Plants for Better Health: Plant Biotechnology in the Generation of New Vaccines and Therapeutics

Jukka Kervinen, July 16th 2013
Many Expression Hosts Are Available but How to Choose the Most Suitable for Production?

- Bacteria
- Insect cells
- Yeast
- Mammalian cells
- Eggs
- Fungi
- Plants
- Transgenic animals
Interest in New Biopharmaceutical Production Technologies

Some concerns with current systems:

• **Safety**: potential to harbor mammalian viruses
• **Capacity**: limited facilities for worldwide demand
• **Response time**: clone selection, line development and scale up
• **Cost**: expensive infrastructure and facility overhead
• **Complexity**: intricate systems with multiple points for failure
Recombinant Protein Production in Plants

Benefits & Challenges:
• Lack of human or animal pathogens
• High scalability
• Low upstream costs
• Production with desired structure and biological function
• Post-translational modifications (glycosylation, phosphorylation, proteolytic cleavages, etc.) can be beneficial or detrimental
• Level of expression and yield
• Response time
• Uniformity of expression

Field production  Confined production  Contained production
Recombinant Protein Production in Plants

Transgenic plants
- Long lead time
- Low yield
- Non-uniform expression
- Gene silencing
- Field production
- Containment
- Regulatory issues
- Commercialization

Plant viral vectors
- Genetic stability of vectors
- Non-uniform expression
- Silencing
- Containment
- Regulatory issues
- Commercialization

Agrobacterial vector
- Dependence from nuclear machinery
- Dependence from ssDNA
- Regulatory issues
- Commercialization

Hybrid Systems

Fraunhofer’s non-Transgenic Expression Systems
Fraunhofer Institutes and Centers

- Nonprofit applied research organization: focused on industry partnerships and the commercialization of technology through licensing and spin offs
- ~1.6 billion Euro annual budget & ~17,000 staff
- 59 German Institutes: spanning many fields of science and technology
- 7 US Centers: CMB focused on plant-based production of biopharmaceuticals
Fraunhofer Center for Molecular Biotechnology

The Center was established as a Partnership between Fraunhofer Germany and the State of Delaware in 2001. Today ~100 employees.

Newark, DE
FhCMB’s approach is based on transient expression using virus-based vectors introduced into plant tissues by vacuum infiltration:

- Launch vectors for high level target expression
- Vacuum infiltration of *Agrobacteria* for synchronized vector delivery to non-transgenic plants
- Hydroponic system for confined, soil free and uniform biomass generation
Plant Growth, Infiltration & Harvesting

Plants...  4-6 weeks

Agrobacterium...

Vector Technology...

Infiltration

3-8 days
Plant Growth, Infiltration & Harvesting

Film
Plant Growth, Infiltration & Harvesting

Not Infiltrated

Infiltrated with GFP
From Laboratory to a Pilot Plant Facility

- Process development is carried out in the R&D laboratory for up to 5 kg of plant biomass
- Technology is transferred to a Pilot Plant Facility for up to 50-100 kg of plant biomass
Equipment design and work was carried out in collaboration with the Fraunhofer Center for Manufacturing Innovation
cGMP Pilot Plant Production Facility

Film
## cGMP Pilot Plant Production Facility

<table>
<thead>
<tr>
<th>Production host:</th>
<th><em>Nicotiana benthamiana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed used per batch:</td>
<td>~1 g (10,000 seed)</td>
</tr>
<tr>
<td>Growth period:</td>
<td>4-6 weeks</td>
</tr>
<tr>
<td>Average aerial biomass:</td>
<td>~50 kg</td>
</tr>
<tr>
<td>Biomass increase:</td>
<td>~50,000 fold</td>
</tr>
<tr>
<td>Seed per plant:</td>
<td>~15 g</td>
</tr>
</tbody>
</table>

One plant provides enough seed for ~0.75 metric tons of biomass
cGMP Pilot Plant Production Facility:
Pre-Infiltration Plant Growth
cGMP Pilot Plant Production Facility:
Vacuum Infiltration
cGMP Pilot Plant Production Facility:
Post-Infiltration Plant Growth
cGMP Pilot Plant Production Facility: Harvest
FhCMB Pilot Facility – Process Chromatography for Purification
How can we use this technology?
**Bacillus anthracis**, a bug with attitude!

Anthrax is a top choice for use as a biological warfare agent.
- Bacteria spores can be processed to become airborne
- Spores remain dangerous for decades
- Can be produced in large quantities with basic technology

Anthrax was used as a biological weapon in the United States.
- Post 9/11 terror attacks, US citizens contracted cutaneous and inhalational anthrax
- Delivering weaponized anthrax was as simple as putting it in an envelope and mailing.
Anthrax Vaccination Strategies

Potentially vaccination targets:

- Live attenuated spores
- Spore components (BclA, BxpB)
- Live attenuated bacilli
- **Protective antigen (PA)**
- Edema factor (EF)
- Lethal factor (LF)
- Capsular antigen

Mol Aspects Med 30, 490-502, 2009
## Examples of Industrially Produced Plant-Based Biologics

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>System</th>
<th>Product</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protalix</td>
<td>Carrot (root cell suspension)</td>
<td>Elelyso™ (taliglucerase alfa) (Gaucher Disease)</td>
<td>FDA Cleared</td>
</tr>
<tr>
<td>InVitria</td>
<td>Rice (seed)</td>
<td>Cell Culture Products (rh-albumin, rh-lysozyme, rh-transferrin, rh-lactoferrin)</td>
<td>Marketed</td>
</tr>
<tr>
<td>Medicago</td>
<td>Tobacco (whole plant)</td>
<td>Influenza Vaccines</td>
<td>Phase II</td>
</tr>
<tr>
<td>Planet Biotechnology</td>
<td>Tobacco (whole plant)</td>
<td>Dental Caries</td>
<td>Phase II</td>
</tr>
<tr>
<td>Ventria Bioscience</td>
<td>Rice (seed)</td>
<td>VEN100 (rh-lactoferrin) VEN200 (rh-albumin)</td>
<td>Phase II Phase I</td>
</tr>
<tr>
<td>Synthon</td>
<td>Duckweed (whole plant)</td>
<td>Interferon Alpha (Hepatitis C)</td>
<td>Unknown*</td>
</tr>
</tbody>
</table>

*Synthon acquired intellectual property related to this and other products during the June 2012 liquidation of Biolex Therapeutics. The terms of the acquisition were not published and the status of these products is not currently available.

Fraunhofer-CMB, Newark, DE

Thank you!