Breakthroughs in Plant Based PHB Production: Harnessing Nature to Heal Nature

Kristi Snell
CSO and VP of Research
www.yield10bio.com

3rd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology. October 4th-6th, 2019
The statements made by Yield10 Bioscience, Inc. (the “Company,” “we,” “our” or “us”) herein regarding the Company and its business may be forward-looking in nature and are made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. Forward-looking statements describe the Company’s future plans, projections, strategies and expectations, including statements regarding future results of operations and financial position, business strategy, prospective products and technologies, timing for receiving and reporting results of field tests and likelihood of success, and objectives of the Company for the future, and are based on certain assumptions and involve a number of risks and uncertainties, many of which are beyond the control of the Company, including, but not limited to, the risks detailed in the Company’s Annual Report on Form 10-k for the year ended December 31, 2018 and other reports filed by the Company with the Securities and Exchange Commission (the “SEC”). Forward-looking statements include all statements which are not historical facts, and can generally be identified by terms such as anticipates, believes, could, estimates, intends, may, plans, projects, should, will, would, or the negative of those terms and similar expressions.

Because forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified and may be beyond the Company’s control, you should not rely on these statements as predictions of future events. Actual results could differ materially from those projected due to our history of losses, lack of market acceptance of our products and technologies, the complexity of technology development and relevant regulatory processes, market competition, changes in the local and national economies, and various other factors. All forward-looking statements contained herein speak only as of the date hereof, and the Company undertakes no obligation to update any forward-looking statements, whether to reflect new information, events or circumstances after the date hereof or otherwise, except as may be required by law.
Three potential revenue streams each with different commercialization paths

**Performance Traits (Licensing to Ag Majors)**
- Seed yield
- Oil content
- Canola, soybean, corn - field trials
- Licensing to Ag majors to maximize acreage

**Cash Cover Crops (Camelina - Product Sales)**
- Nutritional oils
- PHA biomaterials
- Business participation
- Product sales, partners along the value chain

**R&D Services**
- Funded R&D
- Milestone payments
- License revenue e.g. CBD medicinals

**Agreements**
- Bayer CropScience
- Forage Genetics
- Future

**Multiple paths to revenue**
Polyhydroxyalkanoate biomaterials

- Renewable, biodegradable class of biomaterials produced by some microorganisms as reservoir of stored carbon and energy
- Fully degradable in all biologically active environments
  - Soil, rivers, oceans, compost, sewage, etc.
  - Intracellular and extracellular depolymerases that degrade the polymer
- Unique features of polymers will allow use in multiple applications
  - Plastics, renewable chemicals, water treatment
    - Plastics applications have historically been targeted
    - Pellets for water treatment simpler first commercial product
- Barriers:
  - Market adoption has been severely restricted by high cost
    - Production by fermentation: cost too high for most applications
    - Production in plants: high level production has often impacted plant growth or seedling emergence/survival

Water treatment application

Denitrifying bacteria naturally present in nitrate polluted water consume PHA and convert nitrate (NO$_3^-$) to inert nitrogen (N$_2$) gas.
**Human health**

“Considering all studies, the strongest evidence for a relationship between drinking water nitrate ingestion and adverse health outcomes (besides methemoglobinemia) is for colorectal cancer, thyroid disease, and neural tube defects. Many studies observed increased risk with ingestion of water nitrate levels that were below regulatory limits.” Mary H. Ward et., al. 2018, International Journal of Environmental Research and Public Health, 15, 1557.

Production of Polyhydroxybutyrate (PHB) in Oilseeds

**PHB cover crop: Harnessing nature to heal nature**

*Cover crop mitigates nutrient runoff in field, produces product for water treatment*

**Choice of crop – Camelina**
- Seed oil levels typically 40% of seed weight (depends on cultivar and growth conditions)
- Does not outcross with *Brassica napus* or other crop Brassicas
- Both spring and winter varieties available
  - *winter varieties cover crop after corn and soybean to prevent nitrogen runoff*

---

**PHB granules in seed**
- Camelina producing PHB

**Winter cover crop**
- Remove excess nitrate from soil
- *Mitigates nitrate issue at source*

**Metabolic Engineering**

---

**PHB**
- Seed oil
- Seed meal
- *Water treatment*
  - Energy
  - Aquaculture feed
  - Industrial oils

**Products generating revenue for farmer**
- Energy
- Animal feed

---

Yield10 Camelina Review: Malik et al., 2018, Plant Cell Rep., 37, 1367
Today

PHB pathway – substrate acetyl-CoA well suited to oilseeds

**PHB biosynthetic pathway**

![Diagram of PHB biosynthetic pathway]

**Targeted sites for production of polymer in seeds**

![Diagram showing targeted sites for PHB production]

**Chloroplasts/plastids** have yielded the highest levels of PHB in plants
- Little production in cytosol and peroxisome
- No production demonstrated in mitochondria

**Yield10 Reviews**
*Somleva et al., 2013, PHA bioplastics, biochemicals, and energy from crops Plant Biotechnol. J., 11, 233*
Prior Work: Production of PHB in Oilseeds

Prior work in seed plastids

Highest levels of PHB Production - Seed Plastids

- Monsanto Canola seed
- Yield10 Camelina seed

Chlorotic seedlings - low survival in soil. Seedlings capable of growth in tissue culture medium supplemented with sugar prior to transfer to soil

