Influence of Growth Conditions on the Production and Composition of Extracellular Vesicles from *Listeria monocytogenes*

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Background

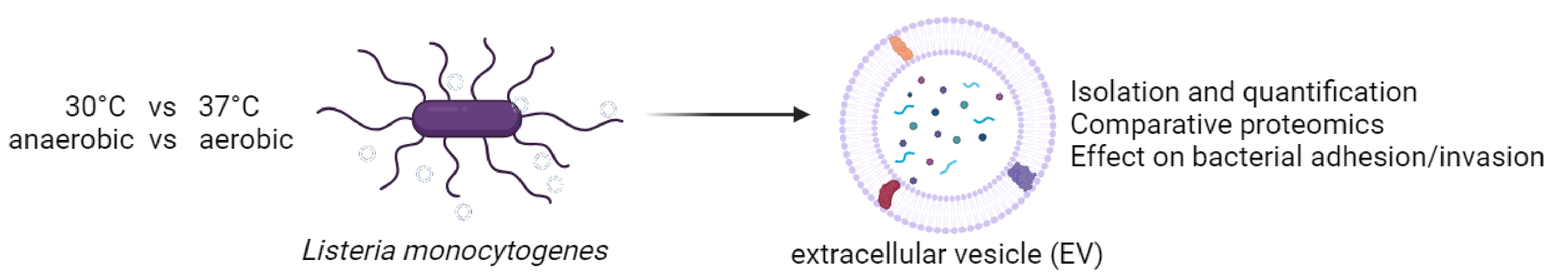
Listeria monocytogenes produces extracellular membrane vesicles (EVs), which are nano-sized, spherical particles originating from the cell membrane, containing various biomolecules such as proteins, lipids, and nucleic acids. The production and composition of EVs are not only a reflection of the physiological state of the producing bacterium, but also in return influence bacterial ecophysiology by modulating bacteria-bacteria, bacteria-environment, and bacteria-host interactions.

Aim and strategy

In this study, we aim to explore the influence of growth conditions on the production and protein composition of *L. monocytogenes* EVs.

L. monocytogenes EGDe was cultivated in LB medium supplemented with 10 mM glucose to late-exponential phase before harvesting EVs for examination, at the following conditions:

(1) 30°C anaerobic (AN); (2) 30°C aerobic (AE);
(3) 37°C anaerobic (AN); (4) 37°C aerobic (AE).



EV isolation and quantification

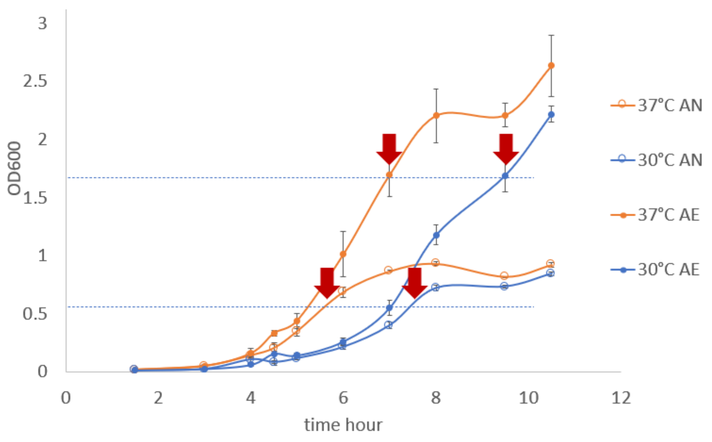


Figure 1. Growth curves and sampling points (red arrows).

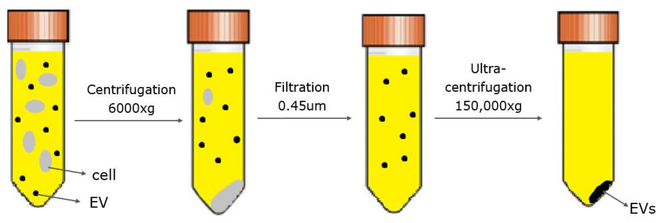


Figure 2. EV isolation procedure.

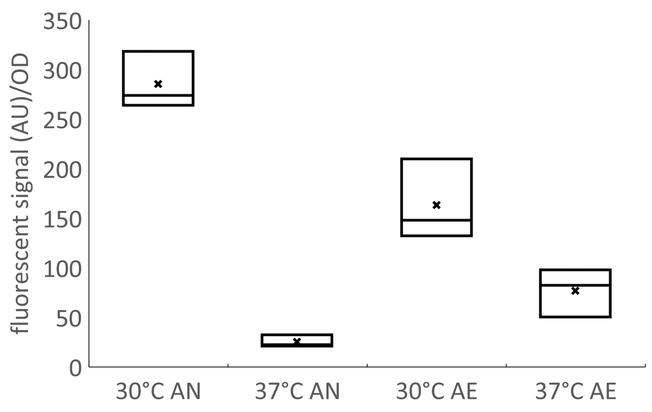


Figure 3. Quantification of EVs from *L. monocytogenes* cultured under different conditions. Isolated EVs were stained with FM4-64 membrane-specific fluorescent dye, and fluorescent signal (arbitrary unit) was measured at wavelength 515/640nm as a reflection of EV quantity. EV quantity was corrected for OD values of the bacterial cultures.

Comparative proteomics

Protein content of EVs and cells obtained from the four cultivation conditions were compared by tandem mass tag (TMT) proteomics.

