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The phosphoproteome signature of Listeria monocytogenes dormancy

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Background

Several bacteria can adapt to stress by entering a viable but non-culturable (VBNC) state (1), where they remain alive and metabolically active but cannot grow in standard culture media (Fig. 1).

Listeria monocytogenes (Lm), a deadly foodborne pathogen for immunocompromised or elderly individuals and newborns, can survive in various stressful environments by transitioning to a VBNC state (2, 3). However, the molecular mechanisms governing this transition are not well understood.

We have recently shown that during starvation in mineral water, Lm progressively transitions to a VBNC state (Fig. 2A-B) (4). Phase contrast microscopy observation revealed that Lm rods become progressively shorter to transform into coccoid forms after 2-3 weeks (Fig. 2C).

Cryo-electron tomography (Fig. 2D) and peptidoglycan profiling (not shown) indicated that this transformation is due to a shedding process of the peptidoglycan, resulting in cell wall-deficient bacteria.

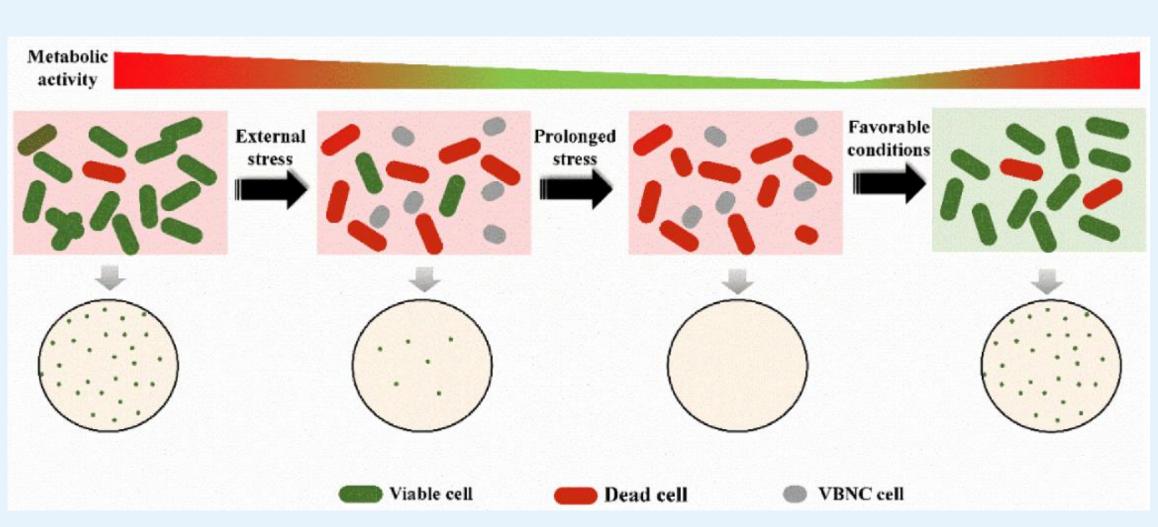


Figure 1. During prolonged stressful conditions, a proportion of viable bacteria will enter into a VBNC state, under which they cannot develop into colonies on agar medium, but cellular metabolic activity is retained. When provided with favorable conditions, VBNC cells can resuscitate to the culturable state.

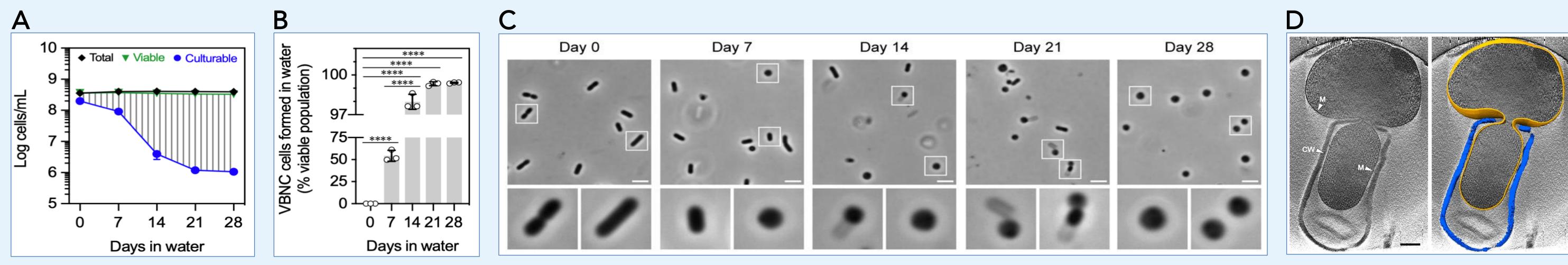
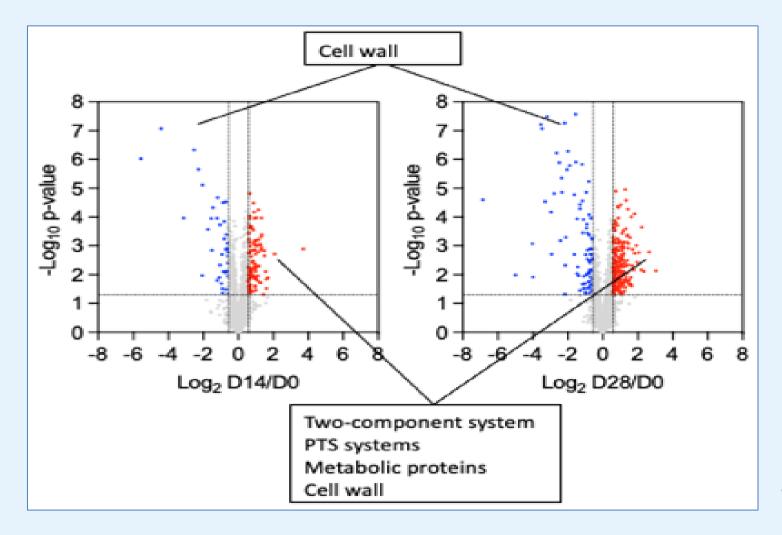


Figure 2. (A) Total, viable and culturable cell profiles of Lm in mineral water. (B) Fraction of bacterial population consisting of VBNC formed in mineral water. highlighted by white squares are shown enlarged in bottom panels. (D) Cryo-electron tomography of Lm cells sampled after 7 days of incubation in mineral water.



