin, M. D. & Acuff, G. R. Heat resistance of *Escherichia coli*  
386 O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and  
387 holding temperatures. *J. Food Prot.* **59**, 230–237 (1996).
- 388 5. The European Union One Health 2019 Zoonoses Report. *EFSA J.* **19**, (2021).
- 389 6. Kaper, J. B., Nataro, J. P. & Mobley, H. L. T. Pathogenic *Escherichia coli*. *Nat. Rev.*  
390 *Microbiol.* **2004 22 2**, 123–140 (2004).
- 391 7. Delignette-Muller, M. L. & Cornu, M. Quantitative risk assessment for *Escherichia*  
392 *coli* O157:H7 in frozen ground beef patties consumed by young children in French  
393 households. *Int. J. Food Microbiol.* **128**, 158–164 (2008).
- 394 8. Devleeschauwer, B., Pires, S. M., Young, I., Gill, A. & Majowicz, S. E. Associating  
395 sporadic, foodborne illness caused by Shiga toxin-producing *Escherichia coli* with  
396 specific foods: a systematic review and meta-analysis of case-control studies.  
397 *Epidemiol. Infect.* **147**, (2019).
- 398 9. Lobete, M. M., Fernandez, E. N. & Van Impe, J. F. M. Recent trends in non-invasive in  
399 situ techniques to monitor bacterial colonies in solid (model) food. *Frontiers in*  
400 *Microbiology* vol. 6 (2015).
- 401 10. Schröter, L. & Dersch, P. Phenotypic Diversification of Microbial Pathogens—  
402 Cooperating and Preparing for the Future. *Journal of Molecular Biology* vol. 431  
403 4645–4655 (2019).

- 404 11. Stewart, P. S. & Franklin, M. J. Physiological heterogeneity in biofilms. *Nature*  
405 *Reviews Microbiology* vol. 6 199–210 (2008).
- 406 12. Rantsiou, K., Mataragas, M., Alessandria, V. & Cocolin, L. Expression of virulence  
407 genes of *Listeria monocytogenes* in food. *J. Food Saf.* **32**, 161–168 (2012).
- 408 13. Costello, K. M. *et al.* Modelling the microbial dynamics and antimicrobial resistance  
409 development of *Listeria* in viscoelastic food model systems of various structural  
410 complexities. *Int. J. Food Microbiol.* **286**, 15–30 (2018).
- 411 14. Rowbury, R. J. & Goodson, M. Extracellular sensing and signalling pheromones  
412 switch-on thermotolerance and other stress responses in *Escherichia coli*. *Sci. Prog.* **84**,  
413 205–233 (2001).
- 414 15. Bhattacharjee, T. & Datta, S. S. Bacterial hopping and trapping in porous media. *Nat.*  
415 *Commun.* **10**, (2019).
- 416 16. Antwi, M. *et al.* Influence of a gel microstructure as modified by gelatin concentration  
417 on *Listeria innocua* growth. *Innov. Food Sci. Emerg. Technol.* **7**, 124–131 (2006).
- 418 17. Kabanova, N., Stulova, I. & Vilu, R. Microcalorimetric study of the growth of bacterial  
419 colonies of *Lactococcus lactis* IL1403 in agar gels. *Food Microbiol.* **29**, 67–79 (2012).
- 420 18. Skandamis, P. N. & Jeanson, S. Colonial vs. planktonic type of growth: Mathematical  
421 modeling of microbial dynamics on surfaces and in liquid, semi-liquid and solid foods.  
422 *Frontiers in Microbiology* vol. 6 (2015).
- 423 19. Antwi, M., Bernaerts, K., Van Impe, J. F. & Geeraerd, A. H. Modelling the combined  
424 effects of structured food model system and lactic acid on *Listeria innocua* and  
425 *Lactococcus lactis* growth in mono- and coculture. *Int. J. Food Microbiol.* **120**, 71–84  
426 (2007).
- 427 20. Smith, D. K., Kassam, T., Singh, B. & Elliott, J. F. *Escherichia coli* has two  
428 homologous glutamate decarboxylase genes that map to distinct loci. *J. Bacteriol.* **174**,  
429 5820–5826 (1992).
- 430 21. Cotter, P. D., Gahan, C. G. M. & Hill, C. A glutamate decarboxylase system protects  
431 *Listeria monocytogenes* in gastric fluid. *Mol. Microbiol.* **40**, 465–475 (2001).

