JOYN BIO

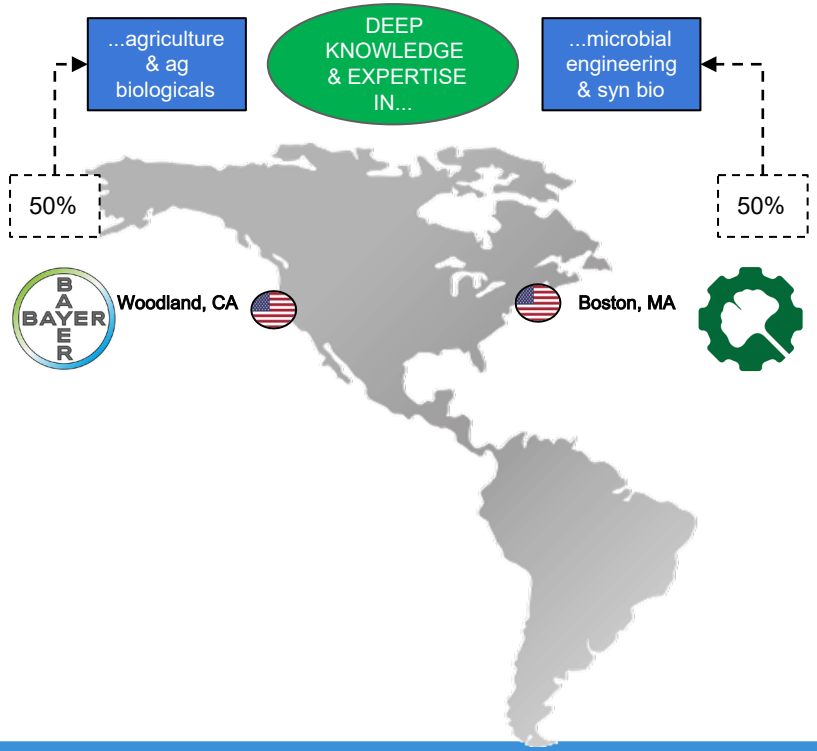
Hypothetical Case Study:

Engineered Microbial Biopesticide

Outline

- Joyn Bio Overview
- Hypothetical Case Study Overview
- Host Strain information
- Genetic elements
- Strain Build
- Regulatory Pathway

Joyn Bio is a JV between **Bayer** and **Ginkgo Bioworks**



- Joint Venture leverages Bayer's agricultural expertise & Ginkgo's organism engineering expertise
- Primary location in Boston + second location at Bayer Woodland, CA site

Bayer and Ginkgo Bioworks Unveil Joint Venture, Joyn Bio, and Establish Operations in Boston and West Sacramento

- Joyn Bio's initial efforts focus on nitrogen fixation to enhance sustainability in agriculture

- Researchers will broadly explore application of beneficial microbes to plants for next generation solutions to agriculture's biggest challenges

Bayer And Ginkgo Bioworks, A Startup, Aim To Make Crops Produce Their Own Nitrogen Fertilizer

Engineering Microbes for Sustainable Solutions for Agriculture

Joyn Bio is Using Biotechnology to develop high -performing microbes

- Agricultural inputs for crop protection and fertility that meet or exceed the performance of their chemical counterparts
- Sustainable solutions to address impacts of climate change
  - Reduce use of synthetic fertilizers and pesticides
  - Increase nutrient use efficiency
  - Increase crop resilience to abiotic stress
  - Enhance soil health

Hypothetical Case Study

Product concept: engineered fungal strain expressing an endotoxin to provide protection against corn rootworm (CRW) throughout the growing season

- **Target Crop:** Corn
- **Application Method(s):**
  a. Sporulated engineered fungi coated on seeds (minimum of 10^6 CFU/seed)
  b. In-furrow application, 1qt/acre
     - solution concentration: minimum of 10^8 CFU/ mL
- **Host Strain:** *Trichoderma harzianum* strain T-22
- **Gene Expressed:** *cry3Bb1*
- **Mode of action:** *cry3Bb1* binds to specific sites in the cells of the CRW digestive system and form ion-selective channels in the cell membrane, resulting in lysis of the cells and death of the pest larvae

