
Details of CSC proposal 2020

Title:

Heat resistance, spore regulatory networks of *Bacillus cereus* and *Clostridioides difficile.*

Supervisor details

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Composition of the research group

Name and title	Specialization	Institution	Involvement
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Dr. Bhaghyashree Swarge	mass spectrometry	SILS	daily guidance (post-doc)
dr. ir. Huub Hoefsloot	data analysis	SILS	data analyst
Dr. Timo Breit	transcriptomics	SILS	advisor
Dr. Gertien. J. Smits	cell biology	SILS	advisor
Prof. dr. Theodorus Gadella Jr.	advanced microscopy	SILS	advisor
Dr. Leo de Koning	mass spectrometry	SILS	advisor
Dr. L de Jong	Biochemistry	SILS	advisor
MSc Richard de Boer	molecular biology	SILS	technician
MSc Winfried Roseboom	mass spectrometry	SILS	technician
MSc Henk Dekker	mass spectrometry	SILS	technician
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Prof. dr. Sander Woutersen	spectroscopy	HIMS*	advisor
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8 PhD students and 1 extra post-doctoral fellow

Top 10 publications of the applicant and research group related to the proposed research

1. Breedijk R.M.P., Wen J., Krishnaswami V., Bernas T., Manders E.M.M., Setlow P., Vischer, N.O.E., **Brul S.** A live-cell super-resolutiontechnique demonstrated byimaging germinosomes in wild-type bacterial spores, Scientific Reports (2020) 10:5312

2. Abhyankar W.R. ,Zheng L.,**Brul S.**,de Koster C.G., de Koning L.J. Vegetative Cell and Spore Proteomes of Clostridioides difficile Show Finite Differences and Reveal Potential Protein Markers. Journal of Proteome Research 2019 18 (11), 3967-3976

3 Swarge B.N., Roseboom W., Zheng L., Abhyankar W.R., **Brul S.**, de Koster C.G., de Koning LJ. 2018. "One-Pot" Sample Processing Method for Proteome-Wide Analysis of Microbial Cells and Spores. Proteomics Clin. Appl. 12:e1700169.

4. . $Omardien, S., Drijfhout, J.W., Zaat, S.A. and **Brul, S.** 2018. Cationic amphipathic antimicrobial peptides perturb the inner membrane of germinated spores thus inhibiting their outgrowth. Front. Microbiol. 9:2277.

5. $Omardien, S.,Ter Beek, A,m Vischer, N., Montijn, R., Schuren, F. and **Brul, S**. 2018. Evaluating novel synthetic compounds active against *Bacillus subtilis* and *Bacillus cereus* spores using Live imaging with SporeTrackerX. Sci. Rep. 8: 9128.

6..Stelder S.K., Benito de Moya C., Hoefsloot H.C.J., de Koning L.J., **Brul S.**, de Koster C.G**.** 2018. Stoichiometry, Absolute Abundance, and Localization of Proteins in the *Bacillus cereus* Spore Coat Insoluble Fraction Determined Using a QconCAT Approach. J. Proteome. Res. 2018. 17:903-917.

7. @Abhyankar, W.R., Kamphorst, K., Swarge, B.N., van Veen, H., van der Wel, N.N., **Brul, S.** , de Koster, C.G., and de Koning, L.J. 2016. The influence of sporulation conditions on the spore coat protein composition of *Bacillus subtilis* spores. Front. Microbiol., 7, 1636.

8. $Zheng, L.; Abhyankar, W.; Ouwerling, N.; Dekker, H.L.; van Veen, H; van der Wel, N.N.; Roseboom, W.; de Koning, L.J.; **Brul, S.**[*]; de Koster, C.G.[*] 2016. *Bacillus subtilis* Spore Inner Membrane Proteome. J. Proteome Res. 15, 585-594.

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10.@Abhyankar, W.R., Pandey, R., Ter Beek, A., **Brul**, S., de Koning, L.J. and de Koster, C.G. 2015. Reinforcement of *Bacillus subtilis* spores by cross-linking of outer coat proteins during maturation. Food Microbiology *45* , 54-62.

Scientific summary

The species of Bacillus and Clostridioides form endospores that can withstand a variety of chemical and environmental assaults. The resistance properties are in part attributed to spore surface proteins. Thermal resistance and germination heterogeneity are key features of spores against which no effective solution has yet been found. Thermal resistance of endospores is of major concern to the food industry as well as healthcare and is partly attributed to their proteinaceous outer layer, the coat. Little is known about the regulatory modules coupling gene expression to protein synthesis and ordered coat assembly.

