# Understanding GE Microbes & U.S. Regulations – Case Study

## Nuts & Bolts of U.S. Regulatory Dossiers for GE Products, SCRA

Nik Evitt, VP of Operations, Switch Bioworks

September 20, 2023

# Disclaimer + Conflict of Interest

- This case study describes a hypothetical GE microbe product based on industry trends, not an actual product of Switch Bioworks.
- No assumptions about Switch Bioworks, its performance, or its research and development pipeline should be made on the basis of this presentation.
- No investment decisions should be made on the basis of this presentation.
- Nik is a stockholder of Switch Bioworks.

# Acknowledgements

**Switchforce**

**Eversole Associates**

- Kellye Eversole
- Dusti Gallagher
- Isabelle Caugant
- Rolanda Young

**US Government**

**Public Comments**

- Docket ID APHIS-2023-0030

**Graphic Design**

- BioRender.com

# Goal: Clarify how product features influence regulatory path

**Detailed Objectives**

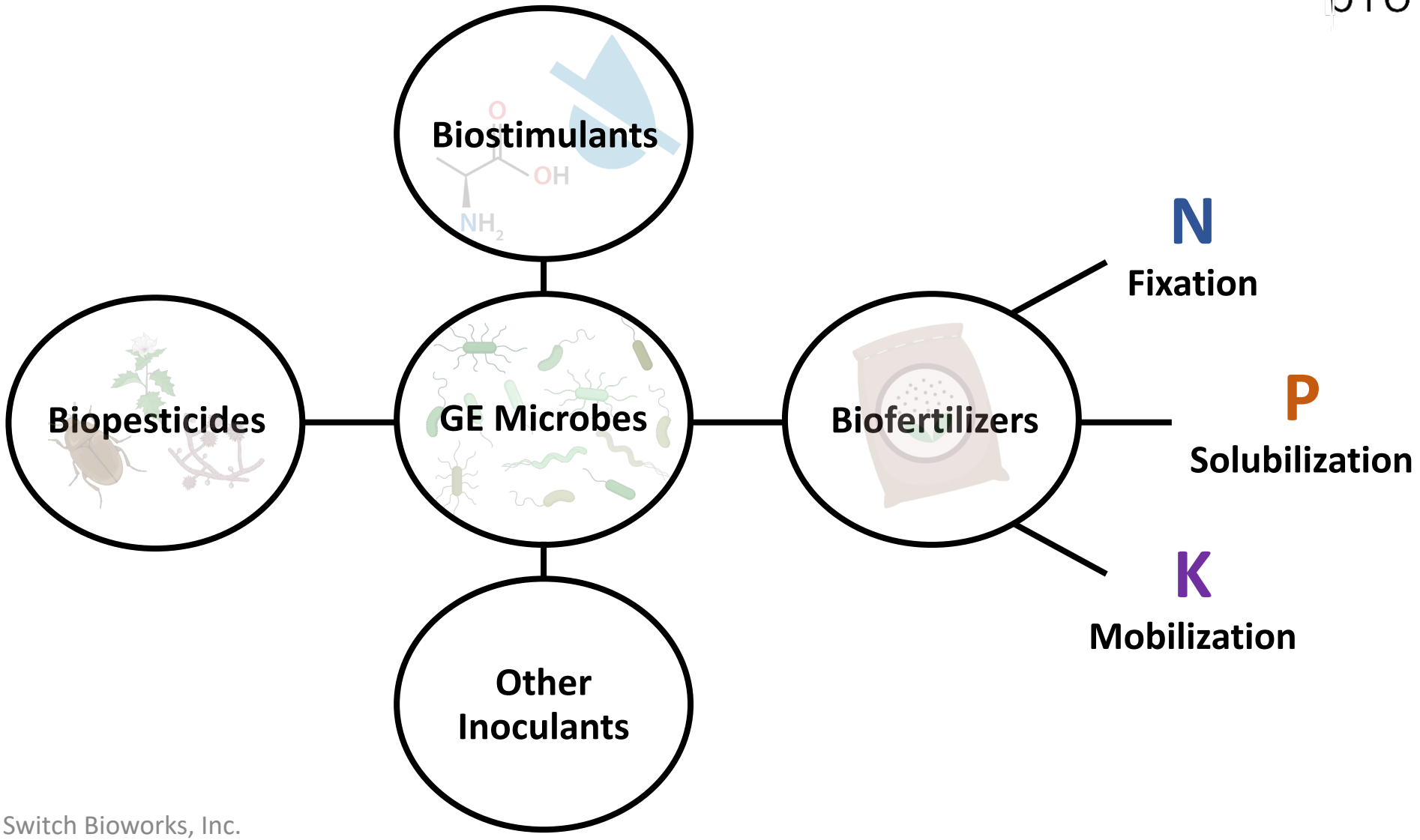
- Review common use cases and product features among agricultural GE microbes
- Describe how industry trends could intersect to create a complex next-generation biofertilizer product
- Explore how technical nuances affect regulatory requirements surrounding initial field release
- Determine where developers could benefit from additional guidance to accelerate grower access to safe, effective microbial products

