

FHM-2.1 M: Fight or flight: improving starter culture production and functionality

Keywords:	starter culture, dairy, bioreactors, adaptive evolution
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Project duration:	MSc (with specialisation Food Biotechnology) – 6 months
Specialisation:	MBT A/B/C, MFT C/D

Project description

Background:

Lactic acid bacteria (LAB) are widely used as starter cultures to make fermented dairy products, such as cheese and yoghurt, in which they cause acidification by production of lactic acid and contribute to texture and flavour formation. However, production of these starter cultures is not optimal yet as indicated by incomplete lactose utilisation despite control of the pH.

Objective:

This research project aims to explore novel methods that can improve starter culture production of LAB and their functionality.

Approaches:

The three proposed methods (see figure 1) that will be investigated are i) cell recycling cultivation, ii) fermentation at alkaline conditions and iii) adaptive evolution for increased lactic acid resistance.

Relevance:

The results of this project are relevant for producers of starter cultures, for the companies that use these cultures to make for instance fermented dairy products like cheese and yoghurt, for producers of LAB as probiotics and in general for fermentation processes that suffer from end-product inhibition, especially from inhibition by organic acids.

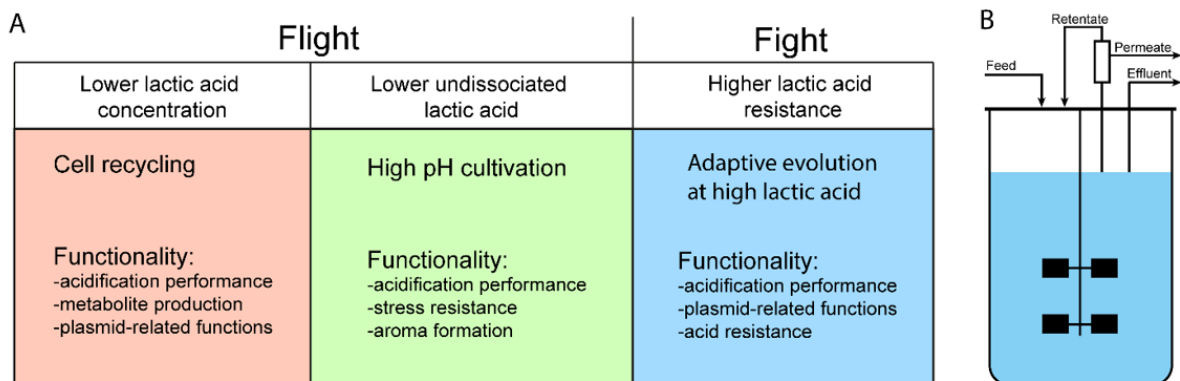


Figure 1: Overview of the project *fight or flight*. **A:** Overview of the proposed methods to improve the production of LAB. The coloured boxes represent the work packages of the postdoc (WP1, orange), PhD student 1 (WP2, blue) and PhD student 2 (WP3, green). **B:** Cell recycling chemostat with 2 effluents: one with biomass (effluent) and one with complete biomass retention (permeate) (9).

FHM-2.2 M: Exploring novel dairy applications for under-exploited food grade *Propionibacterium* species

Keywords: *Propionibacterium* sp., vitamins, fermentation, metabolism, dairy

Supervisors: Alexander Dank (alexander.dank@wur.nl)
Eddy Smid

Project duration: MSc - 6 months

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

Project description:

Background

Propionic acid bacteria (PAB) have some remarkable metabolic properties. These organisms possess the Wood-Werkman cycle to produce propionic acid, acetic acid and CO₂ from lactic acid although also sugars like lactose or glucose can be used to feed the Wood-Werkman cycle. Mixtures of organic acids, secondary metabolites and peptides produced by PAB found their way into application as food grade antifungals (1). Furthermore, PAB produce aroma compounds derived from catabolism of amino acids (2) and by lipolysis (3). The catabolism of amino acids in PAB bears similarities with the pathway found in LAB. However, PAB seem to produce higher amounts of branched-chain acids which leads to the synthesis of important cheese flavour compounds (2).

Aim

The aim of this project is to select the most potent aroma producing food grade species of the genera *Propionibacterium* and *Acidipropionibacterium*. Furthermore, the ultimate objective is to produce concentrated aroma blocks using appropriate high cell density cultivation techniques.

