Ecology of pathogens in enrichments

Keywords:	foodborne pathogens, enrichment, competition, modelling
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Project duration:	MSc – 6 months
Specialisation:	MBT B, MFT A, MFS A/C

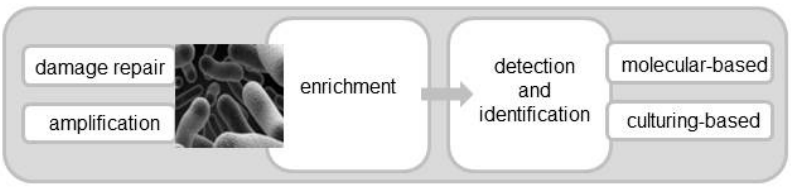
Project description:
The presence of pathogenic bacteria in food products poses a significant threat to global health and with the increased popularity of ready-to-eat products this is only expected to increase. Microbiological testing of foods is therefore an essential activity to check for the presence of pathogens or to verify that control measures are effectively reducing the prevalence of pathogens to acceptable levels. Detection efficacy is however hampered by the fact that pathogens in food are often damaged due to low storage temperature or food preservation and may only represent a small fraction of the total microflora in food. Analytical testing methods therefore incorporate a time-consuming enrichment procedure to recover and selectively amplify damaged target pathogens to much higher concentrations allowing subsequent detection. It is generally recognized that the selective enrichment procedure is still based on trial-and-error practices, and can be qualified as a 'black box' that often suffers from a false negative outcome. Insight into the recovery of stressed pathogens is essential in order to optimize the enrichment protocol and to form the basis of successful detection of foodborne pathogens using both molecular-based and culturing-based methods. Two projects are available that focus on relevant food pathogens:

1. *Enrichment ecology of* Listeria monocytogenes

The devastating consequences of *Listeria monocytogenes* infection from food is exemplified by the recent outbreak in South Africa. For detection in food, the enrichment procedure for *L. monocytogenes* suffers from under-representation of specific (pathogenic) serotypes, thereby reducing its reliability to detect all serotypes of this pathogenic species. This project therefore aims to investigate and model the growth kinetics during enrichment of various *L. monocytogenes* serotypes after relevant food stress to test the hypothesis that some serotypes can repair damage more efficiently, or initiate growth faster during the enrichment. This can provide knowledge about stress recovery mechanisms that can be used to improve the current enrichment protocol.

2. *Enrichment ecology of* Campylobacter

The enrichment procedure for *Campylobacter* is hindered by antibiotic resistant microorganisms, such as ESBLs that outcompete *Campylobacter* during enrichment. These antibiotic resistant microorganisms are naturally present in especially poultry, and prevent reliable detection of *Campylobacter* in these foods.
This project aims to investigate and model the growth kinetics of (sublethally damaged) *Campylobacter* and competitors in enrichment broth in order to improve the selectivity of the medium.



LOD_{50} of *Campylobacter*

Keywords:	Campylobacter, ESBLs, detection, competition, food
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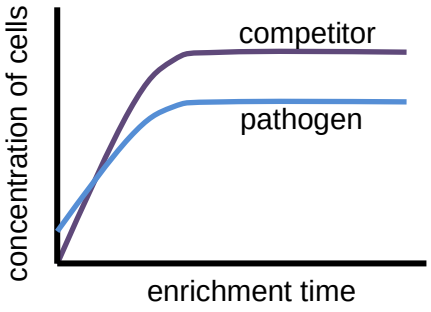
Project duration:	BSc and MSc – 4-6 months (start mid August)
Specialisation:	MBT B, MFT A, MFS A/C

Project description:
Campylobacter jejuni is the number one bacterial cause of gastroenteritis. Detection of this pathogen in food is difficult due to growth of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* during enrichment and isolation of the organism which hampers isolation of *Campylobacter*. Therefore, in the current revision of the ISO protocol (ISO 10272-1; 2017), next to Bolton Broth (BB), Preston broth (PB) is suggested as enrichment broth to inhibit competitive flora in samples where high levels of background flora such as ESBLs are suspected. To validate this revised ISO 10272-1, an Inter Laboratory Study (ILS) was performed where different matrices were used in the enrichment procedures: frozen spinach, minced meat, raw milk and chicken skin. Each matrix was inoculated with a different strain of *C. jejuni* or *C. coli* and the results were expressed as LOD_{50} (Level of Detection) which is the lowest contamination level that can be detected with a probability of 50%.
Since different strains were used for each matrix, results of the ILS were possibly influenced by the strains' characteristics. Therefore, the aim of this project is to test the enrichment procedures for *Campylobacter* in spinach, minced meat, milk and chicken skin with each of the strains used in the ILS. Furthermore, the effect of the presence of ESBL-producing *E. coli* is determined.