**Contents**

1. Introduction: Intended uses and features of GE microbes
2. Case: Consortium of GE microbes applied in-furrow as biofertilizer
   a. Product review + regulatory questions
   b. Regulatory steps to field trial readiness

# Introduction: Intended uses and features of GE microbes

# GE microbes have many use cases



# Diverse microbes offer solutions to each use case


| Number of Microbes | Microbial Taxa & Physiology | GE Parts | GE Methods | Microbial "Metadata" |
|---|---|---|---|---|
| One | Gram + vs. – (spores or no) | Number / Type | Directed Evolution | Geographic Source |
| Two to a Few | Rhizosphere vs. Endophyte | Phenotype (e.g. GOF vs. LOF) | *in situ* | Literature + Data Volume + Quality |
| Consortium | | Source Organism(s) | Cut & Paste | |

# Each agency regulates specific product types

| Agency | Statutes | Regulations | Regulated Products |
|---|---|---|---|
| USDA | • Plant Protection Act (**PPA**) | • **7 CFR 330** (no GE + unknown risk); **7 CFR 340** (GE); **7 CFR 360** (noxious weeds) | • Actual and ***potential*** plant pests (includes certain GE modification); noxious weeds |
| EPA | • Toxic Substance Control Act (**TSCA**)<br>• Federal Insecticide, Fungicide, and Rodenticide Act (**FIFRA**) | • TSCA: **40 CFR 700, 720, 721, 723, 725**<br>• FIFRA: **40 CFR 158** (and others) | • TSCA: catch-all for "new chemical substances" incl. **intergeneric** GE microbes<br>• FIFRA: pesticides incl. **microbes** (GE or no) & **PIPs** |
| FDA | • Federal Food, Drug, and Cosmetic Act (**FD&C**) | • **21 CFR 170.3(i) and 570.3(i)**; Details also in USC | • Food & food additives |

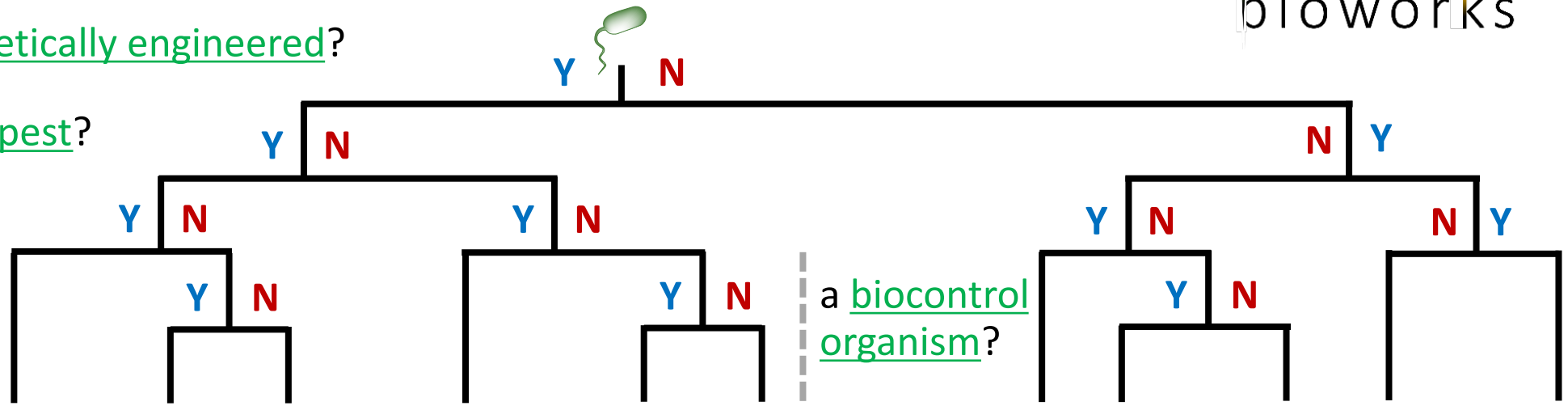
# Determine which federal agency(ies) may regulate the product

Is the microbe genetically engineered?

a (potential) plant pest?

a pesticide?

with intergeneric GE parts?




