

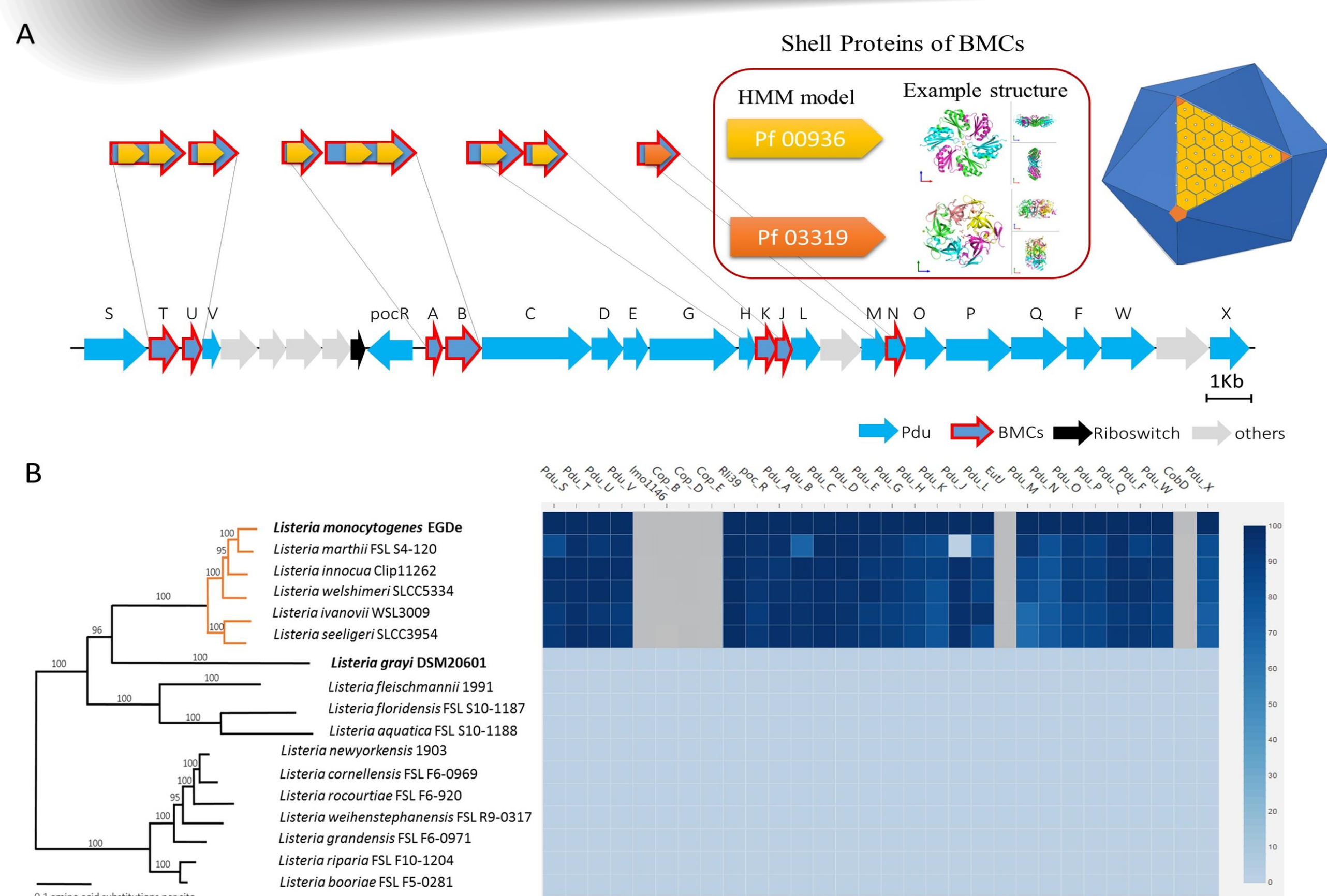
Bacterial microcompartment-dependent 1,2-propanediol utilization stimulates anaerobic growth of *Listeria monocytogenes* EGDe

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Background

Bacterial microcompartments (BMCs) are proteinaceous organelles that can optimize metabolic pathways with toxic volatile intermediates. Previous bioinformatics analysis predicted that BMCs exist in 23 bacterial phyla including *L. monocytogenes*. BMCs are linked to pathogenesis of certain bacteria because they confer a growth advantage by utilizing specific substrates such as 1,2-propanediol (Pd; Pdu cluster). However, the physiological role of BMCs in *L. monocytogenes* is still unexplored. Here we present evidence for a physiological role of BMCs in this pathogen.