Topics:

1. In this project the LOD_{50} of *Campylobacter* in food will be determined, in presence and absence of ESBL-producing *E. coli*. Five reference strains of *Campylobacter* will be tested and several food products will be used, such as chicken, minced meat, spinach or milk.

2. Since it is known that Preston broth (PB) may not only be selective to competing flora, but may also inhibit the growth of specific strains of *Campylobacter*, growth of this pathogen in PB will be followed and compared to growth in Bolton broth, in absence and presence of ESBL-producing *E. coli.*



Variability of *Salmonella* during enrichment

Keywords:	*Salmonella*, spoilage flora, growth rate, competition, enrichment, detection
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Project duration:	BSc and MSc – 4-6 months
Specialisation:	MBT B, MFT A/E, MFS A/C

Project description:
For the detection of pathogens usually enrichment steps are applied, during which the pathogen can recover and multiply. Both pathogens and spoilage flora, however, grow simulaneously during enrichment and competition between pathogens and spoilage flora during enrichment is imminent. After non-selective enrichment, usually a second selective enrichment is applied. This selective step aims to allow the target organism (usually a pathogen) to proliferate while the growth of other microorganisms will be limited by inhibitory compounds and non-optimal growth conditions. During the enrichment steps pathogens and competitive flora compete for the available nutrients and might affect each other.

This study aims at determining the kinetics of *Salmonella* serovars during pre-enrichment and enrichment and the variability thereof. In addition, we will study the effects of sublethal stress and competitive flora on the recovery and detection of *Salmonella*.

How diverse are fungal species?

Keywords:	moulds, yeast, growth limits, heat resistance
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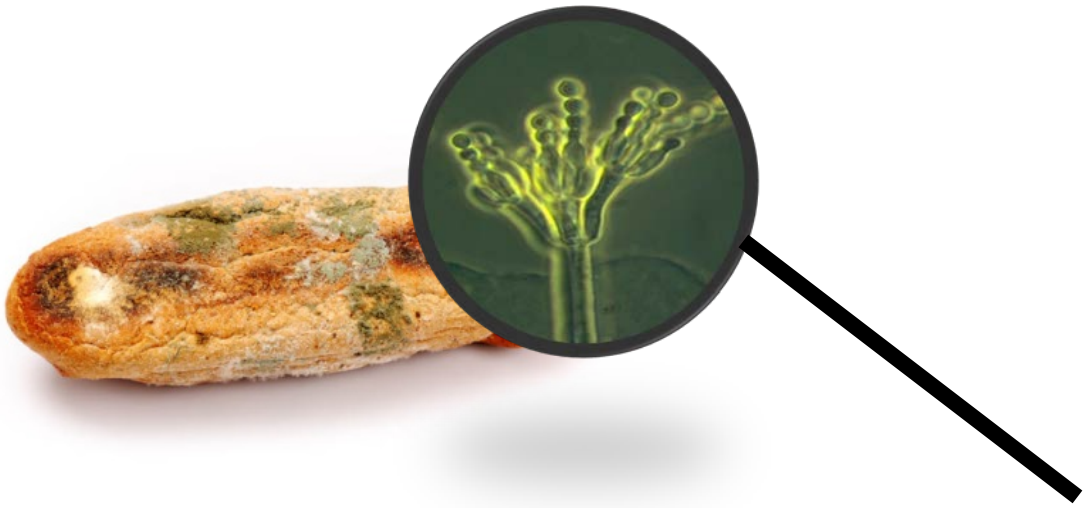
Project duration:	BSc and MSc – 4-6 months (start mid August)
Specialisation:	MBT B, MFT A, MFS A/C

Project description:
Reducing post-harvest food spoilage is needed to feed the growing human world population in a more sustainable manner. At the moment, 25% of the food is spoiled, a significant part due to fungal contamination. Fungal food spoilage can be found in all food categories. For instance, *Aspergillus niger* and *Paecilomyces variotii* are important spoilage fungi of fruits and processed foods. *Penicillium roqueforti* is a spoilage fungus of airtight stored grain and dairy products, while *Saccharomyces cerevisiae* subsp. *diastaticus* is the main cause of spoilage of alcohol-free beer and beer-mix beverages. Fungal spoilage not only affects visual and organoleptic properties of food but can also result in the production of toxins. Fungal food spoilage often starts with a contamination with spores. These reproductive structures are abundant in the environment and germinate and grow out when conditions are favourable. Information of growth limits and heat resistance of moulds and yeast is key to make models to predict growth and inactivation of fungal contaminants in foods.

The aim of this project is to determine growth limits and heat resistance of the above listed fungal species and to quantify differences in growth limits and heat resistance between and within fungal species.

This study will include a literature study combined with experimental analysis. The literature study will give a first scienfic estimation of growth limits and heat resistance parameters and the corresponding variability. Subsequent fungal growth and heat resistance studies in the laboratory will help to quantify differences between strains belonging to the same species is more detail. This study covers four subprojects, focussing each on one fungal species.



