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.*

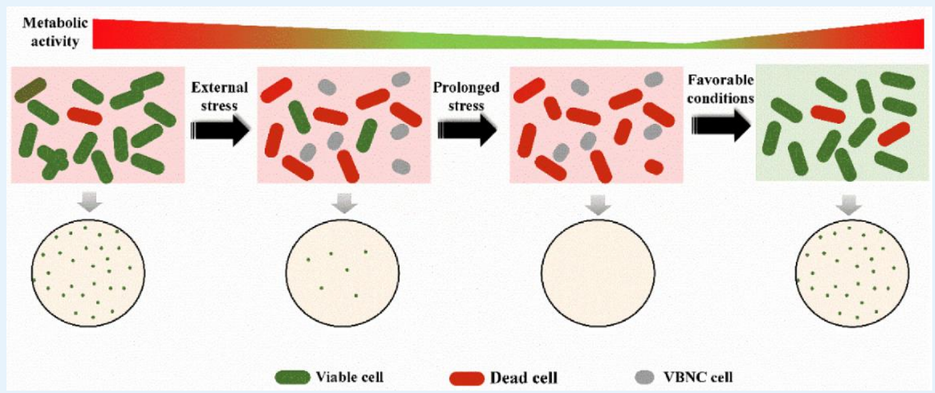


Figure 1. During prolonged stressful conditions, a proportion of viable bacteria will enter into a VBNC state, under which they cannot develop into colonies on agar medium, but cellular metabolic activity is retained. When provided with favorable conditions, VBNC cells can resuscitate to the culturable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

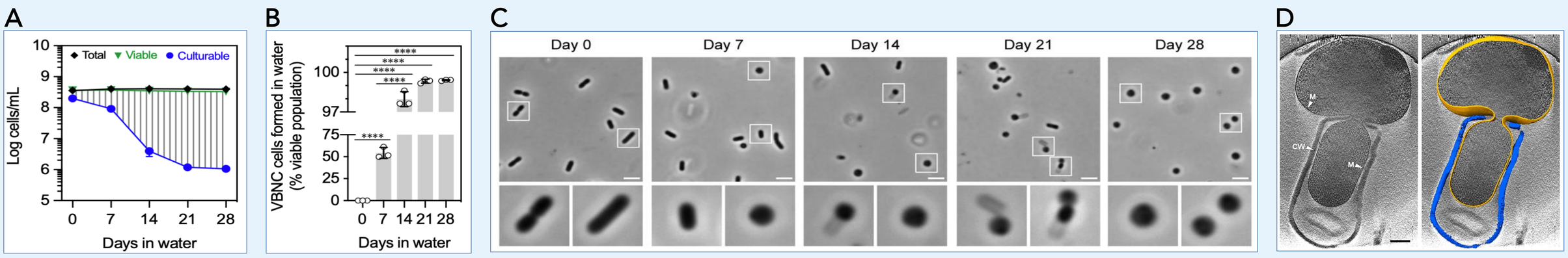
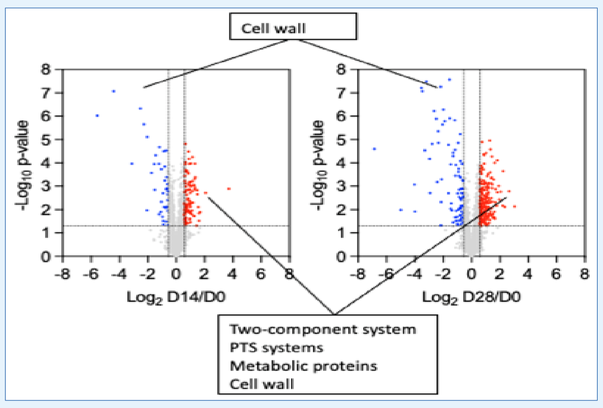


*Figure 2. **(A)** Total, viable and culturable cell profiles of Lm in mineral water. **(B)** Fraction of bacterial population consisting of VBNC formed in mineral water. **(C)** Phase-contrast micrographs of Lm cells during transition into a VBNC state.* Bacteria highlighted by white squares are shown 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.

