The Fascinating Journey from Discovery to Product

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From idea to market, there are many steps to get to a consistently performing Biological product.

Development from discovery to market is complex but with approximately one tenth of costs and 5-8 years faster in the market than chemical pesticides.
So what’s involved?

Discovery and Research

- Identification
  - Discovering a lead is the first step in the process
- Characterization
  - Identification / confirmation of mode of action
  - Testing in predictive assays
- Design
  - Strain Optimization and Process Improvement
- Evaluation
  - Broad greenhouse and Worldwide Field Testing

Discovery and Research
Discovery and Research followed by Development

**Discovery**
- Bioinformatics
- Metagenomics
- Microbial genetics
- Metabolomics and Proteomics

**Understanding Biology**
- Plant microbe interaction
- Mode of action of biologicals
- Microbiome
- Early field characterization
- Safety

**Fermentation & Formulation**
- High throughput fermentation
- Production (submerged and solid state)
- Shelf life & stability
- Compatibility

**Field Testing**
- Registration trials
- Positioning trials

**Moving towards Commercial**
- Regulatory
- IP / Freedom to operate
- Supply chain
- Commercial

Discovery and Research followed by Development
Safety in the Research Phase

Safety
- Human and environmental safety evaluation
- Omics analysis – genome & biological chemistry
- In vitro and in planta characterization
- Field performance profiling
- Formulation development
- Strain & Fermentation development

Efficacy
- Safety studies
- Registration & positioning field trials
- Stability studies
- Process scale-up

Stability

Quality

Discovery & Research
Development
Commercialization

Intellection Property – Protecting your investment
Safety during Research phase

Before we spend money on trials, can we get genomic information that will help?

Before we go to field or scale up a fermentation process…
  Non-GLP mouse oral tox?
  Some countries require bee testing before any field trials

Have we determined Plant Safety (phytotoxicity)?
Let’s focus on Efficacy…

- Safety
- Efficacy
- Stability
- Quality

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- Process scale-up
- Authority evaluation

- Intellection Property – Protecting your investment
Characterizing Efficacy
Making the jump from lab to field can be a big hurdle

Primary in vitro screening
Screening on plants
Field Trials on final target

Photos from Bayer Crop Science, West Sacramento
What conditions will give your discovery the best chance of success?

Understanding mode of action aids in field transfer

**Foliar** Contact?

Environmental conditions?

**Soil** Colonization?
Efficacy during Research
Proof of Concept – moving to the field

QUESTIONS TO ADDRESS:

• Best crop and target based on research results
• Efficacious dose
• Spray intervals needed
• Can adjuvants help?
• What are the right controls?

Field trials, what’s important
   Proof of concept, stand alone, programs
   Regulatory trial
Quality and profitability or cost of goods (COGs)

Safety
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- *In vitro* and *in planta* characterization

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Stability
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Quality
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Application Optimization
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Authority evaluation

Discovery & Research
- Intellection Property – Protecting your investment

Development

Commercialization
Integrated Approach to Developing a Microbial Fermentation Process

**Goal:**
To develop **cost-effective**, robust process for commercial production of microbial active ingredients

**Integrated Approaches:**

**Fermentation**
- Physiology, differentiation, biosynthesis
- Growth media (C, N ...)
- Growth conditions (pH, temp, DO)

**Strain Improvement**
- Eliminate genetic bottlenecks

**Engineering**
- Robustness
- Reproducibility
- Scalability

**Biomass**
- cell density
- morphology
- sporulation
- CFU

**Biological chemistry**
- analytical
- metabolomics

**Bioactivities**
- *in vitro*
- *in planta*

**Physi/chem properties**
- pH
- viscosity
- solids

- Yield $\uparrow$
- Cost $\downarrow$

- **Small Fermenter**
- **Flask**
- **Pilot plant**

- 2-4x
- 5x
- 3-30x
Process Flow Diagram

1. **Fermentation ingredients**
2. **Seed**
3. **Air**
4. **Spores**
5. **Biological Chem**
6. **Solids**
7. **Centrifuge (or filtration)**
8. **Formulation**
9. **Spray Dryer**
10. **Liquid Product**
11. **Heated air or gas**
12. **Cyclone Powder Collector**
13. **Collection Vessel**
14. **Solid Product**
Scaling up the process during Development

The Critical Link between Research and Manufacturing

Convert microbial discoveries into commercial products

Manufacturing process: fermentation & formulation

Lab scale → Pilot scale → Production

10-100x

Creation
Innovation
Optimization

Scale up
Validation
Specifications

Implementation
Reproducibility
Production

10-100x
Process Development

Transforming microbial discoveries into commercial products
Create manufacturing process & formulation

Fermentation ➔ Downstream ➔ Formulation

Active Ingredients ➔ Concentration/stabilization ➔ Final product
How to interest a partner in development and commercialization

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**Application Optimization**