- 432 22. Nomura, M. *et al.* Lactococcus lactis contains only one glutamate decarboxylase gene.  
433 *Microbiology* **145**, 1375–1380 (1999).
- 434 23. Waterman, S. R. & Small, P. L. C. The glutamate-dependent acid resistance system of  
435 *Escherichia coli* and *Shigella flexneri* is inhibited in vitro by l-trans-pyrrolidine-2,4-  
436 dicarboxylic acid. *FEMS Microbiol. Lett.* **224**, 119–125 (2003).
- 437 24. Waterman, S. R. & Small, P. L. C. Transcriptional expression of *Escherichia coli*  
438 glutamate-dependent acid resistance genes *gadA* and *gadBC* in an *hns rpoS* mutant. *J.*  
439 *Bacteriol.* **185**, 4644–4647 (2003).
- 440 25. Capitani, G. *et al.* Crystal structure and functional analysis of *Escherichia coli*  
441 glutamate decarboxylase. *EMBO J.* **22**, 4027–4037 (2003).
- 442 26. De Biase, D., Tramonti, A., Bossa, F. & Visca, P. The response to stationary-phase  
443 stress conditions in *Escherichia coli*: Role and regulation of the glutamic acid  
444 decarboxylase system. *Mol. Microbiol.* **32**, 1198–1211 (1999).
- 445 27. De Roy, K., Marzorati, M., Van den Abbeele, P., Van de Wiele, T. & Boon, N.  
446 Synthetic microbial ecosystems: an exciting tool to understand and apply microbial  
447 communities. *Environ. Microbiol.* **16**, 1472–1481 (2014).
- 448 28. Verheyen, D. *et al.* Food microstructure and fat content affect growth morphology,  
449 growth kinetics, and preferred phase for cell growth of *Listeria monocytogenes* in fish-  
450 based model systems. *Appl. Environ. Microbiol.* **85**, (2019).
- 451 29. Saint Martin, C. *et al.* Spatial organisation of *Listeria monocytogenes* and *Escherichia*  
452 *coli* O157:H7 cultivated in gel matrices. *Food Microbiol.* **103**, 103965 (2022).
- 453 30. Gobert, A. P. *et al.* Shiga Toxin Produced by Enterohemorrhagic *Escherichia coli*  
454 Inhibits PI3K/NF- $\kappa$ B Signaling Pathway in Globotriaosylceramide-3-Negative Human  
455 Intestinal Epithelial Cells. *J. Immunol.* **178**, 8168–8174 (2007).
- 456 31. Chagnot, C. *et al.* Colonization of the meat extracellular matrix proteins by O157 and  
457 non-O157 enterohemorrhagic *Escherichia coli*. *Int. J. Food Microbiol.* **188**, 92–98  
458 (2014).
- 459 32. Graveline, R. *et al.* Monitoring F1651 P-like fimbria expression at the single-cell level  
460 reveals a highly heterogeneous phenotype. *Infect. Immun.* **83**, 1929–1939 (2015).

