New tools and insights in physiology and chromosome dynamics of Clostridioides difficile

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Stellingen behorend bij het proefschrift

New tools and insights in physiology and chromosome dynamics of *Clostridioides difficile*

1. The extracellular location of TcdC is not compatible with direct binding of the OB-fold domain to intracellular nucleic acid or protein targets and suggests a mechanism of action that is different from previously characterized anti-sigma factors. *(This thesis, Chapter 3)*

2. The overall origin organization and mechanism of DNA replication initiation is likely to be conserved within the Firmicutes. *(This thesis, Chapter 4)*

3. *C. difficile* autofluorescence might result from direct oxidation of specific cell components, which may vary in abundance dependent on growth phase or cell cycle stage. *(This thesis, Chapter 6)*

4. Since HU-family proteins can act differently *in vivo* despite high amino acid sequence similarity, it is necessary to study the role of these proteins in each organism. (*This thesis, Chapter 4 and 5*)

5. Bacteria are not bags of randomly distributed molecules but demonstrate a complex and highly structured subcellular organization. *(Cambré A and Aertsen A. Microbiol Mol Biol Rev. 2020)*

6. *C. difficile* was originally called that way owing to its "slower growth and less striking physiologic properties" *(Hall IC and O'Toole E. American Journal of Diseases of Children. 1935)* and is still a difficult and challenging organism to study.

7. Anaerobic microbial systems are underexplored by fluorescence microscopy and a larger effort is needed to engineer genetically encoded substrate-independent fluorophores for use in anaerobic bacteria. *(Chia et al. Curr Opin Chem Biol. 2019 and this thesis)*

8. Though TcdE isoforms and Cwp19 have been implicated in toxin release from *C. difficile*, it is unclear if these proteins are part of a secretion mechanism. *(Govind R. et al. J Bacteriol. 2015 and Wydau-Dematteis S. mBio. 2018)*

9. The microscope opens a door to see and explore the wonders of the microscopic world, but making relevant observations always depends on the eye and experience of the researcher. *(Inspired by the work of Antonie van Leeuwenhoek and Thomas Bonney)*

10. Collaboration and improvisation are tools for successful research. *(Inspired by Charles Darwin and MacGyver series)*

11. No matter where we are and no matter what the question is, the answer is always "coffee first".

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