

Identification of Genes Differentially Expressed During Sclerotium Formation in *Sclerotium rolfii*

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Objectives:

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

Project Overview:

In a procedure previously employed by our laboratory, we have used Suppression Subtraction Hybridization PCR (SSH) to create two gene libraries for a peanut infecting *S. rolfii* strain 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 are being selected, sequenced, and compared to sequences available in the National Center for Biotechnology Information (NCBI) database.

Progress:

We identified a method using cellophane filters to cleanly isolate sclerotia at each of three sclerotium developmental stages; early, mid and late development. Using this method, we collected over a gram of tissue from each developmental stage and over a gram of mycelial tissue. RNA from these tissues was isolated and purified and used in the SSH gene library kit (Clontech). Following proper protocols, JT generated two libraries each with approximately 1000 clones and has shown that they contain appropriate sized inserts suggesting good library quality.

Now that the libraries have been created sequence analysis of the individual clones can be just underway.

Original Budget:

Supplies:	\$2000.00
Travel:	<u>\$1000.00</u>
Total:	\$3000.00

To date, \$1000 of the supply budget has been used to buy lab reagents and a Clontech SSH kit. A \$1000 was used from the travel budget by JT for travel to the 23rd Fungal Genetics Conference in March 2005 to present results from this research project.

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