- 461 33. Lim, H. N. & Van Oudenaarden, A. A multistep epigenetic switch enables the stable  
462 inheritance of DNA methylation states. *Nat. Genet.* **39**, 269–275 (2007).
- 463 34. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in  
464 *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 6640–  
465 6645 (2000).
- 466 35. Hartmann, R. *et al.* Quantitative image analysis of microbial communities with  
467 BiofilmQ. *Nat. Microbiol.* **6**, 151–156 (2021).
- 468 36. Pin, C., Sutherland, J. P. & Baranyi, J. Validating predictive models of food spoilage  
469 organisms. *J. Appl. Microbiol.* **87**, 491–499 (1999).
- 470 37. Smith, S. & Schaffner, D. W. Evaluation of a *Clostridium perfringens* predictive  
471 model, developed under isothermal conditions in broth, to predict growth in ground  
472 beef during cooling. *Appl. Environ. Microbiol.* **70**, 2728–2733 (2004).
- 473 38. Baka, M., Noriega, E., Van Langendonck, K. & Van Impe, J. F. Influence of food  
474 intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model  
475 systems at suboptimal temperatures. *Int. J. Food Microbiol.* **235**, 17–27 (2016).
- 476 39. Baka, M., Verheyen, D., Cornette, N., Vercruyssen, S. & Van Impe, J. F. Salmonella  
477 Typhimurium and *Staphylococcus aureus* dynamics in/on variable (micro)structures of  
478 fish-based model systems at suboptimal temperatures. *Int. J. Food Microbiol.* **240**, 32–  
479 39 (2017).
- 480 40. Baka, M., Vercruyssen, S., Cornette, N. & Van Impe, J. F. Dynamics of *Listeria*  
481 *monocytogenes* at suboptimal temperatures in/on fish-protein based model systems:  
482 Effect of (micro)structure and microbial distribution. *Food Control* **73**, 43–50 (2017).
- 483 41. Bury-Moné, S. & Sclavi, B. Stochasticity of gene expression as a motor of epigenetics  
484 in bacteria: from individual to collective behaviors. *Research in Microbiology* vol. 168  
485 503–514 (2017).
- 486 42. Caniça, M., Manageiro, V., Abriouel, H., Moran-Gilad, J. & Franz, C. M. A. P.  
487 Antibiotic resistance in foodborne bacteria. *Trends in Food Science and Technology*  
488 vol. 84 41–44 (2019).

- 489 43. Nielsen, M. B., Knudsen, G. M., Danino-Appleton, V., Olsen, J. E. & Thomsen, L. E.  
490 Comparison of heat stress responses of immobilized and planktonic *Salmonella*  
491 *enterica* serovar Typhimurium. *Food Microbiol.* **33**, 221–227 (2013).
- 492 44. Su, L. K. *et al.* Lysogenic infection of a Shiga toxin 2-converting bacteriophage  
493 changes host gene expression, enhances host acid resistance and motility. *Mol. Biol.* **44**,  
494 54–66 (2010).
- 495 45. Cinquin, B. & Lopes, F. Structure and fluorescence intensity measurements in biofilms.  
496 in *Methods in Molecular Biology* vol. 2040 117–133 (Humana Press Inc., 2019).
- 497 46. Lenz, A. P., Williamson, K. S., Pitts, B., Stewart, P. S. & Franklin, M. J. Localized  
498 gene expression in *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* **74**,  
499 4463–4471 (2008).
- 500 47. Floyd, K. A. *et al.* Adhesive Fiber Stratification in Uropathogenic *Escherichia coli*  
501 Biofilms Unveils Oxygen-Mediated Control of Type 1 Pili. *PLOS Pathog.* **11**,  
502 e1004697 (2015).
- 503 48. Klauck, G., Serra, D. O., Possling, A. & Hengge, R. Spatial organization of different  
504 sigma factor activities and c-di-GMP signalling within the three-dimensional landscape  
505 of a bacterial biofilm. *Open Biol.* **8**, (2018).
- 506 49. Ross, T. & McMeekin, T. A. Modeling Microbial Growth Within Food Safety Risk  
507 Assessments. *Risk Anal.* **23**, 179–197 (2003).
- 508 50. Hernández-Galán, L. *et al.* Effect of dairy matrices on the survival of *Streptococcus*  
509 *thermophilus*, *Brevibacterium aurantiacum* and *Hafnia alvei* during digestion. *Food*  
510 *Res. Int.* **100**, 477–488 (2017).
- 511 51. Heumann, A. *et al.* Intestinal release of biofilm-like microcolonies encased in calcium-  
512 pectinate beads increases probiotic properties of *Lactobacillus paracasei*. *npj*  
513 *Biofilms Microbiomes* 2020 61 **6**, 1–12 (2020).
- 514 52. Chamignon, C. *et al.* Evaluation of the Probiotic Properties and the Capacity to Form  
515 Biofilms of Various *Lactobacillus* Strains. *Microorg.* 2020, Vol. 8, Page 1053 **8**, 1053  
516 (2020).
- 517 53. Lee, J. *et al.* Structure and Function of the *Escherichia coli* Protein YmgB: A Protein  
518 Critical for Biofilm Formation and Acid-resistance. *J. Mol. Biol.* **373**, 11–26 (2007).

