Supplementary material

*Listeria monocytogenes* sub-lethal injury and VBNC state
induced by acidic conditions and disinfectants

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Figure S1. Population (log CFU/ml) of *L. monocytogenes*, strain Scott A, after incubation for 18h in TSBYE. Single colonies (three biological replicates tested in duplicate) from TSAYE stock culture were transferred by a loop in 10 ml TSBYE and incubated for 24h at 30°C. Following, 0.1ml of the 24h cultures were transferred in 10ml TSBYE and incubated for 18h at 30°C. Growth curve indicates that after 18h the population remains constant – in stationary phase.
## Supplementary Tables

Table S1. Comparison of *L. monocytogenes* mean population (log CFU/ml) at different pH values (3.0, 2.7, 2.5) at the same time of exposure and temperature (20°C or 4°C) after exposure to AA.

| Exposure Time (h) | Total culturable population; enumerated on TSAYE | Non-injured population; enumerated on TSAYE+5%NaCl |
|-------------------|--------------------------------------------------|--------------------------------------------------|
|                   | pH 3.0   | pH 2.7   | pH 2.5       | pH 3.0   | pH 2.7   | pH 2.5       |
| AA                |          |          |              |          |          |              |
| 20°C              |          |          |              |          |          |              |
| 0                 | 6.90<sup>A</sup> | 6.99<sup>A</sup> | 6.43<sup>A</sup> | 6.85<sup>a</sup> | 6.99<sup>a</sup> | 6.30<sup>a</sup> |
| 1                 | 6.88<sup>C</sup> | 6.45<sup>B</sup> | <LOD<sup>A</sup> | 6.82<sup>b</sup> | 5.98<sup>b</sup> | <LOD<sup>a</sup> |
| 2                 | 6.56<sup>C</sup> | 3.28<sup>B</sup> | <LOD<sup>A</sup> | 6.54<sup>c</sup> | 2.41<sup>b</sup> | <LOD<sup>a</sup> |
| 3                 | 5.86<sup>B</sup> | <LOD<sup>A</sup> | <LOD<sup>A</sup> | 5.42<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |
| 4                 | 4.83<sup>B</sup> | <LOD<sup>A</sup> | <LOD<sup>A</sup> | 3.81<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |
| 5                 | 3.08<sup>B</sup> | <LOD<sup>A</sup> | <LOD<sup>A</sup> | 2.26<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |
| 4°C               |          |          |              |          |          |              |
| 0                 | 6.76<sup>A</sup> | 6.90<sup>A</sup> | 6.81<sup>A</sup> | 6.75<sup>c</sup> | 6.91<sup>b</sup> | 6.41<sup>a</sup> |
| 1                 | 6.68<sup>B</sup> | 6.19<sup>AB</sup> | 5.94<sup>A</sup> | 6.73<sup>b</sup> | 5.59<sup>b</sup> | 1.66<sup>a</sup> |
| 2                 | 6.61<sup>C</sup> | 3.72<sup>A</sup> | 5.09<sup>B</sup> | 6.65<sup>c</sup> | 2.66<sup>b</sup> | <LOD<sup>a</sup> |
| 3                 | 6.53<sup>B</sup> | 3.04<sup>A</sup> | 3.61<sup>A</sup> | 6.48<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |
| 4                 | 6.46<sup>B</sup> | 2.66<sup>A</sup> | 2.32<sup>A</sup> | 6.23<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |
| 5                 | 6.27<sup>B</sup> | 1.46<sup>A</sup> | <LOD<sup>A</sup> | 5.76<sup>b</sup> | <LOD<sup>a</sup> | <LOD<sup>a</sup> |

Statistically significant differences (p < 0.05, CI 95%) are indicated with different uppercase (TSAYE) and lowercase (TSAYE-5%NaCl) letters, respectively.
Table S2. Comparison of *L. monocytogenes* mean population (log CFU/ml) at different pH values (3.0, 2.7, 2.5) at the same time of exposure and temperature (20°C or 4°C) after exposure to HCl.

| Exposure Time (h) | Total culturable population; enumerated on TSAYE | Non-injured population; enumerated on TSAYE+5%NaCl |
|-------------------|-----------------------------------------------|--------------------------------------------------|
|                   | pH 3.0 pH 2.7 pH 2.5                           | pH 3.0 pH 2.7 pH 2.5                             |
| HCl               |                                               |                                                 |
| 20°C              |                                               |                                                 |
| 0                 | 7.13A 6.93A 7.06A                              | 7.10a 6.92a 6.96a                               |
| 1                 | 7.17C 6.78B 6.24A                              | 7.07b 6.73b 5.85a                               |
| 2                 | 7.12C 5.65B 2.72A                              | 7.07c 5.39b 2.64a                               |
| 3                 | 7.09C <LOD<LOD A                               | 7.02c 4.36b <LOD<LOD A                          |
| 4                 | 7.12C <LOD<LOD A                               | 6.99b 3.52b <LOD<LOD A                          |
| 5                 | 7.08C 3.09B <LOD<LOD A                          | 6.97c 2.94b <LOD<LOD A                          |
| 4°C               |                                               |                                                 |
| 0                 | 6.99A 7.15AB 7.07B                              | 7.01a 7.16ab 7.05b                              |
| 1                 | 6.93A 7.03A 6.51A                               | 6.94a 7.05a 6.22a                               |
| 2                 | 6.91B 7.06B 5.19A                               | 6.91b 7.05b 4.61a                               |
| 3                 | 6.81B 6.98B 3.69A                               | 6.82b 6.98b 3.22a                               |
| 4                 | 6.78C 6.95B 2.79A                               | 6.83b 6.90b 2.69a                               |
| 5                 | 6.82B 6.90B 2.09A                               | 6.72b 6.84b 2.01a                               |

