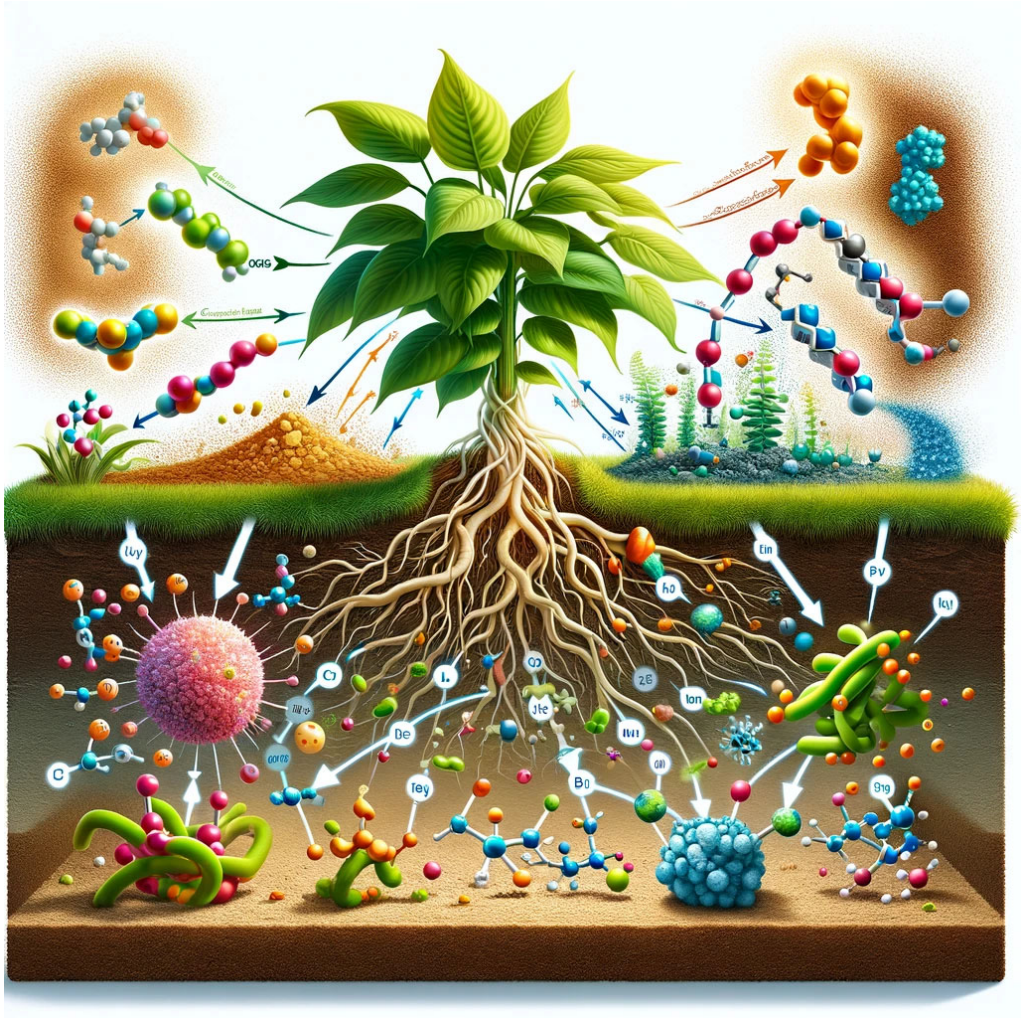


The Hidden Helpers:

Microbial Modes of Action in Crop Production

UltraClear.com

ABI Inc.

5350 Transportation Blvd.,
Suite 18 B
Garfield Hts., OH 44125

800-847-8950

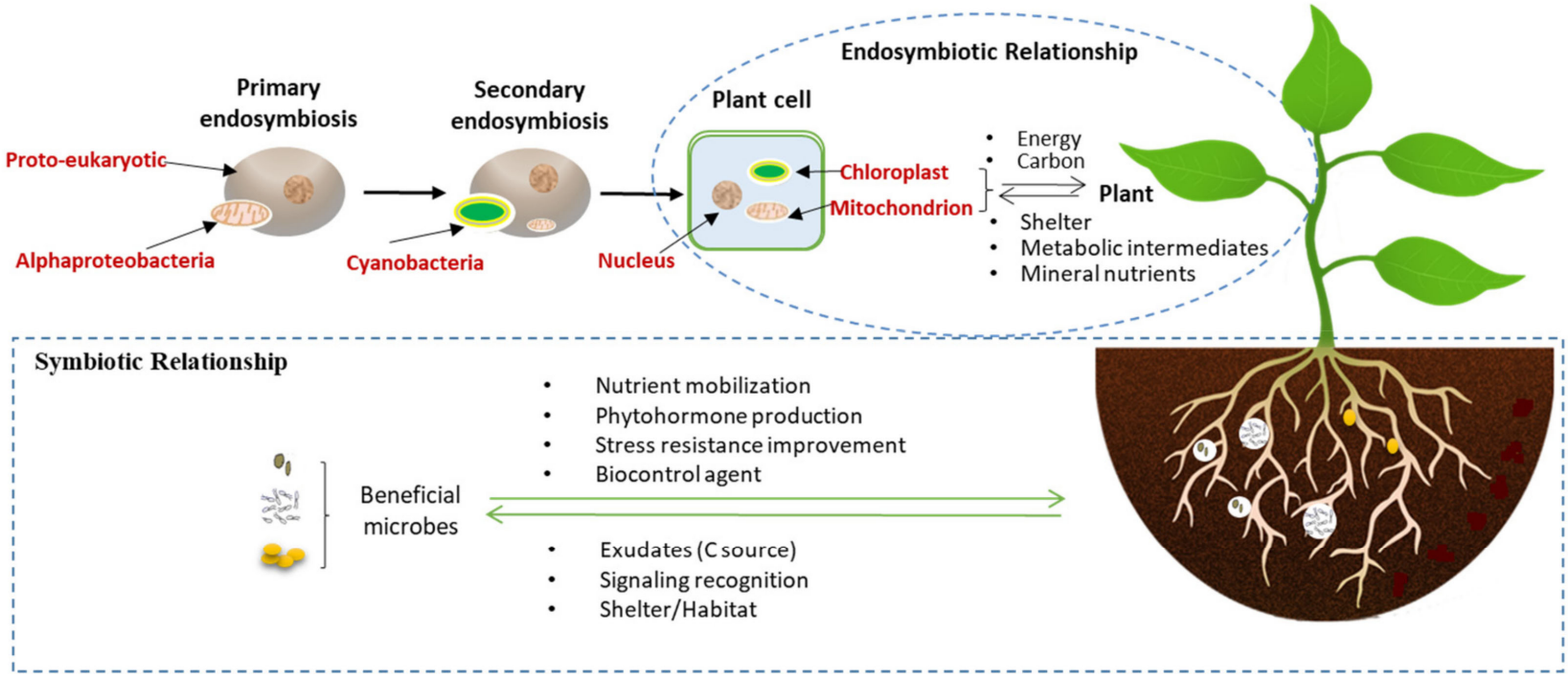
Outline of Topics

- ❑ Plant as a Superorganism
- ❑ Plant growth-promoting bacteria (PGPB)
- ❑ PGPB – modes of action
- ❑ TLC Products laboratory and Eurofins Agroscience field trials
- ❑ Examples of Ohio Organic Farm Data 2023 – Stephen Lapp
- ❑ Take-home messages

Outline of Topics

- ❑ Plant as a Superorganism
- ❑ Plant growth-promoting bacteria (PGPB)
- ❑ PGPB – modes of action
- ❑ TLC Products laboratory and Eurofins Agroscience field trials
- ❑ Examples of Ohio Organic Farm Data 2023 – Stephen Lapp
- ❑ Take-home messages