Techniques and methods

In this project you will have the opportunity to use bioreactors, HPLC, GC-MS, fluorescence microscopy, various selective plating techniques and advanced data processing methods.



Picture from: <https://bio.pisceswebdesign.com/propionibacterium-freudenreichii> Picture from: <https://www.health.harvard.edu/blog/dairy-health-food-or-health-risk-2019012515849>

References

1. Lind H, Sjögren J, Gohil S, Kenne L, Schnürer J, Broberg A. 2007. Antifungal compounds from cultures of dairy propionibacteria type strains. *FEMS Microbiology Letters* 271:310-315.
2. Thierry A, Maillard MB. 2002. Production of cheese flavour compounds derived from amino acid catabolism by *Propionibacterium freudenreichii*. *Lait* 82:17-32.
3. Kerjean J-R, Condon S, Lodi R, Kalantzopoulos G, Chamba J-F, Suomalainen T, Cogan T, Moreau D. 2000. Improving the quality of European hard-cheeses by controlling of interactions between lactic acid bacteria and propionibacteria. *Food Research International* 33:281-287.

FHM-2.3 M: Thiamine as precursor for meat aroma compounds

Keywords: Meat aroma, yeast, thiamine, fermentation, vegan products

Supervisors: Rebecca Rocchi (rebecca.rocchi@wur.nl)
Eddy Smid

Project duration: MSc - 6 months

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

Project description:

Background

The overall meat flavour depends on various variables such as species of animal, feeding regime and cooking time. The actual meat aroma compounds are products of various biological and chemical processes such as lipid oxidation, Strecker reactions, Maillard reactions and thiamine degradation. Especially the compounds derived from thiamine degradation are of interest for the production of plant-based meat aroma's which can be used for flavouring novel vegetarian and vegan products. Thiamine (also referred to as vitamin B1) is degraded by heat into products like 2/3-mercapto-3/2-pentanone and 2-methyl-3-furanthiol which have a meaty aroma. Several food grade bacteria and yeast are known to produce thiamine. These microbes potentially serve a role as thiamine cell factories for the production of natural non-animal derived meat aroma's.

Aim

The aim of this project is to produce highly concentrated meat aroma's for plant based meat replacers. The approach will be based on the use of thiamine as precursor for meat aroma production.

Techniques and methods

In this project you will have the opportunity to use bioreactors, GC-MS, fluorescence microscopy, various selective plating techniques, adaptive evolution and advanced data processing methods.

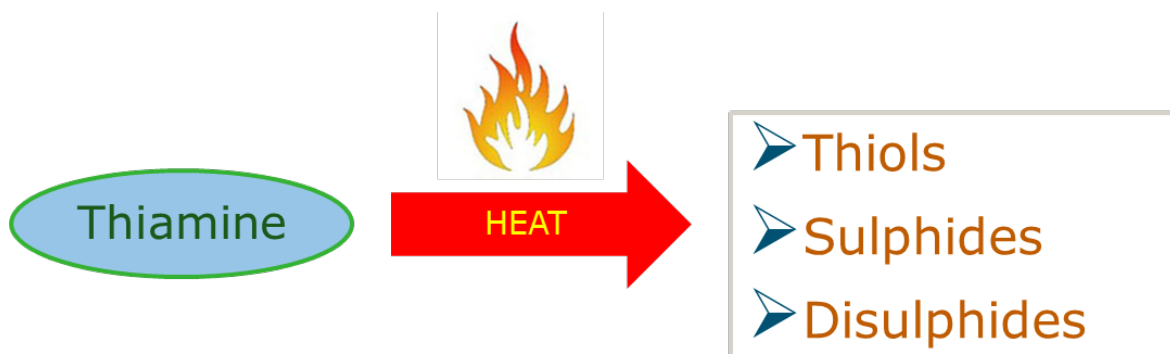


Figure 1. Heat driven generation of meat aroma compounds (thiols, sulphides and disulphides) from thiamine.

FHM-2.4 M: 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/C/D, 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 B₁₂ 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 B₁₂ in lupin tempeh by *in situ* fortification. LWT - Food Sc. & Technol. 96:513-518



FHM-2.5 M: Wild-card project as follow-up of PToFF project

Keywords:	Novel fermented foods
Supervisors:	Judith Wolkers-Rooijackers (judith.wolkers-rooijackers@wur.nl) Eddy Smid
Project duration:	MSc - 6 months
Specialisation:	MBT A/B/C, MFT C/D Note: only for students who did a PToFF project-bachelor thesis at FHM

Project description:

This project can only be executed by students who did a non-wet lab PToFF project as bachelor student at FHM. The project entails that students will develop a novel fermented foods along the lines that were set-out in their Bachelor-thesis report.

