SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels

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

Clémence Whiteway

to obtain the degree of Doctor of Sciences

Title of the PhD thesis:

The polysaccharide capsule of *Acinetobacter baumannii*: roles and regulation

Promotor:
Prof. dr. Charles Van der Henst

The defence will take place on
Friday, December 1 2023, at 16h00 in auditorium D.2.01 at the VUB (Etterbeek), pleinlaan 2, Building D, 1050 Brussels (BE)

Members of the jury
Prof. dr. Dominique Maes (VUB, chair)
Prof. dr. Joske Ruytinx (VUB, secretary)
Prof. dr. apr. Thomas Demuyser (VUB)
Prof. dr. Tom Coenye (UGent)
Prof. dr. Eduardo Rocha (Institut Pasteur, Frankrijk)

Curriculum vitae

In 2019, Clémence Whiteway obtained her master's degree in Molecular and Cellular Biology with a specialization in Fundamental Microbiology at the Sorbonne/ Pierre et Marie Curie University in Paris, France. Next, she started her PhD journey at the VUB under the supervision of Prof. Charles Van der Henst in the Microbial Resistance and Drug Discovery group (VUB-VIB Center for Structural Biology). During these four years, her work led to the publication of one first-author paper and of a quick guide on *Acinetobacter baumannii.* She also collaborated and contributed to diverse now-published studies.
During her PhD, she guided one bachelor, and two master's students and participated in the organization and supervision of research rotations in the MRDD group.

Abstract of the PhD research

Acinetobacter baumannii is a Gram-negative bacterium considered one of the most concerning bacterial pathogens for human health and for which research and development of new antimicrobials are a priority. Despite its clinical relevance, knowledge about its virulence and non-antibiotic resistances is still limited. However, among its identified virulence factors, the production of the polysaccharide capsule is known to be critical to protect it from the host immune defenses, diverse antimicrobials, and desiccation. Most of the genes required for capsule biosynthesis and export are regrouped in the chromosomal capsule locus (K-locus). Over 237 unique K-loci sequences (KL) have been identified so far with a wide diversity in the composition and structure of the capsule between isolates. In addition, the regulation of capsule production itself generates phenotypic heterogeneity in homogenous clonal populations.

I characterized a natural mutant of *A. baumannii* showing a stable translucent phenotype on a solid medium. Bioinformatic analysis of the K-locus revealed the presence of an IS*Aba13* insertion sequence, interrupting the gene encoding for the initial glycosyltransferase ItrA, a key protein for O-linked protein glycosylation and capsule biosynthesis. By using transmission electron microscopy and a colloidal silica gradient to semi-quantify capsule production, I showed that this mutant is non-capsulated and by using *Galleria mellonella,* that it is not virulent *in vivo*. Using a genetic approach, I demonstrated that this insertional inactivation is fully responsible for the observed phenotypes. Finally, I showed that the non-capsulated and avirulent *itrA::ISAba13* mutant can revert to a capsulated and virulent state upon scarless excision of IS*Aba13* under stress conditions. Next, I investigated how the presence or absence of the capsule can positively or negatively impact different biological processes of *A. baumannii* such as resistance to phagocytosis, natural transformation, and adhesion. I generated and characterized different capsule mutants for capsule production, virulence *in vivo,* and growth.

Finally, I assessed how these mutants and several clinical isolates with different capsulation levels adhere to polystyrene plates and epithelial cells using microscopy. I determined that capsule production can negatively impact adhesion to biotic and abiotic surfaces in our experimental conditions.

This thesis aimed to build a better understanding of capsule regulation and its involvement in the pathogenesis of *A. baumannii* and will contribute to the design of new tools and strategies against its resistance arsenal.