Plant as a Superorganism



Lyu et al. The Coevolution of Plants and Microbes Underpins Sustainable Agriculture. Microorganisms 2021, 9(5), 1036; https://doi.org/10.3390/microorganisms9051036

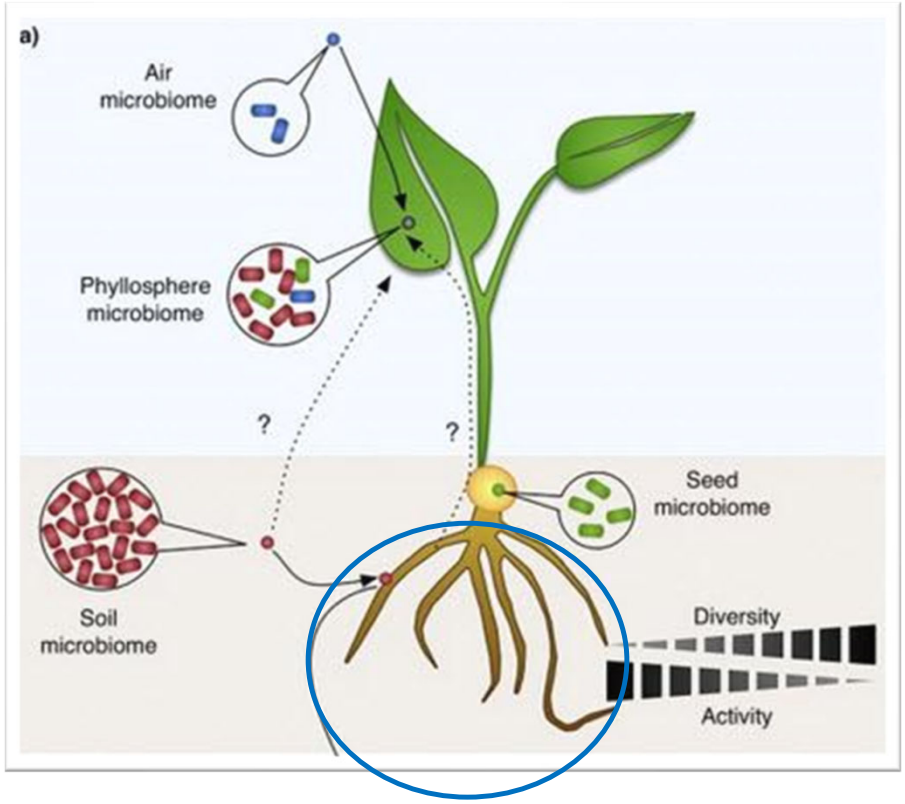
Plant as a Superorganism

Plant-microorganism interactions: since the colonization of land by ancestral plant lineages **450 million years ago**.

Plant + Microorganisms-symbionts = Superorganism

Selective pressure: host-adapted microorganisms that impact plant fitness

High microbial densities are detected on and in plant tissues



Microbiome of rhizosphere

Sánchez-Cañizares et al. Understanding the holobiont: the interdependence of plants and their microbiome. Curr Opin Microbiol. 2017;38:188-196. doi: 10.1016/j.mib.2017.07.001.

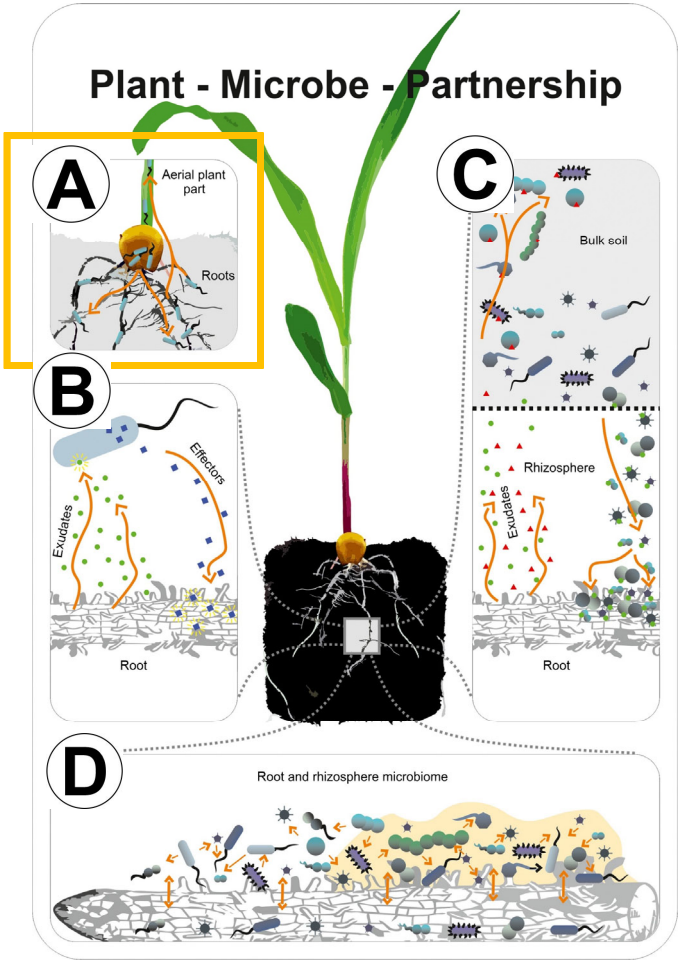
Plant as a Superorganism

A: Microbes associated with the seed

B: With root growth, plant EXUDATES begin and this initiates interaction with microbes OUTSIDE of the seed

C: These interaction result in the RHIZOSHERE

D: With rhizosphere established, the plant and microbiome interact DYNAMICALLY.



https://doi.org/10.1016/j.tplants.2016.01.008

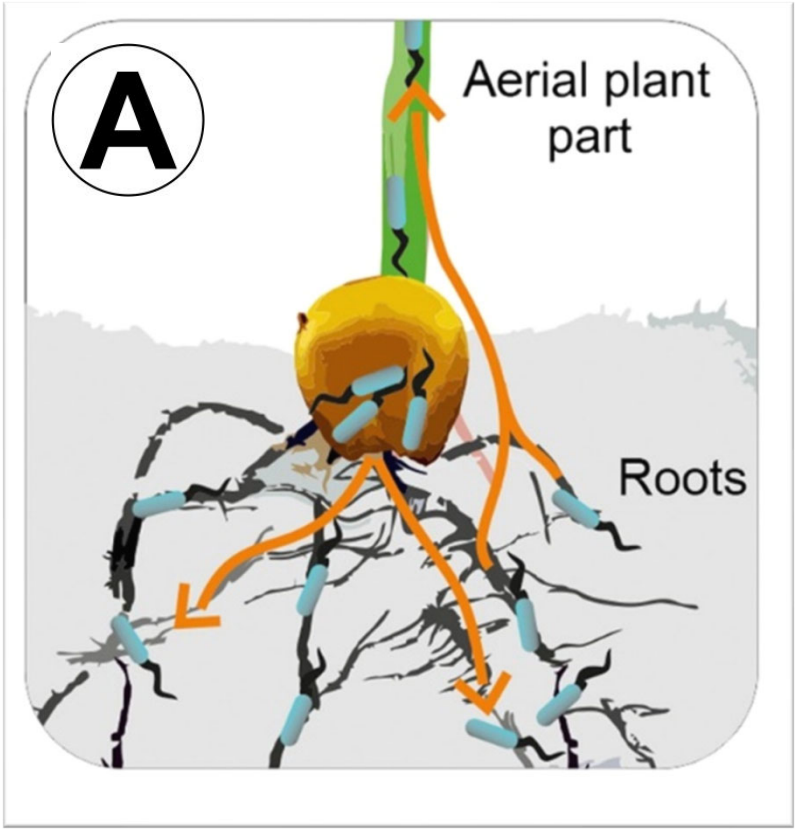
Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

Plant as a Superorganism

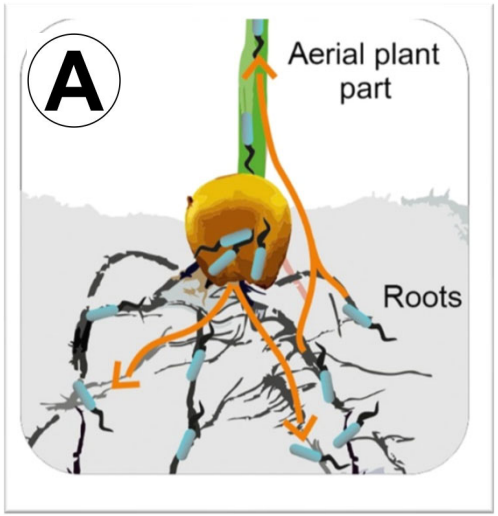
Plants are colonized initially by microbes originating from the **seed**.