To understand the molecular events leading to the VBNC transition and cell wall loss, we analyzed the proteome of bacteria transitioning to a VBNC state (Fig 3).

Proteomic analysis identified up- and down-regulated proteins (red and blue, respectively) involved in cell wall metabolism and remodeling, cell metabolism (PTS systems), and cell signaling (two component systems). Since many of these proteins are known to be phosphorylated, we hypothesize that phosphorylation cascades regulate the VBNC transition.

Figure 3. Volcano plot showing the intensity fold change (in \log_2) of proteins at day 14 (D14) and day 28 (D28) compared to day 0 (D0) on the x axis. The experiment was performed 4 times and a t-test was performed to calculate –log P values for each protein, indicated on the y axis

Objectives

Hypothesis: Phosphorylation/dephosphorylation events regulate VBNC transition

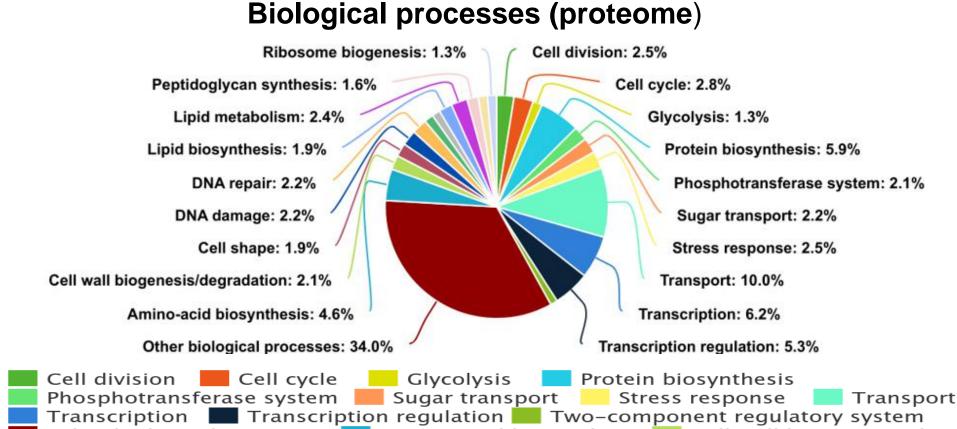


- Characterize the phosphoproteome of Lm transitioning to the VBNC state
- Identify differentially-phosphorylated targets that might regulate VBNC transitions

Experimental pipeline Mineral Transition to VBNC state Day 14 **Bacterial proteins Proteomics** Phosphoproteomics Digestion (Lys-C/ trypsin ratio 1:50) 1D gel (short migration) 10 µg of ----proteins Dimethyl Gradient: 64 min, Flow: 250 nl/min, labelling column C18: 50 cm Phosphopeptide In-gel digestion enrichment (trypsin) 6 enrichments with TiO₂ and IMAC Identification with

Preliminary results

To set up phosphopeptide enrichment we started from bacteria grown in BHI until the stationary phase or incubated in water for 1 or 2 days. We used the pipeline shown beside. Phosphopeptides were purified via TiO₂ beads.



Certain functions such as glycolysis, PTS

sustem, sugar transport and stress response

are over-represented compared to the

rRNA processing Ribosome biogenesis

phosphoislands.

proteome.

Shotgun proteomics led to the identification of 861 proteins (ca 30% of the Lm proteome). We report the biological processes associated to the identified proteins.

Other biological processes Amino-acid biosynthesis Cell wall biogenesis/degradation Cell shape 🔃 DNA damage 📒 DNA repair 🔃 Fatty acid metabolism Ion transport Lipid biosynthesis Lipid metabolism Peptidoglycan synthesis Other biological processes: 26.5% Phosphopetide enrichment via TiO₂ beads, revealed 104 phosphoproteins and 130

Two-component regulatory system: 1.5

Biological processes (phosphoproteome) Cell division: 5.9% Cell cycle: 5.9% Glycolysis: 4.4% Protein biosynthesis: 4.4% Phosphotransferase system: 10.3% Transcription regulation: 5.9% Transcription: 8.89 Sugar transport: 8.8%

Stress response: 4.4%

Glycolysis Protein biosynthesis Phosphotransferase system Sugar transport Stress response Transport Transcription Transcription regulation Two-component regulatory system Other biological processes

Transport: 13.2%

Conclusion and pespectives

i2MassChroQ (1.0.9)

- ✓ These results show that our protocol is suitable to isolate phosphoproteins.
- ✓ We were able to identify 104 phosphoproteins and 130 phosphoislands.
- ✓ We are currently optimising our experimental pipeline with the aim to profile the Lm phosphoproteome during VBNC transition and identify molecular players and signalling pathways involved in this physiological state

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