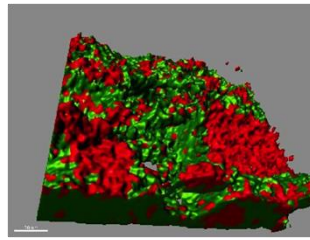


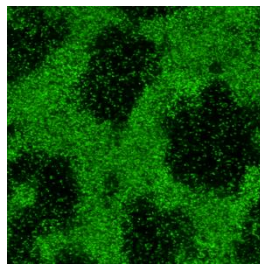
1 **SUPPLEMENTAL MATERIAL**



2

3 **MOVIE S1:** Journey to the center of a honey-comb-like biofilm. Channels potentially acting  
4 as feeders of the biofilm were visualized. Propidium iodide staining showed the presence of  
5 red pockets of materials formed by a mixture of dead cells and released eDNA. The movie  
6 was realised from a 3D reconstruction of CLSM acquisitions using IMARIS software.

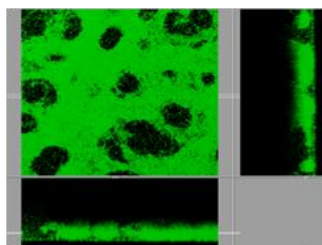
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9 **MOVIE S2:** Both motile and sessile bacteria are visualized in a 48h honey-comb-like  
10 biofilm. Swimming bacteria are preferentially located in the hollow voids. Real time  
11 recording was achieved by XY images acquired every 1.6 s for 90 s using CLSM acquisitions.  
12 Real time is indicated in the lower left-hand corner.

13



14

15 **MOVIE S3:** Planktonic 10403S-GFP bacteria progressively adhere to the surface to form  
16 microcolonies and a network of sessile bacteria after 48h. Time-lapse confocal imaging was  
17 achieved by acquisition every 30 min for 48h.

18 **TABLE S1:** Characteristics of *Listeria monocytogenes* isolates used in this study (ListRA  
 19 collection).

20

Strain	Origin	Serovar	Reference	Lineage <sup>1</sup>	Clonal complex <sup>1</sup>	Swimming Diameter <sup>2</sup>	Mean thickness <sup>3</sup>	Roughness <sup>3</sup>	Biovolume <sup>3</sup>
<b>10403S gfp</b>	human clinical isolate/modified	1/2a	This study	II	CC7	+	26.49	0.36	251050
<b>1<sup>E</sup></b>	Machine cheese making plant	1/2b	(1)	I	CC3-CC6	+	14.01	0.44	122621
<b>1F</b>	Cheese	1/2a	(1)	II	CC121-101	+	21.25	0.19	310290
<b>1S</b>	Brine	1/2a	(1)	II	CC121-101	+	28.23	0.15	385557
<b>2F</b>	Cheese	1/2a	(1)	II	CC121-101	+	17.76	0.18	262023
<b>370P-Lm</b>	Food	ND	INRA UMR 1319	I	CC3-CC6	+	20.47	0.28	230764
<b>3<sup>E</sup></b>	sink cheese making plant	1/2b	(1)	I	CC3-CC6	+	17.68	0.26	211170