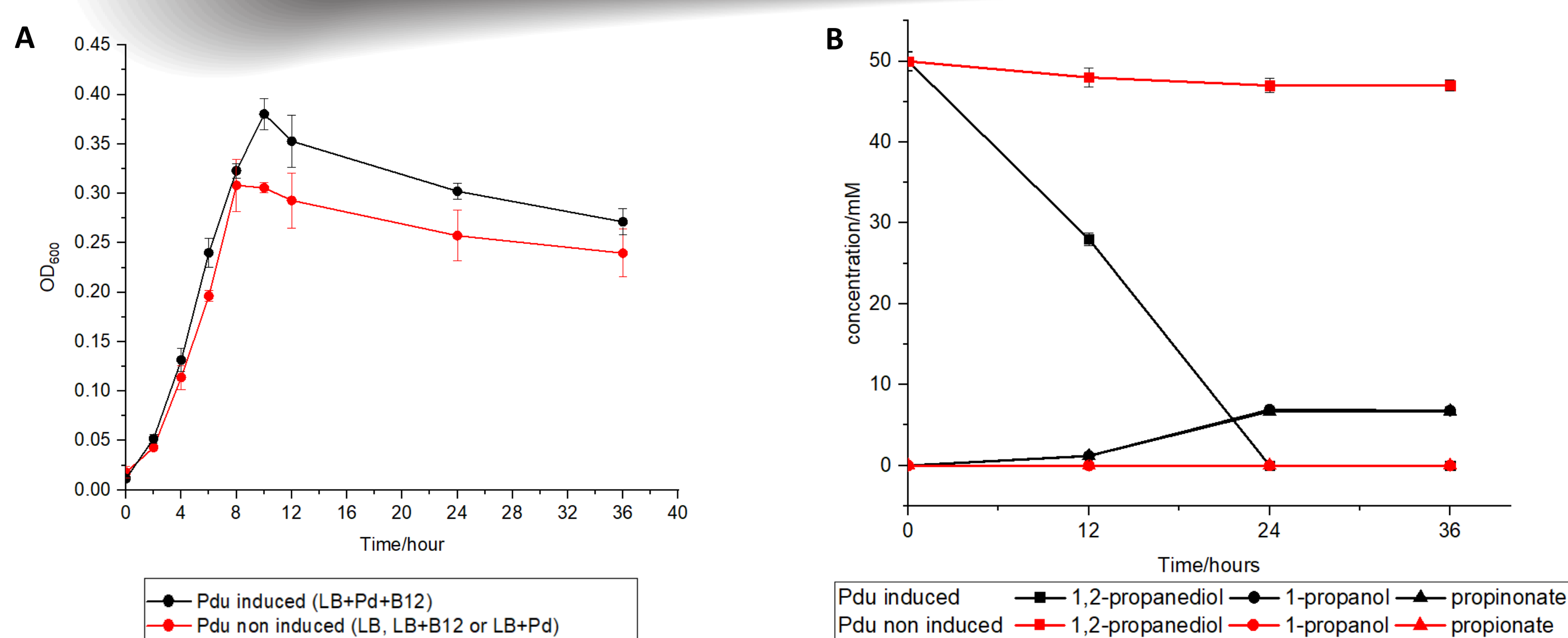
1. Pdu cluster Alignment



1,2-propanediol utilization (Pdu) gene cluster with BMC shell proteins highly conserved in *Listeria sensu stricto* but absent in *Listeria sensu lato*

- A) Scheme of the Pdu cluster with BMC presented as an icosahedron. BMC shell proteins domain: Pf00936 and Pf03319
B) Phylogenetic tree and corresponding similarity heat map of the Pdu cluster in 17 *Listeria* species. Orange lines *Listeria sensu stricto*, black lines *Listeria sensu lato*

