Cell Wall Biology in Oomycete Pathogenicity (CBOP)

The oomycete phylum is a group of eukaryotic microorganisms that comprises plant pathogens responsible for severe environmental damage and economic loss. Members of the genus Phytophthora infect woody plants in natural ecosystems and a wide range of agriculturally important crops such as potato, tomato and soybean. *Phytophthora infestans* is the causal agent of potato and tomato late blight and, as such, one of the most significant agricultural pathogens. This species was responsible for the Irish potato famine in the mid-19th century and has had a tremendous impact on the history of human kind, resulting in malnutrition, famine and population displacement. It continues to impact world agriculture by causing the most destructive disease of potato. When left unchecked, the pathogen can destroy a potato field in a few days. Annual crop losses due to *P. infestans* represent billions of dollars globally. Potato is the fourth most important food crop in the world and an alternative to the major cereal crops for feeding the increasing world population. The area used for potato production is rising faster worldwide than for any other crop due to its high yield potential and excellent nutritional characteristics. The global production of potato, however, is seriously challenged by the susceptibility of the cultivars to *P. infestans*. Management of the pathogen is challenged by its remarkable speed of adaptation to control strategies such as genetically-resistant cultivars. Thus, the development of alternative strategies to tackle *P. infestans* has become a priority for the agriculture industry. The main objective of the CBOP program was to identify specific protein targets for disease control, with a particular focus on enzymes involved in cell wall formation. The pathogen cell wall is vital for the microorganism and blocking its biosynthesis leads to cell death, thereby protecting the host crop from the disease.

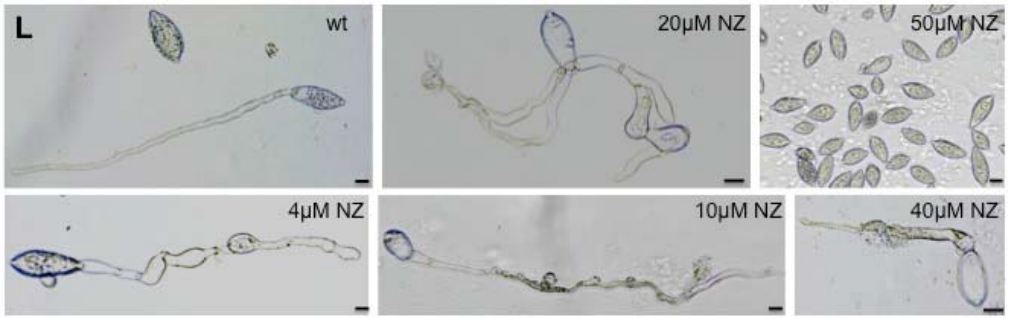
The CBOP program was successful in identifying several targets and could demonstrate the efficiency of chemicals blocking cell wall biosynthesis for disease control, as illustrated in the Figure below.

Summary of the main results obtained for each identified cell wall protein target:

Cellulose synthases: Understanding the precise role of individual cellulose synthases is essential to our understanding of cell wall biology in the oomycetes.

Cellulose synthase genes (CesA) were identified and their expression profile analysed at different developmental stages, thereby providing a map of the key genes involved in the overall biological life cycle of the pathogen. Gene silencing experiments were performed demonstrating the importance of cellulose biosynthesis in pathogenesis. Silencing of individual genes by RNA interference revealed a different function for each gene. Silencing of CesA 1 and 2 led to a 40-50% reduction in cyst germination and an increased in appressoria bursting (i.e. cell death of specialized infectious cells). Silencing of the two other genes, CesA 3 and 4, does not lead to a loss of cell integrity, thereby demonstrating a different function than for CesA 1 and 2. ***In conclusion, CesA 1 and 2 represent the best target for disease control since their function in cell wall stability is more important than for other CesA genes, in particular in infectious cells. These data were corroborated by pathogenicity tests performed on each RNAi silenced strains.***