Prior work in seed cytosol

Highest levels of PHB Production - Cytosol

- Arabidopsis leaf cytosol
- Cotton fibers cytosol

No prior reports of engineering PHB into cytosol of seeds

Revisit production of PHB in cytosol

Two genetic constructs created that differ in targeting of polymerization enzyme

- **Construct 1.** All enzymes targeted to cytosol
- **Construct 2.** Thiolase (PhaA) and reductase (PhaB) enzymes targeted to cytosol; PHA synthase (PhaC) anchored to the cytosolic face of the endoplasmic reticulum (ER)

Camelina plants transformed, T1 generation seeds isolated, T1 plants grown in soil in greenhouse, T2 generation seeds harvested
T_2 Generation Seed: PHB Content and Survival of lines

**Cytosolic PHA synthase**
*(top 20 PHB producers)*

**ER anchored PHA synthase**
*(top 20 PHB producers)*
Cytosolic PHB producers, healthy seedlings with narrow cotyledons

**Phenotype of 7 day old seedlings**

<table>
<thead>
<tr>
<th>Type</th>
<th>PHB</th>
<th>Emergence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>4.4%</td>
<td>92%</td>
<td>87%</td>
</tr>
<tr>
<td>Cytosolic PhaC</td>
<td>4.5%</td>
<td>53%</td>
<td>33%</td>
</tr>
<tr>
<td>ER PhaC</td>
<td>4.4%</td>
<td>92%</td>
<td>87%</td>
</tr>
</tbody>
</table>

**Light microscopy of cotyledon cross sections**

- **20X magnification**
  - IS = Intercellular space
  - VB = vascular bundle
  - PM = palisade mesophyll
  - SM = spongy mesophyll
  - UE = upper epidermis
  - LE = lower epidermis

Visible differences in:
- size and presence of intercellular spaces (IS)
- elongation of palisade layer
Spatial Distribution of PHB Granules in Seeds

Transmission electron microscopy (TEM) of cotyledon in imbibed T2 seeds

- Polymer accumulates as granules within seed
Polymer Production in Advanced Generations

ER targeted synthase showed clear advantage over cytosolic targeted synthase in homozygous lines

- **Cytosolic targeted synthase**: PHB production dropped from high of 4.5% PHB (T2 seeds) to 2.9% PHB (T3 seeds)
- **ER targeted synthase**: Homozygous lines producing T3 seeds with 9.1% and 6.8% PHB isolated

Only lines with ER targeted synthase pursued in later generations
ER Targeted Synthase: PHB Production in T₄ Seeds

Lines grown in greenhouse and controlled environmental chamber programmed to simulate average spring growth conditions¹

- Results for best line shown

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Greenhouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Controlled Environmental Chamber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>20</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

- Day length (h)
- Day temp (°C)
- Night temp (°C)

<table>
<thead>
<tr>
<th>T₄ seed PHB range</th>
<th>3.9 - 7.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% survival T₃ seedlings</td>
<td>86</td>
</tr>
<tr>
<td>Avg T₄ seed yield (g)</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td>Number of plants</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T₄ seed PHB range</th>
<th>4.6 -10.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>% survival T₃ seedlings</td>
<td>78</td>
</tr>
<tr>
<td>Avg T₄ seed yield (g)</td>
<td>14.7 ± 3.9</td>
</tr>
<tr>
<td>Number of plants</td>
<td>4²</td>
</tr>
</tbody>
</table>

¹Temperature settings in the controlled environmental chamber adapted from average weekly historical data between early May and late July for Saskatoon, Saskatchewan, Canada, an area suitable for Camelina growth. ²Size of growth chamber limited number of replicates
Partitioning of Carbon in PHB Producing Lines

Oil and protein content measured in top producing plants from greenhouse and controlled environmental chamber (simulated spring conditions) growth

- Production of PHB reduces seed oil content
  - PHB has more value than oil
  - Looking for genes to increase carbon to seed to boost oil using GRAIN modeling platform
- Little difference in protein content observed with PHB production
- Higher levels of PHB and oil observed in plants grown under simulated spring conditions (chamber growth)
Conclusions and Future Directions

• Stable PHB production achieved in homozygous Camelina seeds by anchoring polymerization enzyme PHA synthase to endoplasmic reticulum (ER)

• Levels up to 10.2% of mature seed weight achieved when grown under controlled conditions simulating spring in Canadian prairies

• PHB produced at expense of oil but not protein

• Cotyledons healthy and narrower than wild-type controls
  - Significant improvement over plastid PHB producers that had chlorotic cotyledons with low survival\(^1\)

• Field trials planned

• Investigating use of PHB producing cover crop to address nutrient runoff
  - Cover crop mitigates nutrient runoff at source, product PHB used to treat contaminated water
  - Example of agricultural product to correct the ills of agriculture

\(^1\)Malik et al., 2015, Plant Biotechnol. J., 13, 675
Breakthroughs in Plant Based PHB Production: Harnessing Nature to Heal Nature

Kristi Snell
CSO and VP of Research
www.yield10bio.com

3rd International Conference on Plant Synthetic Biology, Bioengineering, and Biotechnology.
October 4th-6th, 2019