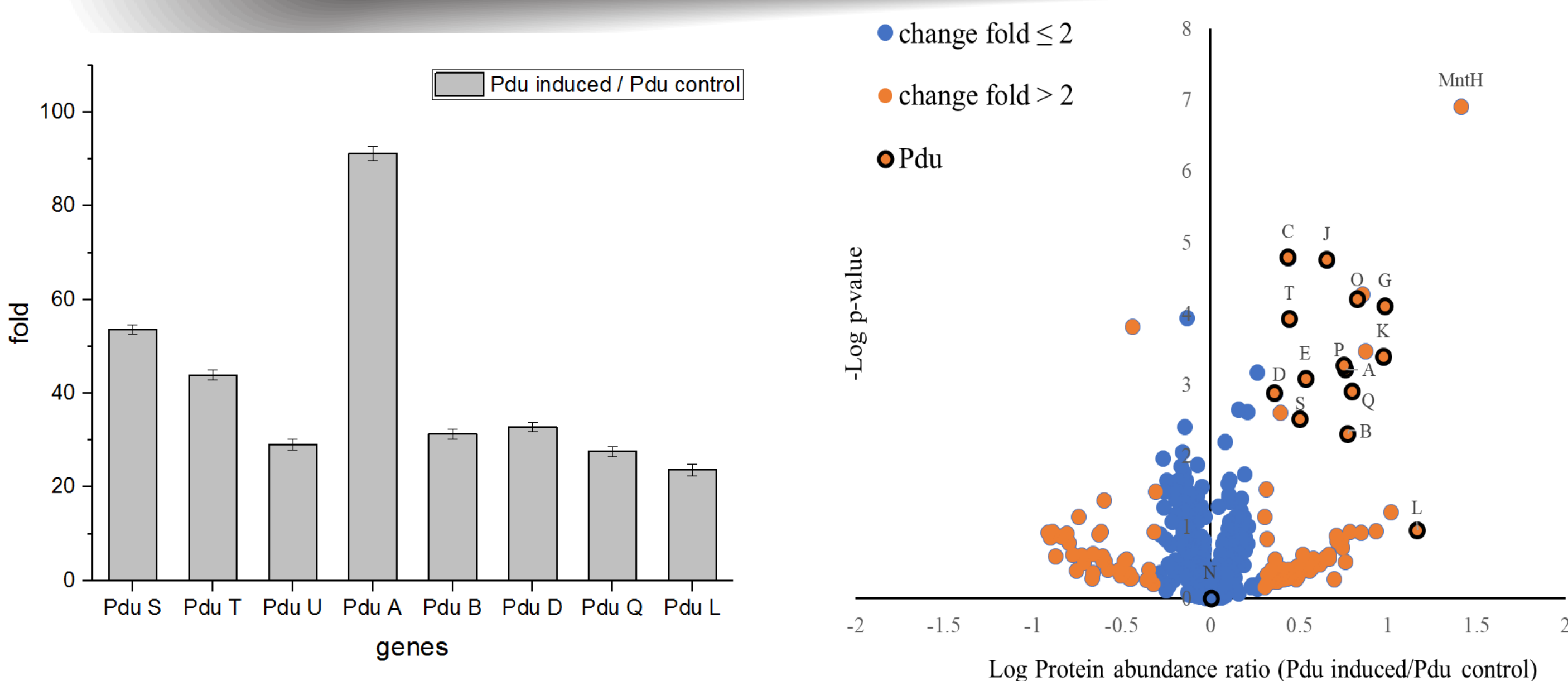
2. Growth and Pd utilization



Pd utilization with production of 1-propanol and propionate confers an anaerobic growth advantage to *L. monocytogenes* EGDe