Our aim is to elucidate these mechanisms in a two-pronged approach. First by assaying the spore contents of a panel of *Bacillus cereus* and *Clostridioides difficile* strains and isolates with differing heat resistance properties by high throughput proteomics and metabolomics we will exhaustively characterize their constituents. Through comparison of the spore proteome and metabolome to that of corresponding vegetative cells and the relative resistance of spores to stresses we can find proteins and small molecules that correlate with increased or decreased resistance phenotype.

Second, the complete sporulation process of *B. cereus* will also be analysed by comprehensive proteomics and transcriptomics enabling us to build a mathematical model of the regulatory network of coat biosynthesis. We will test the hypotheses that, during sporulation, coat biosynthesis is regulated at the post-translational level and that the processes involved are conserved among two different spore forming bacteria and may modulate spore thermal resistance. Time-resolved data from proteomics and genome-wide transcript analyses of sporulating wild type *B. cereus* will be combined with existing literature to infer the molecular sporulation network.

Through integration of these two datasets, this study will shed light on the mechanisms regulating coat biosynthesis and help elucidate the contribution of coat composition to heat resistance. Thus, we will contribute both to the establishment of novel insight in the mechanisms that govern the developmental biology in endospore forming bacteria as well as establish strategies to control bacterial spore formers in the food chain and healthcare settings.

Description of the proposed research

Objectives

Introduction

Within the genus Bacillus, the gut commensal or even probiotic *Bacillus subtilis* is the model organism for Gram-positive bacteria. The genus also contains a group of pathogenic bacteria known as *Bacillus cereus* sensu lato, which, upon ingestion of their toxins or endospores, can cause vomiting and diarrhea.[6] All species from the genus Bacillus form endospores, which are dormant and resistant cellular structures, through the process of sporulation (Figure A).[7] *C. difficile* on the other hand is a gram positive obligate anaerobic pathogen which causes *C. difficile associated infection* (CDI). This is a health-care-associated infection mainly transmitted via highly resistant endospores from one person to the other[55].

Endospores have a layered structure. The proteinaceous outer layer, called the spore coat, consists of a basement layer, inner coat, outer coat and crust, and is mostly assembled from components present or synthesized in the mother cell.[8-10] *B. cereus* endospores are also enveloped by an exosporium; a loose-fitting sac-like structure.[11] Endospores can resist much higher temperatures than vegetative cells[1,2,12] and are also remarkably resistant to other stressors, such as chemical agents. The level of resistance is influenced by the conditions under which endospores are formed, such as temperature during sporulation or composition of the sporulation medium, and varies between endospores from different Bacteria.[13,14] High tolerance towards stressors has been attributed to the unique properties of the dehydrated endospore core [8,9,12,15]. In addition, spore coat proteins seem to provide cellular integrity as well as resistance towards extreme environmental conditions. This is supported by (our own) observations that the thermal resistance of released endospores increases simultaneously with changes in the structure of the spore coat layers over time.[1,2]

Highly resistant endospores of pathogens, such as *B. cereus* and *C. difficile* are of major concern to the food industry and healthcare. These spores can persist in food processing lines and contaminate hospital environments.[55,1,2,12] A comprehensive analysis of the mechanisms controlling protein assembly in the spore coat in *B. cereus* as well as the profiling spore composition of various strains of *B. cereus* and *C. difficile* will provide new leads for the extreme resistance towards stressors observed for endospores.[2,16,17]

Sporulation and spore coat biosynthesis

Viable endospores can only be formed when gene expression and translation of proteins involved in spore biosynthesis are regulated in time and space (Figure A). Gene expression is controlled by sigma and transcription factors expressed by the pre-divisional cell, mother cell and forespore, and is initiated by the master transcriptional regulator of sporulation, Spo0A~P.[18] This regulator is activated by a phosphorelay pathway that includes autophosphorylating kinases KinA and KinB, which sense changes in environmental conditions. Only a fraction of an isogenic population of *B. Subtilis* will enter sporulation. This has been correlated to the dynamic transcription of several phosphorelay genes. Stochasticity of phosphorelay genes could be an important regulator for sporulation heterogeneity and might lead to variation in sporulation time between cells.[19] We will induce KinA expression to synchronize sporulation for our analysis.

