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Proteomic Analysis of Witches' Broom Disease, caused by Moniliophthora perniciosa

Towards a greater understanding of Witches' Broom Disease of Cocoa

Witches' Broom Disease (WBD) of cocoa is a significant factor affecting productivity of cocoa in South America and the Caribbean. The causal organism, the basidiomycete fungus *M. perniciosa* (formerly known as *Crinipellis perniciosa*) has a complex and unusual lifecycle which involves initial biotrophic infection within living host tissues, stimulating the production of characteristic green "brooms", followed by a saprotrophic phase synchronous with the death of the plant tissues. The rate of development and final size of the green brooms, and the subsequent production of spore-producing mushrooms on the dead broom tissues are important factors in the epidemiology of the disease. Whilst it is known that there are interactions between the strain of the fungus and the variety of plant causing differences in symptom expression and rate of disease spread, the reasons for this variation are not understood. This research set out to investigate gene expression patterns in strains of *M. perniciosa* which differ in their pathogenicity, to develop a model system to study differences in symptom expression in the host and to develop a quantitative PCR (qPCR) technique to quantify the amount of fungal DNA *in planta* so that the extent of infections can be tracked even where no symptoms are expressed.

Proteomic analysis of diverse isolates of *M. perniciosa* showed that those collected from cocoa (C-biotype) were very similar to those collected from members of the Solanaceae family (S-biotype), whereas the types collected from lianas (L-biotype) were distantly related. Phylogenetic analysis of the rRNA operon ITS (internal transcribed spacer region) DNA sequences provided further evidence that the C- and S-biotypes are closely related, and the lack of diversity in the C-biotype suggests that it has diverged from the S-biotype very recently. Detailed proteomic analysis enabled the identification of several stress-related proteins and some potential elicitors capable of acting as virulence factors. Within the C-biotype isolates there were remarkably few proteomic differences but three of the specific spots were associated with the more pathogenic isolates, raising the possibility that even small changes in the proteome could have implications for the epidemiology and evolution of the disease.

Using a model S-biotype x tomato pathosystem, differences in symptom expression where found between normal and mutant types which are ethylene or auxin insensitive, confirming that both of these plant growth regulators are disrupted during infection.

qPCR showed that pathogen DNA in tomato increased with the severity of the symptoms and decreased away from the point of inoculation. The detection of fungal DNA in asymptotic tissues raises the possibility that the technique could be developed for use as part of a quarantine/disease screening procedure.

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Moniliophthora perniciosa: proteome, host colonization and hormonal alterations in tomato

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Abstract:

Moniliophthora perniciosa, causing Witches' Broom Disease (WBD), is a major pathogen on *Theobroma cacao*. Proteomic comparisons and ITS sequences indicated that the C- and Sbiotypes, pathogenic respectively on cacao and solanaceous hosts, are very closely related, whereas the L-biotype, saprotrophic on rainforest lianas is more distinct.

The S-biotype x tomato pathosystem proved a valuable model to quantify WBD symptom morphology. Three ethylene mutants, an auxin mutant and WT of tomato showed significant symptom differences. Inhibition of adventitious roots, delayed senescence, gigantic leaves/flowers, suggested high cytokinin and low ethylene levels. The loss of apical dominance suggested a low auxin/cytokinin ratio in the stem, but auxin is likely present in high amounts in hypertrophied tissues.

qPCR showed that pathogen DNA in tomato increased with the severity of the symptoms and decreased away from the point of inoculation. Small levels were also detected in asymptomatic tissues and in atrophied roots. qPCR in potato callus dual culture showed a slow rate of colonization during biotrophy, followed by acceleration after the switch to saprotrophy. Microscopic observation showed swollen structures at hyphal tips suggesting potential involvement in the switch and/or in plant cell penetration.

Proteomic analysis of the fungal saprotrophic phase allowed identification of 22 proteins, providing clues to its physiology and potential virulence factors. Four members of the aldoketo reductase (AKR) family were present. One was specific to a Bahian isolate. Another was related to plant auxin-induced member of the AKR subfamily aryl alcohol dehydrogenases (AAD), which contains proteins involved in lignin degradation such as aryl alcohol oxidase (AAO). A nitrilase could be involved in the indole-acetonitrile (IAN) pathway of auxin synthesis.

There are few proteomic comparisons of fungi and this is the first application in *M. perniciosa*, showing that overall proteomes are highly conserved; thus rare protein changes may have a strong physiological/evolutionary meaning.