<b>50-4</b>	Soil	ND	INRA, UMR 1347	I	CC1	+	27.31	0.25	288587
<b>51-8</b>	Soil	ND	INRA, UMR 1347	I	CC1	+	20.32	0.21	280228
<b>51-9</b>	Soil	ND	INRA, UMR 1347	I	CC1	+	20.99	0.21	292682
<b>6<sup>E</sup></b>	wall cheese making plant	1/2a	(1)	II	CC7	+	17.94	0.19	280911
<b>7F</b>	Cheese	3b	(1)	II	CC121-101	+	20.28	0.20	302147
<b>CIP 103573</b>	Milk	1/2c		II	CC9	+	48.53	0.30	232544
<b>CIP 103574</b>	Milk	1/2a		II	CC37	+	42.15	0.19	566971
<b>CIP 104794</b>	guinea pig	1/2a		II	CC7	+	48.87	0.25	608339
<b>CIP 78.38</b>	unknown	4b		II	CC1	+	31.44	0.31	221140
<b>CIP 78.39</b>	Chicken	4c		III	CC131	+	24.08	0.20	328288
<b>CIP 82.110</b>	Rabbit	1/2		II	CC7	-	21.04	0.17	305561
<b>EGD-e</b>	Rabbit	1/2		II	CCnd3	+	34.01	0.25	367226
<b>H1</b>	healthy pregnant woman	1/2 a	(2)	II	CC7	+	20.82	0.48	178460
<b>H10</b>	peripheral swab	4	(2)	I	CC2	+	27.37	0.39	167756
<b>H11</b>	healthy 35 year old man	1	(2)	II	CC121-101	+	20.63	0.24	304188
<b>H13</b>	blood culture	4	(2)	II	CC7	+	21.16	0.22	294800
<b>H14</b>	cephalorachid liquids	4	(2)	I	CC1	+	20.23	0.17	314341
<b>H15</b>	blood culture	1	(2)	II	CC8	+	19.42	0.36	246525
<b>H16</b>	blood culture	1	(2)	II	CC7	+	21.09	0.22	298070
<b>H17</b>	healthy 10 year old child	3a	(2)	II	CC121-101	+	17.90	0.19	259320
<b>H18</b>	blood culture	1/2b	(2)	I	CC3-CC6	+	22.97	0.29	252799
<b>H19</b>	blood culture	4b	(2)	I	CC2	+	17.27	0.27	248559
<b>H2</b>	healthy 3 year old	4b	(2)	I	CC2	+	18.94	0.27	251122
<b>H20</b>	Human	ND	INRA, UMR 1347	I	CC2	+	19.10	0.29	237487
<b>H21</b>	blood culture	4	(2)	I	CC1	+	14.65	0.32	201165
<b>H22</b>	blood culture	4b	(2)	I	CC1	+	19.30	0.23	257321
<b>H23</b>	Liver	1	(2)	I	CC3-CC6	+	20.15	0.17	321584
<b>H24</b>	Placenta	4b	(2)	I	CC3-CC6	+	19.60	0.34	213610
<b>H25</b>	Placenta	1/2a	(2)	II	CC18	+	15.46	0.56	125177
<b>H27</b>	healthy 2 year old	4b	(2)	I	CC2	+	17.29	0.23	240309
<b>H28</b>	healthy 7 year old	1/2b	(2)	I	CC3-CC6	+	17.68	0.25	257533
<b>H3</b>	blood culture	1	(2)	II	CC7	+	23.14	0.21	318024
<b>H31</b>	healthy 55 year old	1/2a	(2)	II	CC18	+	21.53	0.26	322408