Sigma factor σK is crucial for the expression of a large group of coat proteins in the late mother cell (Figure A stages IV-V). Transcription of sigK is activated in a feed forward loop by transcription factor SpoIIID and σE. σK is subsequently activated through cleavage by SpoIVFA, which is under control of σG. The gene-regulatory circuit consists of a linked series of coherent and incoherent feed forward loops, including additional regulatory proteins, that generate successive pulses of transcription in which large numbers of genes are in succession switched on and off.[20-24] The regulatory network module extending from σK via translation of coat proteins to a spatially organized coat structure is not fully known.

Synthesis of spore coat proteins in *B. subtilis* begins several hours before a functional coat appears and is controlled on the level of gene expression and post-translational during assembly of the coat.[9] In *B. anthracis*, transcripts of proteins that make up the spore proteome are overrepresented in an earlier phase of the life cycle as more than half of the spore proteome genes appear to be up-regulated.[25] This suggests that, at least in *B. anthracis*, the majority of the spore proteome is packaged from pre-existing stocks rather than synthesized de novo during sporulation. Unlike the well-studied regulatory network governing the expression of the sigma factors during sporulation, the regulatory modules directing downstream gene expression and ordered assembly of coat proteins in the coat layers have not been fully elucidated.

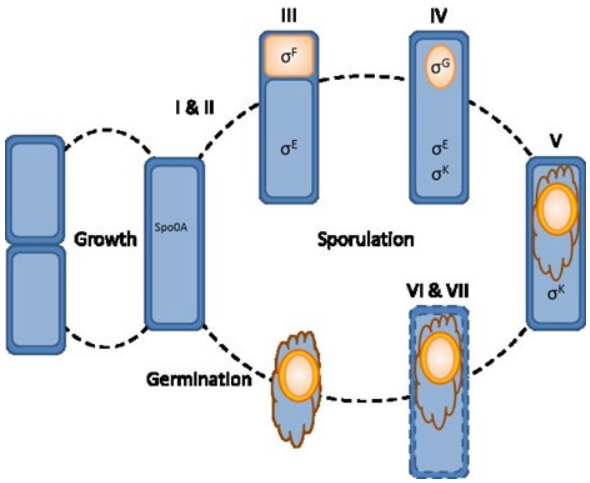

Figure A - Sporulation cycle and sigma factor control. Under control of master regulator Spo0A sporulation is initiated when conditions do not favor growth. In **stage I** the chromosome is duplicated and subsequently in **stage II** segregated to the pre-spore, which is separated from the mother cell by formation of a septum. Under control of sigma factors σ^E and σ^F proteins are synthesized in the mother cell and forespore, respectively in **stage III**, and the forespore is engulfed by the mother cell. During **stage IV** the germ cell wall and cortex layer deposit and sigma factors σ^G and σ^K become active in respectively the forespore and mother cell. In **stage V** the proteins synthesized under sigma factor σ^K control finally deposit onto the spore initiating at the mother cell proximal pole thereby forming the coat and exosporium layers. **Stage VI** entails maturation during which proteins are cross-linked to increase resistance properties. This process continues after **stage VII** completes, in which the mother cell lyses, resulting in a released spore.

Six classes of spore coat proteins have been identified by monitoring the localization kinetics of 41 GFP-fused proteins during sporulation.[26] At the start of spore coat morphogenesis, major components of all four layers of the spore coat are present. The coat proteins show up in temporal waves and the spore coat appears to develop from an organized scaffold on the mother cell proximal pole. Morphogenetic proteins direct coat proteins through protein-protein interactions to the appropriate coat layer during assembly.[10,27] The morphogenetic protein SpoIVA is expressed in the mother cell under control of σE and first localizes at the dividing septum. Later on, SpoIVA forms a shell surrounding the pre-spore. SpoIVA self-assembles into cable like structures that serve as a scaffold for further coat assembly. SpoVID directly interacts with SpoIVA and subsequently recruits SafA which is necessary for further encasement of the spore. The presence of SpoIVD is also a prerequisite for the anchoring of CotE. The model described in Driks and Eichenberger[9] points out several proteins that are key to the formation of the various coat layers, e.g. CotE that serves as a scaffold to construct the outer layer and CotX, CotY and CotZ that are needed to form the crust. The way in which the order of the outer coat protein assembly is regulated is not fully known. At the end of the spore development the coat matures through proteolytic processing, cross-linking and glycosylation of proteins.[2]

