Title

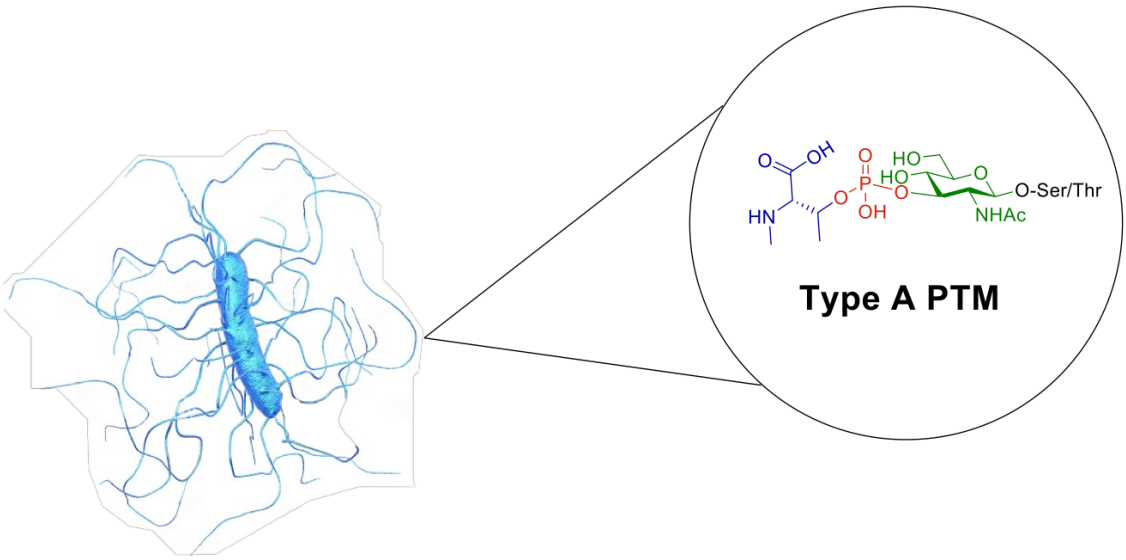
Deciphering the biosynthesis of the type A PTM of the flagellum of the *Clostridioides difficile*

Authors

B. van Puffelen, A.N.A Ammerlaan, Z.X. Su, D.V. Filippov, P.J. Hensbergen (LUMC), J.D.C. Codée

poster presentation

Bacterial flagella play a crucial role in a broad range of processes, varying from motility to adherence. Strain 630 *Δerm* of the *Clostridioides difficile* relies on a unique post-translational modification of flagellin, the protein that makes up the flagellum fragment. This post-translational modification is vital for bacterial motility and virulence, the biosynthesis of this post-translational modification is, however, not yet fully understood. We synthesized proposed bacterial metabolites, cytidine diphosphate (methyl) threonine, and mimetics thereof. Hence, these were submitted to the relevant biosynthesis proteins and thus evaluated with quantitative mass spectrometry-based proteomics to elucidate their roles in the modification process. In addition, the type A PTM was installed on a single serine moiety and compared to biological material using NMR spectroscopy. Our results indicated that CDP-(methyl) threonine is a superior donor substrate for the phosphotransferase (CD2044) compared to CDP-Threonine, suggesting that methylation of threonine occurs prior to the transfer of phosphothreonine.



References (*max 3*)

1. Claushuis, B., et al., *Revised Model for the Type A Glycan Biosynthetic Pathway in Clostridioides difficile Strain 630Δerm Based on Quantitative Proteomics of cd0241−cd0244 Mutant Strains*. ACS. Infect. Dis., 2023, **9**: p. 2665-2674
2. Faulds-Pains, A., et al., *The post-translational modification of the Clostridium difficile flagellin affects motility, cell surface properties and virulence.* Mol microbiol., 2014, **94**: p. 272-289
3. Engelsma, S.B., et al., *Combined Phosphoramidite-Phosphodiester Reagents for the Synthesis of Methylene Bisphosphonates*, Angew. Chem. Int. Ed. 2017, **56**: p. 2955-2959