<b>H32</b>	healthy 19 year old	1/2a	(2)	II	CC121-101	+	19.57	0.27	252390
<b>H34</b>	healthy 8 year old	1/2a	(2)	II	CC121-101	+	18.75	0.20	278454
<b>H35</b>	healthy 11 year old	1/2b	(2)	I	CC3-CC6	+	20.10	0.16	323092
<b>H36</b>	epoisse cheese outbreak, human	4b	(2)	I	CC1	+	17.07	0.21	257681
<b>H37</b>	Human	ND	INRA, UMR 1347	II	CCnd1	+	17.43	0.19	284253
<b>H38</b>	healthy carrier	1/2a	(2)	II	CCnd1	+	16.97	0.18	269703
<b>H4</b>	blood culture	ND	(1)	II	CC8	+	20.47	0.39	196277
<b>H5</b>	cephalorachid fluid	1	(2)	II	CC7	+	23.42	0.22	326862
<b>H6</b>	healthy 30 year old woman	3	(2)	I	CC3-CC6	-	22.02	0.15	349334
<b>H7</b>	genital swab	1	(2)	I	CC3-CC6	+	21.97	0.21	282734
<b>H8</b>	blood culture	1	(2)	II	CC21	+	19.33	0.44	161725
<b>H9</b>	blood culture	1	(2)	I	CC1	+	18.66	0.23	282655
<b>Lis.4.12</b>	Smoked salmon	1/2a	ISHA	II	CC121-101	+	21.67	0.24	280476
<b>Lis.4.15</b>	tartar of salmon	1/2a	ISHA	II	CC121-101	+	23.39	0.20	356604
<b>Lis.4.18</b>	Vegetables salad	1/2a	ISHA	II	CCnd4	+	22.59	0.22	325189
<b>Lis.4.27</b>	Minced meat	1/2a	ISHA	II	CC121-101	+	21.50	0.25	309531
<b>Lis.4.28</b>	Duck legs	1/2b	ISHA	I	CC3-CC6	+	23.57	0.24	245390
<b>Lis.4.30</b>	Raw turkey roll	1/2b	ISHA	I	CC3-CC6	+	28.99	0.27	321275
<b>Lis.4.31</b>	Spicy herring	1/2b	ISHA	I	CC3-CC6	+	20.85	0.24	300578
<b>Lis.4.32</b>	Raw milk	1/2b	ISHA	I	CC3-CC6	+	12.37	0.44	134263
<b>Lis.4.33</b>	Minced meat	1/2c	ISHA	II	CC9	+	16.23	0.23	255104
<b>Lis.4.37</b>	Duck foie gras	1/2c	ISHA	II	CC9	+	16.89	0.26	252276
<b>Lis.4.39</b>	tartar of salmon	1/2c	ISHA	II	CC9	+	15.73	0.26	229681
<b>Lis.4.4</b>	Goat and courgette brochette	1/2a	ISHA	II	CC121-101	+	22.95	0.21	333903
<b>Lis.4.42</b>	Smoked salmon	3a	ISHA	II	CC121-101	+	19.63	0.25	264515
<b>Lis.4.43</b>	Sliced bacon	3a	ISHA	II	CC121-101	+	18.93	0.27	235859
<b>Lis.4.45</b>	Grilled bacon	3a	ISHA	II	CC121-101	+	16.66	0.23	252615
<b>Lis.4.46</b>	Goat sandwich	3a	ISHA	II	CC121-101	+	17.27	0.23	269191
<b>Lis.4.47</b>	Dices of salmon	4b	ISHA	II	CCnd2	+	18.22	0.29	258398
<b>Lis.4.5</b>	vegetables salad with ham	1/2a	ISHA	II	CC121-101	+	22.87	0.21	337272
<b>Lis.4.50</b>	Salmon surface control	4b	ISHA	I	CC2	+	20.54	0.19	301622
<b>lis.4.56</b>	Cow raw milk cheese	ND	ISHA	II	CC18	+	17.53	0.28	247352
<b>Lis.4.59</b>	Goat raw milk	ND	ISHA	I	CC3-CC6	+	19.26	0.21	279741
<b>Lis.4.6</b>	Ham sandwich	1/2a	ISHA	II	CC121-101	+	22.20	0.23	310711
<b>Lis.4.61</b>	buffalo raw milk cheese	ND	ISHA	II	CC37	+	19.69	0.20	295150
<b>Lis.4.62</b>	sheep raw milk	ND	ISHA	II	CCnd4	+	20.90	0.18	335700
<b>Lis.4.8</b>	Tuna, eggs and surimi sandwich	1/2a	ISHA	II	CC121-101	+	21.58	0.20	327759
<b>Lm23</b>	rook feces	4	(1)	I	CC1	+	22.54	0.26	224214
<b>Lm38</b>	rook feces	1	(1)	II	CCnd4	+	19.85	0.20	281559
<b>Lm481</b>	Cheese	1/2b	Aerial collection, Illkirch	I	CC3-CC6	+	24.34	0.17	369953
<b>Lm5945</b>	Soil	ND	INRA, UMR 1347	II	CC21	+	27.64	0.23	395027
<b>Lm5948</b>	Soil	ND	INRA, UMR 1347	II	CCnd5	+	23.68	0.22	360584
<b>Lm6298</b>	Soil	ND	INRA, UMR 1347	II	CCnd3	+	15.99	0.17	281735
<b>Lm6579</b>	Soil	ND	INRA, UMR 1347	I	CC1	+	23.15	0.13	397448
<b>Lm81</b>	rook feces	1	(1)	II	CC26	+	17.54	0.28	179767
<b>Lm97</b>	rook feces	1	(1)	II	CC21	+	19.23	0.25	231722