We take the view that protein cross-linking at cysteins, tyrosines, glutamines and/or lysines is also key to proper coat assembly[2] and take special interest in proteins that have a high percentage of these residues or are capable to interact with these residues, e.g. the kinase CotH or transglutaminase Tgl. An NWO funded post-doc project in our laboratory investigates protein cross-linking in mutants lacking essential coat proteins. From our observation that an increased level of protein cross-linking correlates with enhanced thermal stress resistance we believe that the coat composition and heat resistance are related. Hence, the spore coat provides additional protection to heat stress enabling spores to better survive thermal treatments. Over the past decades, biological sciences and bioinformatics have advanced in the elucidation of the regulation of *B. subtilis* spore layer protein synthesis. Bioinformatics has shown that the sporulation regulatory network and the sporulation sigma factors are fully conserved in endospore-forming bacteria, whereas downstream genes and their regulation appear to be significantly less conserved.[18,27] Conservation of spore proteins and assembly mechanisms in *B. cereus* and *C difficile* is still not fully known.

Innovative aspects

In this project, we will use mass spectrometry and next generation sequencing to monitor, for the first time, the entire sporulation process of *B. cereus* with an integrated differential time-resolved multi-omics-based approach. The application of this method will beyond doubt lead to publications in peer-reviewed journals, whereas the generated genome-wide transcript and protein data along with the constructed model on the regulation of sporulation will lead to methodology and biology publications. In addition, the proteome and metabolome composition of vegetative cells and spores of a panel of *B. cereus* and *C. difficile* with differ in their heat resistance will be acquired. The thorough comparison of the composition of isolates differing in heat resistance sporulation will yield findings that are as such publishable and material for a chapter in the PhD thesis of the candidate, even if no compositional differences between spore coats are observed. Finally, the proposed research will be mostly fundamental and as such will support many researchers in this field of research. Nevertheless,

elucidation of the mechanisms involved in spore coat synthesis and in its structuring, could provide valuable targets for the food industry or medical community to steer desired spore characteristics.

Scientific approach

In this project we will focus on the hitherto unanswered research question of how the order of protein incorporation into the assembling spore coat is controlled. We will investigate whether spore coat biosynthesis during sporulation is regulated both at the level of gene expression and the post-translational level as wells as whether the composition between endospores with varying thermal stress resistance is fundamentally different. Therefore, we will study the mechanisms regulating translation of spore coat proteins and the protein composition of coat layers as well as the mechanisms driving the development of heat resistance of endospores of the food-borne pathogen *B. cereus*. In addition we will collect a comprehensive inventory of the proteins and metabolites of spores and vegetative cells of a panel of *C. difficile* and *B. cereus* strains that differ in heat resistance. This will shed light on how the composition of the endospores relates to its resistance to stress for these two pathogens. Integration of these two datasets will give insights which proteins or metabolites are correlated with increased heat resistance in these two pathogens, and how the spore coat of *B. cereus* is assembled. This in turn will expand the knowledge of spore coat formation for both these organisms as much of the knowledge now hinges on observations in *B. subtilis*.

We will address our main questions via the following sub-questions: (**1**) Are there proteins and metabolites which are spore specific that correlate with increased heat resistance in *C. difficile* and *B. Cereus*? (**2**) Are *B. cereus* coat proteins available as a pre-existing stock waiting for assembly or synthesized de novo in pulsed wave for timely delivery at the surface of the growing spore? (**3**) What mechanism directs the order of protein incorporation during assembly of the spore coat layers? (**4**) How is thermal stress resistance of the *B. cereus* spore affected by regulation of gene expression, assembly and the structure of the spore coat and what effect has temperature (stress) during sporulation on this? (**5**) How well conserved is the regulatory network for gene expression of spore coat proteins between *B. cereus* and *C. difficile*?

Previous studies often use transcriptomics, proteomics or metabolite measurements in isolation to profile spores of various spore forming bacteria. However integrating data on all three major omics levels will paint a clearer picture of spore make-up and dynamics than studying them on their own. Especially using only transcriptome data to infer regulatory interactions leading to spore assembly gives an incomplete picture. As, regulators of gene expression are often proteins and under dynamic conditions, proteins and mRNAs, having different life spans, are weakly correlated as their concentrations evolve on different time scales. Consequently, inference of regulatory mechanisms from time series of mRNA data alone may potentially lead to spurious conclusions. We will therefore address questions **1** by combining spore proteome and metabolome data and **2,3** with a combination of time-resolved transcriptomics and proteomics to set up a quantitative gene expression model.

