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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.

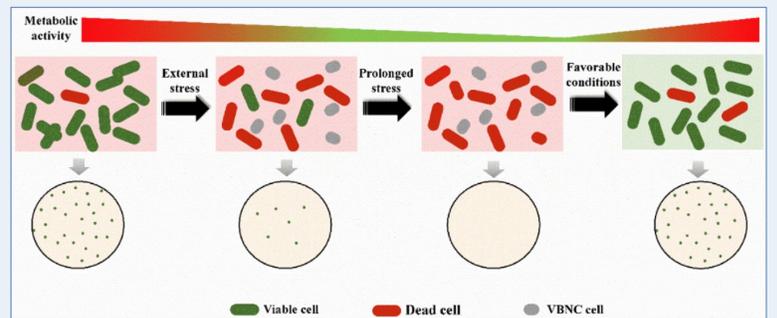


Figure 1. During prolonged stressful conditions, a proportion of viable bacteria will enter into a VBNC state, under which they cannot develop into colonies on agar medium, but cellular metabolic activity is retained. When provided with favorable conditions, VBNC cells can resuscitate to the culturable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

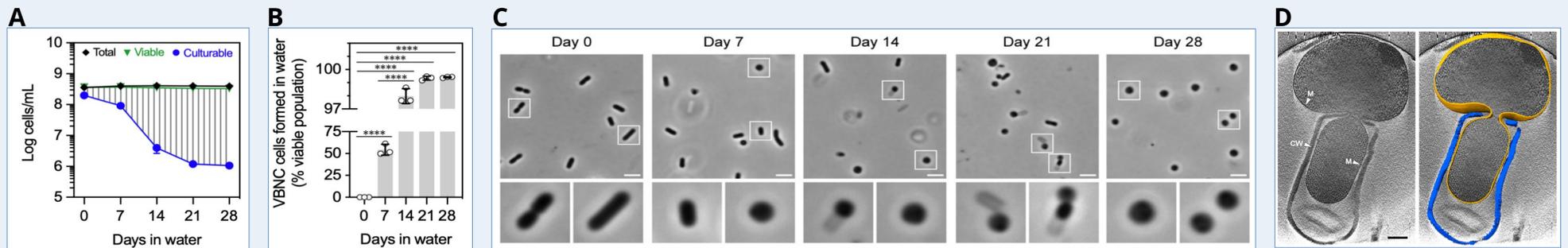
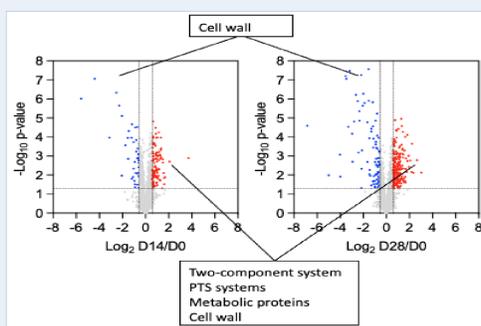


Figure 2. (A) Total, viable and culturable cell profiles of *Lm* in mineral water. (B) Fraction of bacterial population consisting of VBNC formed in mineral water. (C) Phase-contrast micrographs of *Lm* cells during transition into a VBNC state. Bacteria highlighted by white squares are shown enlarged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

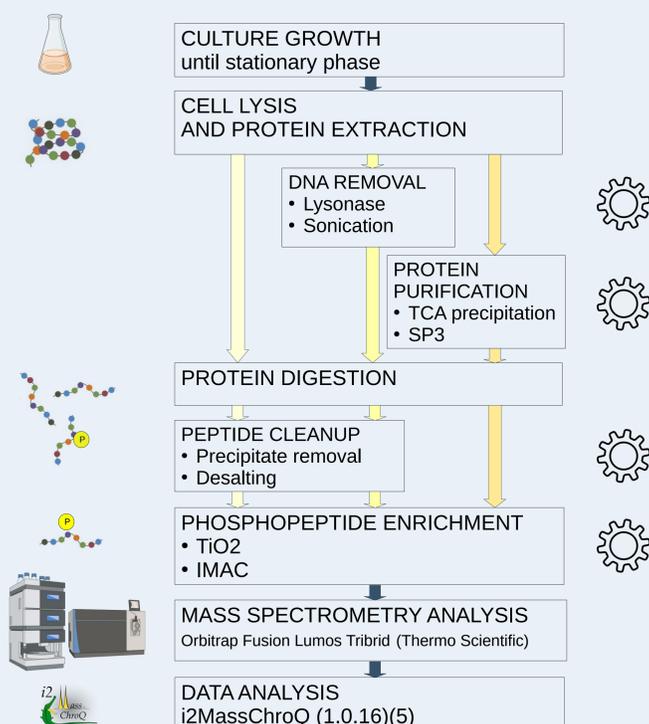
Phosphorylation/dephosphorylation events regulate VBNC transition



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

Phosphoproteomics pipeline optimisation

3 test batches 4 optimised steps



Preliminary results

To set up phosphopeptide enrichment we started from bacteria grown in BHI until the stationary phase. We used the pipeline optimisation shown beside.

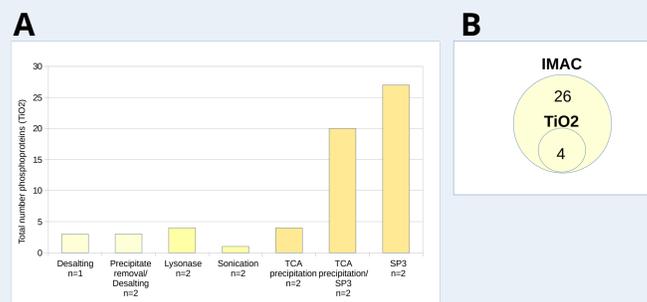


Figure 4 (A) Distribution of phosphoproteins identified during pipeline optimisation. (B) Venn diagram grouping identified phosphoproteins in the 1st test batch comparing TIO2 vs IMAC enrichment.

- ✓ Sample preparation using SP3 protein purification and IMAC enrichment were the best conditions for *Lm* phosphoproteomics (Fig. 4).
 - The two methods will be combined and tested.
- ✓ A total of **59 phosphoisland** were identified on 75 unique peptides of **52 phosphoproteins**.
- ✓ The **Ser/Thr/Tyr** phosphorylation site distribution was **61:39:0**.
- ✓ Certain functions such as glycolysis, PTS system, sugar transport and stress response are over-represented compared to the proteome (data not shown).
- ✓ One of two serine/threonine kinases (PrKA), involved in cell wall remodelling of *Lm*, was identified as phosphorylated.

References

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Conclusion and perspectives

- ✓ These results showed that our protocol is suitable to **isolate phosphoproteins**.
- ✓ We were able to identify **52 phosphoproteins and 59 phosphoislands in stationary phase**.
- ✓ 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.