

How to retool the fungal cell factory-
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Background:

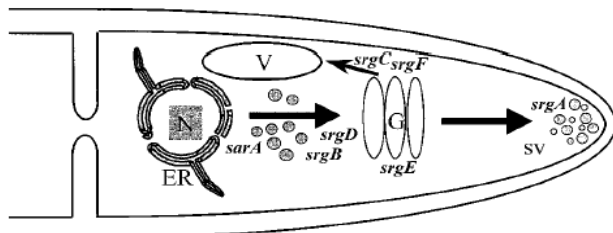
Filamentous fungi are commercially utilised to secrete valuable gene products ranging from industrial compounds to molecules of pharmaceutical importance. Genetically engineered strains can be made to express, for example mammalian antibodies and growth factors. Yields of foreign gene products are low and present methods have been unable to raise them to commercially attractive levels.

Outcomes:

- Method for high throughput screening of pharmaceuticals
- methods for high-throughput screening of organisms for efficient secretion of gene products of interest.
- Method of fast inexpensive manufacture of biologically derived pharmaceuticals (antibodies etc) using the fungi

Progress to date

We have developed a novel approach to boost the yields by finding the molecular bottlenecks in the secretion process. This development is continuing. These bottlenecks can be removed or circumvented by genetic design of improved strains of fungi. Concurrently our research will also produce methods for high-throughput screening of organisms for efficient secretion of gene products of interest.



Key subcellular components (organelles) in the protein

- 1) **A preliminary organelle map** of the fungal secretory pathway has been produced using confocal microscopy and direct fluorescent labelling
- 2) **Genetic tagging of secreted proteins for tracking has been carried out**, We have constructed a series of vectors combining the proteins of interest (CBH1 – native and XynB-foreign) with suitable fluorescent, DNA-based markers.
- 3) **Fluorescence Resonance Energy Transfer (FRET)** experiments have been done confirming interactions of selected proteins in the fungal secretory pathway. Systematic multidimensional high-content and high-resolution cellular fluorescence imaging supports molecular microarray research in pharmaceutical and bioindustry
- 4) **The secretome for yeast is now available**, along with the complete genomes for the filamentous fungi *N. crassa* and *T. reesei*, Using the information available in yeast cells we will validate a more complete picture of the fungal secretory pathway, with emphasis on similarities and differences.

Funding is sought to develop an effective platform technology for producing secretion localisomes, identifying targets and studying protein-protein interactions in a fungal system.

Additionally team has expertise with fungi: genetics, physiology, fermentations, molecular biology and is able offer services in these areas.