RNA isolates will be analysed in-house by the MAD: Dutch Genomics Service and Support Provider, a genome-wide analysis facility with whom we have a longstanding collaboration. Their state-of-the-art equipment and expertise is available to us and will be used to analyse samples by RNA-Seq (http://www.dutchgenomics.nl/).

We will use a differential time-resolved proteomics approach to study the time-course of spore coat development for all coat proteins in wild-type spores as well as heat-resistant and more heat sensitive spores to address question 3. Insight in sporulation of *B. cereus*, which has not been investigated to the extent of B. subtilis, through genetics will require extensive laboratory research. We will therefore acquire time-resolved transcriptomics and proteomics datasets of sporulating *B. cereus* and infer the network using B. subtilis models for quantitative gene expression to address question **4**.

Characterization of B. cereus and C. difficile strains and their heterogeneous sporulation

In this project, the *B. cereus* laboratory strain ATCC 14579, the heat resistant *B. cereus* food product isolates BC(MBMFS) 1-5, of which genomic sequences are available, will be used.[15,28,29] We will start by assessing the growth and sporulation conditions of the *B. cereus* strains, thereby focusing on the synchronization of sporulation within cell cultures. Well-synchronized cultures are expected to produce uniform expression profiles and are, as a result, essential for a detailed analysis of the transcriptome, proteome relation during sporulation.[19,30] Special attention will be paid to the putative importance of heterogeneous sporulation in the (thermal) stress resistance of spores, in particular in relation to the structure of the spore coat. In addition the C. dificile laboratory strain ATCC BAA1382 and the clinical isolates donated by the medical microbiology department of the Amsterdam UMC, CD(UMCA)1-5 will be assayed for growth and sporulation as well as relative thermal resistance before pure spore and vegetative cell samples are generated to address question **1**.

Development of a generic metabolome and proteome quantitative mass spectrometry method for sporulation

Our lab has years of experience with the extraction and identification of proteins from vegetative cells and all spore layers with mass spectrometry. We have developed specific procedures to isolate membrane[31] or insoluble fraction spore proteins[32,33]. These targeted analysis procedures were more recently complemented by a one-pot protein analysis method for proteomics, which enabled us to identify over a thousand proteins from all layers of *B. subtilis*, *B. cereus* and *C. difficile* endospores, including previously unidentified proteins.[3,55]. In this project, we will use our one-pot protein analysis method for proteomics but extend it to allow for fast an efficient extraction of metabolites from the same sample as well.

For the sporulation time series experiments, in the earlier stages of sporulation, before lysis of the mother cell, the entire B. subtilis cytosolic proteome will be analyzed for the presence of spore proteins and particularly coat proteins that are available for assembly, whereas (fore)spores will first be isolated by lysis of the mother cell and subsequently purified prior to analysis.[32] In intermediate stages the fractions of the mother cell and fore spores will be separated biochemically and individually analyzed.[34] Known regulatory proteins of the sporulation regulatory network will also be quantified to fit computational models (vide infra).

Monitoring the process of sporulation

The sporulation process invokes a drastic morphological change of the bacterial cell (Figure A) and each sampling of the sporulation progress will be supplemented with microscopic analysis in order to determine the sporulation stage and accurately synchronize the changes in the proteome and transcriptome.[37] In addition, the process will be monitored with spectrophotometry and viability counts. We plan to let our transcriptome data guide our proteomics experiments as has been done for studies in Escherichia coli by the mass spectrometry group in a previous project[5]. Prof. Brul also has ample experience with transcriptome analyses including transcriptome analysis of sporulating *B. subtilis*[37-40], although a different strain and conditions were used, transcriptome and proteome were not coupled, and sporulation was not synchronized within cell cultures as we intend to do in this PhD project. Furthermore, to probe for protein localization at subcellular level we will also generate fluorescent reporter constructs. For this we will make use of a novel bright monomeric red fluorescent protein, mScarlet, which has been developed at our institute and is less prone to interfere with localization due to steric effects.[41] Our laboratory has experience with state-of-the-art high-resolution imaging techniques16 and currently studies germinosome localization using super-resolution microscopy[17]. An integrated approach of microscopic analysis and (existing) transcriptome data, such as the study by Nicolas et al. 2012[42] and our own data, combined with the literature model[9] as a starting point will be used to derive time points for sampling to capture the essential stages of coat biogenesis during sporulation with our combined transcriptomics proteomics approach.

