Efficient delivery of vitamin K2 by bacteria involved in food fermentation process

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

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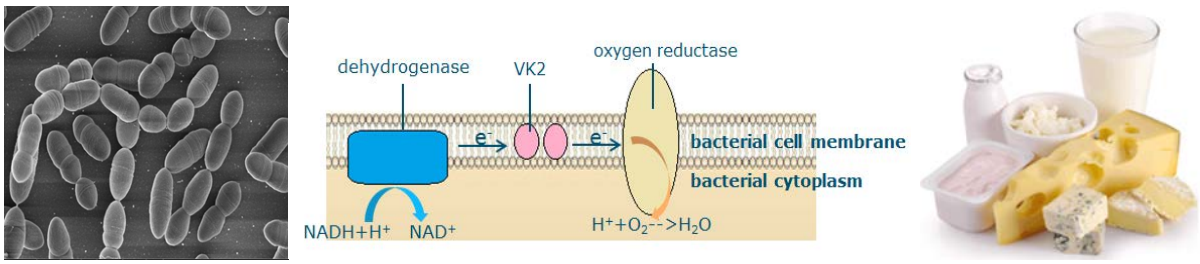
Project duration: BSc and MSc (with specialisation Food Biotechnology) – 4-6 months

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.

Novel concepts for fermented vegetables

Keywords: fermented vegetables; lactic acid bacteria; novel foods

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

Project description:

Background:

Consumption of fruits and vegetables is essential for a healthy human diet. However, in many countries of the Western world, people do not meet the recommended daily intake of vegetables and fruits. Fermentation of plant raw materials offers an attractive way to boost the daily consumption of fresh-like vegetables and fruits.

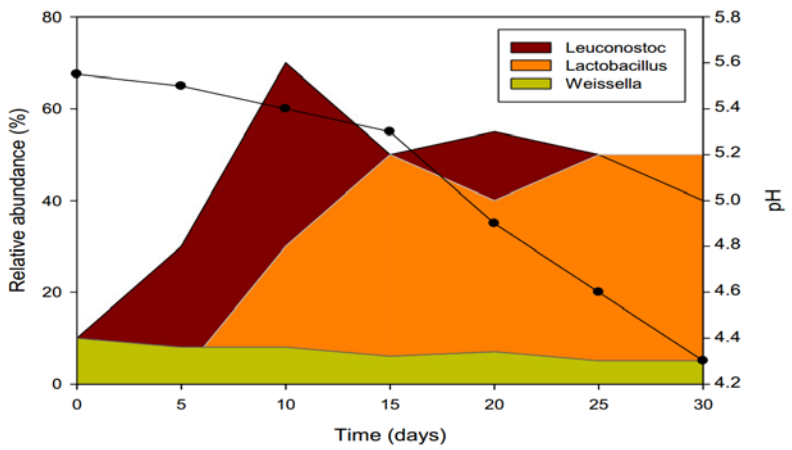
Topic:

This project will focus on the exploitation of lactic acid bacteria for the development of novel fermented (combinations of) vegetables and fruits. Various fermentable raw materials will be considered: tomatoes, cabbage, horseradish, leek, ginger, seaweed, carrots, cucumber and red beets. In addition, new formulations of salts with lower sodium content can be investigated. The succession of lactic acid bacteria will be monitored using various techniques and subsequently linked to product characteristics (pH, texture, nutritional value and microbial safety).

Literature:

Di Cagno, Coda, De Angelis and Gobbetti. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 33: 1-10

Wolkers-Rooijackers, Thomas and Nout. 2013. Effects of sodium reduction scenarios on fermentation and quality of sauerkraut, LWT - Food Science and Technology 54: 383-388



Succession of lactic acid bacteria and change of pH during spontaneous fermentation of kimchi. (from: Di Cagno et al, 2013)

Co-cultivation of *Streptococcus thermophilus* and *Bifidobacterium breve*

Keywords: mixed culture; microbial interactions; dairy ingredients

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Project duration: BSc and MSc (with specialisation Food Biotechnology) – 4-6 months

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

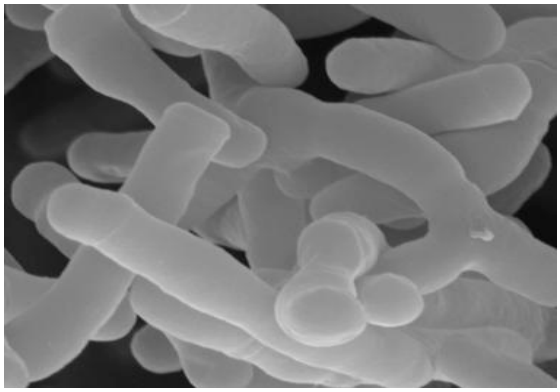
Project description:

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.