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| USDA | BRS (PPA) | BRS (PPA) | BRS (PPA) | -- | -- | -- | === === | -- | PPQ (PPA) | -- | PPQ (PPA) | PPQ (PPA) |
| EPA | OPP (FIFRA) | OPPT (TSCA) | -- | OPP (FIFRA) | OPPT (TSCA) | -- | === === | OPP (FIFRA) | -- | -- | -- | OPP (FIFRA) |

FDA

- Is it food or a food additive (nothing else matters)? If yes, check with FDA to see if it is regulated.
- **Historically, FDA has not regulated fertilizer**, soil amendments, or soil inoculants.

# Case: Consortium of GE microbes applied in-furrow as biofertilizer – Product review + regulatory questions

# Product Overview

**Stage**

- 1st field trial release after contained R&D

**Intended Use**

- Apply to soil surrounding crop plants
- Generate bioavailable nitrogen → plant growth
- Corn → lettuce + sweet potato (used as food)
- **NOT** to be marketed as pesticide/biocontrol

**Formulation**

- Spray dried powder
  - 5 separate microbes
- Aqueous rehydration media
  - No known allergens

**Mechanism of Action**

- High consortium colonization (5% – each 1%)
- "Basal-bolus" nitrogen release → plant growth

**Organisms (all GE)**

| Species | Origin |
|---|---|
| *Gluconacetobacter diazotrophicus* | Rice field (CA, US) |
| *Pseudomonas stutzeri* (Strain #321-β) | Corn field (SW Kenya) |
| *Pseudomonas stutzeri* (Strain #42-γ) | Corn field A (Boone, IA, US) |
| *Kosakonia radicincitans* | Corn field A (Boone, IA, US) |
| *Paenibacillus azotofixans* | Corn field (IN, US) |

# Emerging Questions: Formulation

**Permit count for a consortium product**

- **How many permit applications must be submitted to release the combined product?**
- **Which filing types must be submitted to which agencies?**
- If one application per organism:
  - **Can closely related organisms be grouped into a single application? When?**
  - USDA draft guidance for microbes : “For release permits, only a single species may be submitted on a given permit application.”

**Potential rehydration media additive with broad-spectrum antibacterial activity**

- Technical specifications
  - Confinement → active concentrations only **within 36 inches** of crop plants
  - Persistence → **half-life of 2 days** (biological activity gone @ 20% of initial concentration)
  - Safety → Tolerance in arthropods + plants | Clinical safety in humans, mammals, birds, fish
  - Usefulness → Widespread clinical resistance (human + vet), largely replaced
- **What factors would regulators consider to determine if additive is allowable?**

# Organism Profile: *G. diazotrophicus (Gd)* Strain #12345-α

**Organism Details**

Habitat: Endophyte

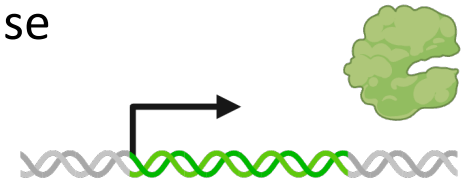
- Inhabits roots, stems, leaves
- Unlikely pathogenicity (mostly promotes growth)

Origin: Rice field in CA

- Yields in 90$^{th}$ %ile for CA
- Microbiome sequencing → normal relative abundances

**GE Modification Details**

- CDS for plant virus protease
- Not independently infectious or mobile
- Tech: Cut & Paste



**Data + Literature**

- **Pro:** Robust maize growth promotion, 10x the quantity of con data, lab + greenhouse
- **Con:** Sick *Arabidopsis*, different *Gd* strain, 5 papers, same academic lab

