Innovation in Microbial Production for Improved Product Viability

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Introduction

A significant problem in the expanded use of microbial products is inconsistent performance in the field. In a similar way to the "disease triangle", in order to effectively inoculate host plants, beneficial microbes must be highly viable, survive and proliferate in a potentially unfavorable environment **(Figure 1)**. Successful microbe colonization of the plant requires delivery of a high concentration of highly viable microbes. Sub-optimal performance in the field can often result because the microbes experience a decline in viability during storage and distribution **(Figure 2)**.

To overcome issues with microbe stability during production and distribution, 3Bar Biologics developed **LiveMicrobe™ (Figure 3)**, an easy to use, low cost, disposable beneficial microbe delivery system, in which the microbes are grown in the final packaging. By pushing fermentation of the microbes just-in-time, the freshest, most viable microbes are consistently applied in the field. The novel delivery technology enables commercialization of many different beneficial bacteria, including harder to stabilize gram-negative bacteria, to improve crop productivity and nutrient use efficiency.

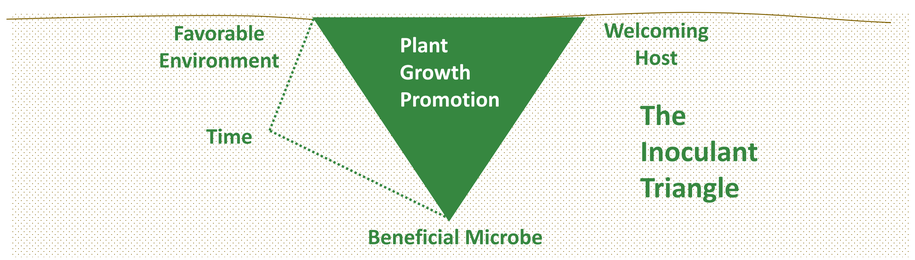

Figure 1. The Inoculant Triangle

Problem: Microbes decline before reaching field

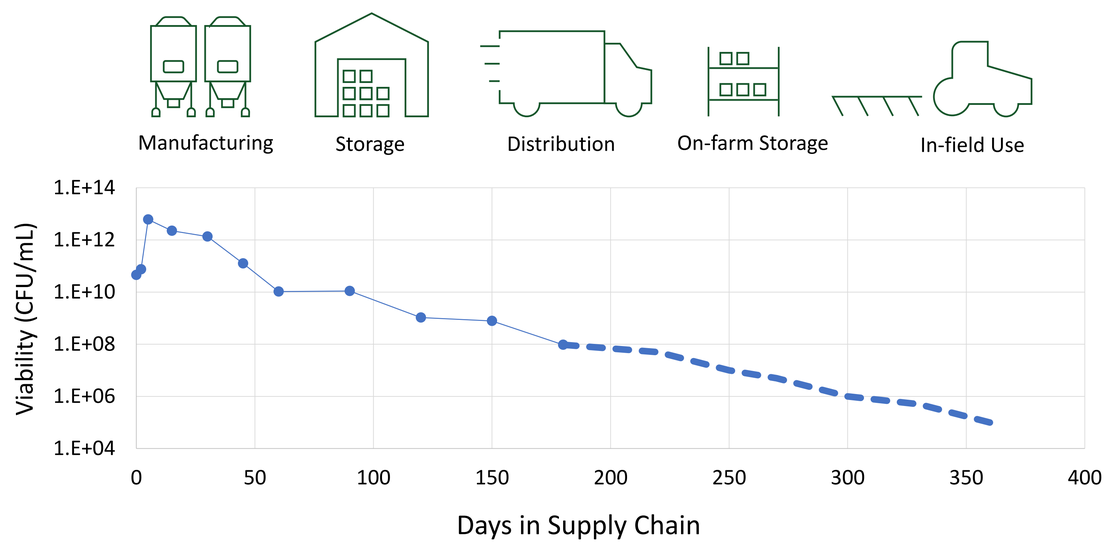

Figure 2. Decline of Microbes During Storage and Distribution

Solution: Microbes grown fresh

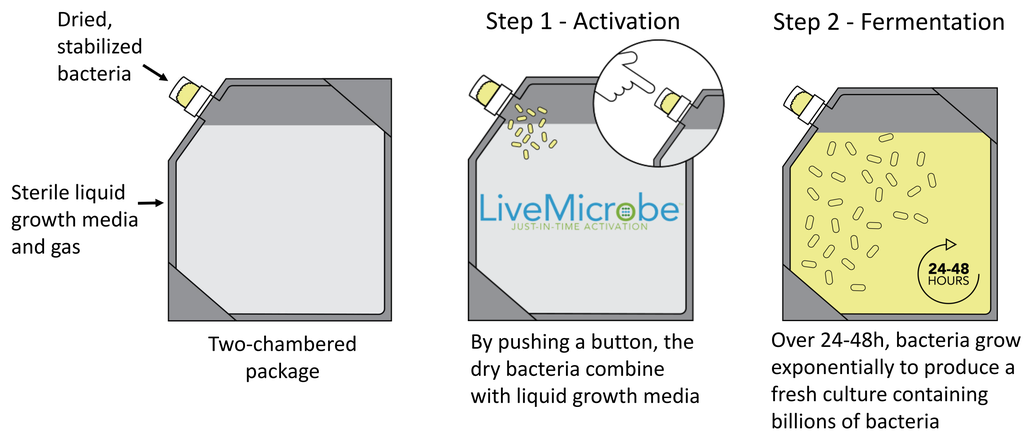

Figure 3. 3Bar Biologics' LiveMicrobe™ Just-in-Time Fermentation Technology

Microbial Product Development Case Study: Bio-YIELD® ST

Bio-YIELD® ST (Figure 4) is a microbial product developed for liquid seed treatment, applied downstream just prior to planting. Bio-YIELD® ST includes *Pantoea agglomerans*, with demonstrated plant growth promoting benefits for enhanced early vigor and greater yield potential.

