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**Secretome Analysis of Plant-fungal Symbiotic Relationship Using
Yeast Signal Trap System**

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**Honors Senior Project
Approval**

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Department: Biological sciences

Degree: B.S.

Full title of project: Identification of Genes involved
in signaling during Ectomycorrhizal
Symbiosis

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ABSTRACT

We describe a technique for simultaneous isolation a number of cDNAs encoding secreted proteins. The technique makes use of the Yeast Signal Trap (YST) system which targets and isolates proteins that are excreted during the interactions of a symbiotic relationship. An YST screen, using a three frame vector system, was adapted to study the symbiotic interaction between ectomycorrhizal fungus (*Laccaria bicolor*) and poplar trees, possibly revealing sets of genes encoding secreted proteins from both organisms. These Proteins are important to understand how the symbiotic signaling leads to formation of mycorrhizae. The *L. bicolor* cDNA library that was transformed into yeast culture strain DBY2005 allowed us to select several clones that grew on the sucrose-containing YP. Some examples of isolated and sequenced clone identifications ranged from GTPase-activating Protein (used in signaling) to Beta-isopropylmalate Dehydrogenase (used for Amino Acid Transport & Metabolism). This project will continue in isolating more clones through the YST system to identify and classify the secreted proteins from *L. bicolor*. To understand these intercellular signaling processes are not only for the purpose of farming and the plant ecosystem, but it is also for any therapeutic potential they may contain.

Keywords: Protein secretion, Aspen trees, ectomycorrhizal fungus, Yeast Signal Trap

INTRODUCTION

Many different important biological events revolve around intercellular signaling processes. Isolation of the genes which encode the signaling proteins is of great interest, not only for the purpose of understanding the process these interactions govern, but also for any therapeutic potential they may contain. One kind of signaling that has not been explored is the interaction between mycorrhizal fungi and trees during a beneficial symbiosis relationship.

Ectomycorrhizae: Tree species dominating forest ecosystems (e.g., poplars, pines, oaks) develop symbiotic associations, so-called ectomycorrhizas (ECM), with soil fungi. Mycorrhizal plants exhibit 5 to 20% higher photosynthetic rates than non-mycorrhizal plants, producing a very large, if poorly understood, contribution to the global carbon cycling budget. The prospecting and absorbing extraradical hyphal web captures soil minerals (P, N, water) and organic nitrogen, assimilates and translocates a large proportion of them to roots of the growing host plant. In return, the fungus within the root is protected from competition with other soil microbes and, provided with plant carbon (Smith and Read, 1997).

To date, the only method reported to predict secreted proteins was by using computational prediction. An equivalent experimental screen for secreted proteins would provide an important complement to such a bioinformatics approach. In this regard, the yeast secretion trap (YST) functional screen represents an attractive option (Jacobs et al. 1997; Lee et al. 2004). The Yeast Secretion Trap (YST) is a valuable technique used to target the secretomes (secreted proteins) which are excreted outside the cell wall by the tree tissue and fungal tissue. A YST screen, using three frame vector system, was

adapted to study the symbiotic interaction between ectomycorrhizal fungus (*Laccaria bicolor*) and poplar trees, possibly revealing sets of genes encoding secreted proteins from both organisms (Figure 1). These Proteins are important to understand how the symbiotic signaling leads to formation of mycorrhizae.

MATERIALS AND METHODS

Construction of L. bicolor secretome cDNA library

The RNA samples were taken from aspen trees infected with ectomycorrhizal fungus (*Laccaria bicolor*). The single-strand cDNA were prepared from a pooled sample of mRNA which was purified from the RNA samples.

After the single-strand cDNA synthesis, second-strand cDNA were prepared by using BD Matchmaker™ Library Construction & Screening Kits (Clontech, CA). To check if the second-strand cDNA had been generated, fragments were run in agarose gel using electrophoresis. The cDNA fragments (0.3 to 1.0 kb) were recovered using Qiagen MiniElute Gel Extraction Kit (Qiagen, CA). After the second-strand synthesis, the Taq Polymerase causes sticky ends to be created. To ligate with the adapter EcoR1, blunt ends are needed. Using Pfu Polymerase, blunt ends were synthesized (Pfu Polymerase Kit). Once the sample has blunt ends, ligation is done for EcoR1 adaptors. After ligation, the sample is phosphorylated and an agarose gel is run using electrophoresis to check sample. The sample is isolated and recovered using gel extraction kit (Qiagen MiniElute Gel Extraction Kit). After the concentration of the sample was measured by NanoDrop, it is ligated to a mixture of the three vectors (pYST-0, pYST-1, pYST-2) that previously had been digested with EcoR1 and Not1. Ligations were transformed into electrocompetent TOP 10 *Escherichia coli* by electroporation and the transformants selected on an ampicillin-containing medium.