**What degree of plant pest risk would be assigned to this strain? Effects on regulation?**

**Since *Gd* is in roots + leaves … it will be in potatoes + lettuce.**

**Does this subject the biofertilizer to FDA regulation? The consortium or just the *Gd* strain? When?**

# Organism Profiles: *P. stutzeri* Strains #321-β and #42-γ

## 321-β Organism Details

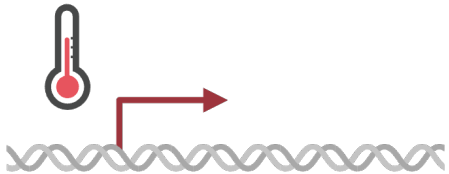
Habitat: Rhizosphere

Origin: Corn field in SW Kenya

- Yields in 90th %ile for Kenya
- Robust plant health over season
- Microbiome sequencing → expected relative abundances

## 321-β GE Modification Details

- Temp-sensitive promoter
- EXACT same sequence as in 42-γ and *Kr*



- **Source material from either 42-γ and *Kr* (but lab records are unclear which)**
- Tech: Cut & Paste

## 42-γ Organism Details

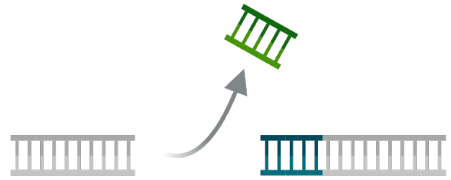
Habitat: Rhizosphere

Origin: Corn field "A" in IA

- Yields in 90th %ile for US
- Robust plant health over season
- Microbiome sequencing → expected relative abundances

## 42-γ GE Modification Details

- Truncation of native N assimilation regulatory protein
- Tech: CRISPR-Cas OR Cut & Paste



**Does method matter? TSCA or no?**

# Organism Profile: *K. radicincitans* Strain #777-ε

**Organism Details**

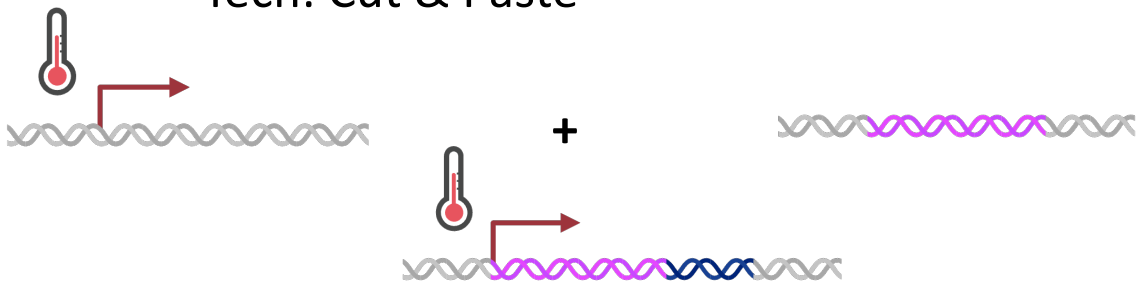
Habitat: Rhizosphere

Origin: Corn field "A" in IA

- Yields in 90$^{th}$ %ile for US
- Robust plant health over season
- Microbiome sequencing → expected relative abundances

