Production and characterization of polygalacturanase from *Aspergillus niger* and its fusions with hydrophobin and ELP tag using *Nicotiana benthamiana* as bioreactor

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Plant molecular farming represents a next generation of plant-made proteins for diverse purposes including industrial applications in bioconversion of lignocellulosic biomass. The use of tobacco constitutes an ideal system for analysis and production of recombinant enzymes due to its low cost of production and an almost unlimited scale-up potential. One of the main polysaccharides of the primary cell wall, pectins are thought to restrict the access of cellulases to their substrate. In this study, we analysed the expression levels of *Aspergillus niger* polygalacturanase I (*An*PG I), an enzyme that catalyze the hydrolysis of 1,4 α-D galacturonic acid linkages in the smooth region of pectin. The recombinant protein was targeted to five different compartments: ER, vacuole, chloroplast, cytosol and apoplast. Accumulation level of 3.0%, 2.5% and 1.9% of total soluble protein (TSP) were observed in the ER, apoplast and vacuole, respectively. Interesting, the recombinant protein did not show accumulation when targeted to chloroplast and cytoplast. This may be due to the need of post-translational modifications which normally occur in the secretory pathway. Ideally, for the saccharification of plant cell walls, crude plant extract containing polygalacturanase should suffice. However, for cases where the protein needs to be purified, fusions of *An*PG I with elastin-like polypeptide (ELP) and hydrophobin (HFBI) were also analysed. Analysis of *An*PGI::ELP fusion targeted to the ER and vacuole showed a significant increase in the level of protein accumulation to 3.6% of TSP in either compartment. However, when hydrophobin was used as fusion partner a negative impact was observed with level of accumulation dropping to 2.4% and 0.2% of TSP for ER and vacuole, respectively. As well, the self release of reducing sugars was observed in plant leaves expressing the recombinant protein. These results will be discussed in the context of enzymatic digestion of cellulosic biomass for biofuel production.