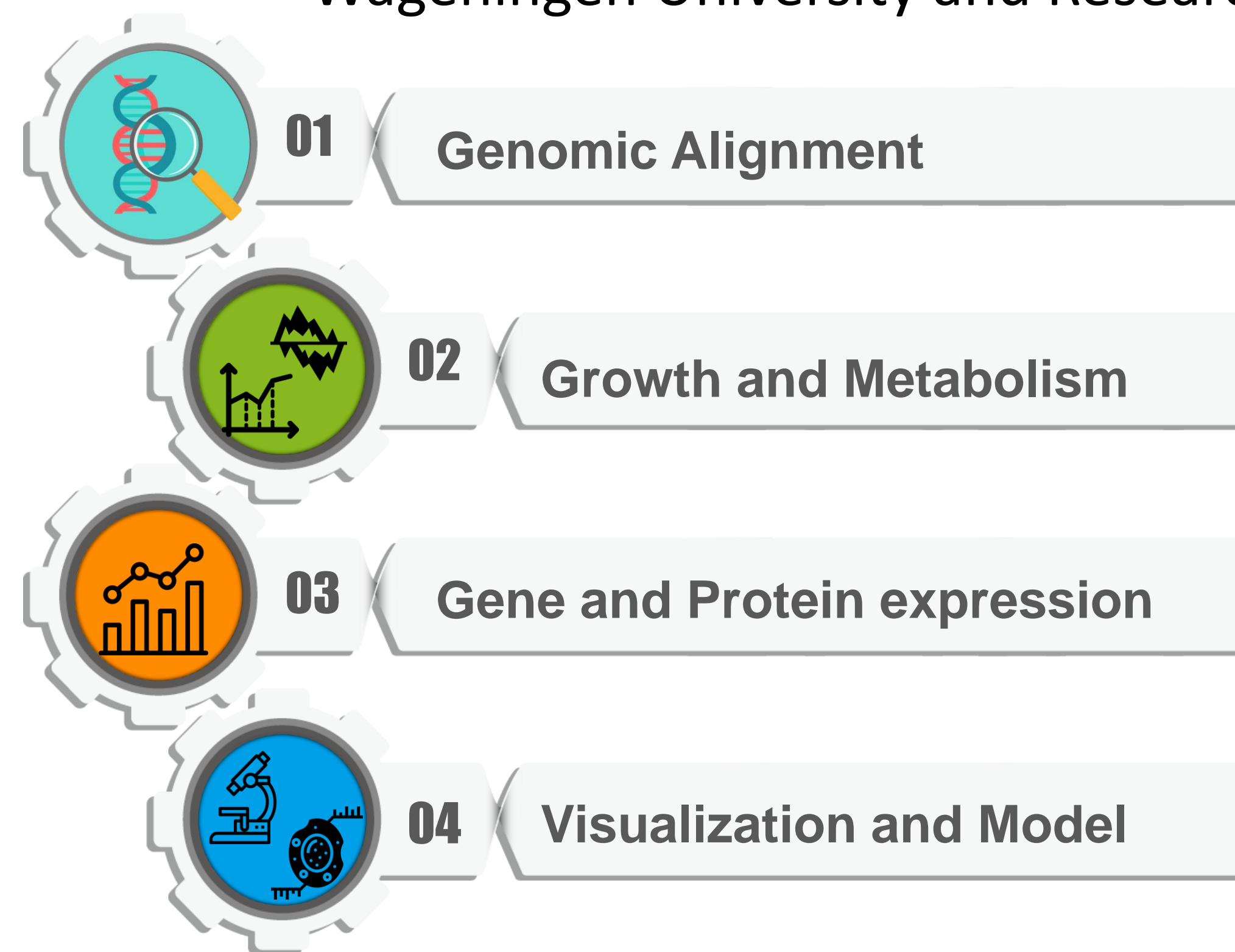
- A) Impact of Pdu activation on anaerobic growth of *L. monocytogenes* EGDe in LB medium.
B) Utilization of 1,2-propanediol (squares), production of 1-propanol (circles) and production of propionate (triangles)
* No significant Pd utilization and No significant growth advantage in aerobic culture

