Aroma production by propionic acid bacteria at near-zero growth rates

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Versatile Propionibacteria

Microbial source
- B12
- Vit K2

Microbial and other sources
- B9/B11
- B2

Propionibacteria (PAB) are sturdy, versatile bacteria that are able to survive prolonged periods of nutrient limitation. Several species are isolated from dairy, including *Propionibacterium freudenreichii* subsp. *freudenreichii, P. freudenreichii* subsp. *shermanii*, *Acidipropionibacterium acidipropionici., A. thoenii* and *A. jensenii*. PAB can ferment lactate into propionate, acetate and CO_2 via the Wood-Werkman cycle (figure 1). Furthermore, PAB produce a variety of vitamins, including vitamin B2, B9/B11, B12 and K2. This makes PAB interesting organisms for aroma production and *in situ* product vitamin fortification.

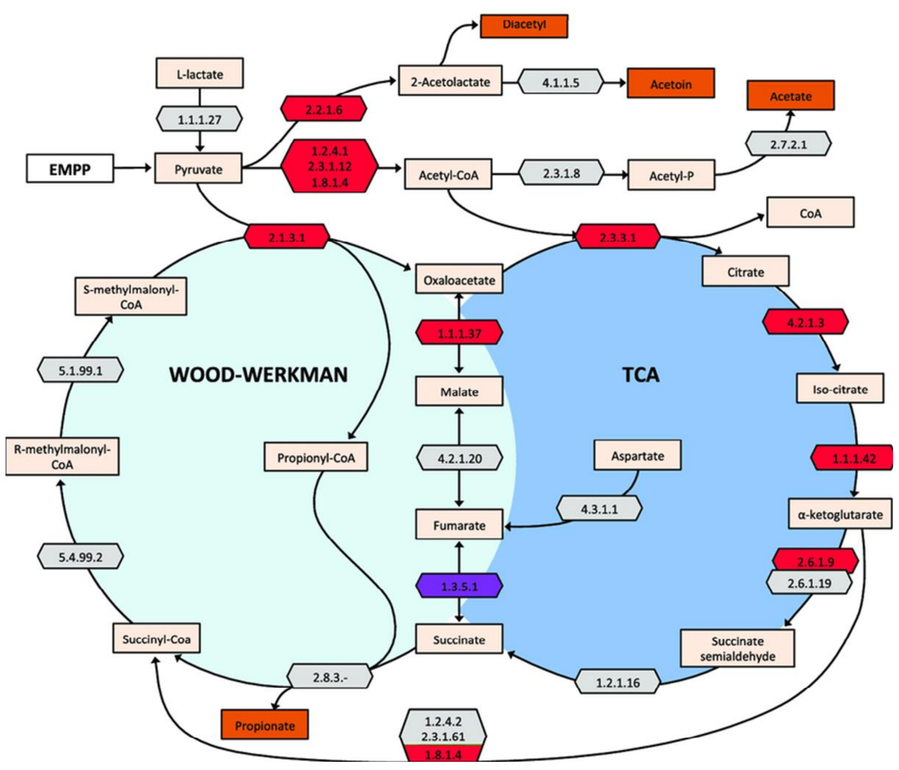

Figure 1. Wood-Werkman cycle in PAB.
Ojala, T., et al. (2017). Functional genomics provides insights into the role of Propionibacterium freudenreichii ssp. shermanii JS in cheese ripening. *International journal of food microbiology, 241*, 39-48.

Screening of aroma production by PAB in batch cultures

To monitor species and strain diversity in aroma production by PAB 16 strains of 5 different PAB species were grown in batch cultures. The produced aroma profiles were analyzed by HS SPME GC-MS.
A principal component analysis showed a tight clustering of commonly used dairy strains of *P. freudenreichii* and *P. shermanii*, whilst aroma production by *A. jensenii* and *A. acidipropionici* species was more strain dependent. Moreover, each species produces different **aroma profiles**.

