Molecular and structural characterization of a ***Vibrio cholerae*** **O1 phosphoporin**

Lery, L.M.S.; Goulart, C.L.; Bisch, P.M.; von Krüger, W.M.A.
Unidade Multidisciplinar de Genômica – IBCCF - UFRJ - Rede Proteoma do RJ

Comparison of 2D protein expression patterns of *Vibrio cholerae* O1 569B and its isogenic *pho**B* mutant, under inorganic phosphate (Pi)-limitation, showed the product of *vca1008* among the differentially expressed by the wild-type. VCA1008, an outer membrane protein (Omp), is essential for the intestinal colonization, what could explain the inability of the *pho**B* mutant to colonize animal models. Electrophoretic analysis of outer membrane preparations under non-denaturing conditions revealed that VCA1008 form trimers. However, when submitted to heat treatments it dissociates in monomers of 33.9kDa e pI 4.76. VCA1008 theoretical sequence has a higher MW (35.9 kDa). The loss of 2.0kDa is compatible with the SignalP analysis, which predicted a signal peptide of 21 residues and was confirmed by the determination of VCA1008 N-terminal sequence by Edman degradation. Further analysis revealed that VCA1008 is a homologue of several phosphoporins, with 28% identity to PhoE, a Pi-starvation induced anionic porin of *E. coli*. COG analysis grouped VCA1008 among porins in agreement Pfam, which placed VCA1008 among the β-barrel forming Omps. Secondary structure analysis revealed 16 beta strands, 7 turns and 8 loops and a large loop L3 containing the domain GTFTGD, a hallmark of classical porins. Furthermore, VCA1008 has four of five conserved lysine residues, responsible for the anionic selectivity of PhoE^{Ec} (K¹⁸, K²⁹, K⁶⁴, K⁸⁴, K¹²⁵). Taken together, these results suggested that VCA1008 is a porin homologue to PhoE^{Ec}. Acknowlegment: FAPERJ, CNPq, FINEP/MCT.