This seed-derived microbiota is ***complemented*** and partly ***substituted*** gradually by rhizosphere microorganisms migrating into the plant via roots.

Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

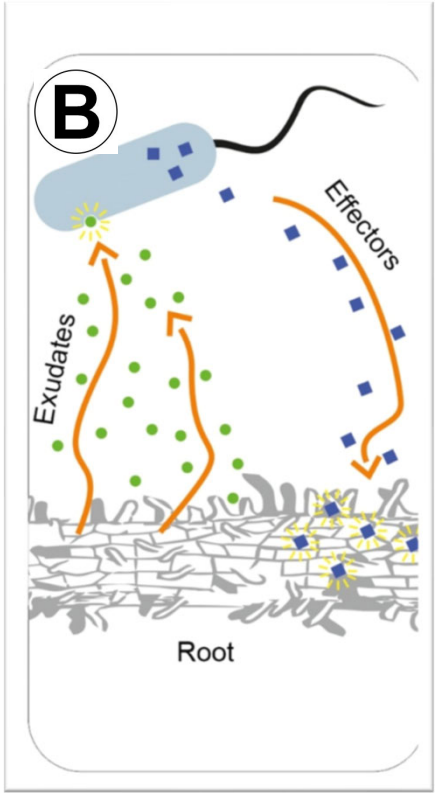


Plant as a Superorganism



TRANSITION from A to B:

During root system growth, the plant starts interacting with other organisms in soil.



Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

Plant as a Superorganism

Essential Definitions:

- **Exudates** are a variety of complex chemicals produced by roots, that travel generally a few millimeters to a few centimeters (2.54 cm per inch). They include organic acids, sugars, amino acids and proteins, phenolics, enzymes, vitamins and hormones. These are all done in order to attract bacteria and fungi and get a desired response. All life fights for exudates. Some microbes are better than others in getting this food (more on this later). There is a real competition for nutrients (exudates and others) in the rhizosphere.
- **Effectors** are compounds made by bacteria and fungi in response to the exudates. They include siderophores, acids or enzymes for P solubilization, plant growth hormones, ethylene reduction, biofilms that protect roots and give the microbes a home, nitrogen fixation, and so on.
- **Phyllosphere** is the above ground plant surfaces, most commonly discussed as the leaves. This is a less nutrient dense region than the rhizosphere, but exudates are produced and effectors are required. This zone is less stable than the rhizosphere, therefore it is likely that microbial inputs to manage the microbiome in this region is very important.
- **Rhizosphere.** The rhizosphere is a zone immediately around the roots, extending out about a tenth of an inch which can look like a jelly or jam under the electron microscope. This microbiome contains a constantly changing mix of soil organisms, including bacteria, fungi, nematodes, protozoa, and even larger organisms.

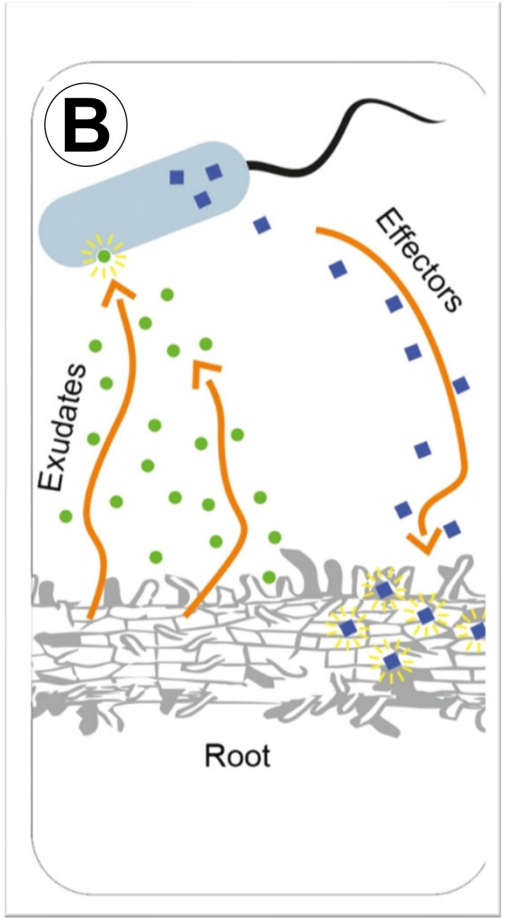
.

Plant as a Superorganism

Taking a closer look at exudates and effectors

The plant produces exudates to attract certain microorganisms, and microorganisms produce effectors, which influence plant immune system and growth

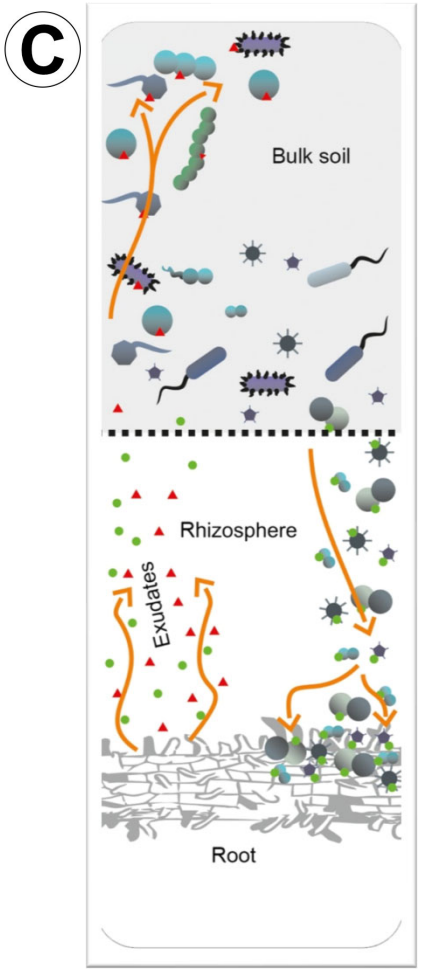
There is a limited supply of exudates, and all life competes for these exudates.



Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

Plant as a Superorganism

Plant exudates attract microbes in the bulk soil thereby directing a subset of them to the root zone forming microbiota of rhizosphere

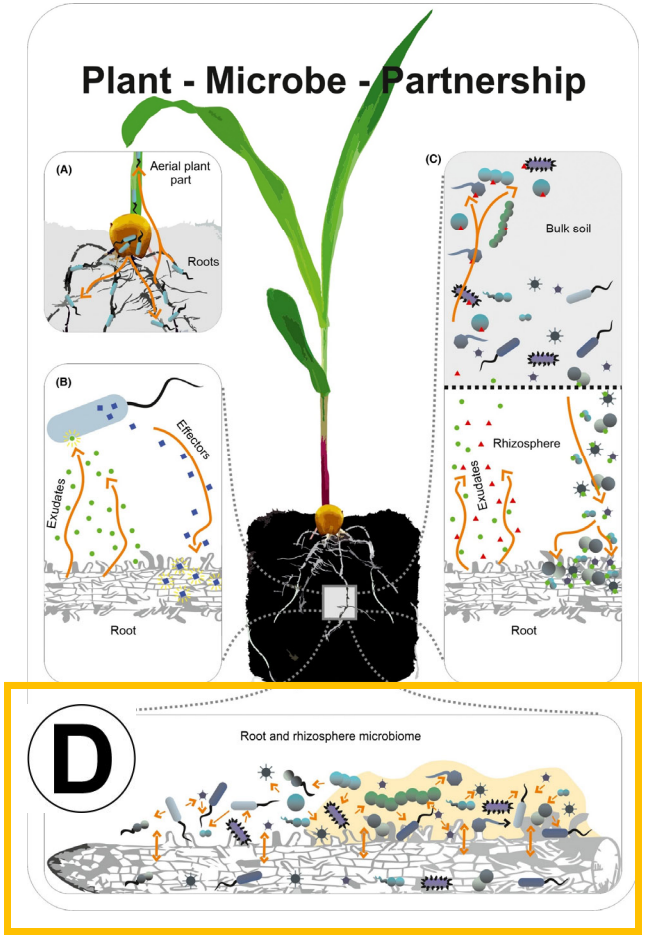


Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

Plant as a Superorganism

Rhizosphere is a dynamic system.

- Chemical interactions and biological diversity change over time
- Environment (water, temp, soil changes) cause changes to rhizosphere
- Plant growth and root morphology change
- Feedback mechanisms exist



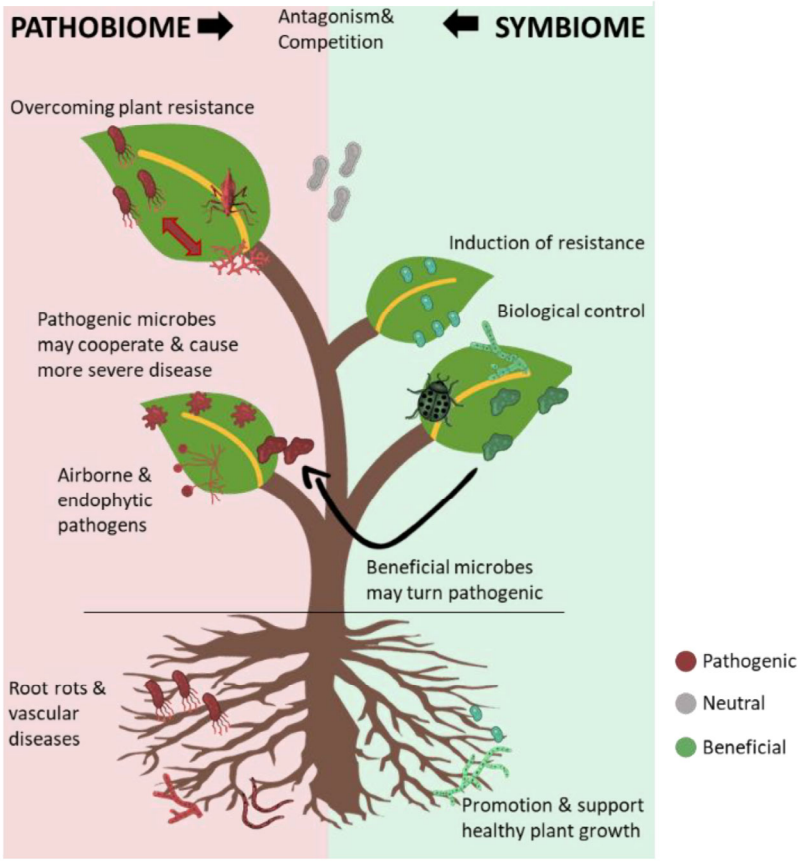
Mitter et al. Plant–microbe partnerships in 2020. Microbial Biotechnology. 2016. 9 (5): 635–640. doi:10.1111/1751-7915.12382.

Plant Superorganism with constant competition

Symbiome — beneficial plant-associated microbes

Pathobiome — collective pathogenic microbes

- The beneficial and pathogenic microbes are ***in continuous antagonism*** and competition for space and nutrients.

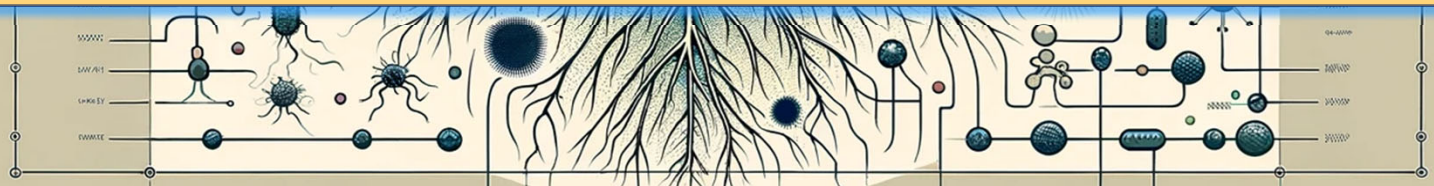


Mannaa et al. Plants under the Attack of Allies: Moving towards the Plant Pathobiome Paradigm. Plants. 2021. 10 (1): 125. doi:10.3390/plants10010125.

In a nutshell:

- **Plants are Superorganisms, with complex interactions between multiple species (phyla) of life forms, both above and below ground. There is fierce competition for limited exudates!**
- **These environments change over time, and applying microbial inputs at the correct times can be beneficial, as we do our best to manage our organic crops**
- **Along with the change, all life competes for exudates above and below ground. *Will the beneficial microbes outcompete the pathogenic microbes?***

That is the key question!



www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Outline of Topics

- ❑ Plant as a Superorganism
- ❑ Plant growth-promoting bacteria (PGPB)
- ❑ PGPB – modes of action
- ❑ TLC Products laboratory and Eurofins Agroscience field trials
- ❑ Examples of Ohio Organic Farm Data 2023 – Stephen Lapp
- ❑ Take-home messages

Plant Growth Promoting Bacteria

Recall from our earlier discussion:

- **Exudates** produced by the plant in both the phyllosphere and rhizosphere, as are **effectors.**
- There is competition by all life for the small supply of exudates that the plant produces, whether above or below ground.
- Beneficial and pathogenic microbes are in competition for these valuable exudates.

What determines who wins? There are two factors that must understand:

- **Competitive Exclusion**

Biology LibreTexts. (n.d.). 6.2.1: Competitive exclusion principle.

- **Functional Redundancy**

Louca, S., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. Nature Ecology & Evolution, 2(6), 936–943.

Plant Growth Promoting Bacteria

Competitive Exclusion:

Two species cannot occupy the same niche in a habitat, different species cannot coexist in a community *if they are competing for all the same resources*.

If either species is unable to evolve to reduce competition, then the species that most efficiently exploits the resource will drive the other species to extinction

Said Another Way:

Survival of the fittest, or what grows fastest on limited exudates will dominate the population, as exudates are truly limited resources!

Plant Growth Promoting Bacteria

Competitive Exclusion and the Rate of Reproduction

Bacteria reproduce by splitting in two (fission)
This chart shows the population changes over 5 hours for a:

- Quick reproducer (30 min)
- Slow reproducer (60 min)

Starting with just one bacterium through 5 hours of growth:

- Quick reproducer - **1024**
- Slow reproducer - **64**

Elapsed Time in Hours	Number of Bacteria "A" Takes 30 Minutes to Duplicate	Number of Bacteria "B" Takes 60 Minutes to Duplicate
0	1	1
0.5	2	1
1.0	4	2
1.5	8	2
2.0	16	4
2.5	32	4
3.0	64	8
3.5	128	8
4.0	256	16
4.5	512	32
After 5.0 hrs	**1024 count of type A**	**64 count of type B**

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Promoting Bacteria

Competitive Exclusion and the Effect of Rate of Reproduction

As seen, at 5 hours of growth, the rapid reproducers dominate the slower reproducers.

