

Diving into bacterial dormancy: emergence of osmotically stable wall-less forms in an aquatic environment

Filipe Carvalho, Alexis Carreaux, Anna Sartori-Rupp, Stéphane Tachon, Anastasia D Gazi, Pascal Courtin, Pierre Nicolas, Florence Dubois-Brissonnet, Aurélien Barbotin, Emma Desgranges, et al.

▶ To cite this version:

Filipe Carvalho, Alexis Carreaux, Anna Sartori-Rupp, Stéphane Tachon, Anastasia D Gazi, et al.. Diving into bacterial dormancy: emergence of osmotically stable wall-less forms in an aquatic environment. 21st International Symposium on Problems of Listeria and Listeriosis (ISOPOL), Sep 2024, Norwich, United Kingdom. hal-04697876

HAL Id: hal-04697876 https://hal.science/hal-04697876

Submitted on 14 Sep 2024

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 - NonCommercial - NoDerivatives 4.0 International License

Diving into bacterial dormancy: emergence of osmotically stable wall-less forms in an aquatic environment

Filipe Carvalho¹, Alexis Carreaux¹, Anna Sartori-Rupp², Stéphane Tachon², Anastasia D. Gazi³, Pascal Courtin¹, Pierre Nicolas⁴, Florence Dubois-Brissonnet¹, Aurélien Barbotin¹, Emma Desgranges¹, Matthieu Bertrand¹, Karine Gloux¹, Catherine Schouler⁵, Rut Carballido-López¹, Marie-Pierre Chapot-Chartier¹, Eliane Milohanic¹, Hélène Bierne¹, Alessandro Pagliuso¹

¹ INRAE, Université Paris-Saclay, AgroParisTech, Institut MICALIS, 78350 Jouy-en-Josas, France; ² NanoImaging Core Facility, Institut Pasteur, 75015 Paris, France; ³ Ultrastructural Bioimaging Facility, Institut Pasteur, 75015 Paris, France; ⁴ INRAE, Université Paris-Saclay, MaIAGE, 78350 Jouy-en-Josas, France; ⁵ INRAE, Université de Tours, ISP, 37380 Nouzilly, France



- In the case of bacterial pathogens, the VBNC state poses an increased public health risk, as bacteria are undetectable by conventional growth-based tests and, given the right conditions, can revert back to a vegetative and virulent state.
- Although the VBNC state is widespread among bacteria (>100 species reported so far), the mechanisms governing this phenotypic switch remain poorly understood.



Context

In-depth characterization of the physiological processes and molecular mechanisms and factors implicated in the formation, maintenance, and reversal of the VBNC state in the human foodborne pathogen *Listeria monocytogenes*

L. monocytogenes undergoes a rod-to-coccus shape shift during transition to a VBNC state in mineral water





Total, viable, and culturable cell numbers of *Lm* EGDe in mineral water. Culturable bacteria were quantified by CFU enumeration; total and viable bacteria were quantified by flow cytometry using CFDA. Dashed area indicates VBNC population.

Phase-contrast micrographs of Lm EGDe in mineral water. Bacteria highlighted in white squares are shown enlarged in bottom panels. Scale bars: 2 µm.





Total, viable and culturable cell numbers of Listeria sensu stricto species L. ivanovii, L. innocua, L. marthii, L. seeligeri, and L. welshimeri during incubation in mineral water. Culturable bacteria were quantified by CFU enumeration; total and viable bacteria were quantified by flow cytometry using CFDA. Dashed area indicates VBNC population.



This morphological transformation is caused by a complete loss of the bacterial cell wall via a molting-like shedding process



Ultrastructure of the stages of *Lm* CW shedding obtained by cryo-electron tomography of *Lm* EGDe sampled from mineral water at day 0 (stage 0) and day 14 (stages 1-5). **Stage 0**: a rod-shaped bacterium with tightly connected plasma membrane (M) and CW layers (white arrowheads). In this example, a septum is forming at the midcell region. **Stage 1**: detachment of the M and CW layers results in the formation of a periplasm-like space (red arrowheads). **Stage 2**: appearance of CW breaches of variable size (yellow arrowheads) exposes the enclosed protoplast to the extracellular environment. **Stage 3**: the protoplast begins bulging through a CW breach at one of the poles. **Stage 4**: the protoplast squeezes out, leaving behind an empty cell wall sacculus. **Stage 5**: the protoplast has fully egressed from its rod-shaped CW encasing, assuming a coccoid morphology as a CWD form. Left panels: tomogram slice; right panels: superposition with 3D rendering of the CW (blue) and M (orange) layers, obtained by manual segmentation of tomogram slice; Scale bars: 200 nm.

