Glycerol metabolism induces *Listeria monocytogenes* biofilm formation at the air-liquid interface

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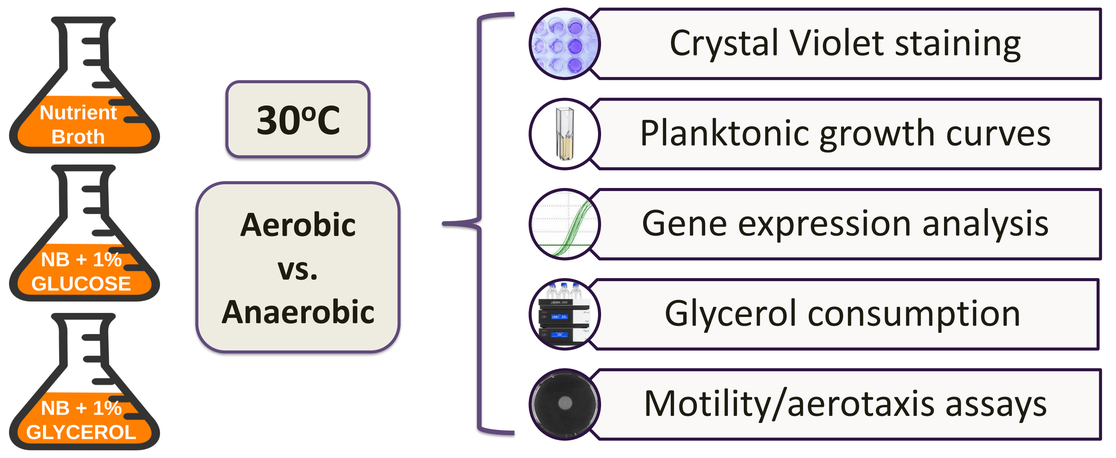
Background

Listeria monocytogenes is a food-borne pathogen that can grow as a biofilm on the surface of food-processing equipment. Biofilms are communities of cells embedded in a self-produced extracellular matrix. This matrix acts as a glue that keeps cells within the biofilm closely attached to each other and to a surface. Biofilm production and characteristics can be affected by a broad range of environmental conditions.

Approach



Results

Supplementation of nutrient broth (NB) with glycerol induced the formation of a biofilm at the air-liquid interface of the culture. This phenotype was not found in NB supplemented with glucose or in plain NB.

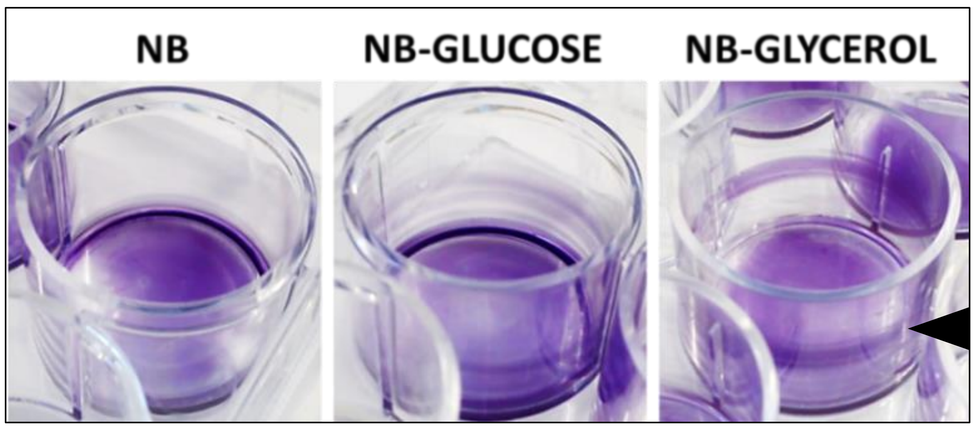


Fig.1: Images of *L. monocytogenes* biofilms after staining with 1% crystal violet. Cells were incubated statically , at 30ºC for 48 hours, in plain NB, NB supplemented with 1% glucose (NB-Glucose) or 1% glycerol (NB-Glycerol).

Total biofilm formation and planktonic growth performance were impaired in NB-Glycerol in anaerobic conditions, compared to the performance of the NB-Glucose control.