Effect of UV-C treatment of breast milk on the inactivation of pathogens

Keywords:	meta-analysis, heat treatment, UV-C treatment, *D*- and *z*-value, variability
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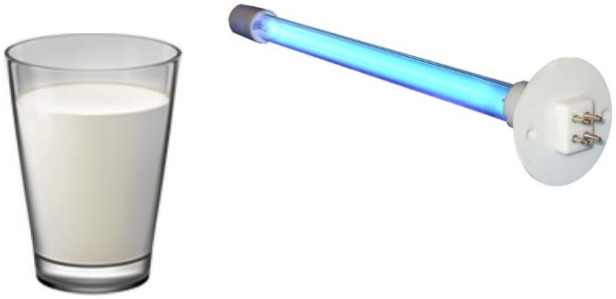
Project description

In hospitals, babies whose mothers are unable to lactate may receive donated breast milk. This donated breast milk is currently always heated to inactivate pathogens that may be present. Several thermal inactivation studies have been done for pathogens in breast milk to determine their thermal resistance. However, heating of milk also leads to damage (e.g. to immune proteins), which is unwanted. Therefore, researchers have looked into non-thermal alternatives to heat treatment. A promising recent development in this area is the use of UV-C to inactive pathogens (Christen et al., 2013).

However, the ability of UV-C to inactivate all relevant pathogens has been insufficiently studied, especially in breast milk. To determine the global inactivation parameters of a pathogen using UV-C, such as the *D*-value and *z*-value, a meta-analysis study can be initiated. In a meta-analysis study, available inactivation data of a pathogens in milk and other foods treated with UV-C are collected, and the global inactivation parameters, as well as the factors influencing UV-C resistance can be extracted. In this way, the differences found in many studies can be explained and the sources of variability can be listed and quantified. This information is critical to realistically predict inactivation of pathogens in samples treated with UV-C.

An important outcome envisaged for the meta-analysis is an overview of the relative resistance of pathogens to UV-C. By combining the outcomes of this meta-analysis with knowledge on occurrence of pathogens in breast milk, a selection of a small number of pathogens of most interest can be made.

Finally you will perform UV-C inactivation experiments with these pathogens of most interest, to study their UV-C resistance. Multiple strains from a same species will be used to determine strain variability in UV-C resistance. This will help us to understand how strain variability affects UV-C inactivation efficiency.



Christen, L., C.T. Lai, B. Hartmann, P.E. Hartmann, and D.T. Geddes. 2013. Ultraviolet-C Irradiation: A Novel Pasteurization Method for Donor Human Milk. PLoS One 8:e68120. doi:10.1371/journal.pone.0068120.

Ecophysiological behaviour of *Listeria monocytogenes* in food production

Keywords:	*Listeria monocytogenes*, ecology, physiology, biofilm, food production
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Project duration:	MSc – 6 months
Specialisation:	MBT A/B/C, MFT A, MFS A/C

Listeria monocytogenes is the causative agent of listeriosis, that can result in severe symptoms as sepsis and meningitis in the susceptible population (young, old, pregnant and the immunocompromised people) with a high mortality rate. Previous foodborne incidents described human listeriosis cases after ingestion of (uncooked) meat products, non-pasteurised cheese, fruit and vegetables, and especially (processed) ready-to-eat (RTE) products. *L. monocytogenes* is able to grow on RTE products which are stored at low (refrigerated) temperatures. This makes it important to study the food pathogen in the RTE production environment and to determine possible transmission routes along the food production chain. For this, samples will be taken from the whole production chain, from raw materials till final products, for microbial analysis with specific focus on *L. monocytogenes*. It is hypothesized that selection of *L. monocytogenes* occurs within the RTE production environment, leading to the evolvement of persistent strains with specific features in the RTE environment and posing a higher risk of product contamination.

This project involves the isolation of *L. monocytogenes* strains from various RTE production environments that will be sub grouped to give information about strains in particular environments. Isolated strains will be compared with each other and with other food and clinical isolates to obtain information on strain differences in genome composition, growth performance, stress and disinfectant resistance and biofilm formation. In addition, biofilm formation capacity on biotic and abiotic surfaces will be assessed in single species and mixed species conditions, i.e., *L. monocytogenes* strains co-cultured with associated microorganisms isolated from food processing environments and foods, to simulate *L. monocytogenes* growth and biofilm behaviour in the RTE production environment. These results will lead to a better understanding of the diversity in the behaviour of *L. monocytogenes* strains, their persistence and transmission routes in RTE production environments.

Abee T, Koomen J, Metselaar KI, Zwietering MH, den Besten HMW. 2016. Impact of Pathogen Population Heterogeneity and Stress-Resistant Variants on Food Safety. Annu Rev Food Sci Technol. 7:439-456.