Abstract

The ascomycete *Fusarium graminearum* is the main causal agent of the head blight disease of small grain cereals and the stalk and ear rot of corn. It is found all over the world and is one of the most important cereal pathogens. The head blight disease causes grain yield losses and reductions in grain quality through the accumulation of non-selective mycotoxins. So far, no cereal cultivars with resistance against *F. graminearum* and no fungicides working efficiently against *F. graminearum* are available.

In the present work, a strategy using mitogen-activated protein (MAP) kinase deletion mutants was used to investigate, which developmental and pathogenic processes of *F. graminearum* are possibly regulated by MAP kinase signal transduction pathways and which mechanisms might be essential for its pathogenicity. First of all, three genes encoding MAP kinases were isolated from *F. graminearum*. They were named Gmap1, Gpmk1 and FgOsm, respectively. Functional characterisation of Gmap1 and Gpmk1 was carried out by producing Δ*gmap1* and Δ*gpmk1* mutants via transformation mediated gene disruption. The function of FgOsm still remains to be analysed. But, according to its amino acid sequence homologies FgOsm belongs to the yeast/fungi stress-activated MAP kinases, which generally regulate responses to hyperosmotic shocks in other fungi.

Gmap1 belongs to the yeast/fungi extracellular-signal regulated MAP kinase subgroup 2 that are known to be involved in cell integrity processes. Characterisation of the Δ*gmap1* mutants revealed a growth defect on solid media, an asexual phenotype, and a drastic reduction of virulence. These results suggested the Gmap1 kinase to regulate cell developmental processes that are important for mating and full virulence. However, the generated Δ*gmap1* mutants exhibited an obvious genetical instability. Therefore, they were not further characterised.

Disruption of the gene encoding the Gpmk1 MAP kinase from *F. graminearum*, a kinase of the yeast/fungi extracellular-signal regulated MAP kinase subgroup 1, led to mutants that displayed a reduced ability to produce aerial hyphae and were unable to form sporodochia, resulting in a reduced conidia production. Furthermore, they were completely unable to form sexual fruiting bodies. Infection tests revealed the mutants to have a drastically reduced virulence towards maize and be completely apathogenic on wheat. These results indicated the Gpmk1 MAP kinase to be involved in developmental processes, such as aerial growth, sporodochia formation, mating processes, as well as in host penetration and invasive growth.

The reduced ability to form aerial mycelia after *gpmk1* disruption suggested a regulation of hydrophobins via the identifed Gpmk1 MAP kinase. Therefore, a gene encoding a putative hydrophobin was isolated from *F. graminearum*. The deduced amino acid sequence showed all characteristics of hydrophobins. The hydrophobin gene was transcribed predominantely in germinating conidia, but could also be found during growth of the fungus on liquid and solid complete and minimal medium, as well as in early stages of perithecial development. No transcription occurred *in planta*, indicating the identified hydrophobin to have no obvious function during plant infection. A regulation of hydrophobin production by the Gpmk1 MAP kinase could however not be verified, so that the growth defects of the Δ*gpmk1* mutants could not be explained by the failure to secrete the identified hydrophobin. But, as most fungi secrete several hydrophobins, it remains to be examined, if a regulatory role of Gpmk1 on other still unknown hydrophobins could be the clue to some of the mutants phenotypes.

Since the trichothecene mycotoxins are so far the only virulence factors known for *F. graminearum*, the mutants were analysed for their ability to produce the trichothecene deoxynivalenol (DON) and additionally the mycotoxin zearalenone (ZON). The experiments revealed DON and ZON to be differentially regulated depending on the substrate the strains were cultivated on. Gpmk1 regulates the induction of ZON production on wheat and on rice, whereas on maize a different pathway is utilized for its induction. Gpmk1 only showed a slight regulatory effect on DON induction during cultivation on rice and no effect during cultivation on maize.

Recently, cell wall degrading enzymes (CWDE) have also been postulated to partake in the head blight disease establishment. In this work, it could be shown that *F. graminearum* can secrete amylolytic, cellulolytic, xylanolytic, pectinolytic, and proteolytic enzymes. Biochemical analysis of the Δ*gpmk1* mutants revealed a regulatory effect of the Gpmk1 MAP kinase on the early induction of an endoglucanase, a xylanase, and a protease activity as well as the overall induction of a lipolytic activity. These results suggest the infection processes of *F. graminearum* to depend on CWDE secretion particularly during the early infection stages. After host penetration the secretion of toxins aids the invasive growth of the pathogen. Hence, differential toxin secretion might result in a varying disease severity. As Gpmk1 influences the ZON induction by far stronger than the DON induction, these results could possibly give a first hint as to a role of ZON in plant infection.