Engineering Overview:

- **Host Strain:** *Trichoderma harzianum T-22*
- **Gene introduced:** *cry3Bb1*
- **Modification Technique:** CRISPR
- **Plant pest genes known:** No
- **Antimicrobial production:** Yes (antibiotic pathway deleted)
- **Product Market:** Biopesticide

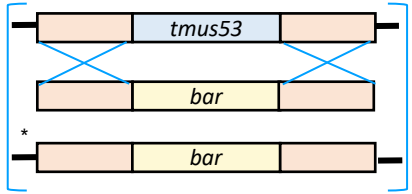
Strain Information and Modification

- **Host Organism:** *Trichorderma harzianum T-22*
  - Naturally occurring fungus used for root disease control in a wide variety of plant hosts
  - EPA Registered, ccommercially available fungicide
- **Modification:**
  - CRISPR: Used to insert *cry3Bb1* and *pry4* genes
  - *pyr4 cre/loxP marker recycling system* for continued selection and marker removal **
- **Genotype:**
  - JB01△tmus53::*cry3Bb1*

** Transformation System for Hypocrea jecorina (Trichoderma reesei) ThatFavors Homologous Integration and Employs Reusable BidirectionallySelectable Markers

Genetic Elements

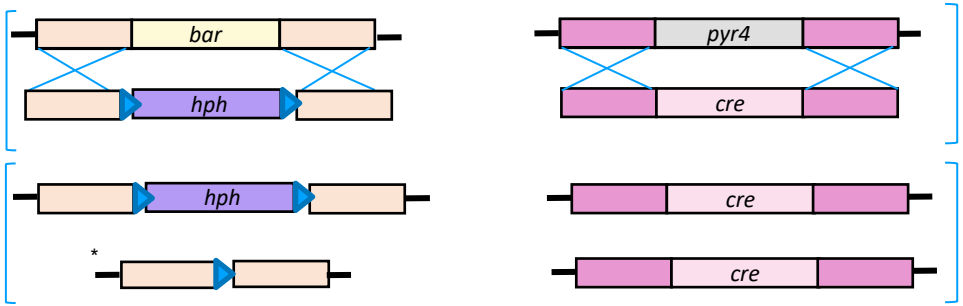
- **Intended Phenotype:** Biocontrol of Corn Rootworm on Corn Plants
  - Promoter: **Peno1**
    - Donor: *Trichoderma reesei*
    - Function: used to drive expression of *cry* gene
  - Gene of Interest: ***cry3Bb1***
    - Donor: *Bacillus thuringiensis*
    - Function: expression of *cry* protein against CRW
  - Selectable Marker:
    - Gene: ***pyr4***
    - Donor: *Trichorderma harzianum*
    - Function: resistance marker used to select engineered strain
  - Terminator: **Teno1**
    - Donor: *Trichoderma reesei*
    - Function: Gene terminator



1. Through transformation the WT *tmus53* gene was replaced with the *bar* gene to both create a NHEJ-deficient strain.

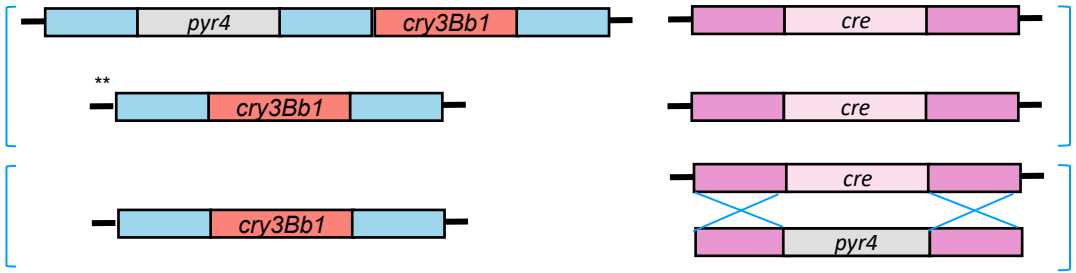
Gene Descriptions:

