

# Genome Sequences and Co-Cultures are Momentous for Present-Day Natural Optimization of Food Fermenting Microorganisms

“In order to naturally optimize growth of microorganisms of interest, it is important to look into their genomes to identify potential constraints in their metabolic potentials impeding optimal growth, where after a simple experimental set-up can establish whether these constraints lead to limitations *in situ* and whether they can be solved by co-culture with other species that do not have these metabolic constraints according to their genome annotations”



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### ABSTRACT

A simple experimental set-up based on genome information can give leads on how to improve the performances of food fermenting microorganisms naturally by means of co-culture.

**Keywords:** Bifidobacterium animalis sub sp. lactis; co-culture; stimulation; proteolysis; genome

Most food fermentations are performed by consortia of microorganisms in which the consortium members interact thereby influencing each other's performance. These interactions can be used to naturally optimize the performance of desired strains, in particular when genome annotations are available. As an example, Cultura is a Danish dairy product fermented by a mixture of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus paracasei* subsp. *paracasei* and *Bifidobacterium animalis* subsp. *lactis*. In particular the latter may have problems growing in milk as genome information shows that it, inter alia, lacks genes for de novo biosynthesis cysteine and dependent on the strain also methionine [1,2], while levels of these amino acids in milk are low [3]. Therefore, it may benefit from supply by consortium members.

In a study, casitone (4g/L), casamino acids (1g/L) and cysteine/methionine (both 325 µM) added to skim milk all significantly improved growth of *B. lactis* in mono culture (Figure 1) when incubated anaerobically for 28h at 37°C (assessed on MRS agar according to Sieuwerts, *et al.* [4]). Similarly, co-culture with *L. acidophilus* and *L. paracasei* stimulated *B. lactis*. There was no additional benefit of adding one of the supplements in the mixed culture with *L. acidophilus*, probably because its protease Prt released sufficient amino acids [5]. With the also proteolytic *L. paracasei* [6], however, there was an additional benefit and this may be due to lower proteolytic activity compared to *L. acidophilus*. *L. bulgaricus*, also Prt+, gave a similar effect as *L. acidophilus*, though in this co-culture casamino acids still stimulated *B. lactis*. Addition of casein to the co-culture with the Prt- *S. thermophilus* increased *B. lactis* growth, though less than in mono culture, and addition of casamino acids or cysteine/methionine led to a reduction. Most likely, *B. lactis* benefitted from the availability of nitrogen source, but got restrained by the much faster growth of *S. thermophilus*. Thus, co-fermentation with the Prt+ *L. acidophilus* and *L. paracasei* may aid *B. lactis* performance by providing amino acids, while the Prt- *S. thermophilus* is more likely to act as competitor.

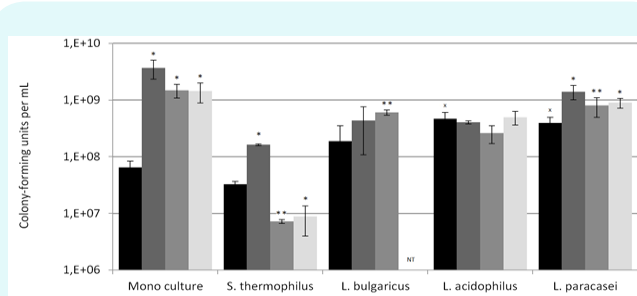
This study showed that, in order to naturally optimize growth of microorganisms of interest, it is important to look into their genomes to identify potential constraints in their metabolic potentials impeding optimal growth, where after a simple experimental set-up can establish whether these constraints lead to limitations *in situ* and whether they can be solved by co-culture with other species that do not have these metabolic constraints according to their genome annotations. It is anticipated that the increasing availability of sequenced genomes will in this way facilitate the application of mixed cultures as natural means to optimize desired microorganisms in fermented food products.

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### CONFLICT OF INTEREST

The author declares no conflict of interest.



**Figure 1:** Growth of *B. lactis* in mono and mixed culture in milk under different conditions. Reference cultures are in plain milk (black bars) and test cultures supplemented with casitone (dark grey), casamino acids (light grey) and cysteine/methionine (white). The test cultures are compared to the reference cultures (\*, significantly different in a pair-wise two-tailed T-test with  $P < 0.05$ ; \*\*,  $P < 0.1$ ) and of the reference cultures the mixed cultures to the mono cultures (x, significantly different  $P < 0.05$ ). NT: not tested.

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