Ζ

L. monocytogenes adjusts its plasma membrane properties to adapt to a cell wall-deficient lifestyle in mineral water





Phase contrast and fluorescence micrographs of *Listeria* sensu stricto species populations incubated in mnineral water. Bacterial cells were fluorescently labelled for DNA (cyan), cell wall (green), and exposed plasma membrane (red). Scale bars: 2 µm.

5 Global and targeted genetic approaches reveal SigB and the autolysin NamA as important regulators of VBNC state transition



a) Total, viable and culturable cell numbers of *Lm* EGDe (WT, dotted line) and isogenic SigB-deficient (Δ*sigB*) mutant (solid lines) strains incubated in mineral water. Culturable bacteria were quantified by CFU enumeration; total and viable bacteria quantified by flow cytometry using CFDA. b) Fraction of the viable cell populations shown in (a) consisting of VBNC cells formed in mineral water. c) Fraction of the cell populations shown in (a) displaying single- or double-labeling of cell wall and exposed plasma membrane by fluorescence microscopy.



a) Fold change of the relative abundance of anteiso-branched (a-BFA), iso-branched (i-BFA), saturated (SFA) and unsaturated (UFA) fatty acids extracted from *Lm* EGDe in mineral water at the indicated timepoints relative to the first timepoint (day 0). b) Laurdan generalized polarization (GP) of *Lm* EGDe in mineral water. Increasing relative GP values suggest reduction of the bacterial membrane fluidity. c) Membrane fluidity of *Lm* EGDe in mineral water. Nile red-stained rod-shaped or coccoid cells were analyzed by total internal reflection fluorescence correlation spectroscopy (TIR-FCS) to determine the membrane-associated dye diffusion coefficient. Each dot represents one measured bacterium, and dots with same color represent bacteria measured from the same suspension. d) Total cell numbers of *Lm* EGDe in mineral water were quantified by flow cytometry in samples treated (red) or not (grey) with mutanolysin. Data are expressed as mean ± standard deviation (a, b, d) or median + interquartile range (c) of three independently prepared bacterial suspensions (white circles in bar plots, differentially colored dots in scatter plot). Statistical significance was determined with two-way ANOVA with Tukey's (a) or Šidák's test (d), and one-way ANOVA with Dunnett's (b) or Tukey's (c) tests.

VBNC L. monocytogenes reverts back to a culturable and virulent form after passage through chicken embryos



a) Phase-contrast and fluorescence micrographs of GFP-expressing *Lm* EGDe before (WT) and after VBNC state entry in mineral water and passage through embryonated chicken eggs (clones V-E1 and VC-E2). Bacteria were fluorescently labelled for DNA (cyan) and cell wall (green). b) Intracellular replication of WT (black circles and line), V-E1 (red down-pointed triangles and line) and VC-E2 (green up-pointed triangles and line) in human epithelial JEG3 and HepG2 cell lines. Intracellular viable bacteria were quantified by CFU enumeration from agar medium-plated cell lysates.

* This study provides new evidence on molecular mechanisms and factors governing the VBNC state transition of a major Gram-positive foodborne pathogen:

6

- Cell wall loss is required for Listeria monocytogenes (and other Listeria species) to enter a VBNC state upon starvation in mineral water.
- Stress response (through transcriptional regulator SigB) and cell wall turnover (through the autolysin NamA) processes are key drivers of VBNC state formation.
- Our findings show that cell wall-deficient bacteria can arise naturally in non-osmoprotective aquatic environments, which may constitute a potential strategy for bacterial survival in hypoosmotic and oligotrophic conditions.