Statistically significant differences (p <0.05, CI 95%) are indicated with different uppercase (TSAYE) and lowercase (TSAYE-5%NaCl) letters, respectively.
Table S3. Comparison of *L. monocytogenes* mean population (log CFU/ml) at different ppm (0.5, 5, 10, 20, 30, 40) at the same time of exposure and temperature (20°C or 4°C) after exposure to PAA.

| Exposure Time (sec) | Total culturable population; enumerated on TSAYE log (CFU/ml) | Non-injured population; enumerated on TSAYE+5%NaCl log (CFU/ml) |
|---------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|                     | 0.5ppm 5ppm 10ppm 20ppm 30ppm 40ppm                          | 0.5ppm 5ppm 10ppm 20ppm 30ppm 40ppm                          |
| 20°C                |                                                               |                                                               |
| 0                   | 7.09A 6.93A 6.76A 6.57A 6.77A 6.25A                           | 6.98c 6.91c 6.75bc 5.79ab 5.37a 5.19a                          |
| 30                  | 7.10B 6.87B 6.80B 5.13A 5.03A 4.89A                           | 7.03c 6.84c 6.70c 4.11b 3.10a 3.50ab                          |
| 60                  | 7.11B 6.83B 6.72B 3.94A 4.56A 3.20A                           | 7.10c 6.80c 6.49c 3.13b 1.19a 1.62ab                          |
| 300                 | 7.16D 4.78C 3.86C 2.37B 4.04C <LOD A                           | 7.08d 3.19c 2.58bc 1.74ab <LOD A <LOD A                       |
| 900                 | 7.07B <LOD A <LOD A 1.44A <LOD A <LOD A                        | 6.95b <LOD A <LOD A <LOD A <LOD A <LOD A                      |
| 1800                | 7.02B <LOD A <LOD A <LOD A <LOD A                             | 6.93b <LOD A <LOD A <LOD A <LOD A <LOD A                      |
| 4°C                 |                                                               |                                                               |
| 0                   | 7.08AB 7.05AB 7.15B 6.59A 6.96AB 6.88AB                        | 7.03a 7.05a 7.14a 6.54a 6.80a 6.60a                           |
| 30                  | 7.10A 7.02A 6.96A 6.81A 6.87A 6.12A                           | 7.11b 6.95a 6.96a 6.32a 6.47a 5.77a                           |
| 60                  | 7.10A 6.99A 7.05A 6.37AB 6.57AB 4.39A                          | 7.09b 6.92b 6.99b 5.21b 5.26b 1.45a                           |
| 300                 | 7.12A 6.77A 6.29A 2.83B <LOD A <LOD A                         | 7.07c 6.74c 4.69b <LOD A <LOD A <LOD A                       |
| 900                 | 7.07C 6.72C 3.59B <LOD A <LOD A <LOD A                         | 7.04c 6.61b <LOD A <LOD A <LOD A <LOD A                      |
| 1800                | 7.07B 6.73B 1.24A <LOD A <LOD A <LOD A                         | 7.01c 6.47b <LOD A <LOD A <LOD A <LOD A                      |

Statistically significant differences (p <0.05, CI 95%) are indicated with different uppercase (TSAYE) and lowercase (TSAYE-5%NaCl) letters, respectively.
Table S4. Comparison of L. monocytogenes mean population (log CFU/ml) at different ppm (20, 30, 40) at the same time of exposure and temperature (20°C or 4°C) after exposure to PAA.

| Exposure time | Total culturable population; enumerated on TSAYE | Non-injured population; enumerated on TSAYE+5%NaCl |
|---------------|-----------------------------------------------|-----------------------------------------------|
|               | 20°C (h) 20ppm 30ppm 40ppm                    | 20ppm 30ppm 40ppm                              |
| PAA           |                                               |                                               |
| 0             | 9.17<sup>C</sup> 5.29<sup>B</sup> 3.05<sup>A</sup> | 8.88<sup>c</sup> 4.14<sup>b</sup> 2.61<sup>a</sup> |
| 1             | 9.14<sup>C</sup> 4.57<sup>B</sup> 2.85<sup>A</sup> | 8.80<sup>c</sup> 3.77<sup>b</sup> 2.14<sup>a</sup> |
| 2             | 9.10<sup>C</sup> 3.72<sup>B</sup> 2.72<sup>A</sup> | 8.86<sup>c</sup> 3.39<sup>b</sup> 1.59<sup>a</sup> |
| 3             | 9.13<sup>C</sup> 1.59<sup>B</sup> <LOD<sup>A</sup> | 8.90<sup>c</sup> 1.39<sup>b</sup> <LOD<sup>a</sup> |

Statistically significant differences (p <0.05, CI 95%) are indicated with different uppercase (TSAYE) and lowercase (TSAYE-5%NaCl) letters, respectively.