3. Gene and Protein Expression

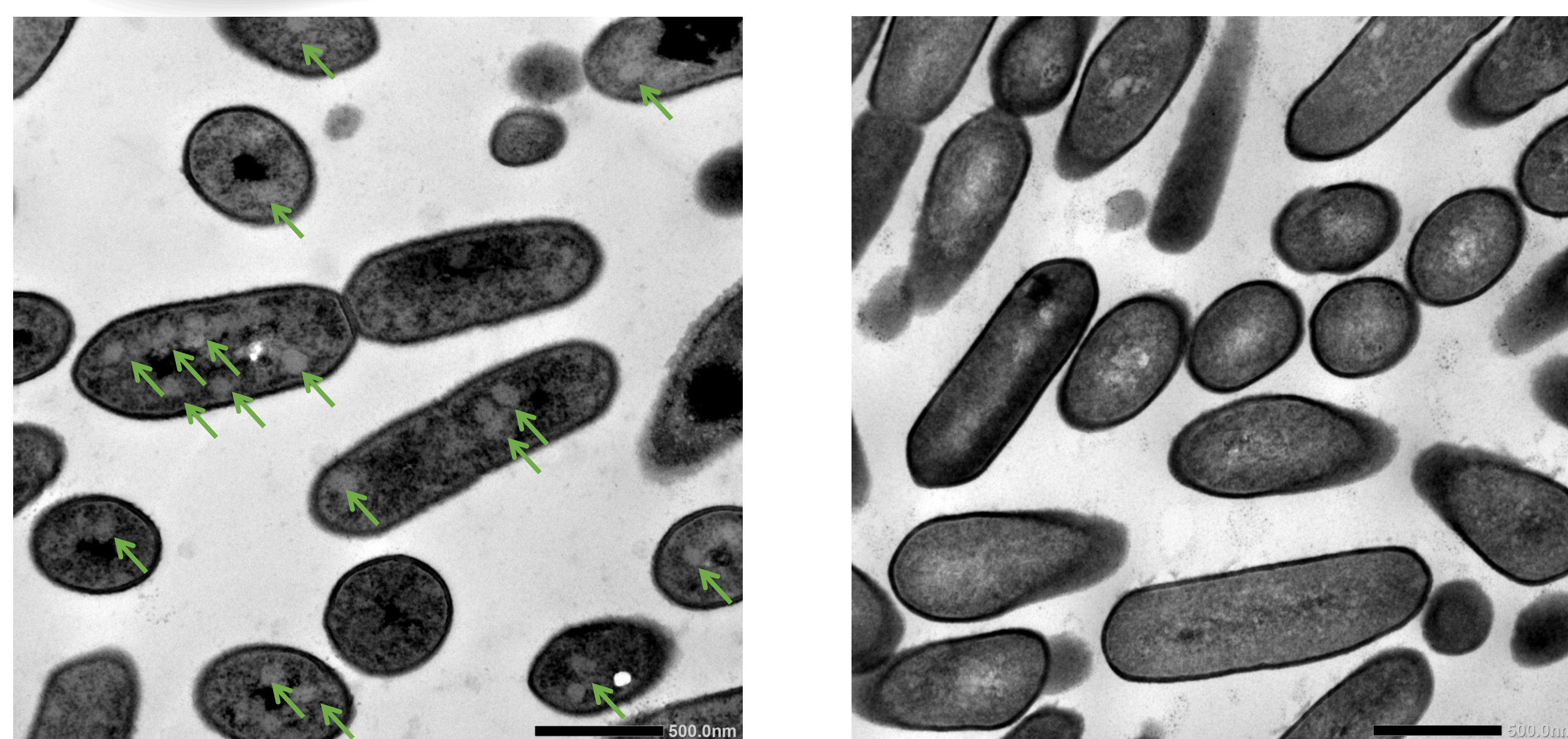


Increased expression of Pdu genes and Pdu proteins (including BMCs shell proteins) in line with Pd utilization

- A) Transcription of *pduSTUABDQL* genes.
B) Proteomic volcano plot of Pdu-induced cells VS non-induced cells; letters indicate Pdu proteins



