Quantitative physiology and proteome adaptations of *Bifidobacterium breve* NRBB57 at near-zero growth rates

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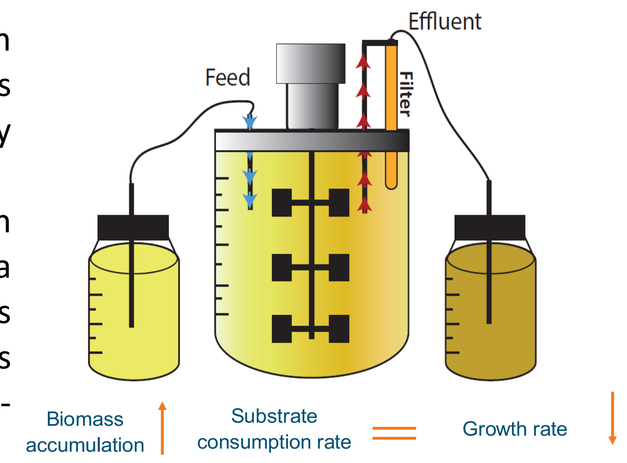
1. Background

- Often in natural environments, nutrients are not readily available for microbial consumption because of low concentrations and competing microorganisms.
- Consequently, microorganisms enter a famine phase where growth becomes slow, while they stay metabolicallv active.
- Extreme nutrient limitation resulting in near-zero growth rates can be obtained in the laboratory using retentostat cultivation
- The retentostat is a bioreactor with continuous feeding of medium at a fixed rate while spent medium is simultaneous removed and biomass is completely retained using a cross-flow filter in the effluent line.

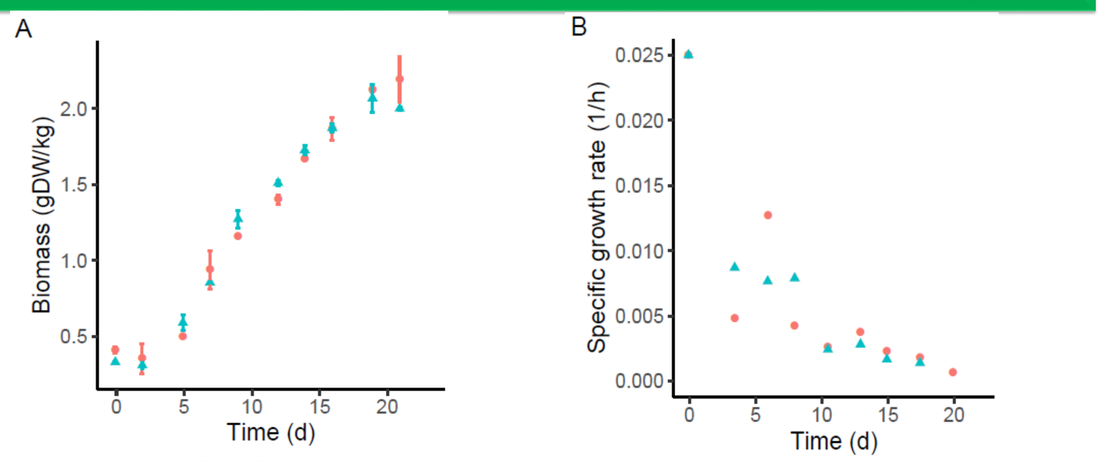


- For this study, we used the probiotic bacterium *Bifidobacterium breve*, simulating the low concentration of nutrients found in its natural ecosystem the human gut.

