

Enzymatic Ligation of Pyruvylated Secondary Cell Wall Glycopolymer to Peptidoglycan as an Essential Step for Bacterial Cell Wall Assembly

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The cell wall macromolecule peptidoglycan of Gram-positive bacteria is modified with anionic secondary cell wall polymers (SCWP) which are essential for bacterial virulence and survival, and serve as a rich source for both validated and unexploited pathways for the design of novel antibacterial strategies. We characterized the structural basis of the cell surface display mechanism of SLH-trimers *via* the β -D-ManNAc epitope of SCWP in the model bacterium *Paenibacillus alvei*.

To understand how the pyruvylated SCWP is transferred to peptidoglycan, four candidate enzymes from the LytR-CpsA-Psr (LCP) protein family encoded on the *P. alvei* genome will be investigated. Notably, LCP enzymes are under current investigation as antibacterial targets against *Staphylococcus aureus*. Using a portfolio of synthetic SCWP intermediates from the *P. alvei* SCWP biosynthesis pathway we will investigate in *in vivo* and *in vitro* approaches the functionality of the *P. alvei* LCPs for transfer of SCWP from lipid-linked precursors to peptidoglycan and the role of pyruvylation. This includes confirming the activity of *P. alvei* LCP proteins in *Bacillus subtilis* and the development of an *in vitro* *P. alvei* LCP assay based on optimized protocols for LCP protein production and purification, using synthetic SCWP-intermediates with different lipid portions and degrees of pyruvylation and testing native and non-cross-linked PGN as acceptors. Select functional *P. alvei* LCP proteins shall be co-crystallized of a with its synthetic substrates

This work will lead to a better understanding of how bacteria functionalize their cell wall with an SCWP other than commonly investigated wall teichoic acids. The outcome of this work can serve to aid drug discovery and development programs targeting LCP enzymes.