- *tmus53:* required for nonhomologous end joining
- *pyr4:* loss of gene causes uridine auxotrophy
- *cre:* encodes for Cre recombinase
- *loxP-hph-loxP: encodes* hygromycin resistance
- * : Designates new parent strain
- ▶ : Designates loxP site

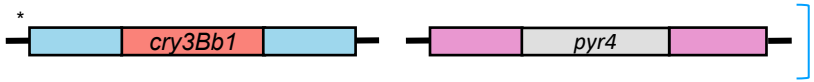


2. In a co-transformation step, the *bar* gene was replaced with the loxP-amdS-hph-loxP cassette and the *pyr4* gene was replaced with the *cre* gene that encodes for Cre Recombinase.
3. The transformants were then selected on Hygromycin.

4. Using a D-xylose inducible promoter, the transformed protoplasts were plated on xylose media causing the *cre* gene to be expressed and the loxP-amdS-hph-loxP cassette to be removed.



5. The CRISPR- Cas9 system was used to transform fungal protoplasts and reintroduce the *pyr4* gene along with our gene of interest, *cry3Bb1*.
6. The pyr4 marker loop out was then induced by plating on counter selective media 5-FOA.
**This strain could then be used for subsequent transformation using the pyr4 cre/loxP system



7. A final transformation was done in order to remove *cre* gene and reintroduce *pyr4* gene.

Field Release Requirements

EPA: A Biotech Notification will be submitted prior to small -scale testing of the organism (40 CFR §172.48)

USDA APHIS BRS: Joyn asserts the engineered microorganism does not pose a plant pest risk given:

- It has NOT been engineered with DNA that is capable of producing an infection agent that causes plant disease or encodes a compound that can cause plant disease
- The host species and the integrated cry protein are both EPA registered pesticides with data demonstrating they are not likely pose to any unreasonable adverse effects to nontarget organisms and therefore are not likely to pose a plant pest risk

https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry3bb1-brad.pdf
https://www.regulations.gov/document/EPA-HQ-OPP-2006-0245-0006

Product Registration

EPA Biopesticide Active Ingredient New Registration for Food Use

Petition to Establish a Tolerance Exemption

PRIA Fee Category B590

Action Code	Description	FY'22-FY'23 Fee	Decision Time (months)
B590	New active ingredient; food use; petition to establish a tolerance exemption (2)(3)	$35,182	18

Product Registration

Trichoderma harzianum strain JB01 is produced through an integrated liquid fermentation process

- Fermentation Whole Broth will be Submitted for Registration as a Manufacturing Use Product (MUP)
- End-use Product (EP) is an Aqueous Solution formulated with Inert Ingredients from EPA Historical List 4a and 4b

Product Composition	MUP	EP
Active Ingredients: *Trichoderma harzianum JB01**	10% (*min 1.0 x 10^9 CFU/mL)	1.5% (*min 1.0 x 10^8 CFU/mL)
Other Ingredients:	90%	98.5%
TOTAL:	100	100

Regulatory Data

Microbial Pesticide Data Requirements	MUP	EP
Product Analysis (40 CFR § 158.2120)	All required studies	All required studies
Toxicology (40 CFR § 158.2140)	● Bridge to host strain pathogenicity data ● Bridge acute tox studies to EP tox data	All required studies
Nontarget Organisms & Environmental Fate (40 CFR § 158.2150)	All required Tier 1 studies	Not required on EP

Regulatory Data

In addition to the required data outlined in the CFR, the following information will be included in the registration data:

- Genetic engineering techniques used
- Identity of the integrated sequence (base sequence data and enzyme restriction map)
- DNA source information
- A description of the characteristics intended to be expressed
- Genetic stability data over multiple generations or growth cycles

Questions

- Would USDA APHIS BRS consider this organism to be a plant pest risk and if so why?
- Does EPA agree with the proposed registration data?