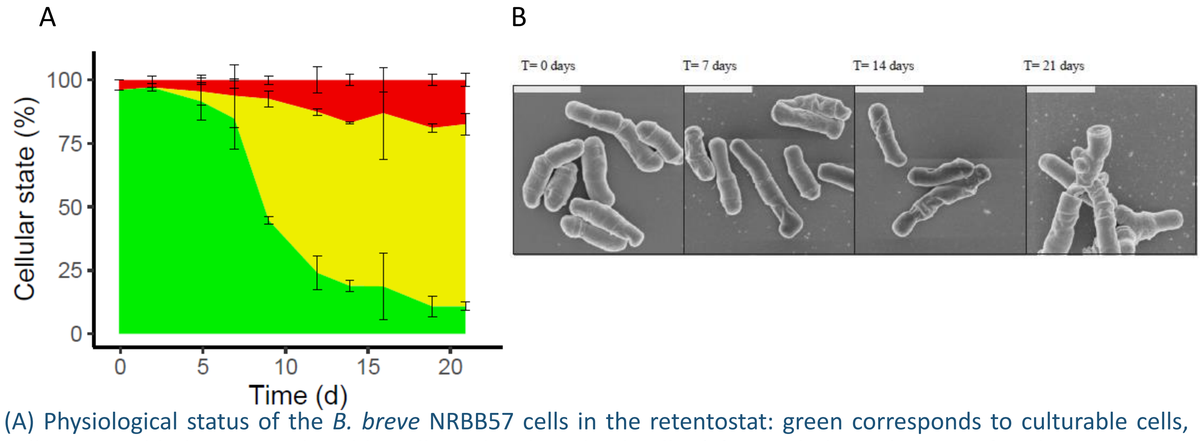
2. Aim

The objective of this study is to investigate the physiological response of *B. breve* NRBB57 at near-zero growth rates using retentostat cultivation. Culturability, metabolism, maintenance requirement and the whole proteome were analyzed to understand the adaptation of *B. breve* at near-zero growth conditions.

3. Results

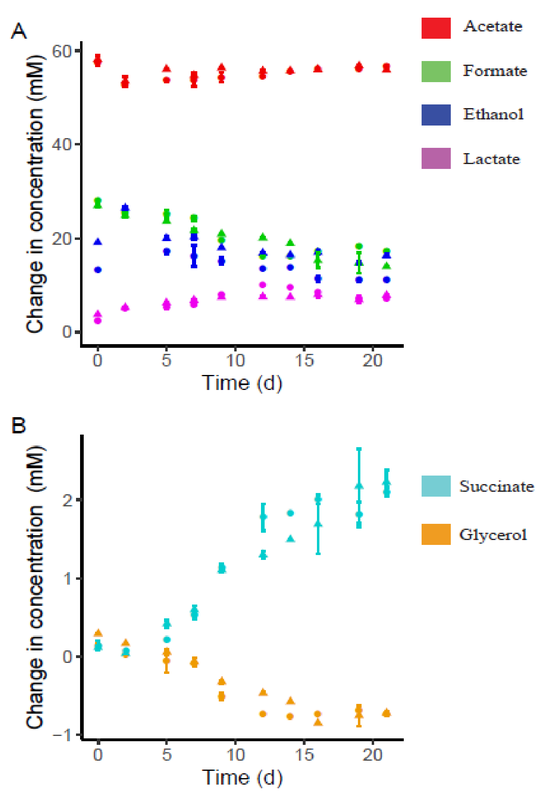


Growth of *B. breve* NRBB57 during the retentostat cultivations. Colors and symbols represent different biological replicates. Time zero corresponds to the last day of the chemostat at a growth rate of 0.025 h^{-1}. (A) Biomass accumulation throughout the retentostat cultivation. (B) Calculated specific growth rates based on cell dry weight measurements.