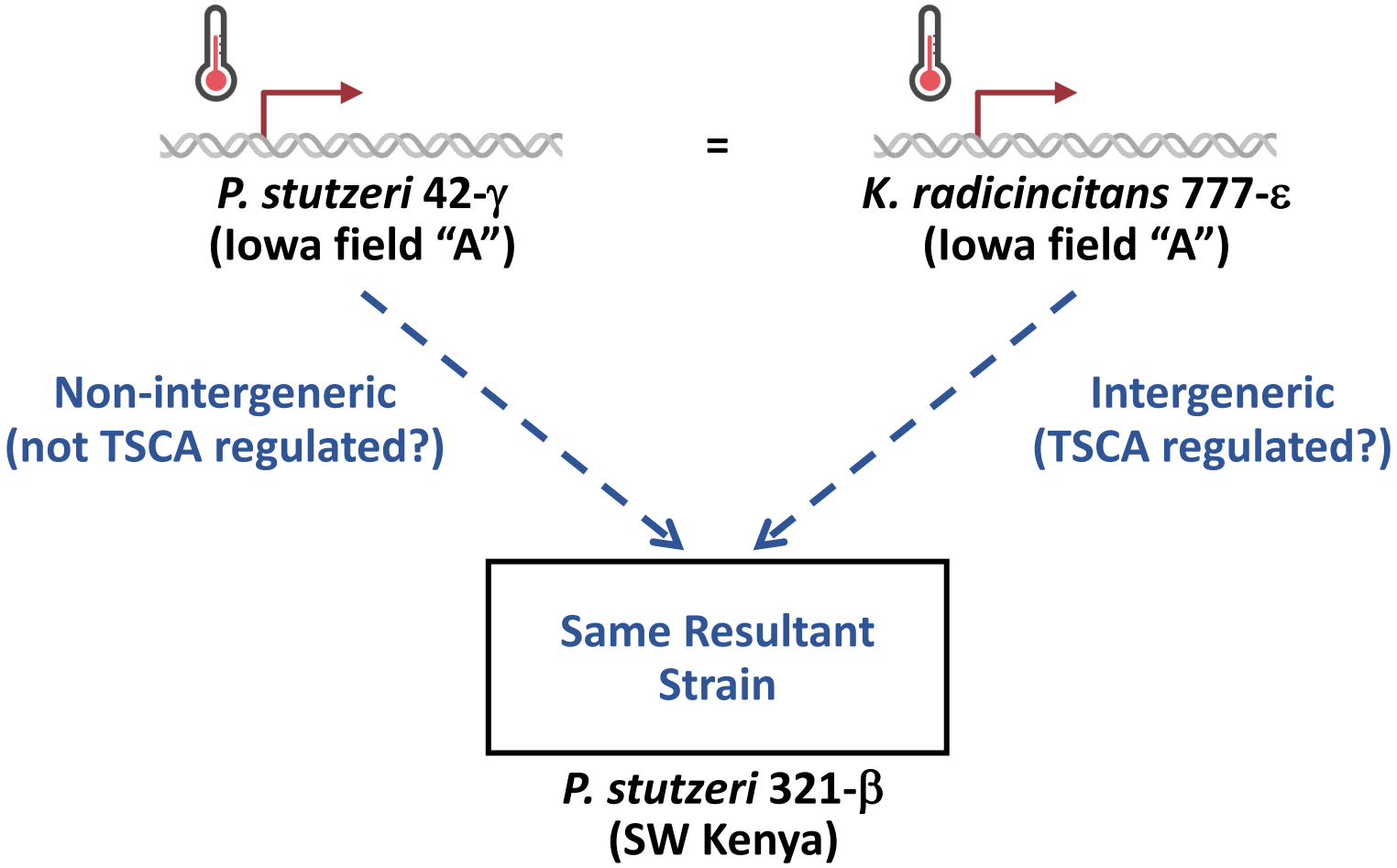
**GE Modification Details**

- Locus-targeted duplication of parts within strain to yield completely novel circuit
- Tech: Cut & Paste

