

P024 - Detection and Characterization of Carbonic Anhydrases in the Outer Membrane Vesicles (OMVs) Released by Helicobacter pylori in the Planktonic and Biofilm Phenotypes

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Introduction: Biofilm represents a successful strategy for *Helicobacter pylori* survival. Current studies report that the EPS matrix of *H. pylori* biofilm is mainly constituted by proteomannans, LPS-related structures, extracellular DNA (eDNA), proteins and Outer Membrane Vesicles (OMVs). The OMVs are spherical bilayered structures (20-250 nm in diameter) which are released by the microorganisms during their growth and have a key role in *H. pylori* biofilm formation. A recent study analyzed the exoproteome of *H. pylori* at different growth phases in the planktonic phenotype. Among the 74 proteins selectively released by OMVs, the Carbonic Anhydrase (CA) was identified, however, no indication of the family was provided. Intriguing, the pathogen uses its CAs belonging to the alpha- and beta-families for the acid acclimation within the human stomach and thus, for the bacterial survival in the host. The aim of the present study was the detection and characterization of CA in the OMVs generated in the planktonic and biofilm phenotypes over time by *H. pylori*.

Materials and Methods: The strains used in the study were *H. pylori* NCTC11637 and 4 clinical strains, *H. pylori* 190, *H. pylori* F1 and *H. pylori* F4 characterized by a different antimicrobial susceptibility pattern. *H. pylori* biofilm formation was evaluated at 2, 6 and 10 days of incubation using Live/dead staining and Fluorescence microscopy. The OMVs from the planktonic (pOMVs) and biofilm (bOMVs) phenotypes were isolated by ultracentrifugation. The detection and quantification of CA associated with pOMVs and bOMVs were evaluated, at each time point, by protonography and mass spectrometry.

Results: *H. pylori* strains developed a mature biofilm already after 2 days of incubation. The presence of CA was detected in both pOMVs and bOMVs, however, the amount of CA in the pOMVs was greater than in the bOMVs except for the *H. pylori* 190. Furthermore, the content of CA increases over time in the pOMVs. The mass spectrometry analysis demonstrated that the CA delivered by OMVs in an isoform of the alpha-carbonic anhydrase family, which has a periplasmic localization.

Discussion and Conclusions: The detection and characterization of CA in pOMVs and bOMVs together with the novel finding of increased production of CA over time, shed new light on the role of this enzyme in the colonization, survival, persistence and pathogenesis of *H. pylori*.