

Bachelorthemen AG Zeilinger SS24

Illuminating them - What are the functions of peroxisomes in the mycoparasitic fungus *Trichoderma atroviride*? (Mario Gründlinger)

This bachelor thesis presents the opportunity to integrate a peroxisomal variant of the green fluorescent protein (GFP) marker into the filamentous fungus *Trichoderma atroviride*. The goal is to create genetically modified strains for the investigation of the elusive functions and dynamics of peroxisomes in *Trichoderma atroviride* through live cell imaging. Techniques such as microbial cultivation, standard molecular cloning, and fungal cell transformation will be employed. The peroxisomes within the fungal hyphae will be observable under varying conditions using a fluorescence microscope.

Dieses Bachelorthema bietet die Gelegenheit, eine peroxisomale Variante des grün fluoreszierenden Proteins (GFP) in den filamentösen Pilz *Trichoderma atroviride* einzubringen. Das Ziel besteht darin, genetisch veränderte Stämme zu entwickeln, um die unbekannt Funktionen und Dynamiken von Peroxisomen in *Trichoderma atroviride* mittels Live-Cell-Imaging zu untersuchen. Dabei werden Techniken wie mikrobielle Kultivierung, standardmäßige molekulare Klonierung und die Transformation von Pilzzellen angewendet. Die Peroxisomen innerhalb der Pilzhyphe werden unter verschiedenen Bedingungen mithilfe eines Fluoreszenzmikroskops beobachtbar sein.

Chemotropism assays with mycoparasitic *Trichoderma* spp. (Siebe Pierson, Susanne Zeilinger)

The student compares chemotropic effects of interesting compounds (e.g. phytohormones, 6-PP, mycotoxins) for different mycoparasitic *Trichoderma* species (*T. atroviride*, *T. virens*, *T. asperellum*) at different developmental stages (germlings, microcolonies, mature colonies). If time allows, the study could be extended to comparisons with how plant pathogens react to these compounds (*R. solani*, *B. cinerea*, *F. graminearum*).

Double knockout transformation in *Trichoderma atroviride* using CRISPR/Cas9 (Siebe Pierson, Susanne Zeilinger)

The student will attempt to create a double knockout with the CRISPR/Cas9 and pTEL vector system. This can be approached in two ways which can then be compared for efficiency. One approach consists of combining the gRNA-Cas9 complexes for both genes and inducing deletion of both genes at the same time. The other approach consists of performing one deletion, cultivating the mutants to dispose of the pTEL vector and then repeating this process to perform the second deletion.

The selected genes show a clear phenotype upon deletion thus allowing easy selection.