

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

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Objectives:

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

Project Overview:

In a procedure previously employed by our laboratory, we will use Suppression Subtraction Hybridization PCR (SSH) to create a gene library for this *S. rolfsii* strain using a CLONTECH PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, CA). This library will be enriched for genes more highly expressed in sclerotia than in hyphae. Individual clones from this library will be 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 to be used in the SSH gene library kit (Clontech). Following proper protocols, JT used the RNA from the tissues in the SSH gene library kit, but the first attempt failed.

New tissue has been collected for each of the growth stages of *S. rolfsii*, and the RNA extraction process is ongoing. A new SSH kit has been ordered, to reduce the likelihood of difficulties in library construction. Once the library has been created, analysis of the individual clones can begin.

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 portion of the travel budget will be used by JT for travel to the 23rd Fungal Genetics Conference in March 2005 to present results from this research project.