Development of a quantitative gene expression model for B. cereus ATCC 14579

The mRNA and protein time profiles will be analyzed with hierarchical cluster analysis (HCA), Principal Component Analysis (PCA) and Singular Value Decomposition (SVD) to detect pulsed-wave events of groups of coat proteins. To detect the time order and time frame shifts between transcription of mRNA and translation of protein, time profiles of mRNA fold changes thus obtained will be correlated with corresponding coat protein concentration profiles. This correlation will unravel post-transcriptional regulation of spore coat gene expression.[43,44] Aside from this, protein translation pulses will also be correlated to the position of known spore coat proteins in coat layers.

For inference of the molecular sporulation network we will follow a middle out approach; we will combine available knowledge from both literature[9,42] and databases, such as computational kinetic models on the initiation of sporulation[45], with our experimental transcriptomics and proteomics data. A quantitative gene expression model will be built for inference of regulatory interactions as well as the identification of quantitative gene regulation functions.[46] Here, we will focus on downstream genes expressing structural and morphogenetic coat proteins.

Heat may influence gene expression through global physiological effects, such as ribosome concentrations, size of metabolic and nucleotide pools effects, as well as the rate of transcription and translation of all genes. Therefore, our datasets will contain quantitative information about ribosome copy numbers and products of housekeeping genes that reflect physiology, which will enable us to incorporate this data in our computational models.[46,47,48]

Differential time-resolved proteomics and transcriptomics experimentation for thermal stress resistance

We will perform differential time-resolved proteomics and transcriptomics experiments to assess the influence of thermal stress and molecular adaptation of heat-resistant isolates on the assembly and the final protein

composition of the spore coat. To this end, the spore coat proteome of spores formed at different sporulation temperatures as well as spores from the wild-type *B. cereus* and the heat-resistant isolate will be quantitatively compared in differential proteomics experiments. If we observe an effect on the proteome, we will elaborate on this using our time-resolved proteomics and transcriptomics experimentation, as described in the above and implement the data obtained in our previously described gene expression model.

Preliminary data

Our preliminary data consists of coat proteins that have been identified for *B. cereus* and *B. subtilis* spores in pilot experiments in our group.[33] Furthermore, we will utilize available computational kinetic models on the initiation of sporulation to build our quantitative gene expression models for spore coat biosynthesis.[21,49-51]

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Timetable of the project.

	Year 1		Year 2		Year 3		Year 4	
Task	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. Physiological characterization of *B. cereus* and *C. difficile* strains, synchronizing sporulation for B. cereus.	■							
2. Extending the 'one-pot' method to a generic metabolome and proteome extraction method.	■							
3. Proteomic and metabolomic profiling of spores and vegetative cells of *B. cereus* and *C. difficile* isolates differing in heat resistance.		■						
4. Inference of heat resistance correlation of metabolites and proteins of *B. cereus* and *C. difficile* spores.		■	■					
5. Time-resolved proteomics & transcriptomics of *B. cereus* sporulation		■	■					
6. Inference of a gene expression model for *B. cereus* sporulation				■	■			
7. Time-resolved proteomics & transcriptomics of heat-resistant isolate sporulation and/or sporulation under heat stress					■	■		
8. Analysis of heat stress and/or heat resistant isolate datasets and inference with quantitative gene expression models.					■	■		
9. Analysis of *B. cereus* datasets and inference with quantitative gene expression models.						■	■	
10. Comparison of the *B. cereus* models to observations in *C. difficile*							■	■
11. Writing of PhD thesis								■

Deliverables							
1. Method for the generic extraction of metabolome and proteome from spores and vegetative cells.		■					
2. List of heat resistance related proteins and metabolites.			■				
3. Time-resolved *B. cereus* spore protein & mRNA concentration profiles*				■		■	
4. Biostatistical model for correlation of mRNA and protein profiles					■		
5. Kinetic model for downstream gene expression of coat proteins*					■		■
6. Molecular network for regulation of *B. cereus* sporulation							■
Milestones							
1. Model for gene expression of *B. cereus* spore coat proteins*					■		■
2. Bacterial sporulation regulatory network of *B. cereus*							■
3. Contribution of spore coat composition to heat resistance				■			■
4. Model for gene expression of *B. cereus* spore coat proteins							■
5. PhD thesis ready for approval							■

