Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 7.09

UNDERSTANDING THE ROLE OF AN EXTRACELLULAR ENDO-1,4-β-GLUCANASE IN THE *PYRENOCHAETA LYCOPERSICI*-TOMATO PATHOSYSTEM

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Pyrenochaeta lycopersici, cell wall degrading enzymes, endo-1,4-β-glucanase, pathogenicity

Many fungal plant pathogens secrete an array of cell wall degrading enzymes mainly involved in the pathogenesis. An extracellular endo-1,4-β-glucanase (named PlEGL1) from the causal agent of the Corky Root Rot (CRR) of tomato, *Pyrenochaeta lycopersici*, was isolated and characterized. *Plegl1* gene is strongly induced during the disease. We are currently investigating its putative role in the pathogenesis and its mechanism of action. We have sequenced *Plegl1* from 18 *P. lycopersici* isolates from different geographic areas of Italy and other world countries. Surprisingly we found that *Plegl1* coding sequence was identical in all the tested isolates, maybe suggesting a key role of this enzyme in the host-pathogen interaction.

In order to obtain preliminary data about the potential of the fungal cellulolytic activity in the virulence on its host, we set up a leaf infiltration assay using *P. lycopersici* culture filtrates with and without cellulolytic activity. The area of the leaf treated with the fungal filtrates becomes chlorotic in 2-5 days which evolves to necrotic lesions, in about 10 days, only with the filtrate having cellulolytic activity. The result shows that both fungal filtrates contain phytotoxins but the cellulase-containing filtrate develops necrosis faster than the other one, suggesting that the cellulolytic activity can be implicate in the pathogen virulence. We investigated the potential hydrolytic activity of *P. lycopersici* cellulolytic filtrate on the bacteria and fungal cell walls by an antibiogram assay for the bacteria and by a spore germination inhibition assay for the fungi. The results were none activity, in the tested conditions, toward the assayed bacteria and fungi, showing that the fungal cellulolytic ability is plant cell wall specific. These data are confirmed by a polysaccharide affinity precipitation assay carried out with the recombinant PlEGL1: chitosan and two types of cellulose, water-soluble and insoluble, were used as substrates and the results showed that the enzyme recognizes only the cellulose-based substrates.

In silico studies showed that PlEGL1 belongs to glycoside hydrolase family 61 (GH61): this enzymes are known to have a poor cellulolytic power but it was shown that they can dramatically enhance the activity of other cellulases in synergism assays. These data are in accordance to the results of the recombinant PlEGL1 acivity *in vitro*, that shows a moderate cellulolytic ability on carboxymethylcellulose substrate. For these reasons the only way to understand the real role of PlEGL1 in the cellulolytic arsenal of *P. lycopersici* is by silencing *Plegl1* expression. We are setting up a gene replacement protocol mediated by *Agrobacterium tumefaciens* transformation. The flanking genomic sequences of *Plegl1* available coding region were obtained by TAIL (Thermal Asymmetric InterLaced) PCR and they will be used for the gene replacement vector construction.