PR4.5

Involvement of *Trichoderma reesei* (*Hypocrea jecorina*) G-alpha protein GNA1 during mycoparasitsm against *Pythium ultimum*

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Trichoderma reesei (Hypocrea jecorina) is widely used in industry and its potential for use in agriculture as a biocontrol agent against phytophatogenic 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 $alfa_i$ group in Cell Wall Degrading Enzymes (CWDEs) production by *T. reesei* during antagonism against *Pythium ultimum*. For that, two mutants were used: Δ*gna1* and *gna1*QL (constitutively activated version of GNA1). The *gna1*QL 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 Δ*gna1* did not grow over *P. ultimum*. Scanning electron microscopy showed that the *gna1*QL mutant promoted more morphological alterations of *P. ultimum* cell wall than the parental TU-6 while the Δ*gna1* caused no effects*.* The mutant Δ*gna1* produced less CWDEs than *gna1*QL and TU-6. The *gna1*QL 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 Δ*gna1*. The intracellular content of cAMP in the strains after 72 hours of incubation was: *gna1*QL (79.85 ± 12), Δ*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*

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D-galacturonic acid (GalA) is the major component of pectin, which can be degraded by plant pathogens; GalA potentially is an important carbon source for microorganisms living on decaying plant material. For bacteria, a catabolic pathway of GalA has been described, which consists of five enzymes converting GalA to pyruvate and glyceraldehyde-3-phosphate. A different catabolic pathway is proposed in filamentous fungi. In *Hypocrea jecorina*, GalA is converted to pyruvate and glycerol via D-galacturonate 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 GalA is performed to determine whether the catabolic pathway from *B. cinerea* can restore the growth deficiency in *E.coli*. Targeted gene replacement of BcgaaC or both BcgaaA and BcgaaC resulted in *ΔgaaC* mutants and *ΔgaaAC* 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 stain B05.10 on tomato leaves, indicating that GalA 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.