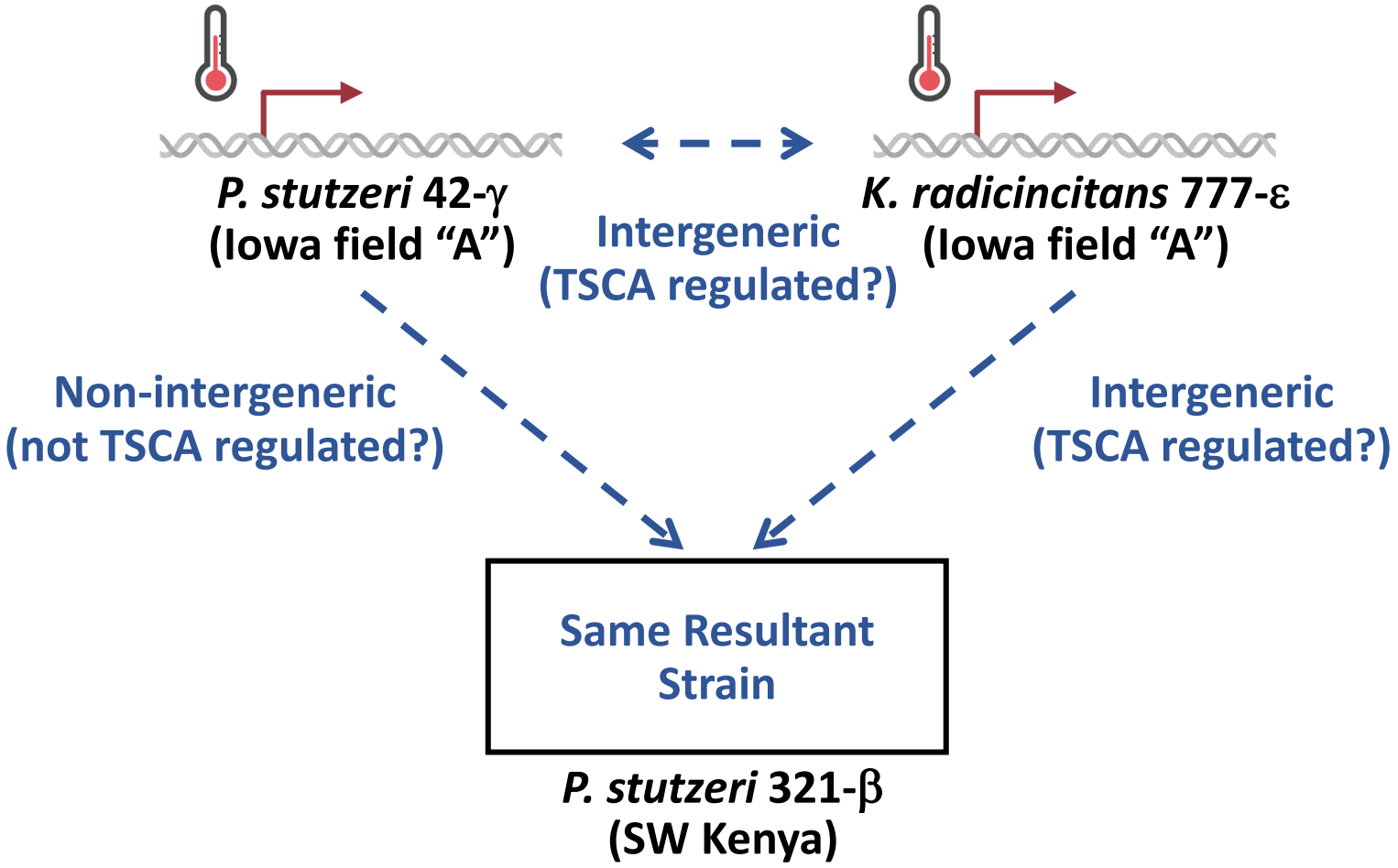


**Who regulates this?**

# Emerging Questions: The Intergeneric Parts Conundrum



# Emerging Questions: The Intergeneric Parts Conundrum



# Organism Profile: *P. azotofixans* Strain #888-ζ

## Organism Details

Habitat: Rhizosphere

Origin: Corn field in IN

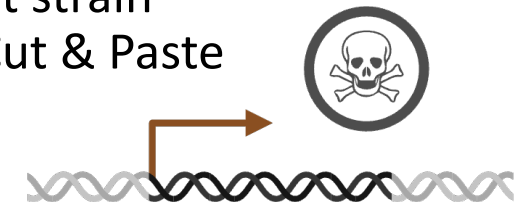
- Yields in 90$^{th}$ %ile for US
- Robust plant health over season
- Microbiome sequencing → expected relative abundances

**Note: Forms dormant endospores, but…**

- **Germinating spores die in absence of other consortia species**

## GE Modification Details

- Heterologous lab suicide gene + promoter from other product strain
- Tech: Cut & Paste

