Stress-response and survival strategies of *Bacillus cereus*

Keywords:	*Bacillus cereus*, sigma factors, polymerase, molecular biology, bioinformatics
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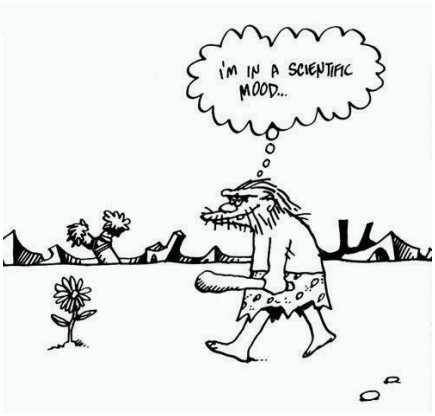
Project duration:	MSc - 6 months
Specialisation:	MBT A/B/C, MFT A/E, MFS A/C

Project description:
Bacillus cereus is a food pathogen that is often involved in outbreaks of food-borne illness. Because *B. cereus* is able to form spores, this organism can survive the production processes used in the food industry. These spores can germinate in the final food product and after that outgrowth might occur. Under these processing conditions the cells are exposed to several types of stress, such as a low or high temperature, high concentrations of salt, a lack of energy sources and/or a low pH. The central question in this research project is to elucidate how vegetative cells of *B. cereus* can withstand the different stresses they can encounter with a special emphasis on the role of regulators of gene expression such as sigma factors.
Sigma factors are subunits of the enzyme RNA polymerase, which takes care of the transcription of DNA to RNA. Sigma factors recognize specific promoter elements and can therefore "switch on" specific sets of genes.

The role of sigma factors in the stress-response of *Bacillus cereus*

In this research project the role of different regulators of of the stress response of *B. cereus* will be characterized using wild type strains and selected deletion mutants. Using different techniques, such as mutant construction, phenotypic analysis, micro arrays, and by promoter-reporter fusions, the expression of genes and roles of corresponding enzymes/proteins will be determined. This molecular biological characterization can be coupled to the analysis of different physiological parameters (such as membrane integrity and energy status), with the aim to obtain insight in the signals that lead to the activation of adpative stress responses. Next, characterisation of the cellular defense systems involved and their impact on adaptation and survival can be determined.



Identification of genes and molecular mechanisms involved in biofilm formation

Keywords:	Biofilm, pathogens, molecular biology
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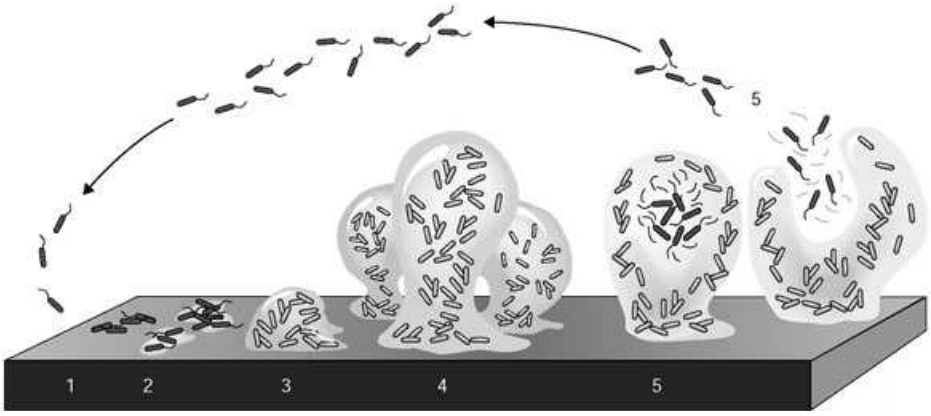
Project duration:	MSc – 5-6 months
Specialisation:	MBT A/B/C, MFT A/E, MFS A/C

