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

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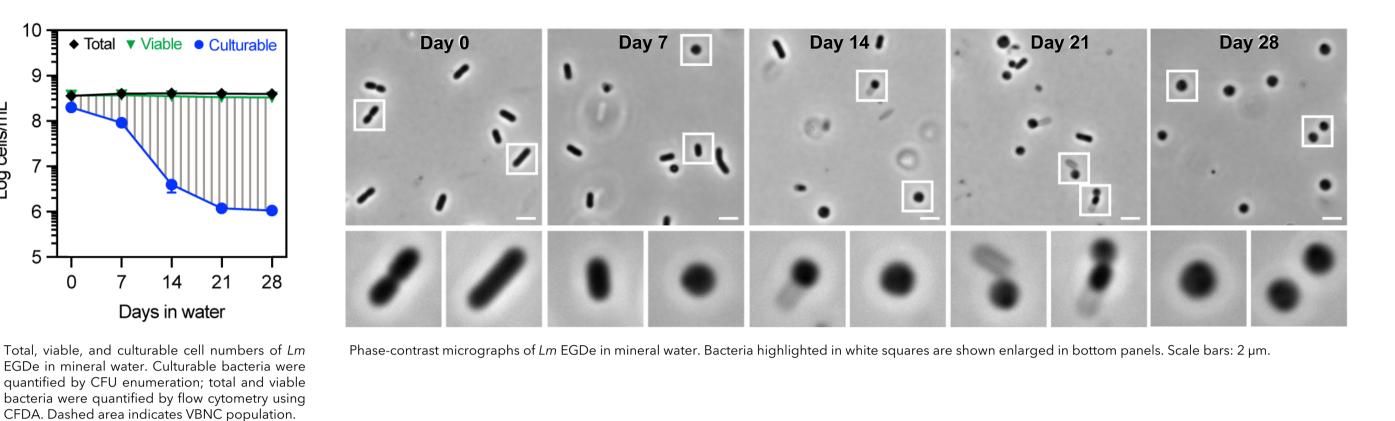
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- Under environmental stress, bacteria may enter a state of dormancy called viable but non-culturable (VBNC) because, as a result of a generalized metabolic slowdown, cells lose their ability to grow in standard culture media.
- ❖ In the case of bacterial 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.