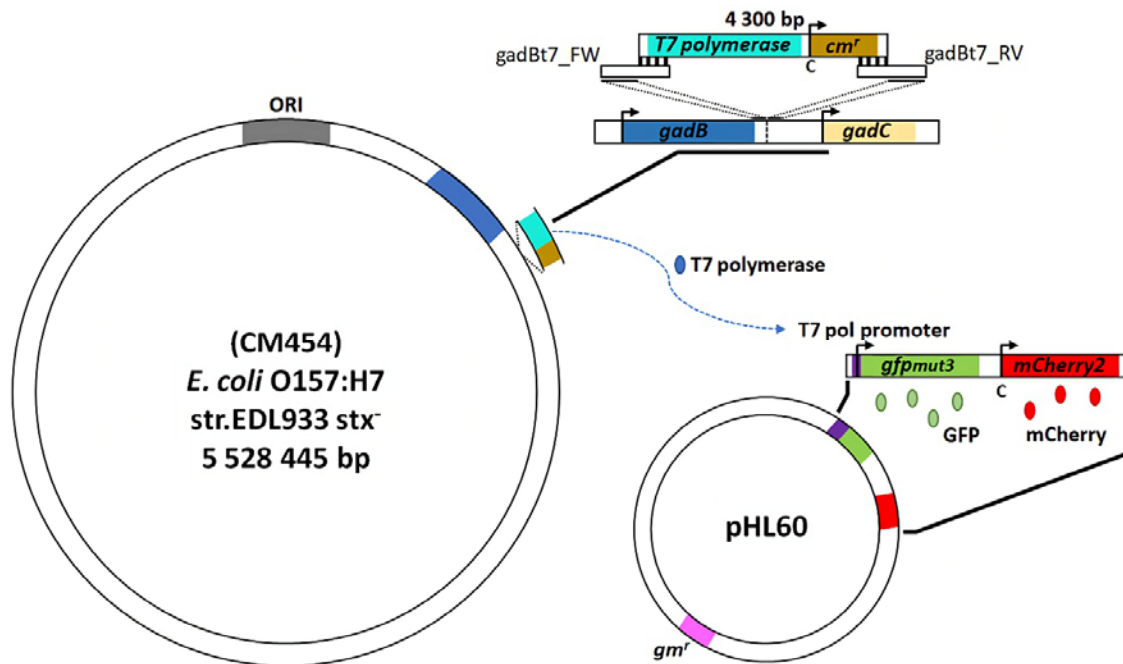
- 519 54. Wood, T. K. Insights on *Escherichia coli* biofilm formation and inhibition from whole-  
520 transcriptome profiling. *Environmental Microbiology* vol. 11 1–15 (2009).
- 521 55. Jeong, K. C., Hung, K. F., Baumber, D. J., Byrd, J. J. & Kaspar, C. W. Acid stress  
522 damage of DNA is prevented by Dps binding in *Escherichia coli* O157:H7. *BMC*  
523 *Microbiol.* **8**, 1–13 (2008).
- 524 56. Sang Ho Choi, Baumber, D. J. & Kaspar, C. W. Contribution of dps to acid stress  
525 tolerance and oxidative stress tolerance in *Escherichia coli* O157:H7. *Appl. Environ.*  
526 *Microbiol.* **66**, 3911–3916 (2000).



