FHM-021M: Efficient delivery of vitamin K2 by bacteria involved in food fermentation process

Keywords: vitamin K2, lactic acid bacteria, delivery, fermentation

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Project duration: MSc (with specialisation Food Biotechnology) – 6 months

Specialisation: MBT A/B/C, MFT A/E

Project description

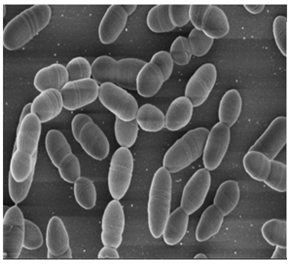
Background:

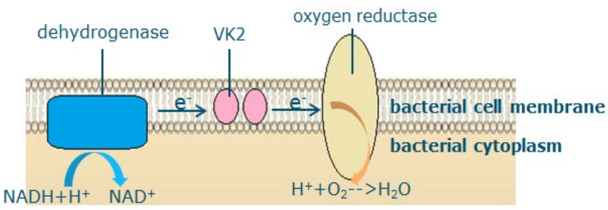
Vitamin K2 is a lipid-soluble vitamin that functions as a carboxylase co-factor for maturation of proteins involved in many vital physiological processes in human body. Vitamin K2 intake is found to be associated with bone and cardiovascular health. Therefore, vitamin K2 fortified food products are highly relevant for a healthy human diet. Vitamin K2 is of bacterial origin and many microbes involved in food fermentation processes were found to be efficient producers of this vitamin. Knowledge of vitamin K2 producing bacteria and relevant mechanisms will contribute to humane health through vitamin K2 enrichment of food via improved fermentation processes and novel delivery methods.

Topic:

Certain strains of lactic acid bacteria, propionibacteria and *Bacillus subtilis* produce vitamin K2 (menaquinones) as electron carriers in the membrane-embedded respiratory electron transfer chain. So which strains exactly are good producers of vitamin K2? What is the level and form of vitamin K2 they produce? Which culturing conditions lead to increased level of vitamin K2 production and what is the mechanism behind it? What would be an efficient method to deliver this lipid-soluble vitamin to the human body? What kind of physiological features of bacteria can be used to improve the delivery?... So many things are waiting for exploration!

In this project you will be investigating (some of) the above mentioned questions, and you will have the chance to use techniques including TLC, HPLC, (real-time) PCR, electron microscopy and flow cytometry.





Left: SEM picture of *Lactococcus lactis* (Alexeeva *et al.* 2015). Middle: Vitamin K2 (VK2) is part of the electron transport chain in the bacterial cell membrane. Right: Examples of fermented foods.

FHM-022M: Co-cultivation of *Streptococcus thermophilus* and *Bifidobacterium breve*

Keywords: mixed culture; microbial interactions; dairy ingredients

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Project duration: MSc (with specialisation Food Biotechnology) – 6 months

Specialisation: MBT A/B/C, MFT A/E, MFS A

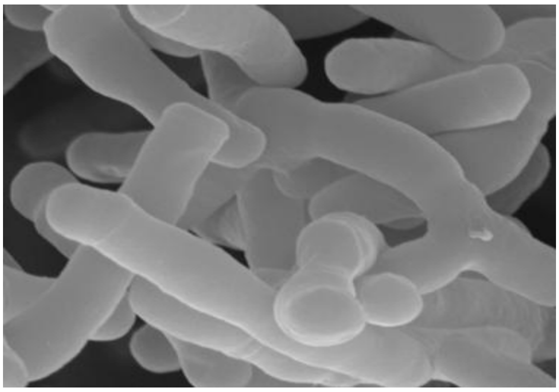
Project description

Background:
Co-cultivation of bacteria is widely used in the production of many fermented foods such as cheese, yoghurt, kombucha, kefir and others. Having two or more species bacteria in the same fermentable substrate can be beneficial for flavour and texture development or to mutually stimulate growth of the two species. In the production of a probiotic milk-based beverage, two species of bacteria are used: a lytic strain of *S. thermophilus* and a probiotic *Bifidobacterium breve* strain. Co-cultivation is expected to deliver a fermented dairy product that contains lactic acid, viable bacterial cells, cell wall components, enzymes such as lactases, and novel oligosaccharides (1). Consumption of these fermented milk-based products can lead to a bifidogenic effect on gut microbiota and a variety of clinically proven health benefits (2). It is however not yet clear how *S. thermophilus* and *B. breve* interact.