<b>LO28</b>	Healthy pregnant woman	1/2c		II	CC9	+	23.66	0.37	257760
<b>NV4</b>	Minced beef	1/2a	(1)	II	CC31	+	21.60	0.26	275012
<b>NV5</b>	Minced beef	1/2c	(1)	II	CC9	+	20.28	0.26	320316
<b>NV7</b>	Bovine carcass	1/2c	(1)	II	CC9	+	14.65	0.38	190097
<b>NV8</b>	Bovine carcass	1/2a	(1)	II	CC9	+	16.40	0.23	236879
<b>R10B24</b>	Soil	ND	INRA, UMR 1347	I	CC3-CC6	+	18.77	0.18	303554
<b>R1B14</b>	Soil	ND	INRA, UMR 1347	II	CC37	+	18.49	0.23	279285
<b>R2B16</b>	Soil	ND	INRA, UMR 1347	II	CC21	+	18.30	0.24	282220
<b>R5A27</b>	Soil	ND	INRA, UMR 1347	I	CC3-CC6	+	17.86	0.19	282822
<b>Scott A</b>	Massachusetts milk outbreak	4b		I	CC2	+	19.23	0.35	245293

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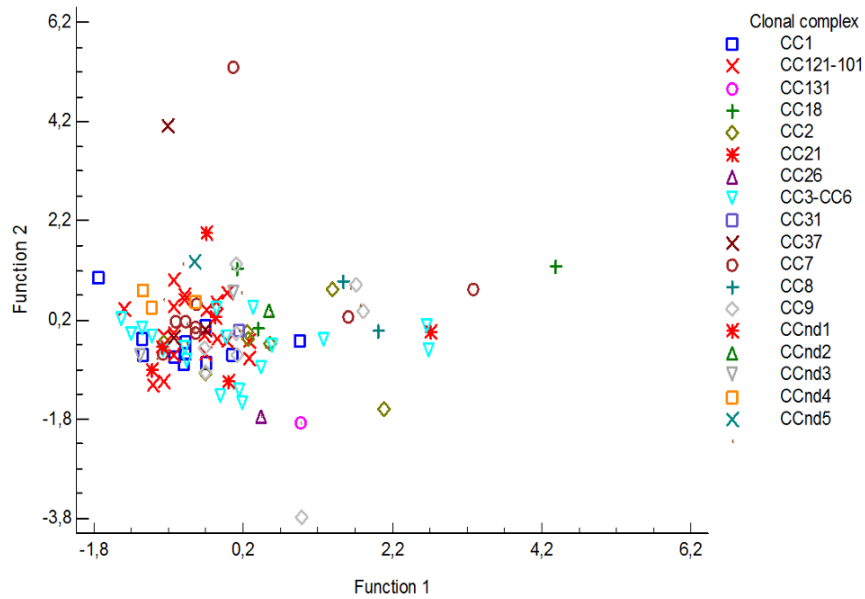
22 INRA : Institut National de la Recherche Agronomique ; CIP: Collection de l'Institut  
 23 Pasteur ; ISHA : Institut Scientifique d'Hygiène et d'Analyse.

24 <sup>1</sup> Genotyping was achieved by sequencing two housekeeping genes: *cat* and *dapE* (see  
 25 Material and Methods section)

26 <sup>2</sup> Swimming tests were performed using 5 µL of each strain subculture inoculated in the  
 27 center of swimming agar (TSB+0.25% agar). After incubation of the plates 24 h at 25°C,  
 28 swimming diameter was measured and motile (+) and non-motile strains (-) were identified.  
 29 Experiments were performed three times independently.

30 <sup>3</sup> Mean thickness (µm), roughness and biovolume (µm<sup>3</sup>) were calculated from the CLSM  
 31 images (means of four acquisitions) using PHLIP, a MATLAB-based image analysis toolbox.

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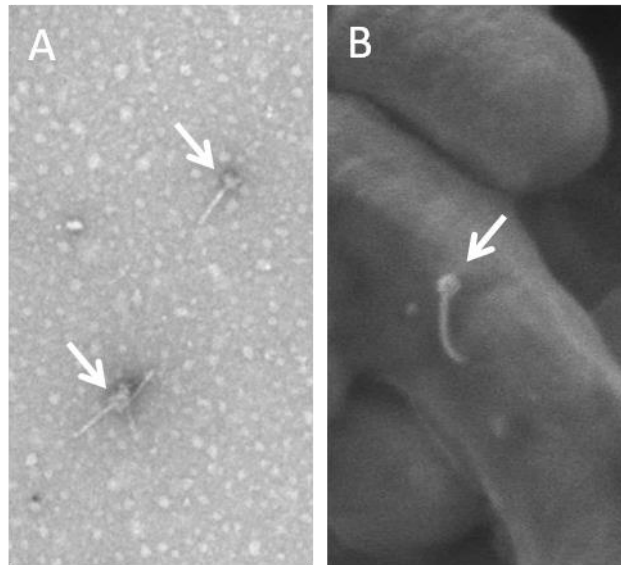


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35 **FIG S1:** Strains classification in the clonal complexes using the biofilm structural parameters  
 36 (thickness, roughness and biovolume). Two discriminating functions were developed using a  
 37 discriminant analysis. Discriminant function 1 was statistically significant with  $P$ -value  $<0.05$   
 38 at the 95% confidence level and improved probability to correctly classify strains in the clonal  
 39 complexes from 0.06 to 0.24.

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45 **FIG S2:** Phages observed in biofilm supernatant after negative staining with Transmission  
46 Electron Microscopy (Zeiss EM902 EELS) (A) and on cells in biofilms with Scanning  
47 Electron Microscopy (B) (white arrows). Concomitant observations of phages and eDNA in  
48 the biofilms hypothesize the occurrence of localized cell death caused by the expression of  
49 prophages.

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52 **REFERENCES**

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