FHM-2.6 M: Stronger together: how complex and simple microbial communities from fermented milk affect the gut microbiota composition in a simulated model system (SHIME)

Keywords: starter culture, dairy, gut microbiota, microbial ecology

Supervisors: Eddy Smid (eddy.smid@wur.nl)
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Project duration: MSc thesis – 6 months

Specialisation: MBT A/B/C, MFT C/D, MFS A, MML A/B, MBI B/C/E, and other relevant MSc programs

Project description

Background:

Human gut microbiota is a complex and highly variable ecosystem, the composition of which is easily affected by a host lifestyle, health condition or a diet. Fermented dairy products harbour high diversity of microorganisms which enrich and stabilise the gut microbial community in humans. Mabisi, a local fermented milk product from Zambia (Fig.1A) with a community of 10 to 20 different fermenting bacterial strains, harbours potentially beneficial microorganisms. In previous studies it has been suggested that mabisi intake can shift the human gut microbiota towards more diverse and healthy community. However, it is still unclear which part of the Mabisi microbial community plays the most important role.

Objective:

This research project aims to test the impact of mabisi intake (contrasting mabisi with full community of 10 strains and minimal microbial community composition of just a single strain) on the composition and functionality of gut microbiota (Fig.1C).

Approaches:

The effect of dietary intake including *mabisi* harbouring either the full or minimized community will be tested in a dynamic *in vitro* simulator model of the human digestion system SHIME (Fig.1B). SHIME will carry the human gut microbiome and shifts in the composition of this gut microbiome as well as the level of short chain fatty acids production will be assessed using molecular methods and HPLC.

Relevance:

The results of this project are relevant to advance or fundamental understanding how microbial ecosystems interact and for nutritionists, producers of starter cultures, for the companies that use complex starter cultures to make fermented dairy products like yoghurt or kefir, and for producers of LAB as probiotics.

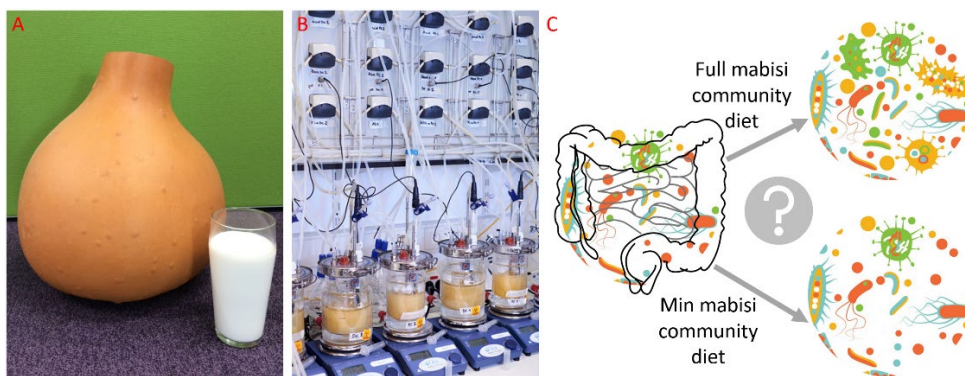


Figure 1. A: Mabisi – a traditional spontaneously fermented milk product from Zambia. B: The organisation of a gut simulation system – SHIME. C: The research hypothesis expecting that the minimal microbial community diet is insufficient to promote the stability and richness of the gut microbiota.

FHM-2.7 M: Bacterial dispersal via fungal "highways" on cheese surface

Keywords: bacterial motility, surface colonization, cheese, fungi hyphae, microbial interactions, water limitation, water potential, dispersal

Supervision: Gamze Gülez (gamze.gulez@wur.nl)
Eddy Smid (eddy.smid@wur.nl)