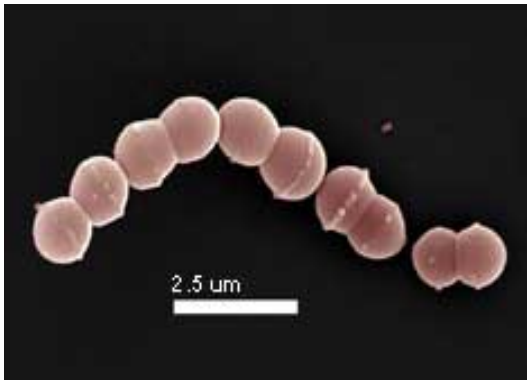
Literature:
1. Agostoni, C., Goulet, O., Kolacek, S., Koletzko, B., Moreno, L., Puntis, J., . . . Turck, D. (2007). Fermented infant formulae without live bacteria. Journal of pediatric gastroenterology and nutrition, 44(3), 392-397.

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/-tWmIqWRrf_o/T2FKbmr4J3I/AAAAAAAAAEM/4UzNMTDALyg/s1600/Bifidobacterium+Brevis.jpg

Bifidobacterium brevi



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Streptococcus thermophilus

Innovation of tempeh using lupine

Keywords: tempeh, fermentation, lupine, soy, novel product

Supervisors: Judith Wolkers-Rooijackers (judith.wolkers-rooijackers@wur.nl)
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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:
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. Under review




Optimizing yeast aroma profiles using CRISPR-Cas genome editing

Keywords: yeast, fermentation, aroma, genome editing

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

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

Project description:

Background:

Genome engineering of food-related microorganisms is an important biotechnological application for the food industry, such as optimizing aroma production for beer making. One of the major breakthroughs in recent years is the development of a genome editing technique called CRISPR-Cas. In the biological context, CRISPR-Cas is a microbial adaptive immune system that degrades invading DNA from, for example, viruses. The system uses a so-called crRNA to guide itself to the invading DNA and subsequently to degrade it. Since crRNAs can be manually designed and produced, the CRISPR-Cas system is a powerful gene editing tool to manipulate genes of interest. Importantly, the genomes of organisms that are edited by CRISPR-Cas are not Genetically Modified Organisms (GMO), hence the technique has far reaching potential for food technology processes.

Topic:

A large number of metabolic pathways are involved in aroma formation by *Saccharomyces cerevisiae* and other yeast species (see figure 1). In previous research projects, we have demonstrated the key-role for esterase activity in the balance between aroma formation and degradation (2). Compared to some non-conventional yeast species like *Cyberlindnera fabianii* or *Pichia kudriavzevia*, *S. cerevisiae* (brewers's yeast) produce relatively low levels of ethyl- and acetate esters.

In this thesis project, the CRISPR-Cas protocol will be setup and validated for industrial and wild strains of *S. cerevisiae* with the objective to engineer the aroma profiles during fermentation of wort. Since this project deals with non-GMO strain engineering, focus will be on using an array of molecular biology techniques.

Literature:

1. Owen W. Ryan, O.W., S. Poddar, and J.H.D. Cate. 2016. CRISPR–Cas9 genome engineering in *Saccharomyces cerevisiae* cells. Cold Spring Harbor Protocols doi:10.1101/pdb.prot086827
2. van Rijswijck, I.M.H., J.C.M. Wolkers – Rooijackers, T. Abee and E.J. Smid. 2017. Diversity analysis of non-conventional yeasts and performance in co-cultures with Brewers' yeast for steering ethanol and aroma production. Microbial Biotechnol. 10:1591-1602

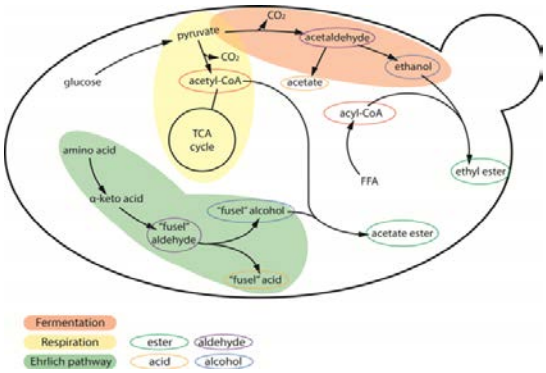

Figure 1. Yeast metabolic pathways involved in the production of acetate and ethyl esters.
From: van Rijswijck, 2017 (PhD thesis Wageningen University).