Objective:
The objective is to unravel the interaction between *S. thermophilus* and *B. breve* during the fermentation of the product.

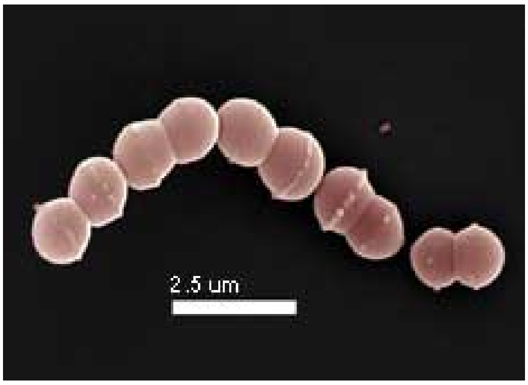
Literature:
1. Agostoni, C., Goulet, O., Kolacek, S., Koletzko, B., Moreno, L., Puntis, J., . . . Turck, D. (2007). Fermented infant formulae without live bacteria. Journal of pediatric gastroenterology and nutrition, 44(3), 392-397.

2. van de Heijning, B. J., Berton, A., Bouritius, H., & Goulet, O. (2014). GI symptoms in infants are a potential target for fermented infant milk formulae: a review. Nutrients, 6(9), 3942-3967



http://2.bp.blogspot.com/-tWmIgWRrf_o/T2FKbmr4J3I/AAAAAAAAAEM/4UzNMTDALyg/s1600/Bifidobacterium+Brevis.jpg

Bifidobacterium brevi



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Streptococcus thermophilus

FHM-023M: Innovation of tempeh using lupine

Keywords:	tempeh, fermentation, lupine, soy, novel product
Supervisors:	Judith Wolkers-Rooijackers (judith.wolkers-rooijackers@wur.nl) Eddy Smid
Project duration:	MSc (with specialisation Food Biotechnology) - 6 months
Specialisation:	MBT A/B/C, MFT A/E, MFS A

Project description

Background:

Tempeh is a traditional soy product where soybeans are dehulled, soaked and fermented with the fungus *Rhizopus oligosporus*. Because of its nutritional value (rich in proteins, dietary fibers and vitamins) it is a popular ingredient in the vegetarian and vegan kitchen. There is little variation in traditional tempeh products because soybeans are the main fermentable substrate. To meet consumers demands, new tempeh formulas must be developed. One of the ways is using a sustainable alternative for soybeans. Lupine, a Mediterranean crop that has a high nutritional value, potentially is such an alternative. Furthermore, lupine tempeh showed good overall acceptability (1).

Topic:

This project will focus on developing new tempeh formulas using lupine seeds as substrate. Lupine tempeh will be compared with soy tempeh with respect to different product characteristics, such as texture, colour, microbial safety. Finally, we will work on developing and improving new mixed starter cultures with vitamin B_{12} producing food grade bacteria (2).

Literature:

1. Bergamaschi 2011. Master thesis, Wageningen University
2. Wolkers–Rooijackers, J.C.M., M. F. Endika and E. J. Smid. 2018. Enhancing vitamin B_{12} in lupin tempeh by *in situ* fortification. LWT - Food Sc. & Technol. 96, 513-518

FHM-024M: Impact of bacterial endosymbionts on *Rhizopus* fermentation performance and implications for food safety

Keywords: bacterial endosymbionts, fungi, fermentation, toxin, safety

Supervisors: Judith Wolkers-Rooijackers
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Project duration: MSc (with specialisation Food Biotechnology) 6 months

Specialisation: MBT A/B/C, MFT A/E, MFS

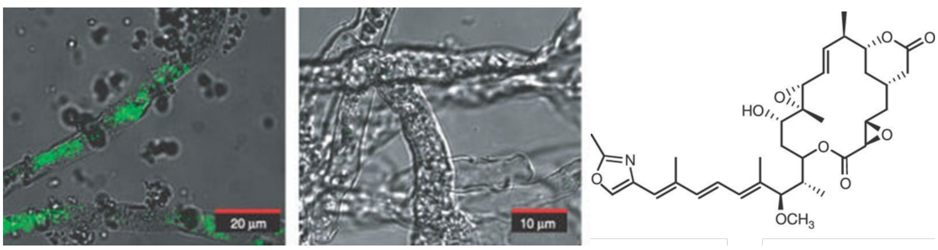
Project description:

Background

The fungal order Mucorales includes fungi which are used for the fermentation of foods. For example *Rhizopus oligosporus* is used to produce tempeh and *R. oryzae* is used to produce Asian alcoholic beverages. However, it was also reported that certain *Rhizopus* spp. could produce toxins, i.e., the antimitotic toxin rhizoxin and the hepatotoxic rhizonin toxin. Notably, the fungus itself is not the producer, but the endosymbiotic bacterium *Burkholderia* spp. Obviously, production of these toxins in (fermented) foods can be regarded as a food safety issue, although currently no data and/or reports are available about food-associated risks. Interestingly, when the bacterium is removed from the fungus, it can still grow but the fungus cannot reproduce because sporangiospores cannot be formed. In this way the endosymbiont-dependency of the fungal sporulation process secures vertical transmission of the bacteria (see references). Notably, it has not been investigated to what extend the endosymbiont(s) affects fungal (food) fermentation performance.

Aim

This project aims to characterize *Rhizopus* strains of the FHM collection that harbour endosymbiotic *Burkholderia* spp. and to compare their performance to endosymbiont-free strains. A range of phenotypic aspects of the endosymbiont-mould lifestyle can be studied such as spore production, growth rate, response to oxygen, metabolite profile and the presence of toxins in tempeh. The comparative analysis of endosymbiont-free and endosymbiont-containing *Rhizopus* strains will add further understanding to the role of endosymbionts in fermentation performance, and quality and safety aspects of *Rhizopus*-fermented foods.
In this project you will have the opportunity to use techniques such as fluorescence microscopy in combination with live/dead staining, (real-time) PCR, electron microscopy, and HPLC.



Fungal mycelium with and without stained endosymbiont, and rhizoxin toxin structure

References

Araldi-Brondolo, S. J., Spraker, J., Shaffer, J. P., Woytenko, E. H., Baltrus, D. A., Gallery, R. E., & Arnold, A. E. (2017). Bacterial Endosymbionts: Master Modulators of Fungal Phenotypes. *Microbiology Spectrum*, *5*(5).

Dolatabadi, S., Scherlach, K., Figge, M., Hertweck, C., Dijksterhuis, J., Menken, S. B., & de Hoog, G. S. (2016). Food preparation with mucoralean fungi: A potential biosafety issue?. *Fungal Biology*, *120*(3), 393-401.

FHM-025B: Global diversity in fermented food products

Keywords: food fermentation, diversity, health benefits

Supervisors: Eddy Smid (eddy.smid@wur.nl)
Judith Wolkers-Rooijackers
Oscar van Mastrigt

Project duration: BSc thesis topics - 4 months

Project execution: This topic does not involve laboratory work

Study programme: BLT and BBT

Project description:

Background:
Fermentation is one of the oldest food processing technologies on Earth. Many fermentation processes we know today are operationally complex, reflecting their long history of use. The skills required for producing good products were passed on for countless generations from mother to daughter and/or father to son, leading to gradual improvements.

In a recently published review paper, Gänzle (2015) produced a "periodic table of fermented foods" (see below). This table provides a compilation of almost all possible categories of fermented foods found on our planet. However, it gives not more than a glimpse of the vast diversity in raw materials, production processes and microbes involved. A thorough, in depth analysis based on published studies for each of the categories found in the periodic table will bring together knowledge and facts that support future research and innovation in the field of fermented foods.

Topic:
In consultation with your supervisors, one fermented food product will be selected. Next, a broad screening of the scientific literature about that selected product will be performed to obtain information about (i) types and diversity of microbes involved, (ii) production schemes, (iii) processing conditions, (iv) country or region of origin, (v) safety and toxicological aspects, (vi) health aspects, (vii) economic implications, (viii) ancient practice versus current practice and (ix) opportunities for innovation. The study is expected to deliver a report and final presentation on the topic and the data will be used later on to build a large data base for fermented foods.