**Commercial Production**

**Authority evaluation**

**Intellection Property – Protecting your investment**
Preparing for Registration

Development Phase
US EPA BIOPESTICIDE
Data Requirements

FIRST REGULATORY AGENCY TO DEVELOP SEPARATE DATA REQUIREMENTS AND STUDY GUIDELINES

• MICROBIAL

• BIOCHEMICAL

SEPARATE DIVISION WITHIN EPA TO REVIEW BIOPESTICIDES

• BUILDS KNOWLEDGE AND EXPERTISE

• TWO DIVISIONS

• MICROBIAL

• BIOCHEMICAL

REVISED DATA REQUIREMENTS ISSUED IN 2007 TO FURTHER CLARIFY THE REQUIREMENTS.
History of Biologics Safety Assessment: US EPA

- 1948 - First microbial pesticides registered in the US
- 1974 – EPA sponsors workshops, symposia and panel discussions to identify relevant safety concerns for microbial pesticides
- 1983 – EPA issues testing guidelines for microbial pesticides (“Subdivision M” of the Pesticide Assessment Guidelines); Subdivision M updated in 1989 after a Scientific Advisory Panel review
- It is in this Subdivision M that the **Tiered testing system** is defined and scientifically justified
Safety…

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- Application
- Optimization
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- Production

- Discovery & Research
- Development
- Commercialization

- Intelllection Property – Protecting your investment
Safety studies to prepare for regulatory submission package
The Tier I “six-pack”, for microbial pesticide registrations at EPA

- Acute oral toxicity
- Acute dermal toxicity
- Acute inhalation toxicity
- Primary eye irritation
- Primary dermal irritation
- Dermal sensitization
US EPA Tier II Tests - Toxicology

When are Tier II data required (per US EPA)?

“(Tier II) Data required when significant toxicity, in the absence of pathogenicity and significant infectivity, is observed in acute oral, injection, or pulmonary studies (Tier I). Route(s) of exposure correspond to route(s) where toxicity was observed in Tier I studies. The toxic component of the TGAI is to be tested.”

[40 CFR 158.2140]

<table>
<thead>
<tr>
<th>Data Requirement</th>
<th>Test Substance</th>
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<tbody>
<tr>
<td>Acute toxicology</td>
<td>TGAI, “toxic component”</td>
</tr>
<tr>
<td>Subchronic tox/path</td>
<td>TGAI, “toxic component”</td>
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### US EPA Non Target Organisms and Environmental Fate

For Microbial Pesticides

<table>
<thead>
<tr>
<th>Testing Category</th>
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<tbody>
<tr>
<td>Avian oral toxicity</td>
</tr>
<tr>
<td>Avian inhalation toxicity/pathogenicity</td>
</tr>
<tr>
<td>Wild mammal toxicity/pathogenicity</td>
</tr>
<tr>
<td>Freshwater fish toxicity/pathogenicity</td>
</tr>
<tr>
<td>Freshwater invertebrate toxicity/pathogenicity</td>
</tr>
<tr>
<td>Estuarine/Marine fish testing</td>
</tr>
<tr>
<td>Estuarine and marine invertebrate testing</td>
</tr>
<tr>
<td>Nontarget plant testing</td>
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<tr>
<td>Nontarget insect testing</td>
</tr>
<tr>
<td>Honey bee testing</td>
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Efficacy during Development Phase

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- Quality
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Process:
- Discovery & Research
- Development
- Commercialization

Additional Notes:
- Authority evaluation
- Application Optimization
- Commercial Production

Intellection Property – Protecting your investment
Efficacy during Development
Registration trial protocols depend on country

REGISTRATION:
US EPA, trials are not required to be submitted
This differs state by state – California requires efficacy

Typically registration trials require stand alone treatments compared to Untreated and standards

POSITIONING TRIALS:
How will the product be used by a grower?
In a program, rotated, tank mixed?
Stability studies also required for Regulatory package

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Advances in the era of Omics
- In vitro and in planta characterization

Intellection Property – Protecting your investment
Many steps from idea to market to deliver consistently performing biological products to growers

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Efficacy

Stability

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Process scale-up

Authority evaluation

Commercial Production

Application Optimization

Intellection Property – Protecting your investment
The Computational Life Sciences group mines the strain collection for genes of interest.

The Microbial Curation team manages and maintains a diverse set of microbial species in our biostore.

Strains that show potential to control pests and diseases, or increase yield are selected for higher-tiered testing and further evaluation by our collaboration-focused project teams.

Microbiology optimizes the selected microbes, identifying variants with enhanced performance.

Microbiology optimizes the selected microbes, identifying variants with enhanced performance.

Biological activity is measured and confirmed in Pest and Disease Management assays.

Our greenhouse team produces plants to be used for testing against diseases/pests in the lab and supports all plant-based research conducted in the greenhouse and microplots.

In various regions, we also have a dedicated metabolism research team that focuses on our large-scale fermentation processes.

Fermentation develops robust, cost-effective and scale-adaptable fermentation processes through media and process optimization at scales ranging from 4 mL to 100 L.

Analytical Services ensure quality and safety of our products by testing each batch for consistency and then transfers these methods to the production site.

Our final product is grown in Production Supply Sites at volumes of 30,000+ liters.

Commercial Sales and Marketing teams collaborate with our stakeholders and customers, such as distributors and growers, to help them use these products in their full program portfolios.

Discovery is an exciting and important first step… But there are many more to follow
Thank you for your attention!

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