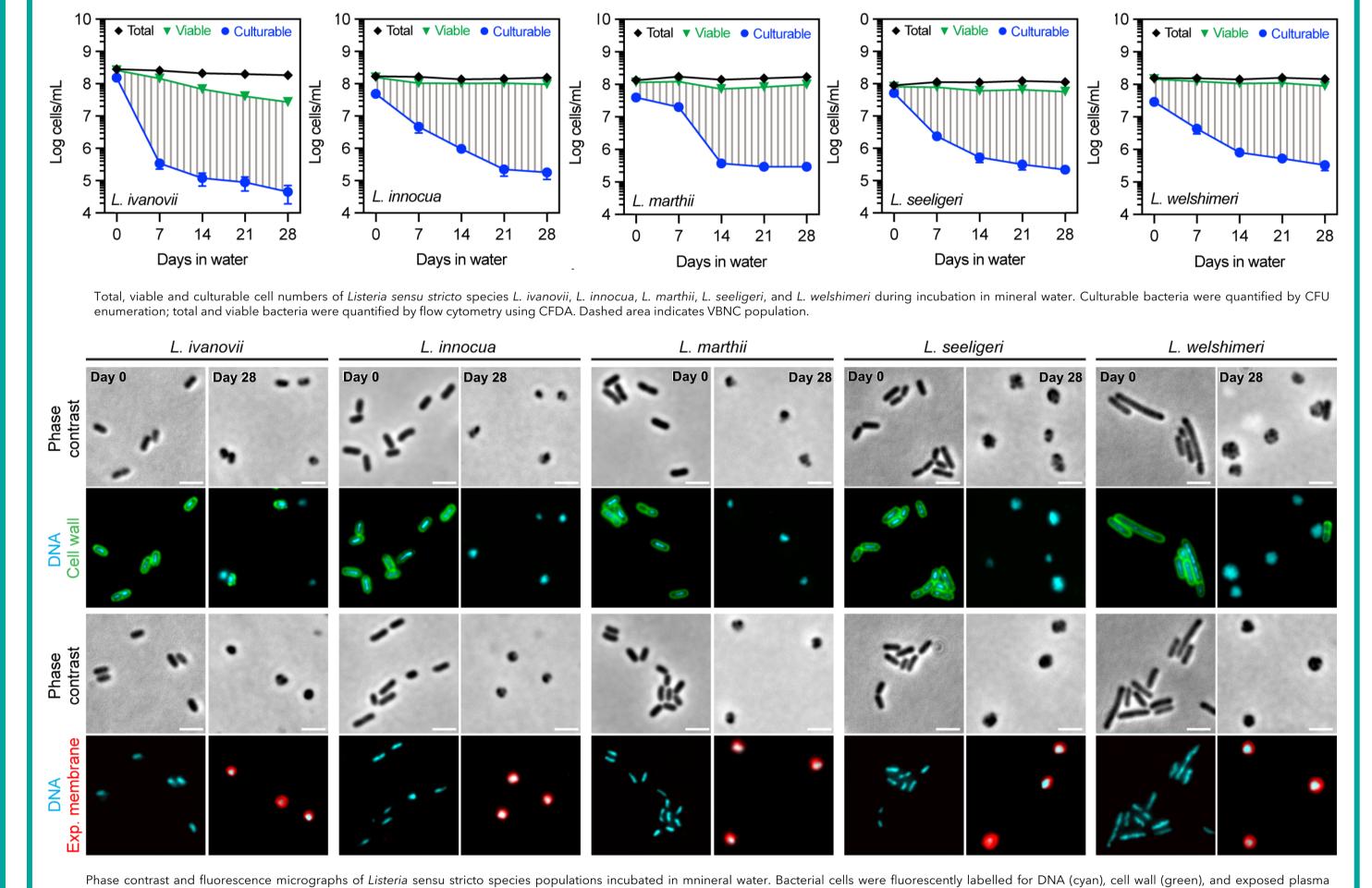
In-depth characterization of the physiological processes and molecular mechanisms and factors implicated in the formation, maintenance, and reversal of the VBNC state in the human foodborne pathogen *Listeria monocytogenes*

