Picea glauca defensin expression by Phage Display as antifungal agent against *Nectria galligena*

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Defensins are small peptides ubiquitously found on eukaryotes that present antiviral and/ or antifungal activities against several pathogens mainly acting on cell membrane permeabillization. A plant defensin from *Picea glauca* (PgD1) has been previously cloned and expressed where preliminary studies have shown its antifungal activity against *Nectria galligena*. *N. galligena* infections are of economic importance for Santa Catarina State, since apple production is an important regional economic activity and apple trees are highly susceptible to *N. galligena* infections, causing fruit rot and canker formation. Defensins are thermal stable peptides due to the presence of at least three disulfide bonds, whose formation hampers significant heterologous expression and therefore its general use as antifungal agent. The goal of this work is to express *P. glauca* defensin PgD1 on M13 phage particle by Phage Display technique. We hypothesize that secreted production of defensing displayed on phage particles will improve disulfide bond production, since viral particles are assembled within the periplasmic region, and protein secretion to the extracellular media will help the peptide purification for subsequent antifungal treatment strategies. PgD1 gene was amplified by PCR and cloned into Pgem-T-Easy cloning vector. The gene was subsequently inserted into phagomyde APIII6 by restriction digestion and ligation using *Hind* III and *Sal* I restriction enzymes. Recombinant clones have been submitted to sequencing in order to assure their identity the absence of inserted mutations on defensing gene. Defesin displayed phage particles are currently being produced in order to test against *in vitro* cultures of *N. galligena.* Control experiments will be performed using M13 helper phage.

Key-words: defensin, *Picea glauca*, *Nectria galligena*, phage display.