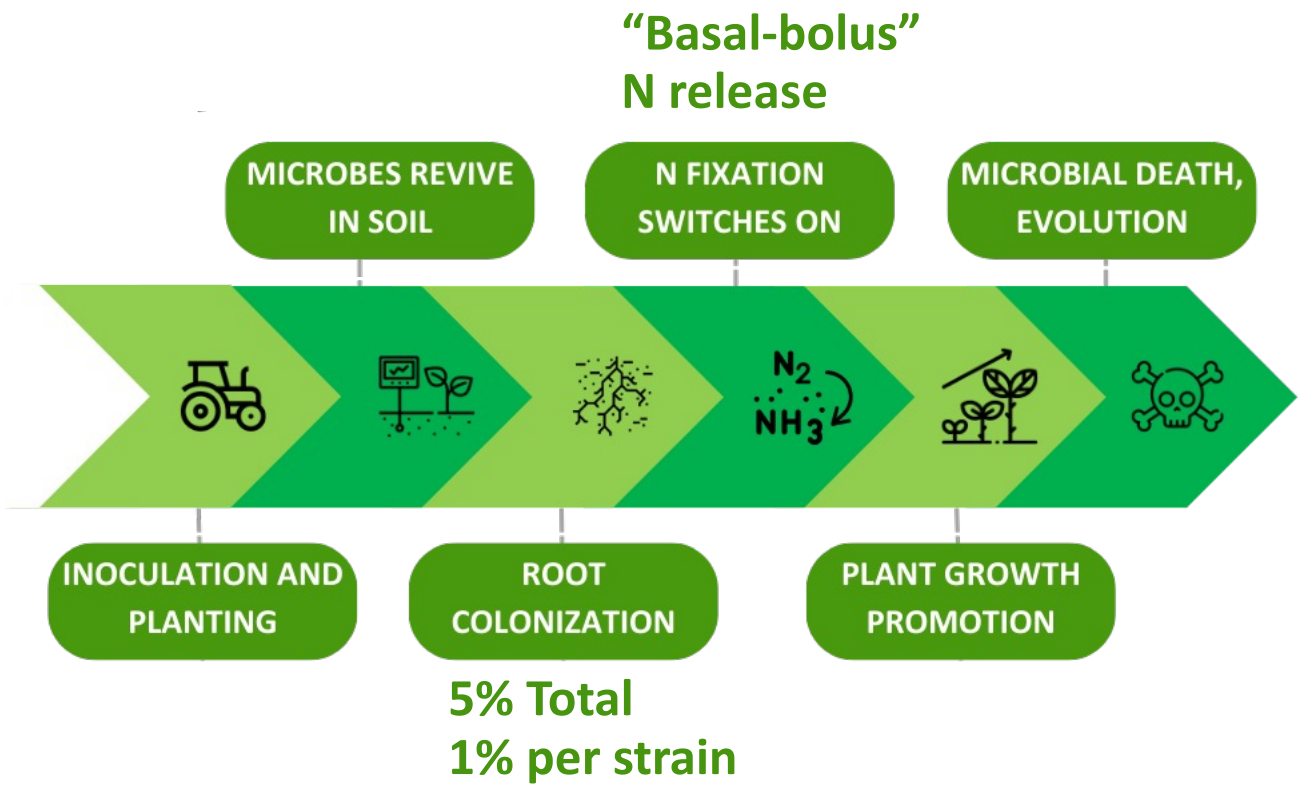


**What pre-release data would satisfactorily abrogate concern for airborne transmission of viable spores?**

# Case: Consortium of GE microbes applied in-furrow as biofertilizer – Regulatory steps to field trial readiness

# What steps must be taken to begin a field trial with full regulatory approval?

## Consortium Product Mechanism of Action




## Completed Data

- *In vitro* $NH_3$ release assay
  - Each strain releases $NH_3$ inducibly or constitutively as predicted
- Maize V4 colonization assays
  - 0.5% colonization of rhizosphere microbiome for each strain **individually**
  - 5% total colonization (1% each) when strains inoculated **as consortium**
  - Relative abundance of other strains not altered
- Plant growth promotion = consistent
- Maize isotope labeling studies
  - Significant chlorophyll N from product fixing
  - Correlated with plant growth promotion

**What additional experiments must be run to secure regulatory approval for release?**
**What quantitative targets (if any) must be met pre-release?**

# Critical aspects of field trial protocols

- **Geographical Location:** Corn field "A"
  - Within 10 miles of Boone, IA
  - Field where *P. stutzeri* 42-γ and *K. radicincitans* 777-ε were isolated
- **Field Characteristics**
  - 9 acres
  - Corn-soy-corn crop rotation for 3 decades
- **Diagnostics**
  - qPCR and flow cytometry assays developed for absolute & relative abundance of product microbes
  - **What quantitative sensitivity and specificity would each agency like to see before field use?**
- **Monitoring**
  - In-soil greenhouse data → all strains below LOD of qPCR and flow by harvest
  - In-soil greenhouse data → 0.1% of gram-negative cells survive simulated winter freeze-thaw
  - Gram-positive spores survive free-thaw well and can be germinated (only in consortium)
  - **What testing frequency, duration, and spatial proximity of persistence/containment sampling sites must occur? What is the monitoring end condition for this product?**
- **Termination**
  - Developer has asked to forego any grower-unfriendly devitalization protocols given cell death above
  - **Is this acceptable?**


switch
bioworks
It’s time to switch.