Project duration: MSc - 6 months

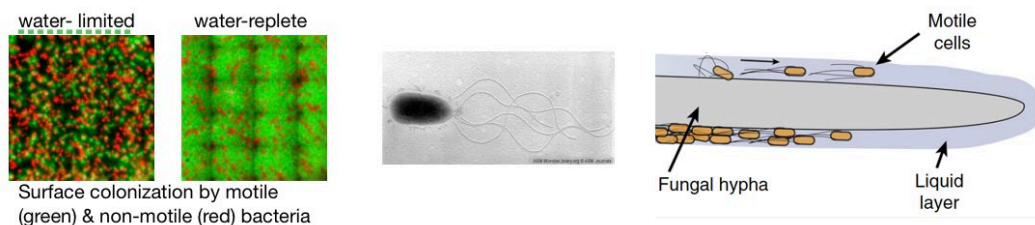
Specialisation: MBT A/B/C, MFT C/D

Project description:

Bacterial colonization of cheese surfaces may have both undesirable (dispersal of pathogenic and spoilage bacteria) or desirable effects (bacteria contributing to aroma/texture). Bacteria can rapidly colonize a surface by exhibiting flagellar motility, which is hindered under water-limited conditions (1). Since cheese surface is a water-limited environment, one might assume that flagellar motility would be limited as well. However, unlike in a lab experiment, bacteria in nature and other settings, like the cheese surface, interact with other microorganisms. Indeed, in water-limited soil, bacteria use thin water films on the fungal hyphae for dispersal through the water deprived pores (2). Despite this aspect of bacterial-fungal interactions and abundance of fungal species on food surface, only one study focused on bacterial dispersal via hyphae on cheese, focusing on how it affected the microbial diversity yet ignoring the effect of water potential which controls water availability (3). Hence, the aim of the study is to quantify flagellar motility mediated dispersal of bacteria via fungal hyphae on surfaces as a function of water potential (both solute and matric potential components) and to determine its effect on bacterial-fungal interactions, adaptive stress responses and underlying mechanisms using model cheese media. Some aspects we can study in this project are listed below.

- Relative fitness: Quantification of colonization areas and CFU counts of motile vs non-motile bacteria
- Molecular level responses: What are the critical traits of bacteria with and without the fungi under controlled water potentials? Does horizontal gene transfer occur? (see 4)
- Metabolic interactions between bacteria and fungal hyphae
- Motility types: Swimming and swarming behavior
- Increasing complexity: How does presence of second bacterial or fungal species affect dispersal, interactions? Can non-motile bacteria be transported by motile bacteria on fungal hyphae?

Study approach may include methods from traditional microbiology, physics, molecular biology, engineering design, 'omics' technologies, microscopy, metabolic assays, surface patterning, etc.



References:

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3. Zhang, Y., Kastman, E., Guasto, J., & Wolfe, B. (2018) Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. *Nat Commun.* 9(1):336
4. Berthold, T., Centler, F., Hübschmann, T. *et al.* (2016) Mycelia as a focal point for horizontal gene transfer among soil bacteria. *Sci Rep* 6, 36390

FHM-2.8 B: Global diversity in fermented food products

Keywords: food fermentation, diversity, health benefits

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

Project duration: BSc thesis topics - 4 months

Project execution: This topic does not involve laboratory work

Study programme: BLT, BBT

Project description:

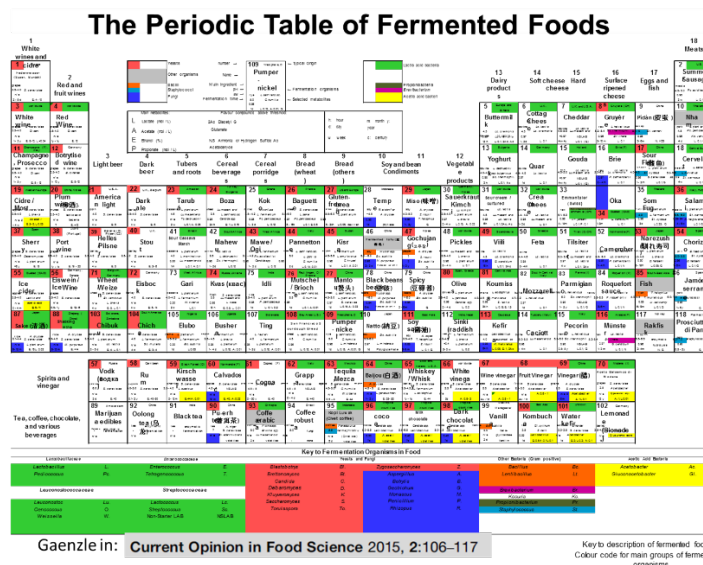
Background:

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.



Gänzle in: Current Opinion in Food Science 2015, 2:106-117

Key to description of fermented foods / Colour code for main groups of fermentation organisms