SCIENCES & BIOENGINEERING SCIENCES

The Research Group
Structural Biology Brussels

has the honor to invite you to the public defence of the PhD thesis of

Brajabandhu Pradhan

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

Structural Characterisation of Endospore Appendages (ENA) and the bacterial functional amyloid curli

Promotor:
Prof. dr. Han Remaut

The defence will take place on
Friday, August 25, 2023 at 16h in auditorium I.0.01

The defence can also be followed through a live stream:
https://us06web.zoom.us/j/82708840601?pwd=bUhJTUNHS1BvSG54a3dHNUE1MEQrZz09, Meeting ID: 827 0884 0601, Passcode: Dr-Braja

Members of the jury
Prof. dr. ir. Geert Angenon (VUB, chair)
Prof. dr. Peter Tompa (VUB, secretary)
Prof. dr. Charlotte Martin (VUB)
Prof. dr. Stephen Matthews (Imperial College London)
Prof. dr. Marina Aspholm-Hurtig (Norwegian University of Life Sciences)

Curriculum vitae

Brajabandhu Pradhan earned his Master of Science in Biology from NISER, Bhubaneswar, an affiliate of Homi Bhabha National Institute in Mumbai, India. Following a brief research stay at NCBS, Bangalore, he pursued his doctoral studies under the guidance of Prof. Han Remaut, focusing on bacterial pili structures using Cryo-EM.
During his PhD, Brajabandhu co-authored 2 peer-reviewed research articles with one co-first authorship, 1 preprint, and contributed to 2 review articles in international journals. His work on endospore appendages led to a patent application. He presented posters and/or short talks at 5 (inter)national conferences and workshops, in one of which he won the best speaker award. Brajabandhu also co-organized an international PhD symposium and assisted in practical courses and summer schools for bachelor's and master's students.

Abstract of the PhD research

Bacteria frequently interact with their environment through fibrous protein appendages on their cell surface. Bacterial appendages like flagella and pili are used in various biological processes including motility, adhesion, multicellular organisation, protein and DNA uptake and export, and/or electron conductance. Besides a general interest as important virulence factors, there is a growing interest in the self-assembling and material properties of bacterial appendages. In his PhD research, Brajabandhu Pradhan studied the structural characteristics and assembly principles of two unique classes of bacterial appendages: the functional amyloid curli, and ENdospore Apendages (ENAs) – a novel classes of pili identified on the spores of pathogenic Bacilli.

Although well studied in vegetative cells, little is known about the presence, function and composition of pili and appendages found on the surface of bacterial endospores. The latter are a dormant differentiation state of many Firmicutes, with remarkable resilience under harsh physicochemical conditions. By analysing the surface of endospores of the food poisoning outbreak strain *Bacillus cereus* NVH 0075-95, this study identified two morphologically distinct proteinaceous appendages: staggered and ladder-like Endospore Appendages, or 'S-ENA' and 'L-ENA', respectively. Using Cryogenic Transmission Electron microscopy (Cryo-EM) and helical reconstruction, the 3D structure of S-Ena was determined to a resolution that allowed unambiguous, partial sequence assignment of composing subunits. Genome mining and experimental validation subsequently allowed the annotation of a three gene cluster – *Ena1A, Ena1B* and *Ena1C* - as responsible for the assembly of S-ENA fibers, uncovering a novel class of bacterial pili unique to pathogenic Bacilli. Although the function of ENA remains unknown, this work provides a discussion and experimental interrogation of some hypothetical functions such as spore aggregation and adherence to biological surfaces.

A second part of the work centers around the structure and assembly of the functional amyloid curli. Curli form a major component of the extracellular matrix in proteobacterial biofilms. The safe self-assembly of curli, i.e. without the formation or build-up of cytotoxic species typically seen in pathological amyloids, forms a major point of interest. Using *de novo* structure prediction and cryo-EM analysis this work establishes the architectural principles of curli, establishing that subunits adopt a β-solenoid structure and fall into four general assembly classes. This is a fundamental property that differentiates functional amyloid curli from their sister, pathological amyloids.

Both ENA and curli fibers have structural properties that make them interesting scaffolds for functionalized protein nanowires, with prospective applications, amongst others, in textile industry, agroindustry and medicine.