In the presence of oxygen, both glucose and glycerol enhance growth and biofilm production compared to plain NB. However, under anaerobic conditions only glucose enhances *L. monocytogenes* performance, whereas supplementation with glycerol produces identical results as plain NB.

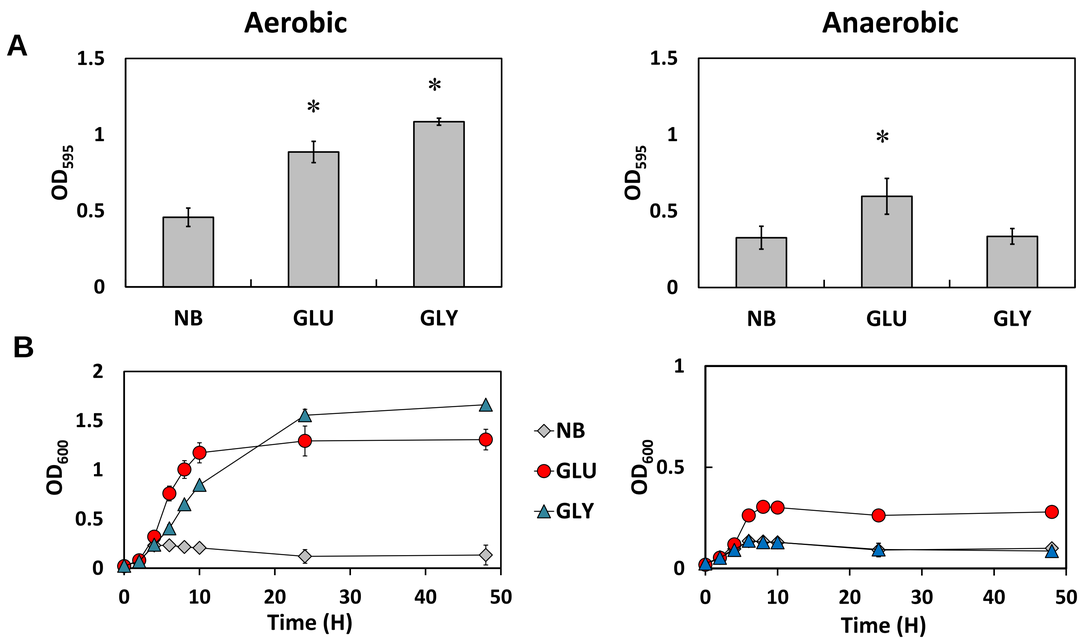


Fig.2: Performance of *L. monocytogenes* under aerobic and anaerobic conditions. Cells were grown in NB, NB supplemented with 1% glucose (GLU) or 1% glycerol (GLY) **A)** Biofilm production as measured by the crystal violet staining technique, after 48 hours of incubation at 30ºC. **B)** Planktonic growth curves based on cell density. Cells were grown at 30ºC for 48 hours. * represents p values < 0.05.

Lack of oxygen induces a downregulation of the genes involved in glycerol transport and metabolism, compared to cells grown aerobically. Moreover, glycerol consumption measurements after 48 hours of incubation show that *L. monocytogenes* does not utilize glycerol at all when cells are grown anaerobically.

