Engineering the biological control and plant growth promotion fitness of *Trichoderma hamatum* GD12 through genetic manipulation.

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I certify that all the material in this thesis which is not my own work has been identified and that no material has been previously submitted and approved for the award of a degree by this or any other University.

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Abstract

Trichoderma species are ubiquitous soil saprotrophs and well-characterized biological control agents. Certain strains have also been shown to stimulate plant growth through the production of bioactive secondary metabolites, and are therefore receiving increased attention as natural plant growth stimulants. Previous research at the University of Exeter (Ryder *et al*. (2012) *Microbiology* 158, 84-97) has shown that the Plant Growth Promotion activity of a biocontrol strain of *Trichoderma hamatum* (strain GD12) can be dramatically enhanced by targeted mutation of the N-acetyl-β-D-glucosaminidase-encoding gene *nag*. However, due to the loss in chitinase activities, the mutant (Δ*Thnag*::*hph)* displays loss of saprotrophic competitiveness and reduced fitness as a biocontrol agent.

We set out to investigate how we can use genetic manipulation to improve strain GD12 in the context of biological control and plant growth promotion. We approached this by firstly sequencing the whole genome of GD12 and then using the information available from this to produce a targeted deletion mutant in the GD12 background disrupting one of the most down regulated proteins in the Δ*Thnag*::*hph,* a branched chain amino acid transaminase (*bcat*), implicated in the production of secondary metabolites. Secondly, we aimed to engineer hyper-secretion and enhanced PGP activities in GD12 without impairing biocontrol activity. Over-expression of the *S. cerevisiae* gene dolichol-phosphate mannose synthase (*dpm1*) in *T. reesei* leads to altered cell wall architecture and increased secretory potential. Using the constitutive promoter ToxA, we over-expressed the *dpm1* gene in *T. hamatum* GD12 and assessed its effects on the biocontrol and PGP activities of the fungus.

The data presented herein, shows, that *bcat* deletion in *T. hamatum* GD12 results in a detrimental effect of germination of lettuce seedlings grown in the presence of Δ*Thbcat*::*hph.* We show that single copy insertions of ToxA-*dpm1* leads to improved PGP activities, while biocontrol fitness is unaffected. However, while multiple copy insertions similarly lead to enhanced PGP, such strains display impaired biocontrol of soil-borne pathogens such as the plurivorous damping-off pathogen *Sclerotinia sclerotiorum*. This work demonstrates that while significant improvements in crop productivity can be achieved through genetic modification of the beneficial rhizosphere fungus *T. hamatum* GD12, it can have important consequences for other aspects of its biology and ecology and competence as a soil-borne microorganism.

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