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CARBON AND PHOSPHORE PROFILES DURING PLANT PATHOGENESIS AND DEVELOPMENT OF THE NECROTROPHIC FUNGUS *BOTRYTIS CINEREA*

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Successful invasion of plants by necrotrophic pathogens depends on their ability to penetrate their hosts and evade defensive mechanisms. To complete their life style *in planta*, pathogenic fungi must be able to gain nutrients from plant cells. The nature of available nutrients supplies metabolized by necrotrophic fungi during infection has received little attention up to now. Our purpose is to characterize metabolic interactions between plants and the necrotrophic polyphage pathogen *Botrytis cinerea*, by using NMR spectroscopy. Metabolic profiles of each partner (*B. cinerea* and sunflower cotyledons as host plant), were drawn up. Natural abundance ^{13}C- and ^{31}P-NMR spectra showed the progressive exhaustion of plant carbohydrates (mainly glucose, sucrose, fructose, inositol, glutamine and glutamate).

Metabolic profiling of lipids (^{31}P-spectra) revealed the presence of plant and fungal specific membrane phospholipids. During the course of infection, a rapid decrease of phosphatidyl glycerol was related to the degradation of plant membrane phospholipids and revealed an other aspect of the degradative strategy of *B. cinerea*.

In *B. cinerea* extracts, mannitol was the only abundant polyol and the main carbohydrate compound. This metabolite, typically associated with fungal, species was detected early during infection and was the sole soluble sugar at the end of the infection course. The metabolic pathway for mannitol biosynthesis and catabolism in fungi takes place through the mannitol cycle, which involves two pathways. Expression analyses of two genes involved in these pathways, mannitol-1-phosphate dehydrogenase (*mpd*) and mannitol dehydrogenase (*mtd*) revealed that both ways are functional in *B. cinerea* and sequentially expressed during sunflower cotyledons infection. TLC and HPLC experiments showed that mannitol was produced at all stages of fungal development. This key stored carbon source could be a regulatory component of carbon flow, rather than a stress response element.

Inactivation of *mpd* and *mtd* is being currently underway and will contribute to establish the role of mannitol during saprophytic development and pathogenesis of *B. cinerea*.

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POSITIVE SELECTION DOES NOT ACT ON ALL ENDOPOLYGALACTURONASE ENCODING GENES OF *BOTRYITIS CINEREA,* IN ACCORDANCE WITH THEIR PUTATIVE ROLE IN PLANT INFECTION.

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Botrytis cinerea secretes multiple endopolygalacturonase isozymes, coded by genes whose transcription is independently regulated. Here we report an attempt to distinguish on an evolutionary basis the polygalacturonases active during either saprophytic or pathogenic growth.

Four of the six endopolygalacturonase encoding genes (*Bcpg1*, *Bcpg2*, *Bcpg3* and *Bcpg5*) were sequenced from 32 strains of *B. cinerea* and three strains of *B. fabae*. The *Bcpg3* gene fragment examined was almost invariable in all strains. *Bcpg5* variability was moderate and similar to that of neutrally evolving genes used by other authors to determine the phylogeny of the *B. cinerea* complex. In contrast, *Bcpg1* and *Bcpg2* were more variable and could be shown to be under positive selection by a likelihood ratio test. Different acting selections are consistent with different roles played by different gene products during the interaction of the pathogen with the host. The product of *Bcpg1* and *Bcpg2* genes are the target of host proteins such as Polygalacturonase Inhibiting Proteins that may cause a positive selection and diversification of the pathogen population. *Bcpg5* and particularly *Bcpg3* were clearly in non-diversifying evolution, to minimize the occurrence of mutations that could compromise function, indicating that high enzyme activity and not host recognition is the driving selection force for these genes.

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ESTABLISHING NEW VECTORS FOR TRANSFORMATION AND GENE-SILENCING IN *BOTRYTIS CINEREA*

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B. cinerea is a necrotrophic fungus capable of infecting over 300 plant species worldwide, attacking various damaged/cracked tissues. In general, *B. cinerea* is becoming more resistant to current fungicides and agrochemical companies are seeking new targets capable of minimizing pathogenicity. The use of gene silencing is a well-established technique in animal, plant and fungal systems. The benefits of down regulating genes are vast, including the potential to identify protein function of targeted genes. Our initial investigations utilized the widely used vector pLOB1 and derivatives to transform and trigger silencing of laccase and superoxide dismutase. Silencing was successful, with low to intermediate levels of silencing observed for both target transcripts, however we also identified unexpected reductions in virulence that did not correlate with the targeted gene. Investigation shows that these were due to specific sequences within the pLOB1 vector. New transformation and expression vectors have been constructed containing the *Aspergillus nidulans oliC* promoter and *trpC* terminator. These alleviate many of the difficulties observed with pLOB1. The new vector system displayed increased transformation and expression rates without the detrimental effects or reduction in pathogenicity observed with the pLOB1 system. Initial investigation may suggest that silencing efficiencies may have decreased using the new transformation vectors, which may be a due to hyper-silencing in the original system. We have strong evidence that gene silencing is possible within *B. cinerea*, giving transformants displaying a range of silencing levels and this may be of benefit when used to assess the amount of a particular gene product necessary for the infection process rather than the all-or-nothing studies achieved through targeted gene disruption.