

Expression of frutalin in *Pichia pastoris* and evaluation of its potential as a cancer marker

Oliveira, C.¹, Duarte, M.², Felix, W.³, Moreira, R.A.^{3,4}, Teixeira, J.A.¹, Schmitt, F.^{2,5,6} and Domingues, L.¹

1. IBB – Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, Braga
2. Life and Health Sciences Research Institute (ICVS), School of Health Sciences, Universidade do Minho, Campus de Gualtar, Braga
3. Departamento de Bioquímica e Biologia Molecular, Centro de Ciências, Universidade Federal do Ceará, Brazil
4. Centro de Ciências da Saúde, Universidade de Fortaleza, Brazil
5. IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto
6. Faculdade de Medicina da Universidade do Porto
carlaoliveira@deb.uminho.pt

One of the most interesting biological properties of frutalin (α -D-galactose-binding jacalin-related lectin from *Artocarpus incisa* seeds) is its potential use in cancer diagnostic due to its ability to interact with galactose complexes of cancer cells surfaces. Hence, the availability of frutalin in large scale will be necessary to facilitate its further application. However, its isolation and purification from plants is time-consuming and results in low yields, as well as in a heterogeneous mixture of different lectin isoforms. To overcome this limitation, frutalin optimized synthetic gene was cloned and expressed in the methylotrophic yeast *Pichia pastoris*. Frutalin was expressed in *P. pastoris* as a single chain protein since the 4-amino-acid linker peptide, that connects α and β chains, was not cleaved. Moreover, the signal sequence used, the *Saccharomyces* α -factor preprosequence, was not completely removed and part of recombinant lectin was highly N-glycosylated. Nevertheless, recombinant lectin ability to bind galactose was maintained. Preliminary immunohistochemical studies for evaluate its potential as a cancer marker revealed that recombinant frutalin is able to recognize prostate cancer cells in the same conditions as native frutalin. It preferentially binds prostate neoplastic cells, rather than hyperplastic and normal cells, showing its potential as tumour marker, underlying its putative role in diagnosis of prostate cancer.