## Abstract

**Human gut bacteria tailor extracellular vesicle cargo for the breakdown of diet- and host-derived glycans**

Seminar Series: Extra-ordinary seminar Infection Biology

Extracellular vesicles (EV) are produced in all three domains of life, and their biogenesis have common ancient origins in eukaryotes and archaea. Although bacterial vesicles were discovered several decades ago and multiple roles have been attributed to them, no mechanism has been established for vesicles biogenesis in bacteria. For this reason, there is a significant level of skepticism about the biological relevance of bacterial vesicles. Bacteroides thetaiotaomicron (Bt), a prominent member of the human intestinal microbiota, produces significant amounts of outer membrane vesicles (OMVs) which have been proposed to play key physiological roles. Here we employed a dual marker system, consisting of outer membrane- and OMV-specific markers fused to fluorescent proteins to visualize OMV biogenesis by time-lapse microscopy. Furthermore, we performed comparative proteomic analyses to show that, in Bt, the OMV cargo is adapted for the optimal utilization of different polysaccharides. We also show that a negatively-charged N-terminal motif acts as a signal for protein sorting into OMVs irrespective of the nutrient availability. Our results demonstrate that OMV production is the result of a highly regulated process in Bt. We performed a screening to identify the machinery involved in vesiculation. We found that mutation of the anti-sigma factor DMA1 leads to hypervesiculation. Dma1 is encoded adjacent to an ECF21 family sigma factor, which is required for the hypervesiculation phenotype. Dma1 presents an unprecedented domain architecture, as it spans both the inner and outer membrane directly connecting the exterior of the cell with the bacteria cytoplasm. Further analysis revealed that orthologs of Dma1 are present throughout Bacteroidota. Our findings provide mechanistic insights into OMV biogenesis in Bacteroides spp.

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