Results

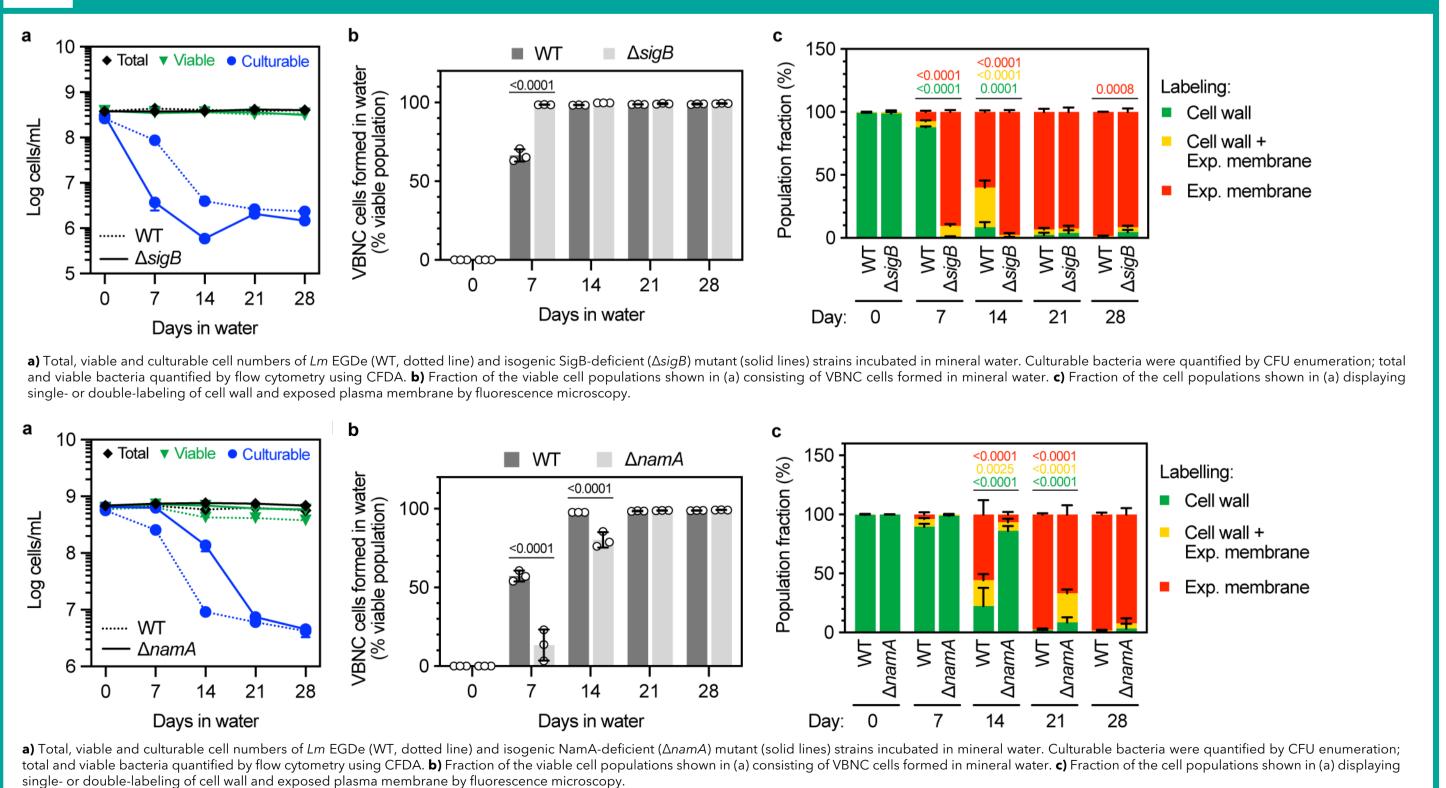
L. monocytogenes undergoes a rod-to-coccus shape shift during transition to a VBNC state in mineral water



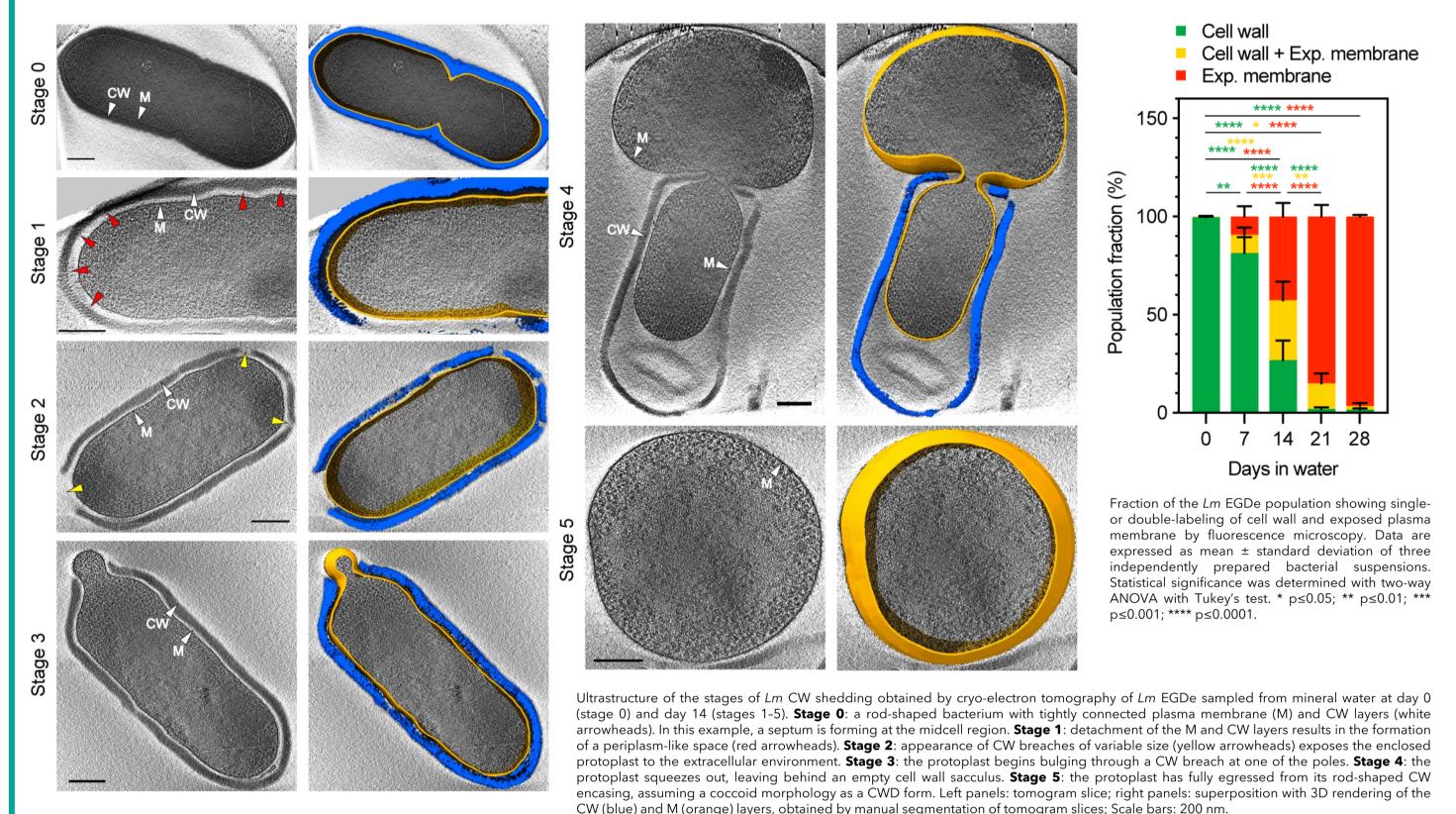
A cell wall-deficient VBNC state is widespread in *Listeria* species