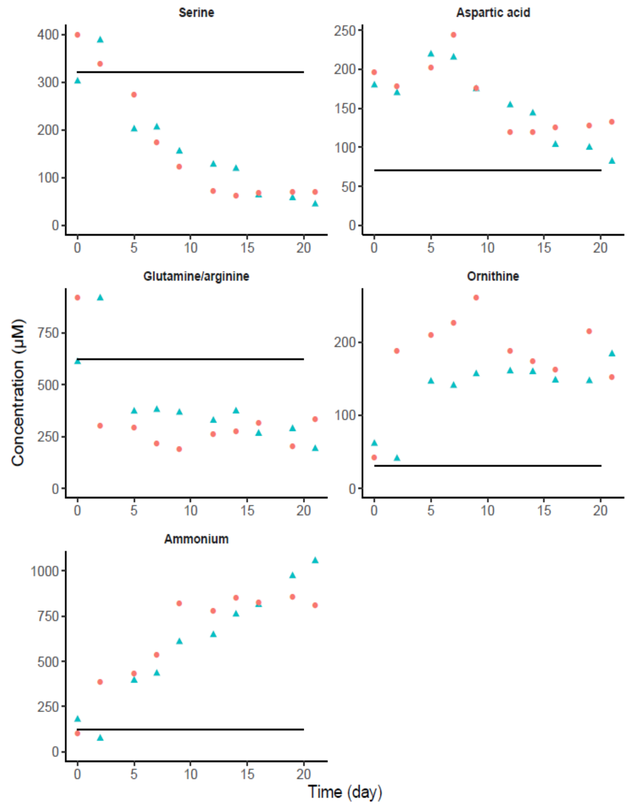


(A) Physiological status of the *B. breve* NRBB57 cells in the retentostat: green corresponds to culturable cells, yellow to viable-but-non-culturable cells and red to dead cells. (B) Morphology changes of *B. breve* NRBB57 cultivated in the retentostat and visualised under scanning electron microscopy. The length of the white bars correspond to 1 µm. Samples were taken at time 0 which corresponds to the last day of the chemostat at a growth rate of 0.025 h^{-1} and after 7, 14 and 21 days of retentostat cultivation.

- Lactose was mainly converted into acetate, formate and ethanol and lactate production showed a slight increase during retentostat cultivation.
- Interestingly, the consumption of several amino acids and glycerol increased over time in the retentostat.
- Likewise, the ornithine concentration increased conceivably as the final product of arginine catabolism. Ammonia production also increased, which correlates with the increased utilization of amino acids.

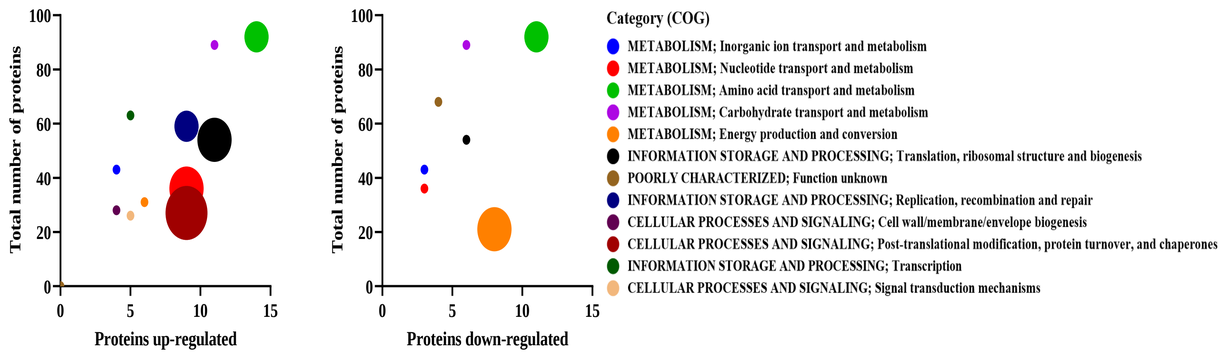


Extracellular metabolite concentrations in the retentostat. Colors represent different metabolites and symbols represent different biological replicates. (A) Main products of the lactose metabolism. (B) Secondary metabolism of succinate and glycerol.



Extracellular amino acids and ammonia in the retentostat measured with UPLC over time. Colors and symbols represent different biological duplicates.

Proteome analysis revealed a down-regulation of ribosomal proteins at near-zero growth rates and an up-regulation of proteins involved in the catabolism of alternative energy sources. In addition, an activation was observed of proteins related to stringent and stress response during the retentostat cultivation.



Enrichment of proteins with significant differences in the abundances during the retentostat cultivation. Bubble size represents the score or significance assigned by the software being 3 the biggest size and 0 the smallest size

4. Conclusions

We demonstrated that *B. breve* NRBB57 can be cultivated in the retentostat system at near-zero growth rates, while remaining viable and metabolically active. Metabolite and proteome analysis revealed that the extreme energy restriction induced a multifaceted response including stress defence and stringent response, metabolic shifts and activation of alternative energy producing pathways.

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