Cloning and expression of frutalin, an alpha-D-galactose-binding plant lectin, in *Pichia pastoris*

C. Oliveira[1], W. Felix[2], R. A. Moreira[2,3], J. A. Teixeira[1] and L. Domingues[1]

[1] IBB Institute for Biotechnology and Bioengineering, Universidade do Minho, Portugal
[2] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Brasil
[3] Centro de Ciências da Saúde, Universidade de Fortaleza, Brasil

Frutalin is the alpha-D-galactose-binding Jacalin-related lectin from *Artocarpus incisa* seeds. This plant lectin was successfully used on the recognition of cancer metastasis, specifically those of breast and thyroid gland, due to its ability to interact with galactose complexes and thus potentially combine with cancer cells surfaces containing these sugars. It can be potentially used as a histochemical probe in the diagnostic of several cancers. However, Frutalin extraction from seeds is a long process with low yields and a mixture of many isolectins can be obtained. Frutalin is thought to be synthesized as a pre-pro-lectin, consisting of a signal sequence, a pro-peptide, a 20-amino-acid beta-chain, a 4-amino-acid linker peptide and a 133-amino-acid alpha-chain. In mature Frutalin, the signal sequence and the pro-peptide may be removed through post and/or co-translational processing and the linker excised to generate two chains, alpha and beta. Active Frutalin consists of a tetrameric protein and each one of its subunits is made of one alpha chain and one beta chain non-covalently linked.

The aim of this work is the production of recombinant biologically active Frutalin in the methylotrofic *Pichia pastoris* KM71H yeast strain. Frutalin synthetic gene, containing codons in preference in *P. pastoris*, was obtained using base synthesis and PCR approaches. Optimized codifying Frutalin sequence was cloned into the pPICZalphaA expression vector that contains the *Saccharomyces* alpha-factor preprosequence to direct recombinant protein into the secretory pathway. Soluble recombinant Frutalin was detected in the culture supernatants after optimized batch culture conditions. Frutalin was expressed as a single chain as the 4-amino-acid linker peptide (T-S-S-N), that connects α and β chains, was not cleaved. Furthermore, incomplete processing of the signal sequence resulted in recombinant Frutalin with one Glu-Ala N-terminal repeat derived from the alpha-factor prosequence, and part of recombinant Frutalin was highly N-glycosylated. Nevertheless, recombinant Frutalin was recognised by native Frutalin antibody and its ability to bind galactose was maintained. The recombinant lectin was purified on an *Adenanthera pavonina* cross-linked galactomannan column taking advantage of the galactose-binding property. Immunohistochemical studies for cancer diagnostic are now being conducted with purified recombinant Frutalin.