Transformation into yeast culture strain DBY2005

Mass extraction of plasmids containing the *L.bicolor* cDNA library was purified using Plasmid Maxi Purification Kit (Qiagen, CA) from a pool of the transformed bacterial colonies. The purified plasmid cDNA library is transformed into yeast culture strain DBY2005 (BD Matchmaker™ Library Construction & Screening Kits – LiAc Method) and selected on sucrose-containing YP. If the cDNA belonged to a secreted protein, then the colonies survived on sucrose containing medium. Plasmids from these yeast cells are isolated and propagated in *E. coli*. These plasmids are used for DNA sequencing to identify the excreted proteins. Online *L. bicolor* genome database was used to identify the proteins.

DNA sequencing and identification of clones: DNA sequencing of positive clones isolated from sucrose plates was done as described (Podila et al., 2002) using the DynamicET sequencing kit (GE Healthcare Systems, NJ) and ABI 310 DNA sequencer (Applied Biosystems, CA). Online *L. bicolor* genome database (<http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html>) was used to identify the proteins.

RESULTS AND DISCUSSION

The *L. bicolor* cDNA library that was transformed into yeast culture strain DBY2005 allowed us to select several clones that grew on the sucrose-containing YP (Figure 2).

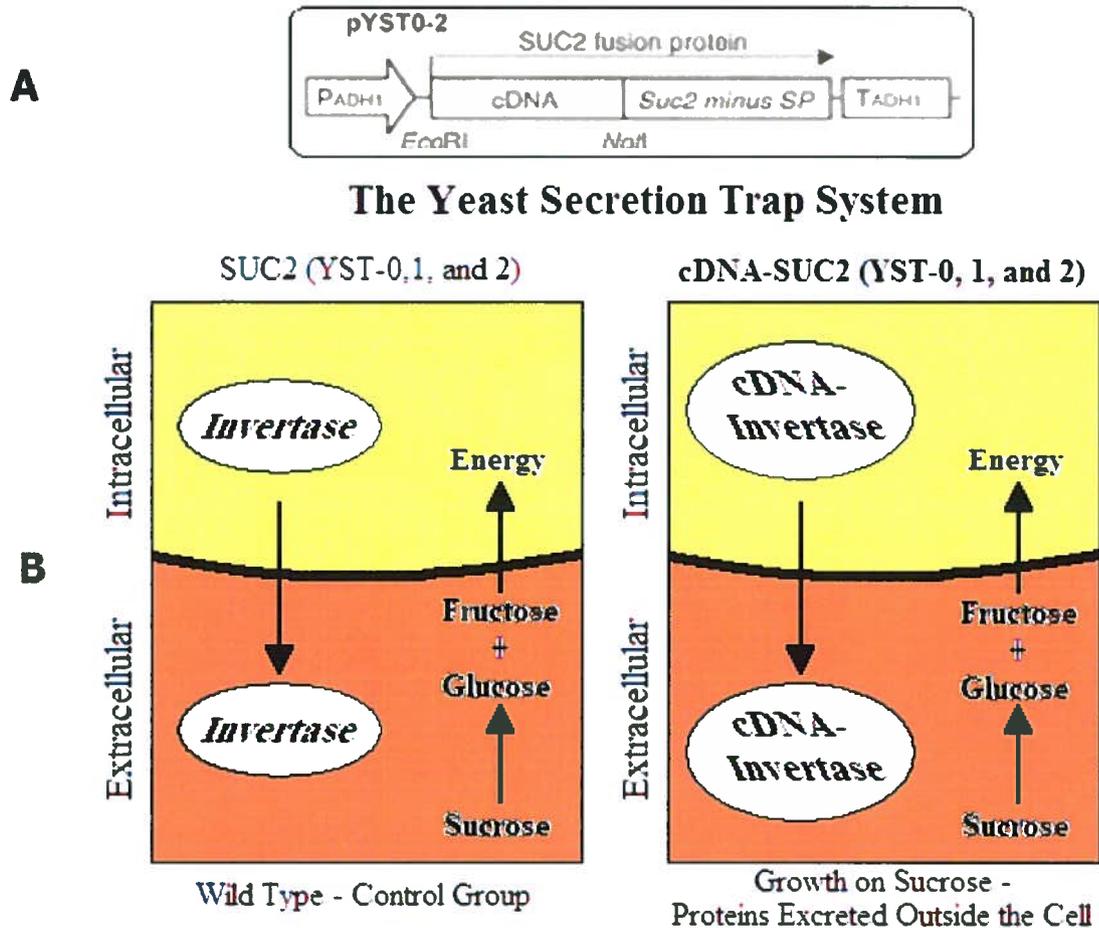