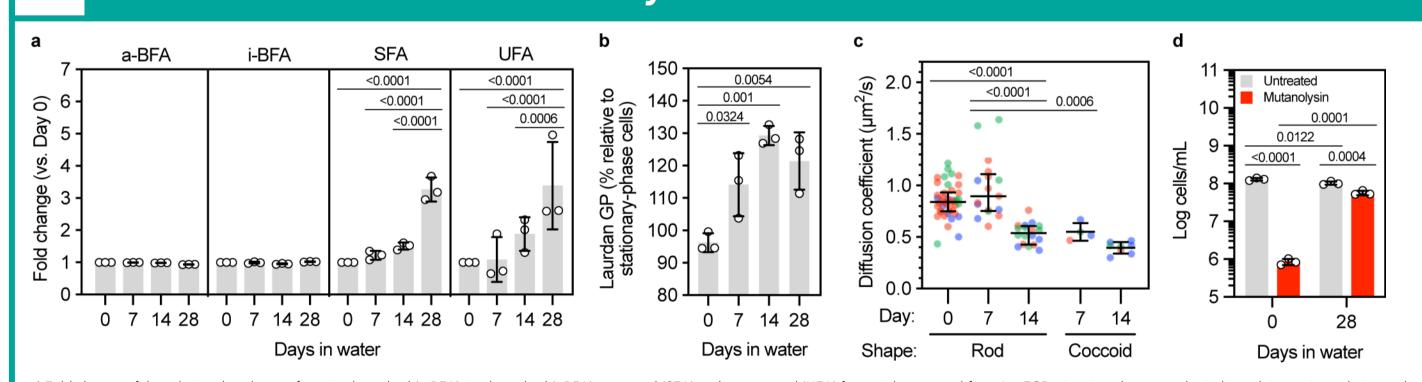
Global and targeted genetic approaches reveal SigB and the autolysin NamA as important regulators of VBNC state transition



