

## **Identification of Genes Differentially Expressed During Sclerotium Formation in the White Mold Fungus, *Sclerotium rolfsii***

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### **Objectives:**

To identify and characterize genes in the *Sclerotium rolfsii* genome that are differentially expressed during sclerotial development as compared to mycelial growth.

### **Project Overview:**

We used Suppression Subtraction Hybridization PCR (SSH) to create two gene libraries for a Georgia peanut *S. rolfsii* isolate using a CLONTECH PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA). One library is enriched for genes more highly expressed in sclerotia than in hyphae while the second is the reverse. Individual clones from these libraries have been selected, sequenced, and compared to sequences available in the National Center for Biotechnology Information (NCBI) database. Currently, efforts are underway to corroborate these results with RNA blot hybridization analysis.

### **Progress:**

We identified a method using cellophane filters to cleanly isolate sclerotia at each of three sclerotium developmental stages; early, mid and late development. We used this method to collect over a gram of tissue from each developmental stage and from mycelial tissue. RNA from these tissues was isolated and purified and used in the SSH gene library kit (Clontech). JT generated two libraries each with approximately 1000 clones. The libraries were arrayed and probed to identify clones containing differential sequences. Differential clones have been sequenced and analyzed using bioinformatics tools. Expression blot analysis is underway to corroborate the differential expression of genes identified in the libraries.

### **Preliminary results:**

Roughly 100 clones from each library were sequenced, which identified over 30 clones that are similar to at least 20 different fungal protein-encoding genes registered in the Center for Biotechnology Information (NCBI) databases. Initial analysis has identified at least 13 clones in the sclerotium-upregulated library that contain sequences similar to protein metabolism genes in other fungal systems, including the model basidiomycete system, *Ustilago maydis*. Just one intriguing example is the identification of 7 sclerotium-specific clones encoding a phosphatidylserine decarboxylase enzyme shown in other systems to be involved in membrane remodeling, including stabilizing integration of proteins in cell membranes. Additionally, a lectin-encoding gene was also identified in the sclerotium-upregulated library, which corroborates published evidence that this same lectin is produced primarily by the sclerotial bodies (1). The sclerotium-downregulated library contains at least 4 clones containing sequences similar to ribosomal proteins required for protein synthesis.

1. Swamy, BM, et al. Immunolocalization and functional role of *Sclerotium rolfsii* lectin in development of fungus by interaction with its endogenous receptor. *Glycobiology* 14(11): 951-957.