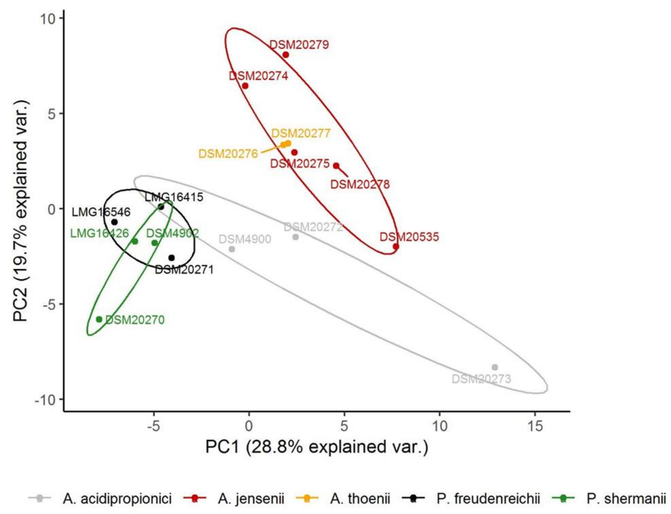

Figure 2. Principal component analysis of aroma profiles produced by 5 PAB species.

This **diversity** in aroma production may be exploited for specific aroma production.

Retentostat cultivations for near-zero growth conditions

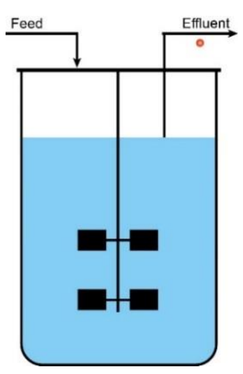

Figure 3. Chemostat cultivation schematic. Medium is fed to the reactor and effluent is removed at the a constant speed.

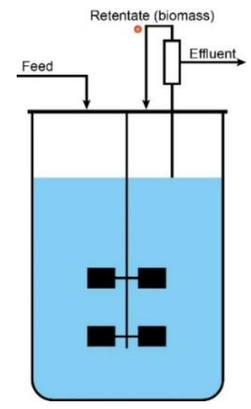

Figure 4. Retentostat cultivation schematic. Medium is fed to the reactor at a constant speed. Effluent is pumped through a filter resulting in retention of biomass.

Background

The most prevailing physiological state of microorganisms on Earth is that of **energy limitation**. Since most research is performed in nutrient-rich batch cultures, this natural physiological state is **poorly represented** in scientific work [1]. Microorganisms indeed show physiological and transcriptional responses at near-zero growth rates related to increased stress tolerance, decreased protein synthesis, morphological changes or secondary metabolite production [2,3]. **Near-zero growth** conditions **resemble** the conditions prevailing during **cheese ripening** and *Lactococcus lactis* produces aroma compounds found in ripened cheese under these growth conditions [4]. Therefore, near-zero growth conditions show great potential for production of typical cheese aroma by Propionibacteria.

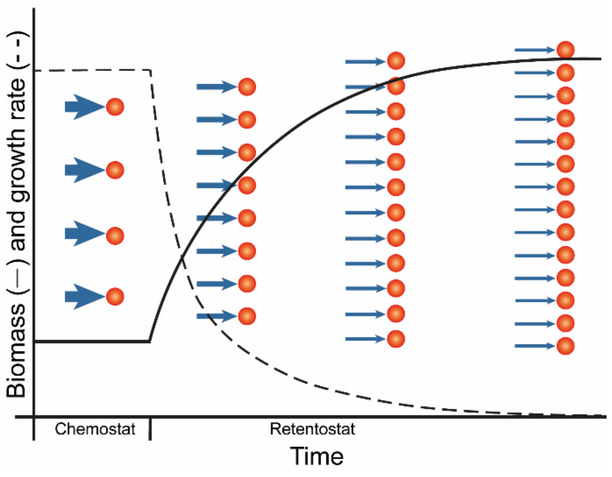

Figure 5. Schematic overview of biomass accumulation and growth rate during retentostat cultivations

Vitamin production and aroma profiles will be determined at near-zero growth conditions achieved in a bioreactor by a retentostat approach [3], i.e., chemostat cultivations in which **biomass is retained** using a filter.
In chemostat cultivations the **dilution rate (D)** determines the growth rate. In retentostat cultivations the growth rate approaches zero due to **retention of biomass**.

Objective

The aim of this study is to grow dairy Propionibacteria at near-zero growth conditions and to study the effect of these conditions on metabolite production (primary metabolism), aroma formation (secondary metabolism) and physiology.

Conclusions

- PAB are promising work horses for production of aroma compounds
- Aroma production is diverse amongst PAB species
- Near-zero growth of PAB in retentostat cultivations offers opportunities for diverse applications such as production of aroma blocks.

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