- Fact: The effectors needed by the plant (biofertilization, growth hormones, various enzymes) all require resources (from the limited material and energy pool within the microbe).
- Fact: When the microbe is synthesizing these effectors, that means material and energy is diverted from reproduction and into effector synthesis, and its reproduction rate is significantly reduced.

The bottom line: the bacteria needed to provide the effectors may not be available in the rhizosphere or the phyllosphere due to the Competitive Exclusion effect.

Ramin, K. I., Allison, S. D. (2019). Bacterial Tradeoffs in Growth Rate and Extracellular Enzymes. Frontiers in Microbiology, 10, 2956.

Seth, E. C., & Taga, M. E. (2014). Nutrient cross-feeding in the microbial world. Frontiers in Microbiology, 5, 350.

This is one valid reason for applying microbial inputs

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Promoting Bacteria

Functional Redundancy:

Functional redundancy as applied to the phyllosphere or rhizosphere means having presence of multiple microbial species within a community that can produce effectors such as biofertilization, growth hormones, or enzymes. This is a key aspect of microbial community resilience that ensure that as many plants as possible get the effectors needed.

Said Another Way:

The rhizosphere and phyllosphere are dynamically changing microbiomes where the fastest reproducing microbes will tend to dominate. This is not ideal for ensuring that the needed effectors will be available. By adding microbes that are ABLE to produce the requested effectors, the Functional Redundancy is improved.

This is another reason for considering microbial inputs. The plant requires bacteria proven capable of supplying various effects in response to exudates.

Louca, S., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. Nature Ecology & Evolution, 2(6), 936–943.

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Promoting Bacteria

Here is a short but accurate summary of how TLC Products ensures that its' product assist with both competitive exclusion and functional redundancy:

- No genetic modification or engineering. Our products are OMRI and EcoCert listed. We never use genetic engineering or modification of any type.
- Careful bench-scale selection of functional characteristics so that our microbes can produce the full range of needed effectors.
- 25+ years of research into stabilizing bacteria in both aqueous and powder form to offer the highest concentration possible and functional capability for at least one year after delivery to customer.
- Highest QC standards in partnership with Vermicon AG (Munich, Germany), employing Fluorescence In-Situ Hybridization, which identifies and counts our bacteria for QC and research purposes
- Unique Plant Growth Hormone synthesis upon request from exudates, using services of Lifeasible (agricultural research services in Shirley, NY) to evaluate the plant growth hormones produced by our bacteria in response to plant signaling request via exudates.

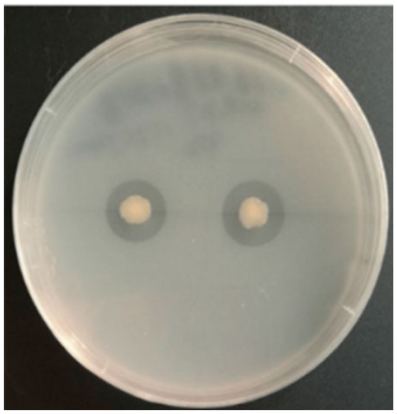
www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Promoting Bacteria

Example: P solubilization

Using specialized plate media to verify P solubilization capability of microbe before using in our products.

This plate includes Tricalcium phosphate, which is not bioavailable. Note the clear zone around the colony. This show TCP solubilized into bioavailable orthophosphate. These colonies are selected to continue through the manufacturing process to ensure P solubilization ability.



Example: Chitinas synthesis

Using specialized plate media to verify chitin digestion, or chitinase production capability of microbe before using in our products.

This plate includes colloidal chitin in the media. Note the clear zones around the colonies. This show the chitinase digestion activity, and the extent and diameter of the clear zone or halo around the colony is both proof of the needed function and its rate of production can be inferred. These colonies are selected to continue through the manufacturing process to ensure chitinase synthesis capability or functionality.

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Promoting Bacteria

Various samples of our product ACF-SR were subjected to growth conditions and exudates (recognition factors that tell the bacteria to produce the plant growth hormones).

Using these special techniquest, we manufacture microbes that are CAPABLE of synthesizing the requested plant growth hormones (effectors) in response to the plant's exudates.

Making sure that our bacteria can produce a variety of plant growth hormones (as evidenced by the table to the right) helps ensure Functional Redundancy (discussed earlier in this presentation)

Lifeasible

Report generated by
Rujane Zhang Ph.D., R&D Manager

Report Reviewed by
Rainy Zhu Ph.D., Project Manager

On September 11, 2023

The amounts in ng/mL of the hormones detected are reported in Table 1.

Table 1 Summary of Phytohormones Content for Each Sample.

Sample Name	Concentration (ng/mL)							
	IAA	MethylIAA	JA	SA	cZ	cZR	tZ	tZR
SR1	244.5	ND	0.26	11.0	0.032	0.086	ND	0.041
SR2	344.2	0.02	0.15	2.4	0.038	0.006	0.023	0.028
SR3	48.6	ND	0.32	1.7	0.056	0.183	0.060	0.092
SR4	12.5	ND	0.10	5.4	0.158	0.008	0.085	0.038
SR5	169.4	1.17	0.13	2.4	0.097	0.005	0.036	0.047

Plant Growth Promoting Bacteria

Partnership with Vermicon AG for State of the Art quality control.

FISH is specialized detective tool to identify and count specific bacteria. It works by using fluorescent probes –glowing markers that attach only to the DNA of the bacteria we're interested in. When these probes bind to their target bacteria, they cause them to light up under a special microscope, allowing us to see and identify these bacteria amongst many others. Special tools (probes are needed for each bacterial species).

FISH primarily detects live bacteria because it relies on the presence of intact RNA, which is abundant in living cells. The fluorescent probes used in FISH bind to RNA within the bacteria. Since RNA rapidly degrades after a cell dies, the probes will not bind effectively to dead bacteria, making FISH an excellent tool for counting and studying bacteria that are alive and active.

The image to the right is one of many Certificates of Analysis provided by Vermicon AG to TLC Products showing exactly what is in our products.

TLC PRODUCTS AGRICULTURE DIVISION

This presentation can be downloaded at:
www.tlc-products.com/case-studies/acres-conference

vermicon
solutions for microbiology

CERTIFICATE OF ANALYSIS

Client Information

Company Name	TLC Products, Inc, dba (doing business as) BluePlant Labs
Client Address	15752 Industrial Parkway. Cleveland, OH 44135. USA
Client Contact	John M. Wong, CEO. johnmwong@tlc-products.com

