

## CELL WALL PROTEOMICS REVEALS A STRONG ASSOCIATION OF ACTA TO THE PEPTIDOGLYCAN PROMOTING *LISTERIA MONOCYTOGENES* INVASIVENESS

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The envelope of Gram-positive bacteria consists of a thick (20-80 nm) peptidoglycan layer decorated with a bulk of proteins and associated polymers. This structure is the interface with the external milieu and constantly readjusts its composition to facilitate adaptation to environmental fluctuations. Here, we used high-accuracy mass spectrometry to monitor the cell wall proteome of the Gram-positive pathogen *Listeria monocytogenes* in growth media containing different nutrients. A surface protein not previously reported to strongly associate to peptidoglycan, the actin-assembly inducing protein ActA, was identified in peptidoglycan purified from bacteria growing in a chemically defined minimal medium. Targeted proteomics of ActA-derived tryptic peptides by multiple reaction monitoring (MRM) revealed that the protein form binding strongly to the peptidoglycan is tethered to the membrane. In this conformation, ActA is exposed on the cell surface and promotes efficient bacterial entry into non-phagocytic eukaryotic cells. ActA was also identified in peptidoglycan of intracellular bacteria upon infection of cultured epithelial cells, implying a strong ActA-peptidoglycan association when actin and other cytoskeleton proteins are recruited by the bacteria. In addition, high-accuracy mass spectrometry allowed the identification of 13 novel *L. monocytogenes* surface proteins that are covalently bound to the peptidoglycan by cleavage of their LPXTG sorting motif. A total of 24 and 15 distinct protein species of this family were identified in peptidoglycan purified from extra- and intracellular bacteria, respectively. New surface proteins related to peptidoglycan metabolism were also identified. Altogether, our data support a novel ActA cell wall association modulating *L. monocytogenes* invasion of eukaryotic cells and reveal high-accuracy mass spectrometry as a powerful proteomic technique for identifying new bacterial surface proteins strongly bound to the peptidoglycan.