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



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



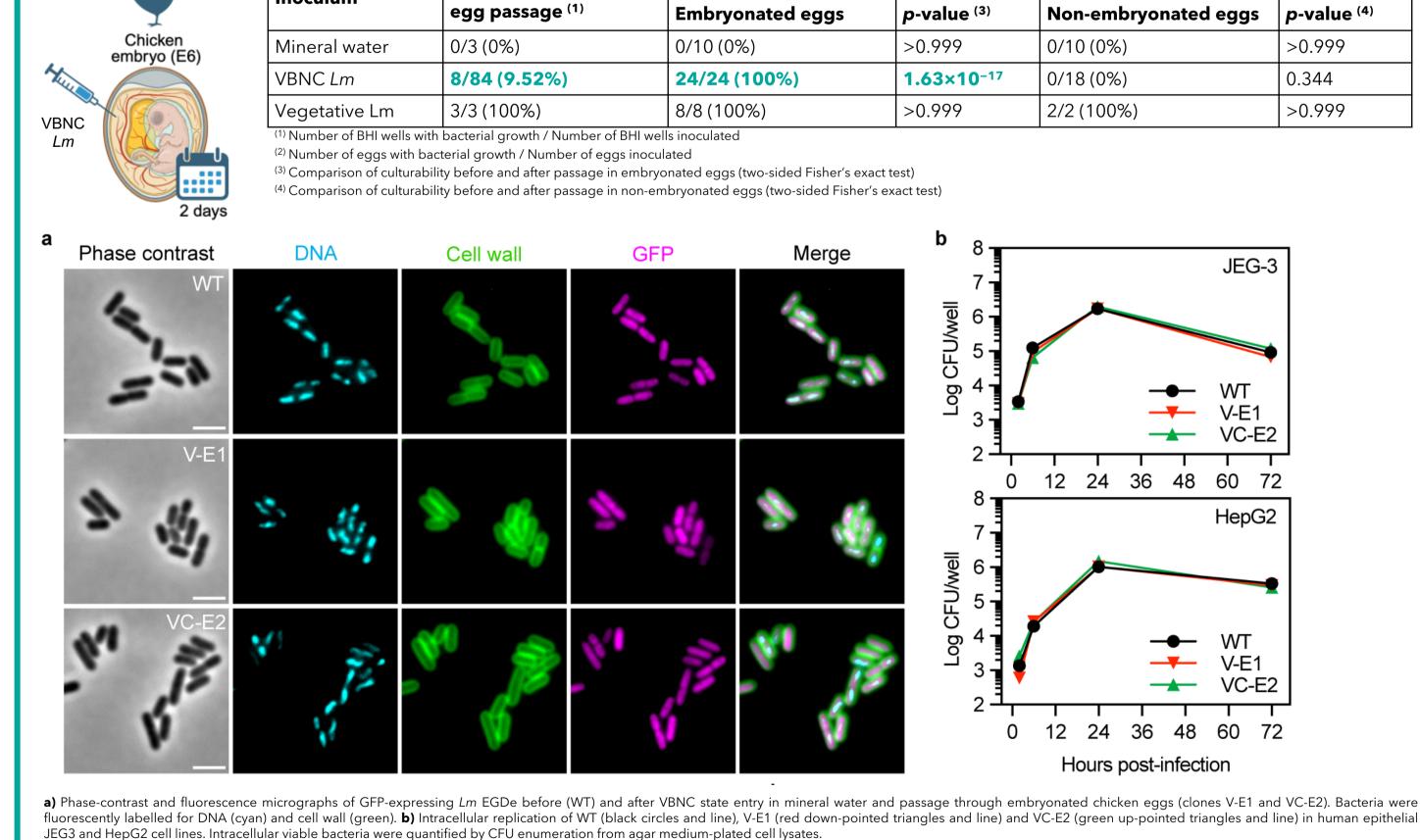
a) Fold change of the relative abundance of anteiso-branched (a-BFA), iso-branched (i-BFA), saturated (UFA) fatty acids extracted from Lm EGDe in mineral water at the indicated timepoints relative to the first timepoint (day 0). b) Laurdan generalized polarization (GP) of Lm EGDe in mineral water. Increasing relative GP values suggest reduction of the bacterial membrane fluidity. c) Membrane fluidity of Lm EGDe in mineral water. Nile redstained 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.

Culturability after egg passage (2)

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

Culturability before

Inoculum



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



Conclusions