Product Information

Product Name	ACF SR
Batch ID	20210714
Date of Manufacture	July 14, 2021

Amount of bacteria

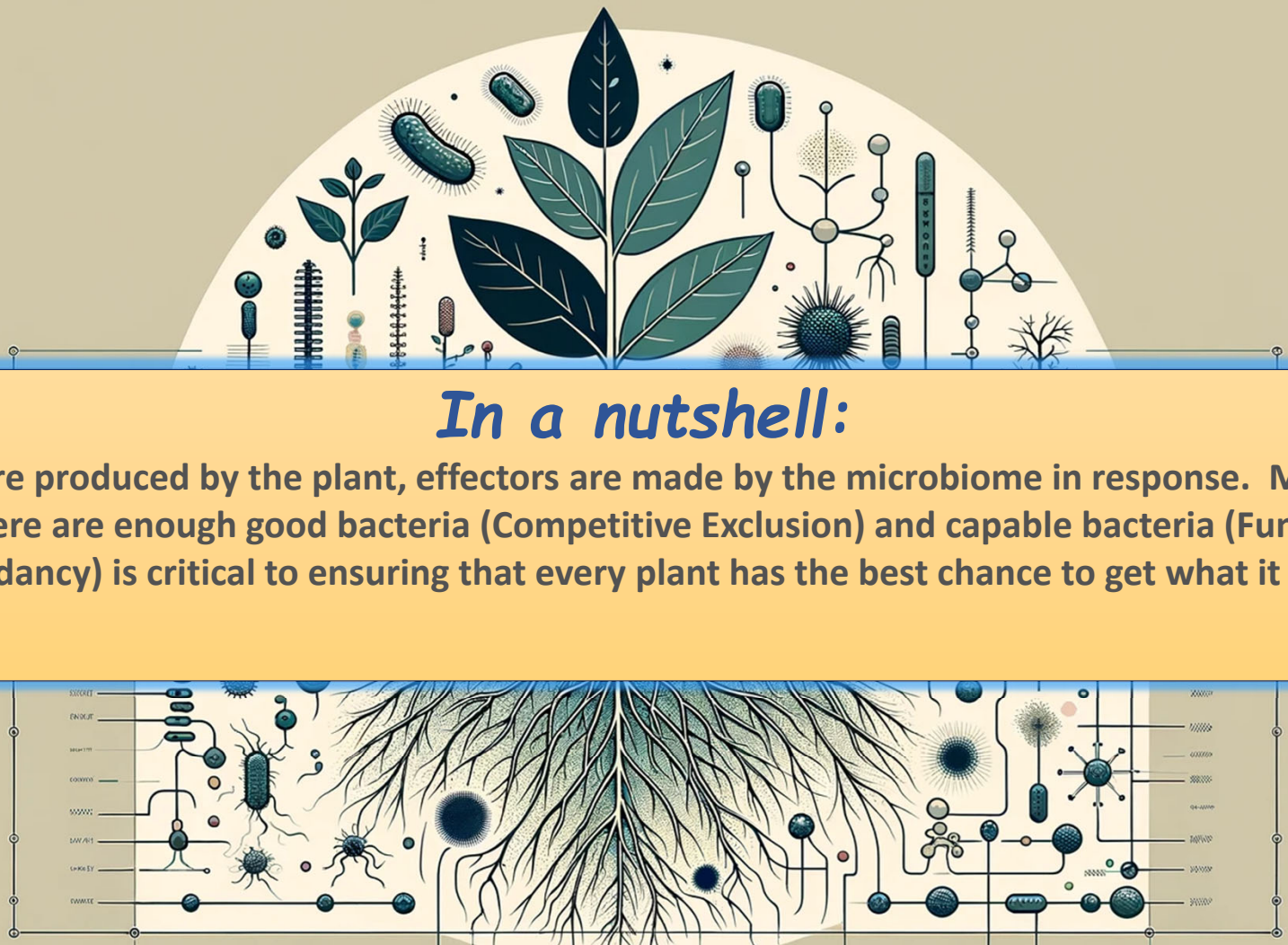
Name	Viable cell count / mL*
Bacillus amyloliquefaciens	7,00E+06
Bacillus licheniformis	6,00E+06
Bacillus subtilis	9,00E+06
Rhodopseudomonas palustris	4,36E+06
AOB: Nitrosomonas eutropha / europaea	6,32E+07
NOB: Nitrobacter spp.	2,18E+05

*Analytical method: qFISH (quantitative Fluorescence in situ hybridization)

Tested by vermicon AG

Name	Signature	Title	Date
Dr. Claudia Beimfohr	C. Beimfohr	Manager QC	20.08.2021

Contact Information: vermicon AG | Emmy-Noether-Str. 2 | 80992 Munich | Germany
phone: +49 89 15882 0 | fax: +49 89 15882 100 | email: support@vermicon.com



In a nutshell:

Exudates are produced by the plant, effectors are made by the microbiome in response. Making sure that there are enough good bacteria (Competitive Exclusion) and capable bacteria (Functional Redundancy) is critical to ensuring that every plant has the best chance to get what it needs.

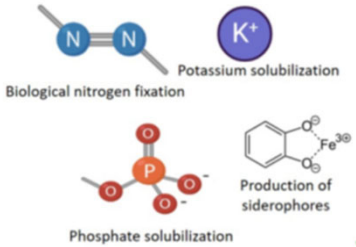
www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Outline of Topics

- ❑ Plant as a Superorganism
- ❑ Plant growth-promoting bacteria (PGPB)
- ❑ **PGPB – modes of action**
- ❑ TLC Products laboratory and Eurofins Agroscience field trials
- ❑ Examples of Ohio Organic Farm Data 2023 – Stephen Lapp
- ❑ Take-home messages

Modes of Action in Brief

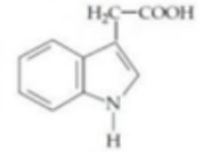
(Biofertilizer Activity, Plant Growth Hormone Production, Enzyme Synthesis)



Biofertilizer activity is performed by microbes and includes the following well known examples:

- Nitrogen Fixation (converts atmospheric nitrogen into ammonia in the soil).
- Another example is P solubilization, in which bacteria convert unusable P into bioavailable P (orthophosphate).

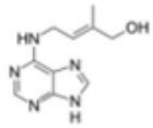
Biofertilization activity is usually the result of plant exudates (signals) and synthesis of the requested function (effectors) by microbes.



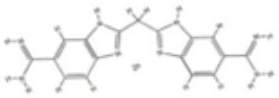
Indoles-3-acetic acid

Plant Growth Hormone Production is performed by microbes and includes these:

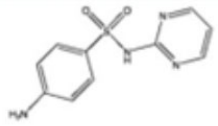
- **IAA (Indole-3-Acetic Acid) and related MethylIAA:** Play a crucial role in regulating plant growth and development, including cell elongation, root formation, and response to light and gravity.
- **JA (Jasmonic Acid) and Salicylic Acid:** These hormones play a key role in plant stress responses, including defense against herbivores and pathogens
- **cZ (cis-Zeatin) and cZR (cis-Zeatin Riboside):** These are forms of cytokinins, a class of plant hormones that promote cell division and growth. They are involved in various plant growth processes, including shoot initiation, leaf growth, and chloroplast development.
- **tZ (trans-Zeatin) and tZR (trans-Zeatin Riboside):** Types of cytokinins, these promote cell division, plant growth, and delaying senescence (aging). They play important roles in shoot development and nutrient mobilization.



Cytokinin



Production of lytic enzymes



Production of antibiotics

Production of Lytic Enzymes and Antibiotics:

Lytic enzymes digest large organic compounds into smaller compounds, resulting in bioavailability. Only small simple molecules pass through cell structures to get ingested. Other lytic enzymes have a biocontrol function (Chitinase)
Production of Antibiotics: Again, plants produce exudates, which attract bacteria, and when the the right bacteria are there, the effector (the antibiotic) is produced. This plays a huge role in plant health, and is an active topic of research.