Microbial product development involved several steps including:

1. Development of dry bioformulation **(Figure 5)**
2. Evaluation of growth kinetics in the LiveMicrobe™ system **(Figure 6)**
3. Evaluation of commercial seed treatment **(Figure 7)**
4. Field trials of seed treated corn and soybean **(Figure 8)**

Figure 5. Bio-YIELD® ST

***Pantoea agglomerans* 3BB1**

- Isolated from Wisconsin soil sample
- Licensed from The Ohio State University
- Demonstrated PGPB in corn and soybean
  - P solubilization (organic acid production)
  - Auxin production
    - Indole-3-acetyl-aspartic acid hydrolase (iaaH)
    - Indole-3-pyruvate decarboxylase (ipdC)
  - Nitrogen assimilation
  - Betaine biosynthesis (osmolyte protective mechanism)

1 Development of Dry Bioformulation

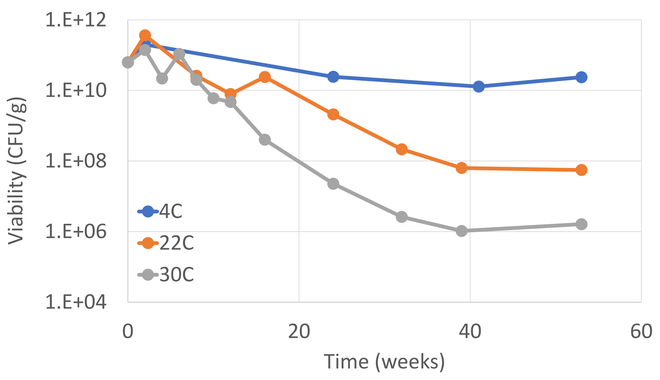

Figure 5. Dry bioformulation stability. Freeze-dried *Pantoea agglomerans* were stored in vials under dessication at 4C, 22C, and 30C. Samples at each timepoint were rehydrated in phosphate buffer solution (PBS) and viable cell counts determined. Stability was maintained above a commercial threshold of 1E6 CFU/g for greater than 1 year shelf-life.

2 Growth in LiveMicrobe™

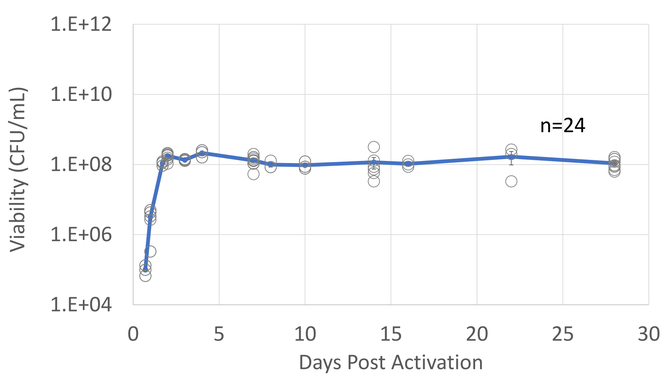

Figure 6. Growth in LiveMicrobe™ system. Individual LiveMicrobe™ product units containing freeze-dried *Pantoea agglomerans* were activated and growth kinetics of bacteria measured. Fermentation was conducted at room temperature (22C). Each product unit (n=24) included 10L liquid nutrient media (10% concentration, tryptic soy broth (TSB)). Bacteria consistently grew to >1E8 CFU/mL within 2 days post activation and maintained stability over the 28 day window of product use.

3 Seed Treatment

Days Post Seed Treatment	Percentage seeds recovered with bacteria: Corn 3 oz, Beginning	Percentage seeds recovered with bacteria: Corn 3 oz, End
0	100%	100%
1	100%	100%
8	90%	90%
29	90%	80%

Figure 7. Recovery of bacteria from commercially treated seed. Corn seed were treated with Bio-YIELD® ST using a commercial seed treater, at a rate of 3 oz/cwt. Seed samples were collected at the beginning and end of treatment and stored at ambient conditions. At each time point, treated seeds were added to an agar assay and emerging roots from germinated seed were washed and bacteria cells measured. After 29 days post seed treatment, *Pantoea agglomerans* bacteria were recovered from 80-90% of seeds.

4 Field Trials

	Soybean	Corn
Bio-YIELD ST (bu/ac)	48.1	245.0
Control (bu/ac)	46.1	242.2
Avg. yield response (bu/ac)	2.0	2.9
Avg. positive yield response (bu/ac)	5.0	6.9
Positive yield response	67%	63%

Figure 8. Field trial results. Replicated small plot trials were conducted across multiple sites in Ohio in corn (n=24 trials, 8 sites; 2015, 2017, 2018) and soybean (n=6 trials; 6 sites; 2017). Compared to control, seeds treated with Bio-YIELD® ST resulted in positive yield responses of 6.9 bu/ac and 5.0 bu/ac (63% and 67% of the time) for corn and soybean, respectively.

LiveMicrobe™ JUST-IN-TIME FERMENTATION TECHNOLOGY

3Bar Biologics is the global leader in customized delivery technologies for living agricultural microbe products. 3Bar's proprietary biomanufacturing and delivery method unleashes the potential for microbe technologies not available for commercial production until now.

To partner with us, contact 3Bar at www.3barbiologics.com

See the power of activating LiveMicrobe™ for yourself!

3Bar BIOLOGICS
Activating the Power of Microbes