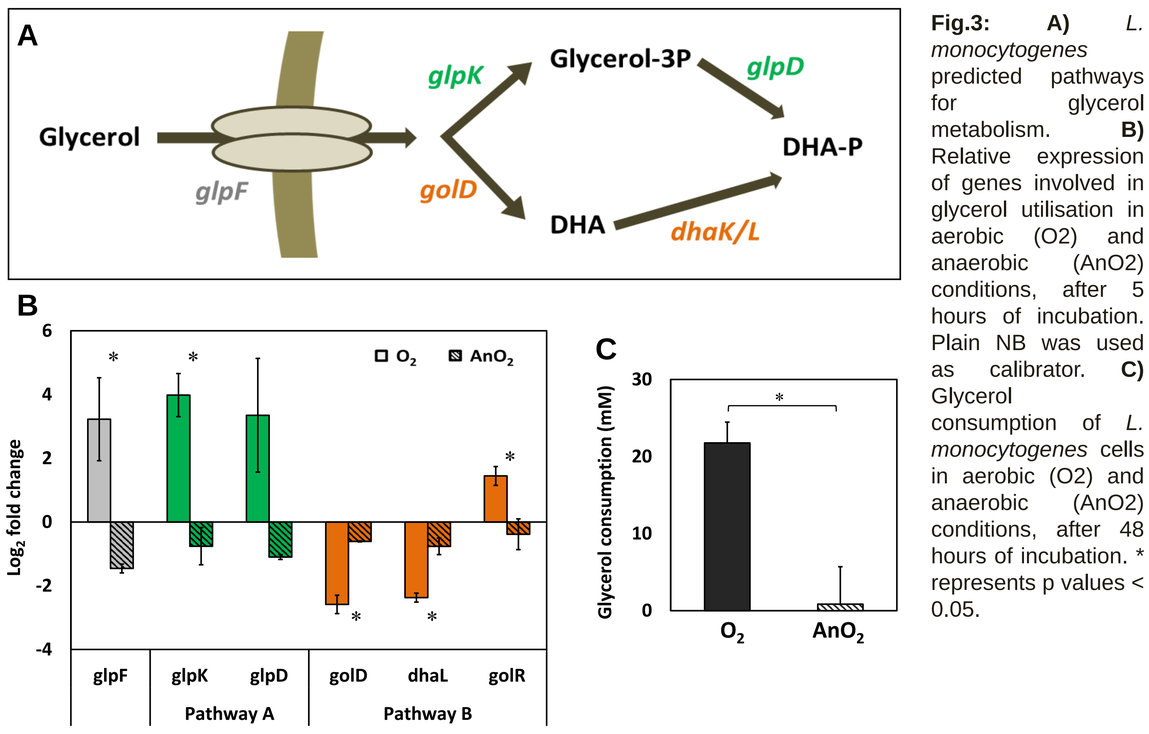


Fig.3: **A)** *L. monocytogenes* predicted pathways for glycerol metabolism. **B)** Relative expression of genes involved in glycerol utilisation in aerobic (O2) and anaerobic (AnO2) conditions, after 5 hours of incubation. Plain NB was used as calibrator. **C)** Glycerol consumption of *L. monocytogenes* cells in aerobic (O2) and anaerobic (AnO2) conditions, after 48 hours of incubation. * represents p values < 0.05.

Cells showed similar levels of motility in plain NA and NA-Glycerol, whereas motility appeared slightly reduced in the presence of glucose.

On the other hand, aerotaxis towards the surface of the tubes was only found in PBS agar supplemented with glycerol.

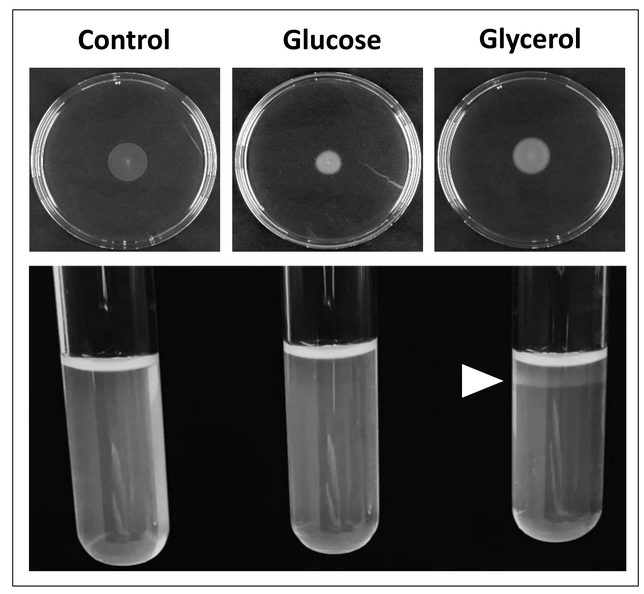


Fig.4: Motility of cells in non-supplemented 0.3% agar, and agar supplemented with glucose and glycerol. **A)** Swimming plates after 20 hours of incubation. **B)** Aerotaxis tubes after 48 hours of incubation.

Conclusions

- ✓ Glycerol induces **biofilm** formation at the **air-liquid interface** of the culture in *L. monocytogenes*
- ✓ *L. monocytogenes* is unable to metabolize **glycerol** without **oxygen** in the conditions tested
- ✓ The presence of glycerol in the media induces **aerotaxis** in *L. monocytogenes*

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