527 **Supplementary materials**

528

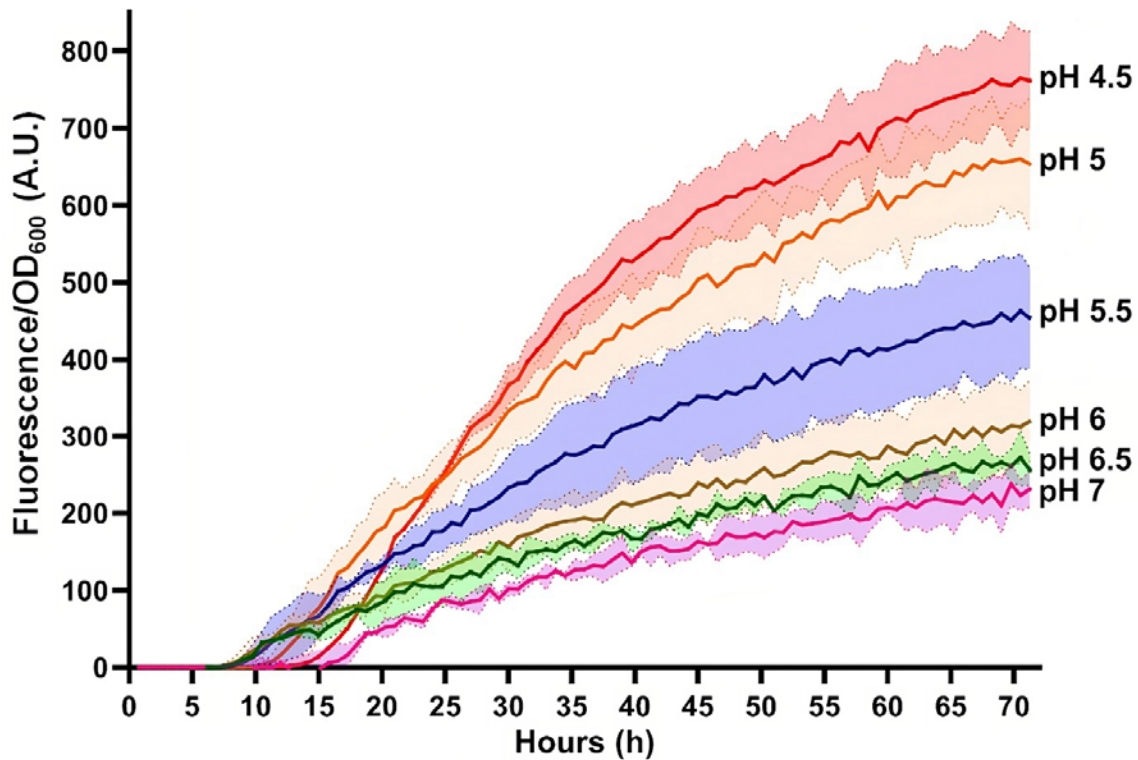
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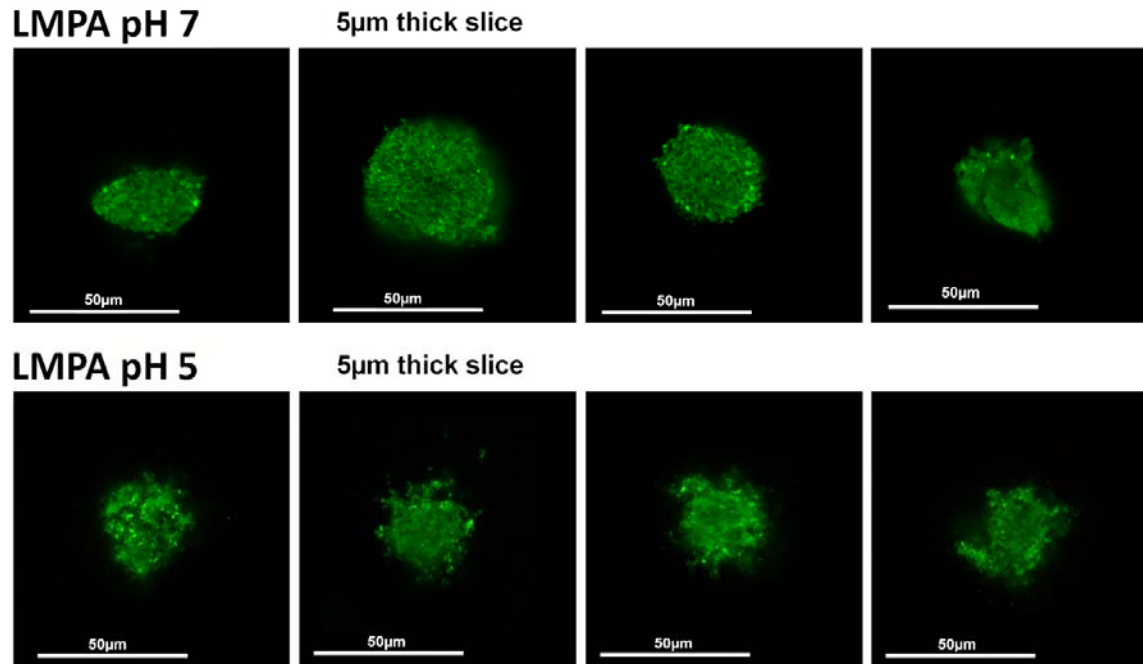
532 **Figure S1: Strategy of the genetic constructions.** (A) chromosome of *E. coli* O157:H7 with  
533 a zoom on the region of the insert and how the different CDS are present in this situation. The  
534 cassette bearing the *T7 polymerase* (in turquoise) and resistance gene *cm<sup>r</sup>* (in brown) is  
535 elongated by PCR with two primers (*gadBt7\_FW* and *gadBt7\_RV*) so that a homology  
536 sequence exists with insertion site. This site is located between the gene interest *gadB* (in  
537 blue) and *gadC* (in yellow). On the right the low copy plasmid pHL60 bearing the fluorophore  
538 encoding genes is represented. It contains the constitutively expressed *mCherry2* (in red)  
539 sequence and *gfpmut3* (in green) under the control of the *T7 pol promoter* (in purple), as well  
540 as a gentamicin resistance gene *gm<sup>r</sup>* (in pink).



