PhD open to successful scholarship candidates

[X] PhD

Proposed research project

Research project Title **Detailed studies of SpoVA Proteins Localization, Interactions and Movement in Bacillus spores**

1. **Description of the project in non-specialist language**

Food poisoning or foodborne disease (FBD) is a major public health problem worldwide. According to the WHO, each year 600 million people around the world become ill after consuming contaminated food. Among these, 420,000 die, including 125,000 young children, due to the susceptibility of this population to a diarrhea, and ~43% of FBDs occur in these patients. About 70% of FBDs result from food contaminated with a microorganism, including bacteria that have virulence factors giving them the ability to cause disease. Recent studies on bacterially caused food spoilage and food borne diseases have revealed that the resistance of many of these organisms to antimicrobials stems is due to aerobic spore-formers found in the human gut microbiota, including members of the Bacillaceae. Among them, *Bacillus subtilis* (BS) is considered the model organism for endospore formation. Spores of *Bacillus* species are extraordinarily resistant to all manner of harsh treatments, and largely because of this resistance, spores of some *Bacillus* species are linked to food spoilage, food borne diseases (anthrax, botulism, or food poisoning), resistance to antibiotics and the development of the resistome. Therefore, it is essential to understand spores' germination mechanisms to develop strategies to combat these dangerous pathogens. This project aims to increase understanding of spores' germination pathways and mechanisms that could be used to activate spores within a host to prevent recurrence. For example, if spores within hosts are forced to germinate during antibiotic therapy, the germinated spores would be killed by the antibiotic and unable to cause deleterious effects.

2. **Project methodology & recent publications of relevance**

Germinant Receptors (GRs) in spore's inner membrane (IM) detect ligands that trigger germination. The multiple IM SpoVA proteins form a channel for release of CaDPA in response to signals from activated GRs, a major germination event. Analysis of GR subunit interactions with domains of SpoVA channel proteins involved in DPA transport may give new insight into signal transduction in spore germination. Consequently, this project will initially investigate SpoVA proteins' localization, Interactions and movement in *B.subtilis* spores using various state-of-art techniques. Fluorescent fusions of SpoVA proteins will be constructed and the fusion proteins functionality in CaDPA uptake and release will be determined? The location of the fusion proteins in the IM will be examined and If the proteins are clustered or evenly distributed or move? Using colocalization with fluorescent GR subunit -fusions as well as FRET analyses, we will determine which other proteins colocalize with SpoVA proteins, even if transiently. Further validation work will be done in *B. cereus* where recently germinosome studies have been initiated by our group. Recent literature of relevance:

-Wang Y 2022. Spore germinosome visualization and dynamics in *Bacillus cereus* PhD thesis University of Amsterdam.

-Wang Y, Vischer NOE, Wekking D, Boggian A, Setlow P and Brul S. 2022. Visualization of SpoVAEa protein dynamics in dormant spores of *Bacillus cereus* and dynamic changes in their germinosomes and SpoVAEa during germination. *Microbiology Spectrum* 10 (3) e00666-22 doi: https://doi.org/10.1128/spectrum.00666-22

3. **Significance of the project**

Bacterial spore formers are major constituents of the microbiota in the human intestine and understanding spore germination is a main thrust of this work. In terms of the adult gut microbiome, as much as 50% of the bacterial community have spore-forming potential and are likely shared between individuals. The project will obtain new detailed knowledge of interactions between an IM complex of GRs plus GerD termed the germinosome with the IM SpoVA channel, as well as interactions among SpoVA proteins and their movements. Given the major roles these two components play in germination, this work will give new insight into mechanism of germination, notably signal transduction, and could allow development of new strategies to either inhibit or promote spore germination. The possible outcomes of the research including the identification of novel antibiotic targets aimed at inhibiting organisms that can cause diseases and promote spore germination and outgrowth for beneficial microbes leading to development of new antimicrobials strategies. This research theme holds immense socio-economic impact in improvising food security and human health for both Pakistan and the Netherlands by increasing knowledge about germination leading to new strategies to modulate germination; this could have major benefits to the food industry, among others.

4. **Resources required to complete your proposed project**

This project will be supervised by Prof Stanley Brul, Director of Biomedical Sciences in the University of Amsterdam and co supervised by Prof Peter Setlow in Department of Molecular Biology and Biophysics at the University of Connecticut Health Centre and will also be benefited from their internal and external research collaborative networks. They will provide access to essential facilities and techniques, such as Phase contrast and fluorescence microscopy, spore tracker, FRET, Molecular Docking etc for completion of this project. The research will be supported by an appropriate PhD Scholarship. The host institute will support this PhD program by providing an outstanding and supportive research environment, intellectual support from world-leading researchers as well as advanced equipment and facilities.

5. **Timeline for completing major research tasks** (for example, gathering data, fieldwork, thesis writing)

Candidature phase and expected duration	**Major Tasks**
Commencement to confirmation (1st year PhD)	Preparation of SpoVA fluorescent fusion Proteins and assessment of their functionality in Ca-DPA uptake
Confirmation to mid-candidature review (2nd year PhD)	Analysis of SpoVA protein colocalization with germnosome constituents by fluorescence microscopy and FRET analyses
Mid-candidature review to thesis review (3rd year PhD)	Analysis of movement of fluorescent SpoVA fusion proteins in dormant spores, further identification of other germination proteins they interact with, and examination of the behavior of the SpoVA protein fusions in germinated spores.
Thesis review to submission (4th year PhD)	Collection all data and prepare to submit the final edited thesis.

6. **Who is the 'owner' of the proposed research project and intended PhD supervisor**?

Title and Name	**Email Address**
Professor Stanley Brul	s.brul@uva.nl
Professor Peter Setlow	setlow@nso2.uchc.edu