Project description:
Many bacteria are capable of colonizing surfaces and grow in structures called biofilms. Biofilms consist of the bacteria, attached to a surface and embedded in an extrapolymeric matrix, which can consist of polysaccharides, protein, DNA etc. Biofilm cells are relatively hard to remove, because they are more resistant to antimicrobial compounds and disinfectants. When food pathogens and spoilage organisms manage to establish biofilm growth in food processing equipment, they will form a persistent source of recontamination. The research in this project focuses on Gram-positive bacteria that can occur in a variety of food products such as *Listeria monocytogenes and Bacillus cereus.*



Different stages of biofilm development

To be able to prevent or fight biofilm formation, more knowledge is required on the genes and molecular mechanism behind this physiological process. The complete genome sequence of representatives of the bacteria listed above are available. The project concerns the identification of cellular parameters and metabolic pathways and signalling compounds involved in biofilm formation using wild type strains and deletion mutants. Differences in biofilm phenotypes will be determined and underlying mechanisms can be further analyzed using techniques like RT-PCR and RNAseq analysis.

Resistant variants in bacterial populations

Keywords:	pathogens, stress resistance, characterization, kinetics
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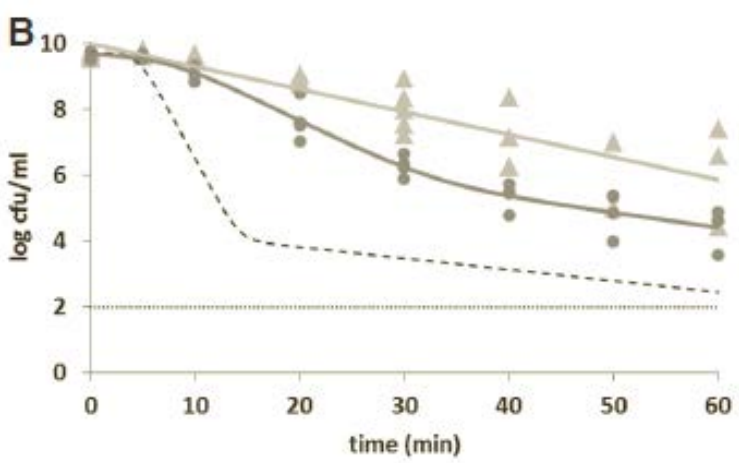
Project duration:	MSc – 5-6 months
Specialisation:	MBT A/B/C, MFT A/E, MFS A/C

Project description:

Listeria monocytogenes is a food-borne pathogen causing the rare but severe disease listeriosis. Its robustness and ability to grow under harsh conditions make it a difficult pathogen to eliminate. An additional feature that can have implications for food safety is the occurrence of subpopulations of stress resistant variants of *L. monocytogenes*. The presence of such subpopulations can lead to tailing of the inactivation curves. This is a major concern for the food industry since tailing can lead to less inactivation than expected when only the linear part of the inactivation curve is taken into account in the design of safe preservation methods. Furthermore also more resistant strains might be selected for in industrial environments. This is especially a concern within the recent trend towards minimal processing. This trend is challenging food producers to design products that guarantee microbiologically safe product by using mild preservation techniques.

The focus of this research is the occurrence of these stress resistant subpopulations. Exposure to low pH [1] results in inactivation curves exhibiting considerable tailing. A set of colonies isolated from the tail were confirmed to be more resistant than the wild type. The resistant isolates with a stable phenotype were further characterized for resistance to other stresses, growth and motility. Phenotyping of a set of resistant isolates revealed population diversity of *L. monocytogenes* LO28.

The aim of this project is to further characterize the obtained set of stress resistant variants including bacteriophage resistance. We want to identify the mutations and corresponding phenotypes in the set of variants in order to get better insight in the mechanisms leading to increased stress resistance in *L. monocytogenes*. Additionally, it is of great interest to investigate *L. monocytogenes* strain diversity (soil, food, clinical isolates) and if the mutations leading to increased stress resistance are also found in other strains of *L. monocytogenes.*



Acid inactivation kinetics of L. monocytogenes LO28 WT and acid resistant variants

1. K.I. Metselaar, H. M. W. Den Besten, T. Abee, R. Moezelaar, M.H. Zwietering (2013). "Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*." International Journal of Food Microbiology **166**(3): 508-514.

