PR4.5
Involvement of *Trichoderma reesei* (*Hypocreanecora*) G-alpha protein GNA1 during mycoparasitism against *Pythium ultimum*

*Trichoderma reesei* (*Hypocreanecora*) is widely used in industry and its potential for use in agriculture as a biocontrol agent against phytophathogenic fungi has just started. We have investigated the involvement of G proteins during mycoparasitism against plant pathogens. Here we described the role of GNA1, a G-alpha protein which belongs to alfa, group in Cell Wall Degrading Enzymes (CWDEs) production by *T. reesei* during antagonism against *Pythium ultimum*. For that, two mutants were used: ∆gna1 and gna1QL (constitutively activated version of GNA1). The gna1QL mutant, like the parental TU-6, inhibited the growth of *P. ultimum* in plate confrontation assay and grew faster than the parental TU-6 while the ∆gna1 did not grow over *P. ultimum*. Scanning electron microscopy showed that the gna1QL mutant promoted more morphological alterations of *P. ultimum* cell wall than the parental TU-6 while the ∆gna1 caused no effects. The mutant ∆gna1 produced less CWDEs than gna1QL and TU-6. The gna1QL mutant showed a better performance in production of CWDEs such as endochitinase, N-Acetyl-β-D-glucosaminidase (NAGase), β-1,3-glucanase, protease, lipase and acid phosphatase, after 72 hours of incubation. However, the parental TU-6 showed higher cellulase activity than gna1QL and ∆gna1. The intracellular content of cAMP in the strains after 72 hours of incubation was: gna1QL (79.85 ± 12), ∆gna1 (268.65 ± 8.5) and TU-6 (109.70 ± 9.2) pmol/mg protein. We therefore suggest that the production of some CWDEs during mycoparasitism by *T. reesei* against *P. ultimum* can be mediated by GNA1 activity or cAMP levels.

PR4.6
Galacturonic acid catabolism in *Botrytis cinerea*

Lisha Zhang, Jan van Kan
*Wageningen University, Laboratory of Phytopathology*
lisha.zhang@wur.nl

D-galacturonic acid (GaA) is the major component of pectin, which can be degraded by plant pathogens; GaA is an important carbon source for microorganisms living on decaying plant material. For bacteria, a catabolic pathway of GaA has been described, which consists of five enzymes converting GaA to pyruvate and glyceraldehyde-3-phosphate. A different catabolic pathway is proposed in filamentous fungi. In *Hypocreanecora*, GaA is converted to pyruvate and glycerol via D-galacturonic reductase, L-galactonate dehydratase, 2-keto-3-deoxy-L-galactonate aldolase, and glycerol dehydrogenase.

The *Botrytis cinerea* genome contains a D-galacturonate reductase gene (*BcgaaA*), a L-galactonate dehydratase gene (*BcgaaB*), and a 2-keto-3-deoxy-L-galactonate aldolase gene (*BcgaaC*). The three genes were cloned into a protein expression vector and the enzymatic activity determined for each gene separately. The heterologous simultaneous expression of BcgaaA, BcgaaB, and BcgaaC in an *E. coli* ΔuxAC mutant which cannot grow on GaA was performed to determine whether the catabolic pathway from *B. cinerea* can restore the growth efficiency in *E. coli*. Targeted gene replacement of BcgaaC or both BcgaaA and BcgaaC resulted in ∆gaaC mutants and ∆gaaAC double knock-out mutants that displayed significantly reduced growth when D-galacturonic acid was used as the sole carbon source. The mutants showed similar virulence as the wild-type strain B05.10 on tomato leaves, indicating that GaA is not the main carbon source for *B. cinerea* growth during infection on tomato leaves. The virulence will be tested on other pectin-rich plants and tissues.