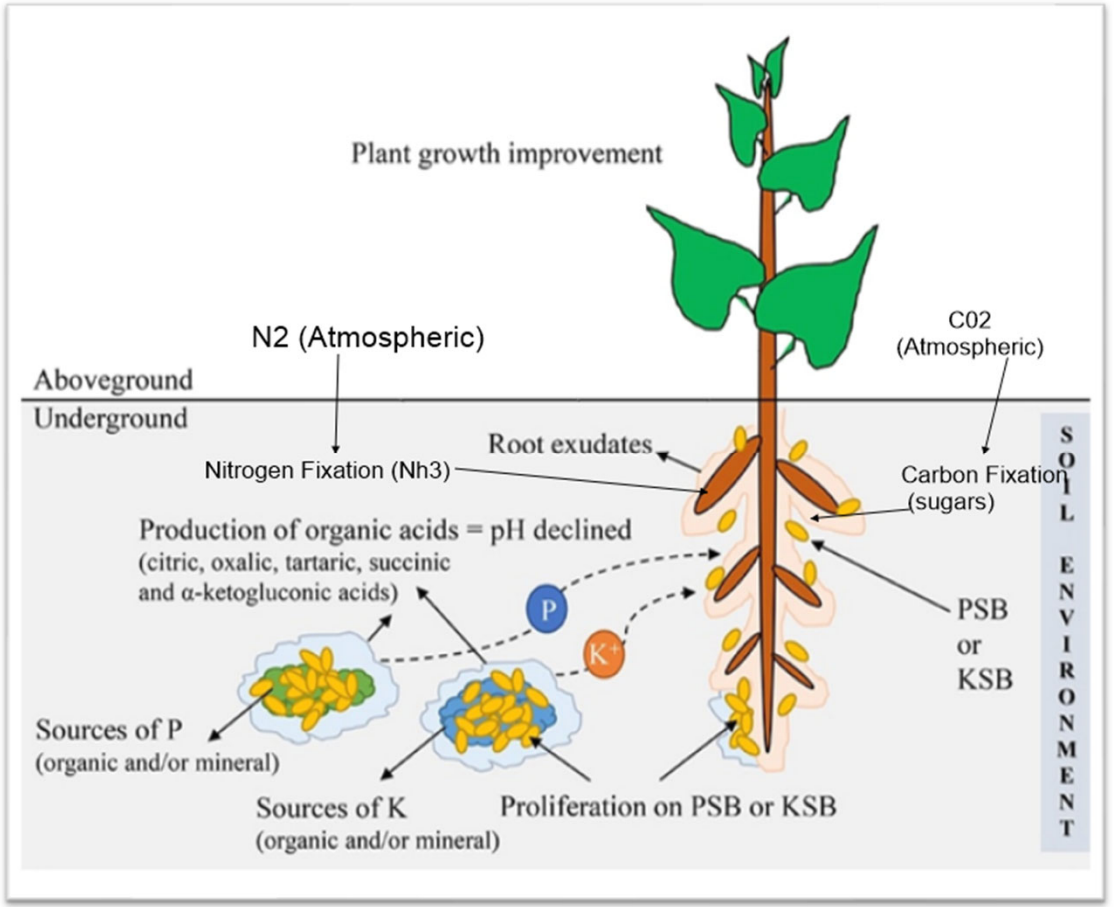
Gómez-Godínez et al. A Look at Plant-Growth-Promoting Bacteria. Plants 2023, 12(8), 1668; https://doi.org/10.3390/plants12081668

Biofertilization

Plants produce **EXUDATES**, which signal microbes to provide biofertilizer activity **(effectors)**

- Nitrogen fixation, carbon fixation, Phosphorous solubilization, and Potassium solubilization are all shown in the diagram
- Other types of biofertilization exist, such as siderophores (small transport vessels for Iron)

Bacteria in TLC formulations are capable of all of the biofertilization functions and will produce the biofertilizer action when in the vicinity of the rhizosphere and when in sufficient numbers.



www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Plant Growth Hormone Synthesis

Plants produce **EXUDATES**, which signal microbes to provide growth hormone synthesis **(effectors)**

- The microbes have to be present (Competitive Exclusion) and have the capability to synthesize the requested hormones (Functional Redundancy)
- Other hormones exist, but those listed in the report from Lifeasible are present in all TLC formulation

Bacteria in TLC formulations are capable of diverse plant growth hormone synthesis and when dosed per directions, are present in the required numbers in the rhizosphere.

Lifeasible

Report generated by
Rujane Zhang Ph.D., R&D Manager

Report Reviewed by
Rainy Zhu Ph.D., Project Manager

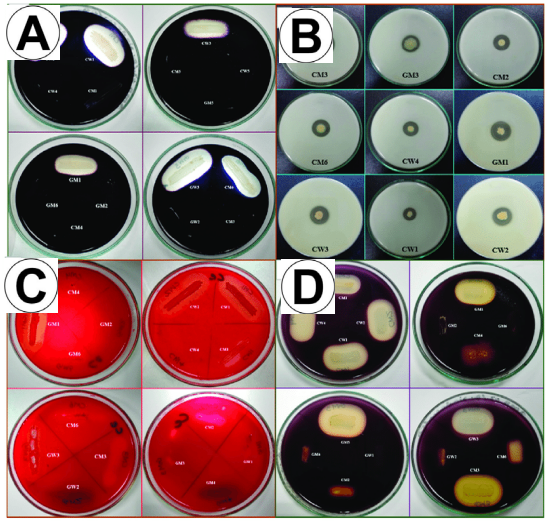
On September 11, 2023

The amounts in ng/mL of the hormones detected are reported in Table 1.

Table 1 Summary of Phytohormones Content for Each Sample.

Sample Name	Concentration (ng/mL)							
	IAA	MethylIAA	JA	SA	cZ	cZR	tZ	tZR
SR1	244.5	ND	0.26	11.0	0.032	0.086	ND	0.041
SR2	344.2	0.02	0.15	2.4	0.038	0.006	0.023	0.028
SR3	48.6	ND	0.32	1.7	0.056	0.183	0.060	0.092
SR4	12.5	ND	0.10	5.4	0.158	0.008	0.085	0.038
SR5	169.4	1.17	0.13	2.4	0.097	0.005	0.036	0.047

Lytic Enzyme Synthesis

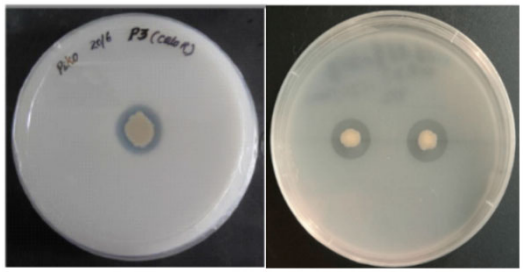


Plants secrete **EXUDATES** that signal microbes to synthesize hydrolytic enzymes **(effectors)**

- In general, these enzyme break large compounds (that are not bioavailable due to their physical size) into simpler, small subunits that are bioavailable to all life in the Superorganism
- Some hydrolytic enzymes are active in the soil away from the rhizosphere, and perform a similar useful role there

Pictured to the right is a series of microbial plates that are used to clearly show hydrolytic enzyme activity of a given bacterium, as follows:

(A) Amylase production (B) Protease production (C) Cellulase production (D) Pectinase production



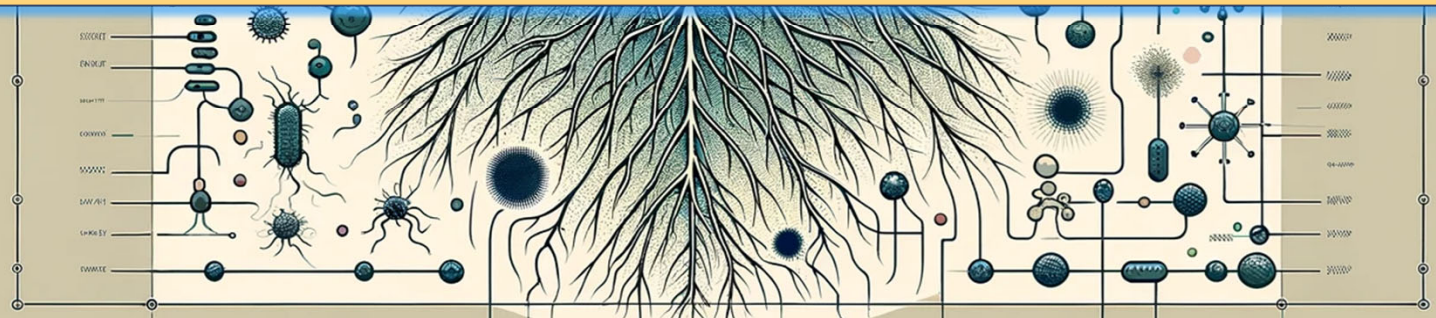
The left plate contains apatite (Tricalcium phosphate, not bioavailable, and opaque white). P solubilization TLC bacteria convert this to bioavailable orthophosphate, (note clear halo) The plate on the right contains a colloidal form of chitin and makes the plate hazy. Chitinase (an enzyme that digests chitin) creates the halo effect seen around the two colonies on the right plate.