Control of fungal spores in food products

Keywords:	fungal spores, mild processing
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Project duration:	MSc – 6 months
Specialisation:	MBT A/B/C, MFT A, MFS A/C

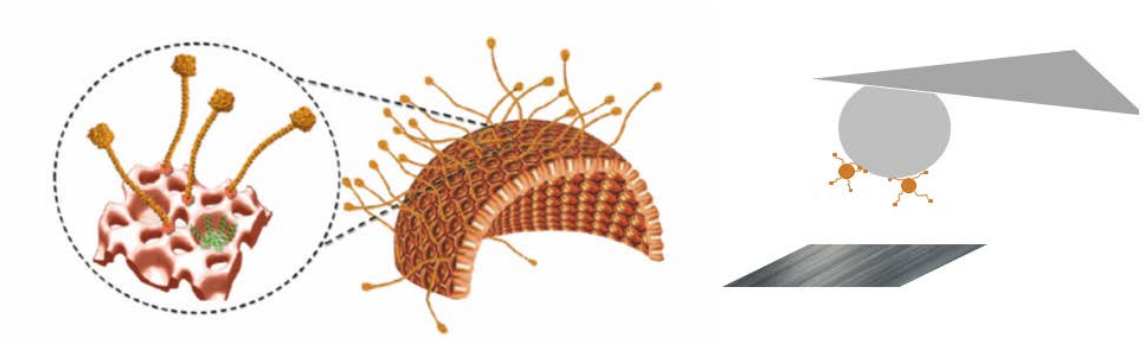
Project description:
An increased demand for healthy and nutritious foods with increased freshness and shelf life and at the same extended shelf life poses new challenges in assuring the stability and safety of a variety of foods. Moulds can grow on cereals, fruits and fruit juices, beverages, dairy products, fermented products and consequently are associated with spoilage of a wide range of products. Not all products tolerate intensive heat treatments required to inactive mould spores as these compomise quality and nutritional aspects. Mild processing techniques in combination with secondary hurdles may be applied to realize stable products.

In this research project, we will focus on the use of mild processing in combination with different matrix composition to control germination and outgrowht of yeast and mould spores. The project involved the use of different yeats species or spores produced by different moulds encountered in food. Mild techniques could involve the use of cold plasma, pulsed electric field (PEF) or high pressure processing in combination with variation in temperature, gas composition, antimicrobial compounds as conditions during storage.

How does a single *Bacillus cereus* spore stick to steel?

Keywords:	*Bacillus cereus*, spores, adhesion, stainless steel, atomic force microscopy
Supervisors:	Tjakko Abee, tjakko.abee@wur.nl Renko de Vries (group leader) and Fabiola Gutierrez (postdoc), PCC Protein Materials
Project duration:	MSc – 6 months
Specialisation:	MBT A/B/C, MFT A, MFS A/C

Project description:



Left: Cartoon of outer layer components of a B. cereus endospore. Right: using colloid-probe AFM we can accurately measure forces of adhesion between the spores and surface materials that are used in the food industry such as stainless steel.

Sporeforming spoilage and pathogenic bacteria are a major headache for the food industry, since the spores are notoriously difficult to remove from food-contact surfaces and to inactivate. An example is *Bacillus cereus*, a pathogen well known to be the cause of food-borne emetic and diarrheal disease associated with rice meals kept at room temperature for too long and a wide range of other foods. In this collaboration between the protein materials group at PCC and prof. Tjakko Abee at Food Microbiology we want to explore whether we can use Atom Force Microscope (AFM) – based measurements to determine the minute forces between individual spores and surface materials relevant to the food industry such as stainless steel. The aim would be to measure what forces are needed to remove them, but also to find out the mechanism of adhesion: which of the surface molecules and/or proteins are responsible for adhesion? This could be addressed for example by studying adhesion efficacy of *B. cereus* endospores producing different types of surface molecules and/or proteins. At this stage, this project is in an exploratory phase: we want to make the first steps (proof of principle experiments) by a comparative analysis of adhesion capacity of spores derived from different *B. cereus* strains and with different sporulation histories. You will first grow *B. cereus* and produce spores at Food Microbiology, and then perform the force measurements at Physical Chemistry and Soft Matter. This is a highly ambitious interdisciplinary project suitable for a Food Technologist or Biotechnologist with an interest in advanced and delicate physical measurements. The AFM-based force measurements produce large amounts of data that need quite extensive processing, so an interest in number crunching with computers is also required. Alternatively, the project could also be interesting for a Molecular Life Scientist with an interest in applications of advanced physical measurements in the food industry.