Figure 1. Details of Yeast Secretion Trap System. A, Schematic diagram of the three yeast vectors (YST-0,1,2) used for cDNA library construction. B, A diagram of how the Yeast Secretion Trap system works.

Figure 2 shows growth yeast cells containing cDNA clones of secreted proteins and growth of selected clones on sucrose-containing YP agar medium. This demonstrates that the *L. bicolor* cDNA library cloned into the three yeast vectors code for secreted proteins and the proteins are excreted outside the cell.

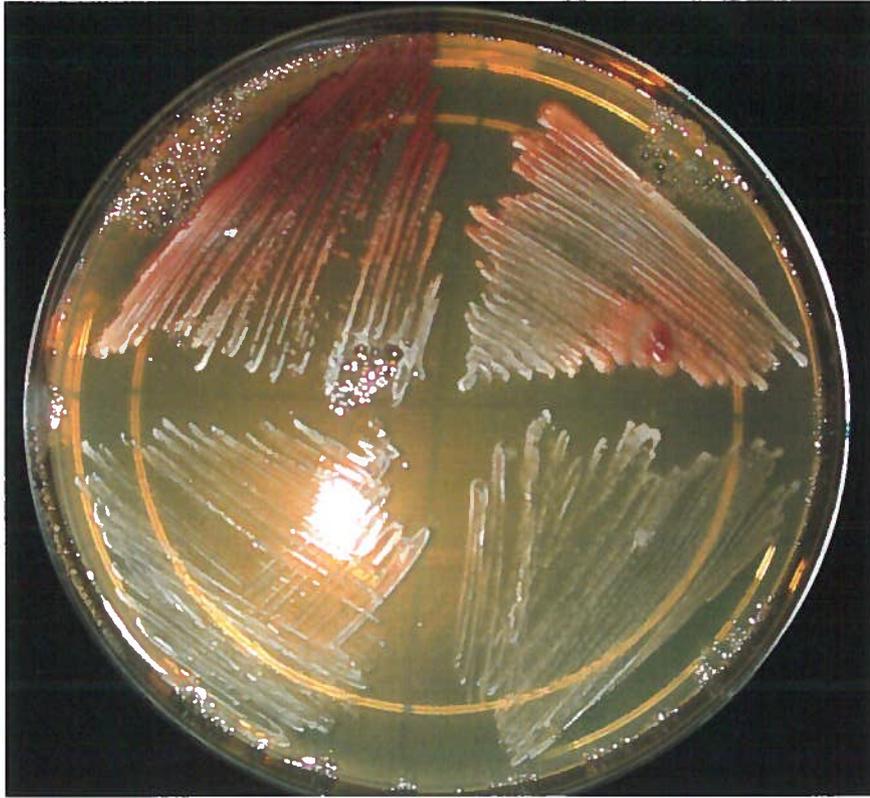


Figure 2. Selected transformed *L. bicolor* cDNA library clones on sucrose-containing YP media.

A number of clones have already isolated and sequenced and online *L. bicolor* genome database was used to identify the proteins. Some examples we have identified can be seen in the table below.

Clones	Subcellular Localization	Function
Fungal Pheromone STE3 GPCR	Plasma Membrane Protein	Signaling
GTPase-activating Protein	Membrane Assoc. Protein	Signaling
Beta-isopropylmalate Dehydrogenase	Cytoplasm	Amino Acid Transport & Metabolism

Table 1. Isolated and sequenced clone identifications, locations within the cell, and their functions within the cell (from online *L. bicolor* genome database).

These results show that the YST system can be adapted to clone and identify secretome proteins of *L. bicolor* involved in symbiotic interaction with poplar trees. In the near future we plan to isolate more clones through the YST system, which will allow

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