## In vitro evolution of sugarcane cystatins

## Flavio Henrique Silva

Department of Genetics and Evolution Federal University of São Carlos, São Carlos, SP, Brasil

Cystatins are natural inhibitors of cysteine proteinases. Their inhibitory activity is explained by the presence of three contact points with the proteinase targets. The first point is an N-terminal glycyl-containing segment that interacts with the S1 and S2 enzyme sub-sites: the second is a hairpin loop containing a QVV motif that is highly conserved in cystatin superfamily and the third is a hairpin loop formed by a tryptophan-containing segment. Plant cystatins present some structural peculiarities and are called phytocystatins. These proteins play a defensive role in plants because of their effect on exogenous proteinases such as those produced by fungi, insects and nematodes. We have described and characterized the first sugarcane cystatin. This protein is able to inhibit plant pathogenic fungi, as well as several cysteine proteinases, such as human cathepsins (e.g. cathepsin K, L, V and others). We performed in vitro evolution experiments using sugarcane cystatins in order to have cystatins with improved inhibitory activities. Some recombined clones obtained after DNA shuffling were analyzed in regards to their activity. These clones showed distinct activity and selectivity when compared with the wild type genes used in the experiment. These experiments are providing us with insights regarding the relationship between the structure and function of cystatins.