Techniques: Basic microbiology (handling *B. cereus* cells and their endospores), Atomic Force Microscopy imaging in liquid, Atomic Force Microscope-based force spectroscopy, Bioconjugation chemistry, Working with large datasets (with for example MatLab).

Growth and survivability of *Campylobacter* in environmental-like conditions

Keywords:	*Campylobacter*, foodborne pathogens, transmission, environment, survival, growth, metabolism
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Project duration:	MSc- 6 months (start in the beginning of August)
Specialisation:	MBT B, MFT A/E, MFS A/C

Project description:

Campylobacter jejuni (C. jejuni) and *Campylobacter coli* (*C. coli*) are one of the major causes of human campylobacteriosis. In both developed and developing countries they cause more cases of diarrhoea than the foodborne bacteria *Salmonella*. In immunocompromised patients there is a chance that the pathogen spreads systemically, leading to more severe chronical complications like the peripheral nervous system disorder, Guillain-Barré Syndrome (Nachamkin et al., 1998).
During the transmission of *Campylobacter*, *Campylobacter* is exposed to a wide variety of environmental stresses. Notably, *Campylobacter* is unable to grow below 30°C, and it is recognized that this pathogen has limited survival chances outside the warm-blooded gastro-intestinal tract of animals. However, the prevalence of campylobacter has dramatically increased, suggesting *Campylobacter* is developing a certain degree of environmental robustness (Kaakoush et al., 2015).
Until recently, it was thought that *Campylobacter* was unable to metabolise carbohydrates. However, this dogma was challenged following a wide whole genome sequencing analysis that revealed the presence of glucose and fucose utilisation clusters, required for glucose and fucose metabolism, respectively. Indeed, addition of glucose and fucose to the media enhanced growth and promoted biofilm formation of *Campylobacters* containing the fucose and glucose gene clusters (Dwivedi et al., 2016; Vegge, Jansen van Rensburg, et al., 2016).

In this project we aim to elucidate the role of these carbohydrate utilisation clusters in *Campylobacter*'s surviving ability outside the warm-blooded host. To do so, survival/growth experiments will be performed at growth-permissive temperatures (T>30 °C) and at non-growth-permissive temperatures (T<30 °C) in the presence of glucose and/or fucose sugars. With the use of a minimal media, containing only essential ingredients for *Campylobacter*, the effect of fucose and glucose will additionally be assessed on relevant transmission parameters such as motility, chemo- and aerotaxis, and biofilm formation.

Dwivedi, R., Nothaft, H., Garber, J., Xin Kin, L., Stahl, M., Flint, A., Szymanski, C. M. (2016). L-fucose influences chemotaxis and biofilm formation in *Campylobacter jejuni*. Molecular microbiology, 101(4), 575-589.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. Clinical microbiology reviews, 28(3), 687-720.

Vegge, C. S., Jansen van Rensburg, M. J., Rasmussen, J. J., Maiden, M. C., Johnsen, L. G., Danielsen, M., MacInture, S., Ingmer, H., Kelly, D. J. (2016). Glucose metabolism via the entner-doudoroff pathway in *Campylobacter*: a rare trait that enhances survival and promotes biofilm formation in some isolates. Frontiers in microbiology, 7, 1877.