

## HAFL Master's Thesis Abstract

Year: 2021

Student's Name: Ulisse Hardmeier

English Title:

**Targeted Sauerkraut fermentations with *Propionibacterium* spp. for natural enrichment in Vitamin B12**

English Summary:

In the present study, the feasibility to carry out to process to obtain a traditional sauerkraut via targeted cobalamin producer bacteria leading a certain amount of vitamin B12, was investigated. A previous study, dedicated to a controlled cabbages fermentation via heterofermentative lactic acid bacteria was conducted to yield a final product with a milder aroma. The inoculation of *Leuconostoc mesenteroides* (FAM 22736) at  $10^5$  cfu/ml as initial bacteria concentration with 1.8% (w/w) NaCl, fermented at 25 °C for 21 days, obtained positive response from sensory analysis. These considerations have been used as basic conditions to co-ferment cabbages with a microbiologically vitamin B12 producer. 17 strains belonging to the genus of *Propionibacterium*, and 12 *L. reuteri* strains, have been first tested by growth assays. In a second step the same strains were tested for the *in situ* production of vitamin B12 in a co-cultivation with *L. mesenteroides* (FAM 22736) in a sterile cabbage juice. To implement sauerkraut production with vitamin B12 biosynthesised by *P. freudenreichii*, a minimal concentration of cobalt (10mg/L), and a discontinuous supply of oxygen (6h : 7 days), have been tested. High performance liquid chromatography (HPLC) was used for the accurate identification and quantification of the synthesized vitamin B12. Compounds present in a sterile cabbage medium have been analysed after the co-fermentation process to evaluate *Propionibacterium* spp. metabolism; furthermore, optimal growth conditions related to the preferential carbon substrates, the pH values; the NaCl, and the lactate content have been assessed by phenotype micro arrays. One strain, belonging to the genus *Propionibacterium* FAM 24252 has been selected for its ability to be metabolically active during the time needed for traditional sauerkraut production, and deliver vitamin B12 in a co-fermentation condition. Phenotype micro arrays shown optimal growth conditions at 5% lactate, 1% NaCl, pH at 5.5 (-), whereas cabbage juice within the co-fermentation has been assessed with 0.4% lactate, 1.8% NaCl; and a decreasing pH value from 4.6 to 3.9 (-). Although, the medium does not match the parameters for *P. freudenreichii* FAM 242522 optimal growth, the strain selected to enrich sauerkraut, gave positive results for the biosynthesis of vitamin B12. Data shown that cyanocobalamin is better produced in a liquid, then in a solid medium. Experiments ran with cobalt and oxygen implementation gave higher amount of vitamin B12, even though cobalt influenced more the process when compared to oxygen. The suited fermentation conditions were evaluated to 25 °C for 28 days with an initial bacteria concentration of  $10^8$  cfu/ml for *P. freudenreichii* FAM 24252, and  $10^5$  cfu/ml for *L. mesenteroides* FAM 22736. In conclusion, enriching sauerkraut with microbially produced vitamin B12 was proven to be feasible, but further investigations concerning cobalamin stability during storage, and the consumer acceptance, are still needed.

Keywords: *Propionibacterium* spp., Sauerkraut, Vitamin B12, co-fermentation

Principal advisor: Prof. Daniel Heine