Putative chitin synthase: CBOP has established an essential role for a putative chitin synthase in the cell wall of *Phytophthora infestans*. The chitin synthase is an attractive target for disease control measures since chitin and related oligosaccharides are not present in plant or animal hosts and would therefore not be affected by chemicals targeted to the production of these carbohydrates. The work performed in CBOP revealed that the only putative chitin synthase gene present in the pathogen is expressed during most developmental stages but that it is certainly not involved in chitin biosynthesis since the wall of the pathogen does not contain any amount of detectable chitin. Most surprisingly, the chitin synthase inhibitor Nikkomycin Z efficiently leads to abnormalities at low concentrations and cell death at slightly higher concentrations, as illustrated in the Figure. This work was confirmed by a completely different approach, based on the silencing of chitin synthase by RNA interference. This approach led to similar abnormalities as represented in the Figure as Nikkomycin Z. ***In conclusion, this work reveals that the putative chitin synthase of the pathogen is vital for the microorganisms and can thus be used as a target for disease control. However, it has a different function than chitin biosynthesis. From the CBOP results, can be speculated that it is most likely involved in GPI-anchor biosynthesis or protein glycosylation.***



Wild-type germling production (top left panel) and germling production after treatment with the chitin synthase inhibitor Nikkomycin Z. Note the morphological abnormalities and cell bursting induced by the inhibitor at low nikkomycin Z concentrations (4-40 micromolar) and the total inhibition of germling production at 50 micromolar. All scale bars = 5µm.

Other important cell wall biosynthetic genes: The project has also produced a thorough comparative analysis of all the available genomes of oomycetes, with a particular focus on cell wall biosynthetic genes. In addition to the abovementioned cellulose and chitin synthase genes, genes involved in the biosynthesis of the cell wall matrix polysaccharides beta-glucans were identified in multiple species and their profile of expression was determined in *Phytophtora infestans* at different developmental stages. This work has also allowed the discovery of domains specific to the oomycetes in some of the glucan synthase enzymes. **This family of enzymes represents yet another interesting target for disease control, as has also been demonstrated in other pathogens such as the human pathogen for candidiasis.**

Small tyrosine rich proteins (STR)*:* The project has identified a potential set of proteins (STR proteins) that act as key regulators of oospore cell wall formation. Maturation of thick-walled sexually derived environmentally resistant oospores will have an important impact on how we treat oomycete diseases in the future. CBOP has established that *Pythium oligandrum* is an excellent model oomycete for the study of the oospore cell wall. *Pythium oligandrum* is a mycoparasite able to quickly kill other oomycetes and fungi that are pathogenic on a range of plants. It therefore has great potential as a biological control agent. Since oospores are produced as environmentally resistant resting spores, oospore-derived oomycete infections have historically been very difficult to control. The project has shown that *Pythium* strains in which the STR proteins are silenced are unable to form fully mature oospores. **Thus, these genes represent an ideal target for the control of oospore derived oomycete infections. The fellow is currently exploiting these data to look for homologues of STR genes in other oomycetes and investigate the usefulness of these proteins as targets for disease control.**

Impact of the research*:* Providing new targets for disease control of late blight and other oomycete and fungal diseases is enormously important for our global society. It is estimated that fungal and oomycete diseases cause over 175M tonnes of damage globally every year in the top five food crops, rice, wheat, maize, potatoes and soybeans. If we could control these diseases in a sustainable manner we could feed an extra 4 billion people per annum. Our research on the cellulose synthases (targets of Mandipropamid and other CAA fungicides used to control oomycete diseases) and the putative chitin synthases (essential genes that represent ideal targets for new control measures) will go a long way towards new sustainable controls. Thus the agrochemical sector, the farming and agriculture and aquaculture sectors will be the target beneficiaries for this research. In addition, the data obtained in the project do not only provide novel targets for controlling late blight and other oomycete diseases, but also, on a wider scale, provide potential drug targets for diseases provoked by other Eukaryotic pathogens. Therefore, it is expected that the European pharmaceutical and agrochemical companies and European farming will also stand to benefit from this research.