Multiple applications of *Neurospora* technology to vaccine development and production

W. Dorsey Stuart PhD
Neugenesis Corporation

New Cells for New Vaccines III
IABS
Wilmington Delaware

28 September – October 1
2008
Filamentous Fungi in Industry

- Filamentous fungi are able to secrete large amounts of protein
- Historically *Aspergillus* and *Tricoderma* species have been utilized for industrial enzyme production
- Host strains and fermentation processes for filamentous fungi have been optimized over many decades
- Product Yields are known to exceed 100 g/L
- Products include
  - Amyloglycosidase, Alpha amylase
  - Proteases, Cellulases, Recombinant enzymes
  - Shoyu, miso and sake
Neogenesis technology platforms were developed using *Neurospora crassa*

*Neurospora*, another filamentous fungus has been the subject of academic research for many decades. Used by George Beadle and Edward Tatum in the 1940’s to demonstrate that genes make enzymes. Nobel Prize in 1958.
Viral Antigens Produced by Neugenesis in *Neurospora*

(a) secreted soluble (membrane anchor truncated) influenza hemmaglutinin
(b) secreted intact neuraminidase with membrane anchor
(c) particles of avian influenza antigens containing hemmaglutinin H5, neuraminidase N1 and matrix protein M1
(c) particles containing H1, N1 and M1 proteins from two seasonal influenza strains (NC and PR8)
How the Neugenesis Technology Works for Single Recombinant Vaccine Antigen Production

*Neurospora* host strain transformed with expression cassette for recombinant HA (membrane anchor removed)

Secreted Soluble HA Antigen
Assay of HA produced in Neurospora

Various buffers

Serial dilutions

Negative control

<table>
<thead>
<tr>
<th>titer</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A Virus vs A Virus Like Particle
Heterokaryon NA Production Strain

Fused Fungal Strains Producing M1 + NA

NA(var₁) decorated particless
Neuraminidase Assay of N1M1 Heterokaryon Strains

Labile enzyme produced at high levels in the platform

Std. Curve mU

1000  500  250

Media dilutions

4x  8x  16x  32x

N1  N2  N3  N4  N6  N7  N8  N9  N10  N11  Host
Hyphal Fusion
The Basis for US Patents 5,643,745, 5,683,899, 6,268,140
Genes for each product subunit are inserted into sibling strains which are then fused and express the multimeric protein.
(c) How the Neugenesis Heterokaryon Technology Works for Multivalent Vaccine production

Fused Fungal Strain Producing $M_1 + HA(v_1;v_2) + NA(v_1;v_2)$
Combinatorial Biology

(d) mix and match by fusions

US PATENT 5,683,399
and Patents Pending
(e) Vaccine antigens expressed on cell surfaces
A potential for HTP screening and perhaps an edible vaccine
Neugenesis has developed *Neurospora*-based technologies that

- Generate new gene sequences *in vivo* which code for commercially valuable proteins

- Immediately produce the complex proteins specified by those new gene sequences

- Screen, on a direct readout basis, the new proteins produced from those new gene sequences

- Assemble combinatorial biological arrays for screening multimeric protein variants
Product Improvement Based on Repeat Induced Point Mutation (RIP)

- RIP specifically targets duplicated DNA sequences in the *Neurospora* haploid genome
- The duplicated DNA is mutated during the sexual cycle before fusion of the nuclei to form a true diploid cell
- Both duplicated genes are mutated at very high frequency
(f) Generation of mutations in HA using RIP

Library of strains producing HA variants

New proteins generated by RIP are immediately expressed for direct screening
Neugenesis Timeline for Vaccine Production

- Storage of host strains
- Fermentation of 250 L startup culture
- Fermentation of 2,000 to 20,000 Liter production culture
- Downstream

2 weeks from freezer stock to harvest of production culture

Neugenesis Platform

Current Platform
In Summary

Neugenesis has proprietary synergistic technology that enables:

- A rapid, low-cost vaccine production system
  - Seasonal flu vaccines
  - Rapid response to new pandemic strains
- Capable of generating
  - Soluble vaccine antigens
  - Virus-like particles (VLPs)
  - Multivalent vaccines and
  - Membrane Immobilized antigens
- A Product Improvement platform to generate new variants
Acknowledgments

Neugenesis
Edward Cambareri  David Jacobson  W. Dorsey Stuart
Yuliya Brudnaya  Rebecca Taylor  Silke Alliger
Ken Takeoka  Kelechi Anoruo

Additional Visualizations
AJF Griffiths, University of British Columbia
Patrick C. Hickey  Lux Biotechnology Ltd
Nick D. Read  University of Edinburgh

Email: dstuart@neugenesesis.com