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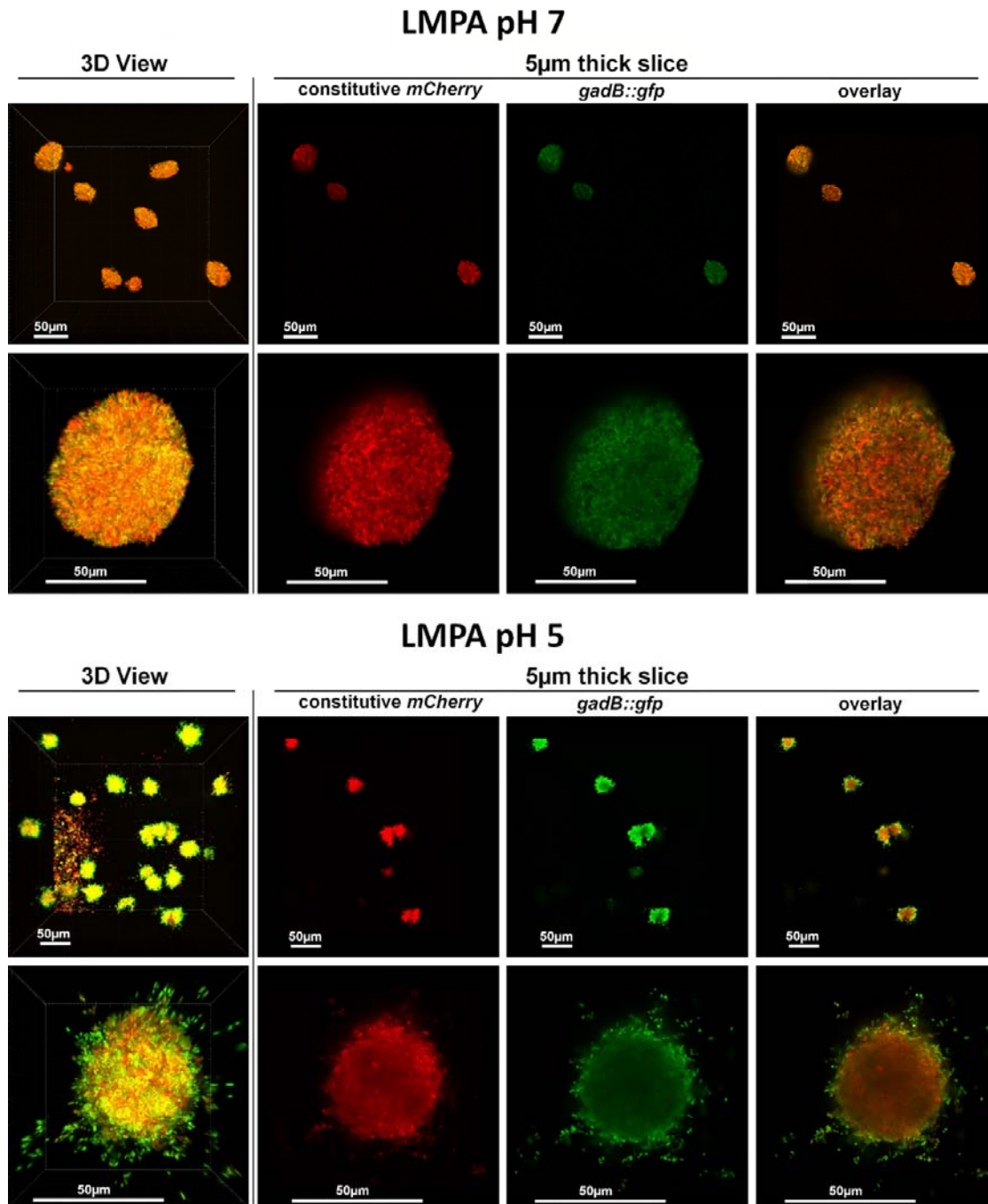
543 **Figure S2: Relative expression of *gadB* in a population in function of time and under**  
544 **different acidity levels.** The fluorescence of planktonic populations of *E. coli* O157:H7  
545 *gadB::GFP* are represented in function of the OD<sub>600</sub>. For each curve representing the mean  
546 signal over time, the standard deviation of values is shown as an area of lighter color. These  
547 curves show that detected fluorescence becomes incrementally brighter with decreasing pHs.  
548 The rise in expressed fluorescence is not linear with greater leaps of intensity below pH=6.0.  
549 Points over time show that fluorescence intensity at pH 4.5 can be 3-4 times higher than  
550 reported values for the pH=7.0 control. The lag time before the green fluorescence is detected  
551 is high at pH 7.0 (15 hours) compared to pH=5.5 where it starts after eight hours. In media  
552 pH=5.0 and pH=4.5, the lag time becomes progressively longer (12 then 14 hours).



