Hes·so
Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Studies to improve vitamin B_{12} titers during fermentation of *Propionibacterium freudenreichii*

Carlo Zupetic

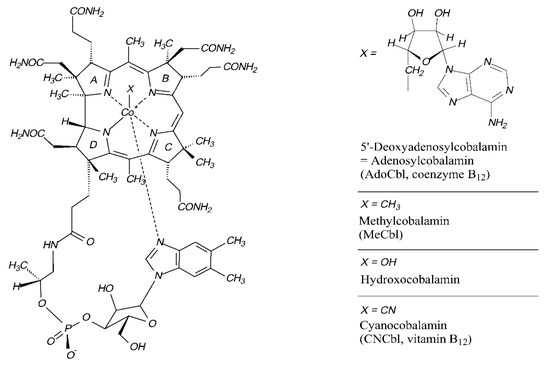
Applied Biosciences

HES-SO Valais

Advisor: Dr. Manfred Zinn, In collaboration with Panvega AG

DESCRIPTION

Vitamin B_{12} is a molecule that is part of the cobalamin compounds group. Cobalamin is a molecule that humans assimilate through foods of animal origin. A deficiency in this vitamin can lead to severe diseases that can even result in death.
To avoid a deficiency, various food supplements are available on the market. The Panvega company offers vegan foods fortified with vitamin B_{12} produced by the microorganism *Propionibacterium freudenreichii*, which has not been genetically modified.

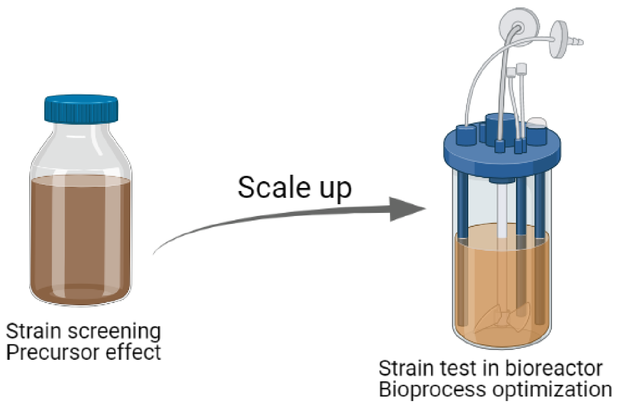


Molecular structures of cobalamin

This organism produces and accumulates the vitamin intracellularly. *Propionibacterium freudenreichii* carries the status of generally recognized as safe (GRAS), which allows its biomass to be directly used on food products as a fortifying agent for this vitamin.

OBJECTIVES

This project's objective was to test different strains of the microorganism normally used by Panvega to identify one that was superior or at least comparable in terms of cobalamin yield. This experiment was carried out on a small scale to reproduce the manufacturing bioprocess used by Panvega.

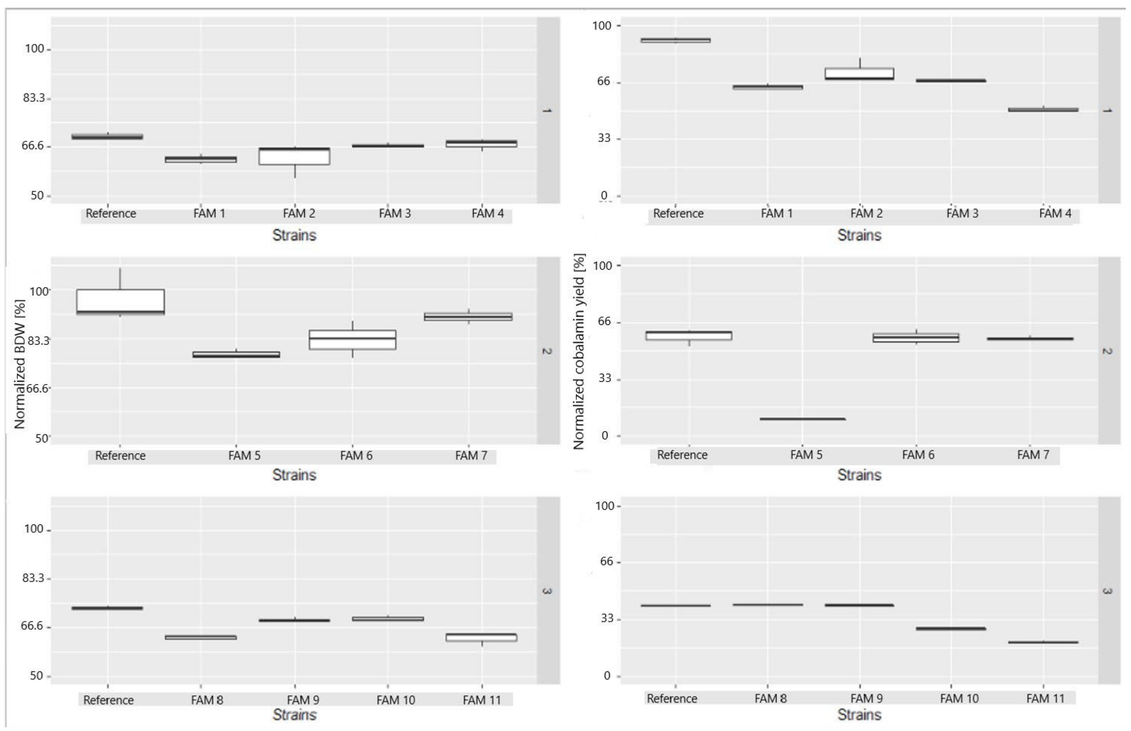


Overview of experiments performed for strain selection

One of these strains tested during the screening was also used for further tests in a bioreactor with a working volume of 2 L and again compared to the reference strain used by Panvega. One of vitamin B_{12} precursors' effect on its yield was tested at different concentrations in the culture medium on the reference strain.

RESULTS

The results obtained from the screening experiment on *Propionibacterium freudenreichii* strains are presented below. This experiment was divided into three different runs where the reference strain was used for comparative purposes.



Biomass dry weight and cobalamin yields for screening experiments with Propionibacterium freudenreichii strains

This led to the selection of at least three strains that were comparable to the reference strain in terms of cobalamin yield and biomass for further study in a bioreactor. The selected strain are FAM 6 and FAM 7 from the second run, and FAM 9 from the third run. The precursor's effect on biomass and cobalamin yield was statistically analyzed by a type-2 ANOVA. Excluding the two hypotheses with a p-value of 0.07 and 0.83 respectively (α=0.95).

Bioreactor during vitamin B_{12} production with PF(left), Products from Panvega AG containing vitamin B_{12} using PF biomass (right)

Experiments in the bioreactor show that the reference strain is superior in cobalamin yield at the end of the bioprocess. This, in fact, obtains at the end of the bioprocess a normalized yield of 72.6 % compared 37.8 % of the strain FAM 7.

CONCLUSIONS

The small-scale experiment was capable of reproducing the bioprocess in a bioreactor. None of the tested strains were superior in cobalamin yield. However, FAM strains 6 and 9 still need to be further studied in a bioreactor. The tested precursor's effect suggests that its presence in the culture media is not a limiting factor for cobalamin production. Further improvements can be made to the bioreactor's bioprocess to increase its productivity.

SWISS UNIVERSITIES APPLIED SCIENCES AND ARTS
MLS | MASTER OF SCIENCE IN LIFE SCIENCES