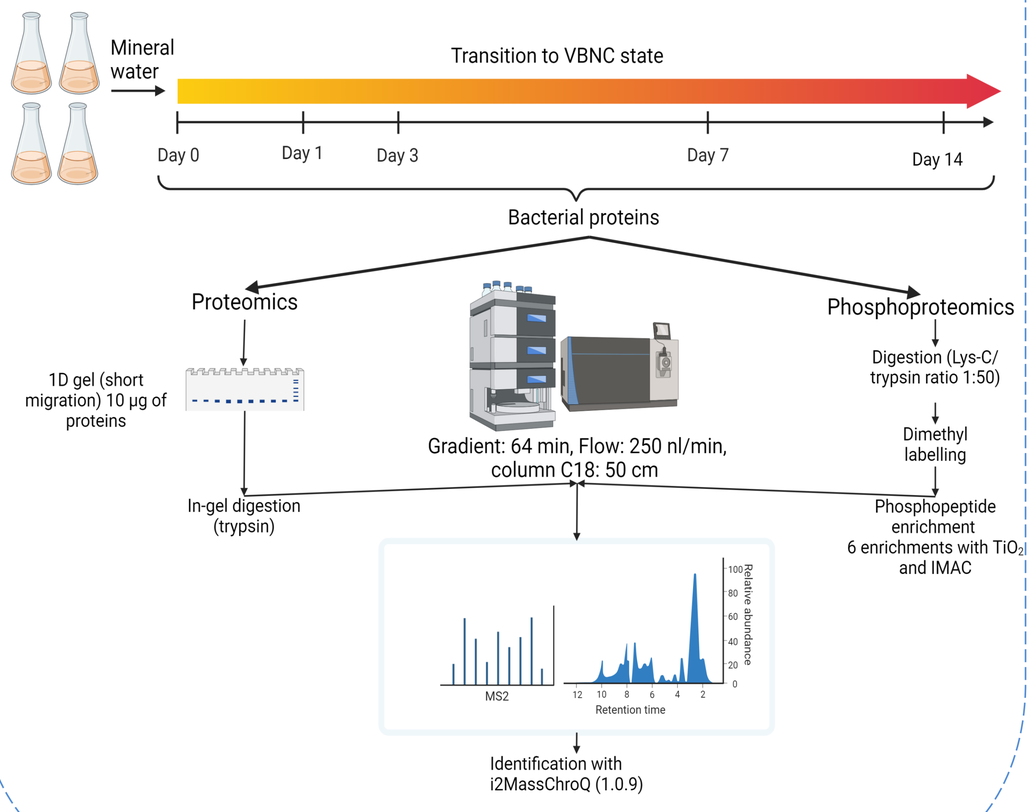
Figure 3. Volcano plot showing the intensity fold change (in log_2) of proteins at day 14 (D14) and day 28 (D28) compared to day 0 (D0) on the x axis. The experiment was performed 4 times and a t-test was performed to calculate –log P values for each protein, indicated on the y axis

Objectives

Hypothesis:
Phosphorylation/dephosphorylation events regulate VBNC transition

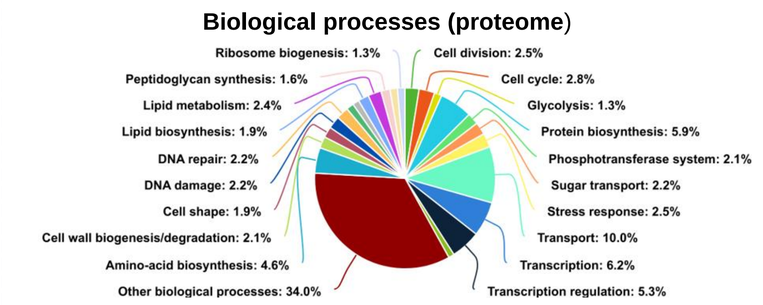
- ✓ *Characterize the phosphoproteome of Lm transitioning to the VBNC state*
- ✓ *Identify differentially-phosphorylated targets that might regulate VBNC transitions*

Experimental pipeline



Preliminary results

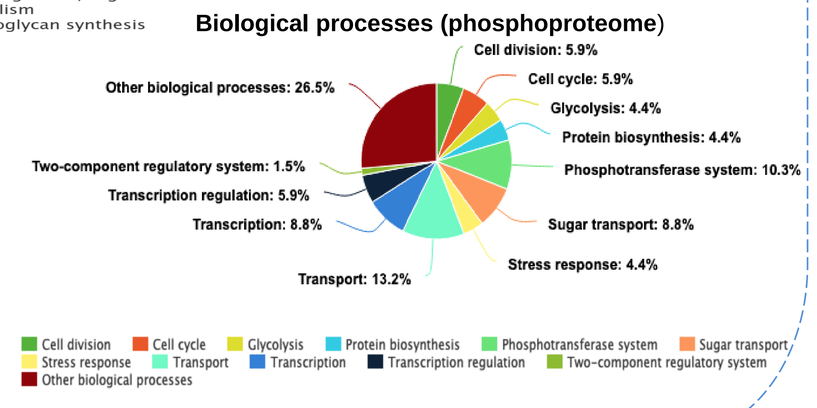
To set up phosphopeptide enrichment we started from bacteria grown in BHI until the stationary phase or incubated in water for 1 or 2 days. We used the pipeline shown beside. Phosphopeptides were purified via TiO_2 beads.



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.

Phosphopetide enrichment via TiO_2 beads, revealed 104 phosphoproteins and 130 phosphoislands.

Certain functions such as glycolysis, PTS sustem, sugar transport and stress response are over-represented compared to the proteome.



Conclusion and pespectives

- ✓ *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*

References

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