FINDING A SUBSTRATE OF THE TYROSINE PHOSPHATASE PtpA FROM *MYCOBACTERIUM TUBERCULOSIS* USING THE SUBSTRATE-TRAPPING METHOD

Villarino, A.[1], **Purificação, M.[1]**; Ferreira, A.M.[2]; Ramos, A.L.[2]; Terenzi, H.[1]
[1]Laboratório de Expressão Gênica, Dep. Bioquímica, CCB, UFSC, Florianópolis, Brazil; [2]Cátedra de Inmunología, Facultad de Ciencias, UR, Montevideo, Uruguay

Protein tyrosine phosphatases (PTPs) of several microorganisms may have an important role in the determination of the pathogenicity by modifying the phosphorylation/dephosphorylation equilibrium in their host. Thus, the identification of PTPs substrates is an essential step to understand their physiological role, and also contribute to the design of new drugs. In this study the substrate-trapping method was used as a tool to identify the putative physiological substrate of PtpA, one of the only two PTPs present in *Mycobacterium tuberculosis*. The mutation of the Asp126 in the active site of PtpA converts the active enzyme into a "substrate-trapping" enzyme: PtpA D126A. The wild-type and mutated PtpA were purified and characterized by kinetic studies. The mutant was shown to be a good "substrate-trapping" tool as its Km value is similar to the Km of the wild-type-PtpA but the Vmax is reduced, allowing the retention of the putative substrate in the active site for a longer period. To identify substrates of PtpA, *in vitro* assays were performed, by incubating the PtpAD126A with different protein extracts from human macrophages THP-1. The enzyme-substrate complexes were isolated by affinity precipitation and analyzed by SDS-PAGE. Assays are in progress to reproduce results and resolve bands by 2D electrophoresis and proceed to mass spectrometry identification of the putatives substrates of mycobacterial PtpA.

Supported by: CNPq/CAPES/MCT/FINEP/FAPESC