Scientific embedding of the proposed research

Physiological characterization, transcriptome analysis and sporulation experiments of B. subtilis and B. cereus will be carried out in the microbiology group of Prof. Brul. Proteomics experiments will be carried out in the labs of Dr. Kramer. State of the art mass spectrometric instrumentation is available, including a new timsTOFpro (installed 08/2019). Both groups have years of experience working together on the molecular physiology of Bacilli, e.g., on method development to detect spore coat proteins.[2,32,33] The laboratories collaborate closely with the Bacterial Cell Biology & Physiology Groups headed by Prof. L.W. (Leendert) Hamoen, who is a Bacillus expert on vegetative growth and cell division. Furthermore, the MAD: Dutch Genomics Service and Support Provider, a genome-wide analysis facility headed by Dr. T.M. (Timo) Breit, is accessible at our university for the transciptomics experiments.[37,52]

Computational and mathematical model building as well as data analysis will be carried out under supervision of and in collaboration with Dr. Hoefsloot. He is a member of the the Biosystems Data Analysis group of Prof. A.K. (Age) Smilde at our university with whom we have a long lasting collaboration most recently as members of the university Research Priority Area Systems Biology of host-microbe interactions (2019-2024).[43,44,53] Application and development of state-of-the-art data analysis techniques to detect clusters of co-regulated genes and to infer molecular networks is a research line of Dr. Hoefsloot.[44,53,54] Consequently, a computer program for hierarchical cluster analysis is available. Furthermore, data analysis of time series from quantitative proteomics experiments and transcriptomics experiments is currently an ongoing effort in our labs for the biostatistical analysis of B. subtilis and Clostridium difficile germination experiments.[31]

Prof. Brul is internationally embedded in the spore community, regularly partaking in the biannual European Spores Conference with both oral and poster contributions.[55] Prof. Brul until recently was an expert international scientific advisor for the research program on Gut Health and Food Safety of the Institute of Food Research (IFR), which integrated into the Quadram Institute for Food and Health.[56] Prof. Brul was lecturers at the invited speaker seminar at the IFR hosted by Prof. M.W. (Mike) Peck, a world renowned expert of the bacterial spore forming organism Clostridium botulinum.[57] Prof. Brul has been co-chairperson of the organizing board and session chair at large FEMS Congresses of European Microbiologists (2015 and 2017) and an invited speaker at the 9th International Congress on Predictive Microbiology in Foods in Rio de Janeiro, Brazil as well as

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Microbial Spoilers in Food (2017) and the European Spore Conference (2018) .[58-61] In 2019 he is an invited speaker at the FEMS Congress of European Microbiologists in Glasgow.

Education:

The PhD student will receive training in research and will also be trained to supervise students. Furthermore it is foreseen that the PhD student can develop herself through courses as appropriate. The candidate will be embedded in an academic research environment. Through participation in work discussions, biweekly spore meeting and scientific conferences the student will be exposed to microbial proteomics, metabolomics and molecular physiology.

In the framework of employability our institute offers training of personal skills to help the student to pursue an adequate position for a career in or outside academia. The student will create an education program together with her supervisor at the start of the PhD. Firstly, this will contain the mandatory Faculty of Science PhD skills development program, including the modules "Mastering your PhD", "Train the lecturer" and "Job and career Planning".[62] She will apply and further develop her teaching skills by supervising undergraduate students during their internships and by teaching in practical courses and tutorials. Secondly, the PhD student will actively participate in the BioSB education program of the Netherlands Bioinformatics and Systems Biology Research School[63] and will have to present and discuss his/her research on (inter)national conferences and presentations. This education program offers, among others, a "Quantitative and predictive modeling" course and various programming and data analysis courses. Finally, the PhD student will be encouraged to attend courses and workshops to further develop general skills obtained during the PhD project in order to maximize the possibility of a successful career inside or outside academia after finishing the PhD, for instance, by attending workshops organized by Innovation Exchange Amsterdam (IXA) on topics such as "How to successfully valorize your initiative" and "Presentation skills in network situations".[64]

Name: Prof. dr. Stanley Brul

Place: Amsterdam Date: 22/04/2020