553

554

555 **Figure S3: Qualitative presentation of the constitutive GFP spatial expression in**  
556 **microcolonies at pH=7 or pH=5.** A control group with a constitutive GFP to verify that at  
557 pH=7 (up) and/or pH=5 (down) the fluorescence of the green fluorescence is not already  
558 spatialized. Each image is a 5µm slice of a stack.

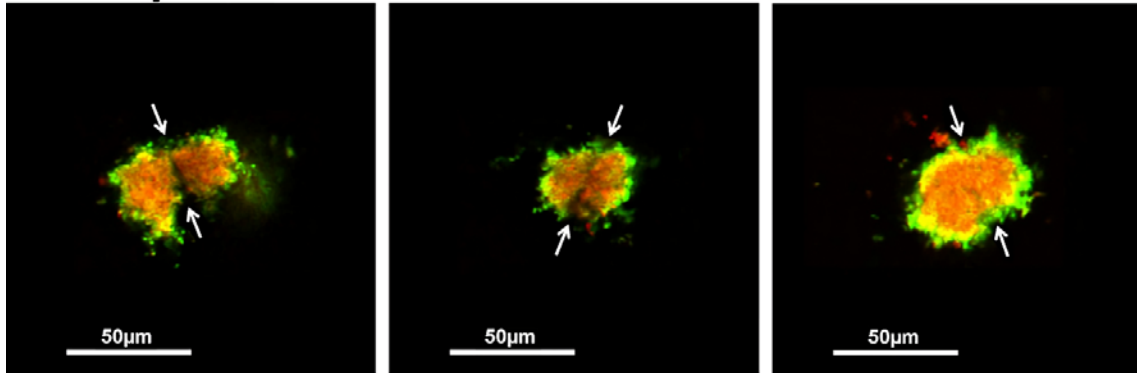


559

560 **Figure S4: Qualitative presentation of *gadB* spatial expression in microcolonies at pH=7**  
561 **or pH=5.** Microscopic observations of the expression of *gadB* in the control LMPA pH=7  
562 (top) and in the acid matrix LMPA pH=5 (bottom). For both groups, the 2 successive rows  
563 use a 40X then a 63X objective to present a collection of microcolonies and a close  
564 observation of a single example. Each image is shown as a 3-dimensional representation  
565 (leftmost column) and in each case a series of 5 µm slices show both fluorescent channels  
566 (middles columns) and the overlay (Rightmost column). The first row of each condition was

567 taken with a 40x air objective (numerical aperture = 0.85) to explore a 290  $\mu\text{m}$  x 290  $\mu\text{m}$   
568 fields.

## LMPA pH 5

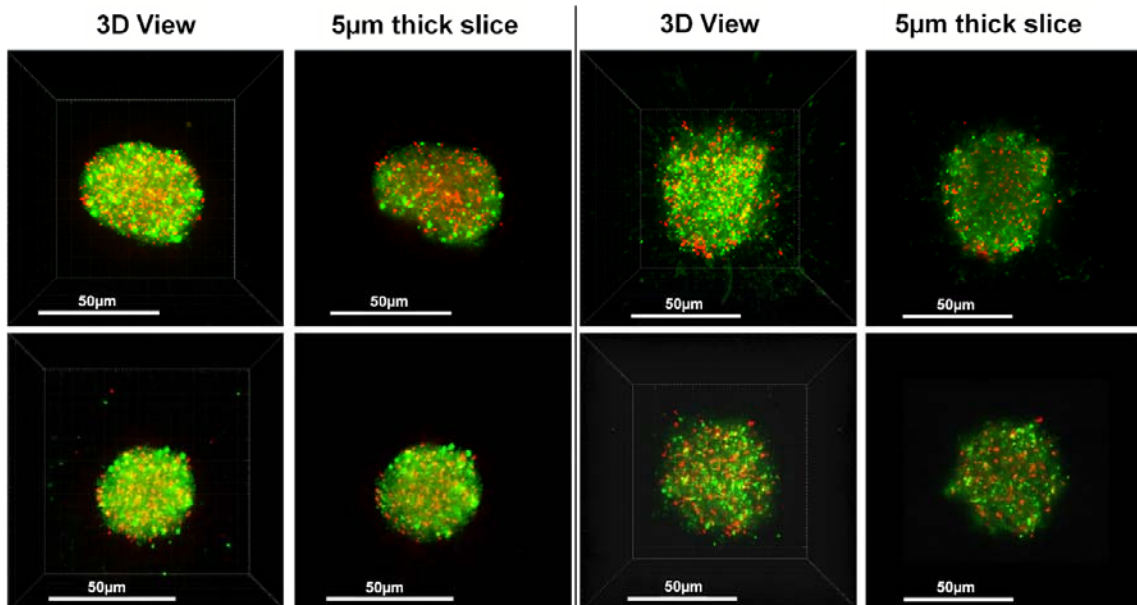


569

570 **Figure S5: The case of joint microcolonies.** Examples where two microcolonies of *E. coli*  
571 O157:H7 are touching or merging in LMPA pH=5. White arrows indicate the separation  
572 between each microcolonies.

## LMPA pH 7

## LMPA pH 5



573

574 **Figure S6: Live/Dead staining in the gel microcolonies.** Bacterial cells in microcolonies  
575 grown in neutral (left side) or acidic (right side) were labeled with the cell impermeant  
576 propidium iodide (red, dead cells) and the cell permeant SYTO 9 (Green, all cells).