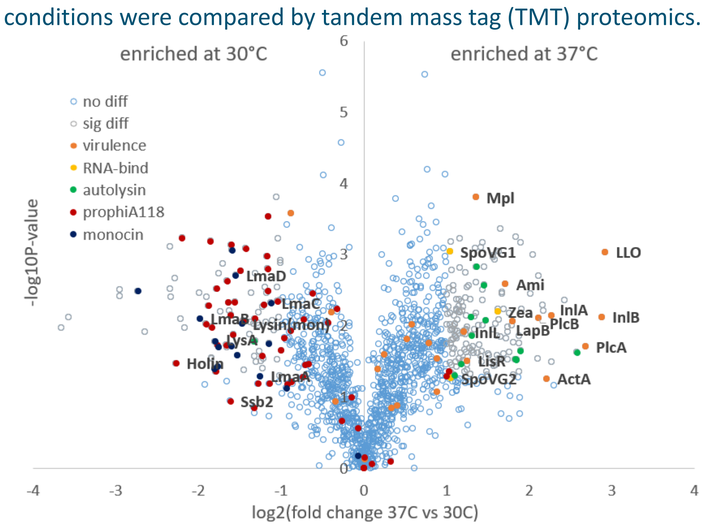


Figure 4. Comparison of EV protein content from 37°C versus 30°C under anaerobic condition. Cut-offs of significant changes are set as fold change > 2 and $p < 0.05$ (three biological replicates).

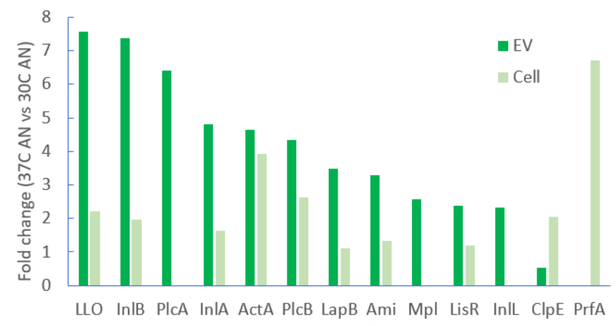


Figure 5. Enrichment of virulence factors at 37°C as compared to 30°C (anaerobic condition), in EVs and in cells.

- Many virulence factors are enriched at 37°C in both EVs and cells, and the enrichment is more distinct in EVs than in cells.
- Several RNA-binding proteins are enriched at 37°C in EVs.
- Autolysins are enriched at 37°C in EVs.
- Prophage/monocin-encoded proteins are enriched at 30°C in EVs, including holins and lysins, which is conceivably linked to enhanced EV production at 30°C.
- Less differences were observed between anaerobic and aerobic conditions, to name an exception: RNA-binding protein Zea was consistently enriched under anaerobic conditions as compared to aerobic conditions (data not shown).

Effect on cell adhesion/invasion

EVs from 37°C, anaerobic cultivation were applied to Caco-2 cell line, and the effect on subsequent *L. monocytogenes* adhesion and invasion was examined.

Caco-2 cells co-incubated with EVs for 6h (FBS-free)
↓ washing
L. monocytogenes applied to Caco-2 cells at MOI=100, 30min
↓ washing
Plate for adhesion count / Incubation 30 min with gentamicin → washing → Plate for invasion count

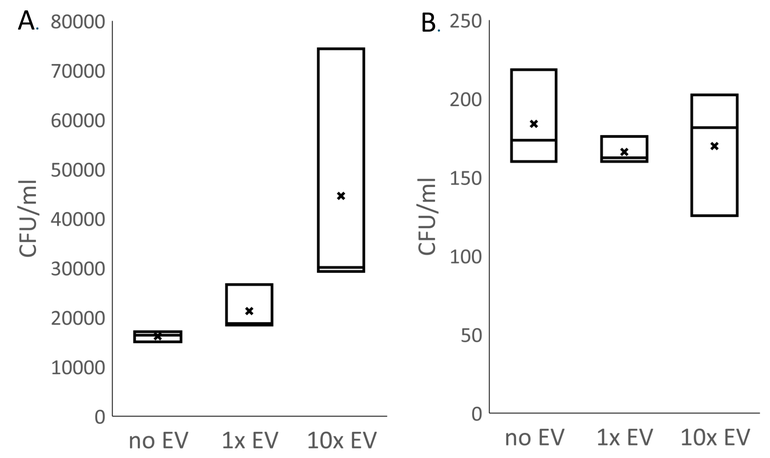


Figure 6. A) Adhesion count. B) Invasion count. Three biological replicates."1x EV" equals to total EV quantity in 20 ml bacterial culture; "10x EV" equals to total EV in 200 ml bacterial culture.

- *L. monocytogenes* adhesion to Caco-2 cells increased on average after incubation with EVs.
- EVs from 37°C are enriched in virulence factors contributing to adhesion, such as LapB, Ami and InlL.
- Caco-2 cell viability and *L. monocytogenes* translocation upon EV treatment are relevant parameters to be determined.

The insights of *L. monocytogenes* EV quantity and protein composition from different cultivation conditions provide promising leads in further investigations, including EV biogenesis mechanism, RNA cargos and roles in pathogen-host interaction.

Relevant literature:
1. Liu Y, Defourny KAY, Smid EJ, Abee T. Gram-Positive Bacterial Extracellular Vesicles and Their Impact on Health and Disease. *Front Microbiol*. 9:1502.
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3. Pagliuso, A., Tham, T. N.,… Cossart, P. (2019). An RNA-Binding Protein Secreted by a Bacterial Pathogen Modulates RIG-I Signaling. *Cell host & microbe.* 26:823–835.e11.

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