4. Visualization and Metabolic Model

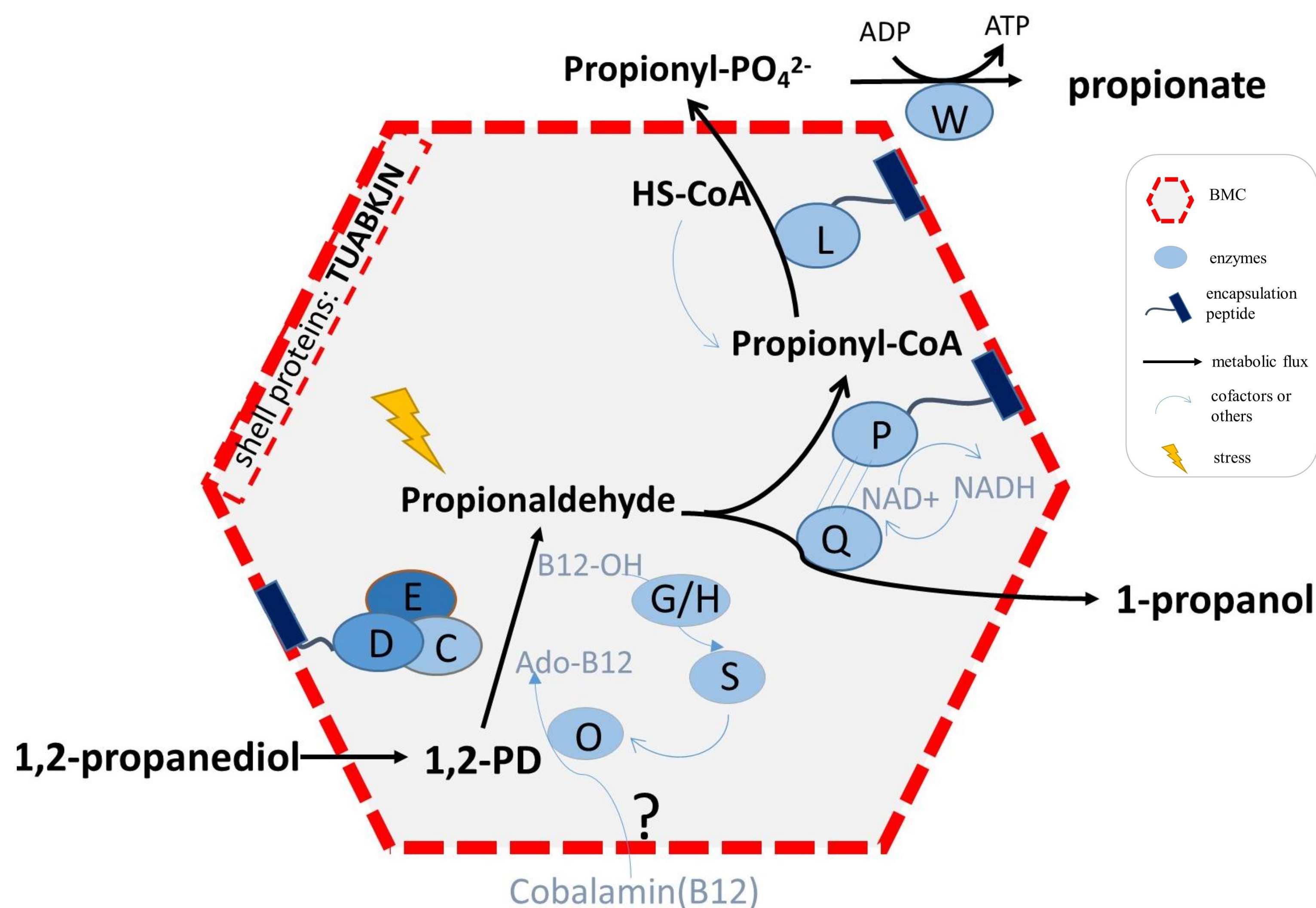


Pdu induced

Pdu non-induced

Visualization of BMCs structures in line with bioinformatics analysis, metabolic phenotyping, transcriptional analysis and proteomics data

TEM images of BMCs in Pdu induced (left) and non-induced control cells (right). Green arrows point to typical BMC structures, with the black scale bars representing 500 nm.

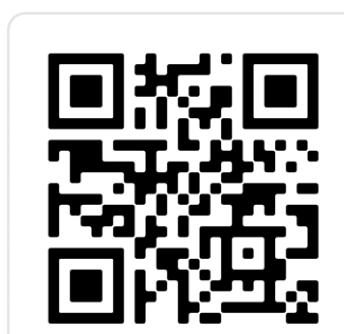


Metabolic model depicting BMC-dependent Pd utilization in *L. monocytogenes*

PduCDE, B12-dependent diol dehydratase; PduP, CoA-dependent propionaldehyde dehydrogenase; PduGH, diol dehydratase reactivase; PduO, corrinoid adenosyltransferase; PduS, cobalamin reductase; PduL, phosphate propanoyltransferase; PduW, propionate kinase; PduQ, propanol dehydrogenase
* Predicted N-terminal encapsulation peptides identified using Jpred4.

Conclusion

- ☐ *L. monocytogenes* EGDe can utilize Pd with concomitant production of 1-propanol and propionate in anaerobic conditions, while the utilization was significantly reduced in aerobic conditions
- ☐ BMCs structures were visualized in *L. monocytogenes* EGDe, in line with bioinformatics analysis, metabolic phenotyping, transcriptional analysis, and proteomics
- ☐ Pd utilization confers a growth advantage to *L. monocytogenes* EGDe in anaerobic conditions



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