Yasmin et al. Identification of New Biocontrol Agent against Charcoal Rot Disease Caused by Macrophomina phaseolina in Soybean (*Glycine max* L.). Sustainability. 2020; 12(17):6856. https://doi.org/10.3390/su12176856

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

In a nutshell:

Biofertilization, Plant Growth Hormone Synthesis, and Enzyme Synthesis are Three Powerful Modes of Action, Regulated by Exudates and Effectors, and Successfully Synthesized by Bacteria in TLC Formulations



www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Outline of Topics

- ❑ Plant as a Superorganism
- ❑ Plant growth-promoting bacteria (PGPB)
- ❑ Application of PGPB in agriculture
- ❑ **TLC Products Laboratory Data and Eurofins Agroscience Field Study**
- ❑ Examples of Ohio Organic Farm Data 2023 – Stephen Lapp
- ❑ Take-home messages

TLC PRODUCTS AGRICULTURE DIVISION

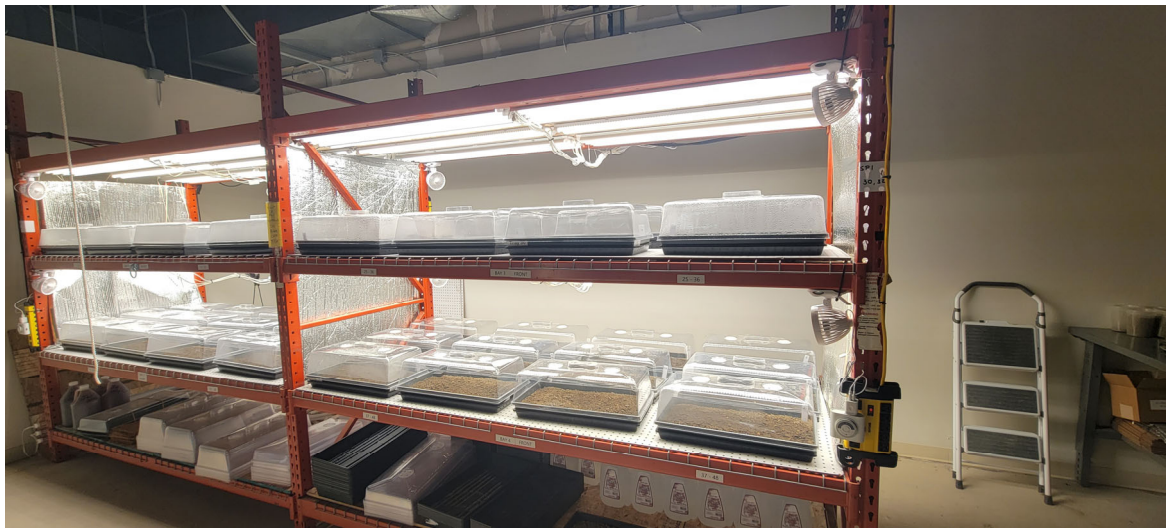
This presentation can be downloaded at: www.tlc-products.com/case-studies/acres-conference

SCAN ME

Laboratory Data

Picture shows four bays in use, 12 trays (1020) per bay. We have a total of 12 bays available for greenhouse studies, which can handle 144 tray experiments simultaneously.

Each bay has lights adjusted to specific PAR values (photoactive radiation) as required for the plant being studied, and fans are located strategically to eliminate “hot spots”



Notes about our greenhouse work. Statistically robust experimental design includes:

- Typically 24 to 32 seeds per tray, and 8 trays per treatment type
  Treatments are assigned to trays randomly, and once trays are set into bays, they are relocated every 48 hours (randomly)
- Precise temperature and humidity control, each tray gets identical treatment
- Each tray is an “observation for statistical purposes”, making ANOVA and TUKEY HSD analysis possible between treatments

www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

Laboratory Data – 2 Soybean Runs

Two recent soybean runs are presented, with these characteristics:

- Seeds were Glycine Max (GLXMA) in the 2 TLC projects and the Eurofins Agrosciences Services study
- In the 2 TLC studies, seeds were planted at the rate of 32 per tray in a 4 x 8 grid equidistantly spaces
- Each tray is an "observation for statistical purposes", making ANOVA and TUKEY HSD analysis possible between treatments
- In the first study, a **low nutrient soil** was used for all trays, while a **high nutrient soil was used in the 2nd TLC soybean greenhouse run**

Soil Analysis Report

Spectrum Analytic
1087 Jamison Road NW
Washingon Court House, OH 43160-8748
www.spectrumanalytic.com

TLC PRODUCTS, INC
26100 1ST STREET
WESTLAKE, OH 44145

Prepared For
TLC PAYS ALL
JOHN M WONG
26100 1ST STREET
WESTLAKE, OH 44145

Sample Information			
Sample	20231120SUMMER	Sampled	11-20-2023
Lab Number	G46170	Tested	11-28-2023
Acres			

Analysis		Result	Optimal	Analysis		Result	Optimal
Soil pH		7.6	6.2-7.0	Nitrate-N	ppm	60	
Buffer pH				Nitrogen, Total	%	0.09	
Organic Matter	%	1.0		Ammonium-N	ppm	1	
CEC		13.1					
K Saturation	%	1.2	2.0-4.0				
Mg Saturation	%	13.7	10-20				
Ca Saturation	%	85.1	50-70				
K/Mg Ratio		0.3					
Ca/Mg Ratio		12.1					
Phosphorus	m3-ppm	22	50-70				
Potassium	m3-ppm	70	160-260				
Magnesium	m3-ppm	244	210-360				
Calcium	m3-ppm	2963	1700-2400				

Low Nutrient Soil (used Summer 2023)

Soil Analysis Report

Spectrum Analytic
1087 Jamison Road NW
Washingon Court House, OH 43160-8748
www.spectrumanalytic.com

TLC PRODUCTS, INC
26100 1ST STREET
WESTLAKE, OH 44145

Prepared For
TLC PAYS ALL
TLC PRODUCTS INC

Sample Information			
Sample	20231025-N	Sampled	10-25-2023
Lab Number	F27188	Tested	10-30-2023
Acres			

Analysis		Result	Optimal	Analysis		Result	Optimal
Soil pH		7.6	6.2-7.0	Nitrate-N	ppm	11	
Buffer pH				Nitrogen, Total	%	0.30	
Organic Matter	%	2.0		Ammonium-N	ppm	3	
CEC		18.1					
K Saturation	%	2.4	2.0-4.0				
Mg Saturation	%	14.7	10-20				
Ca Saturation	%	82.9	50-70				
K/Mg Ratio		0.6					
Ca/Mg Ratio		11.1					
Phosphorus	m3-ppm	582	40-70				
Potassium	m3-ppm	205	190-300				
Magnesium	m3-ppm	362	260-430				
Calcium	m3-ppm	4021	2400-3400				

High Nutrient Soil (used Fall 2023)

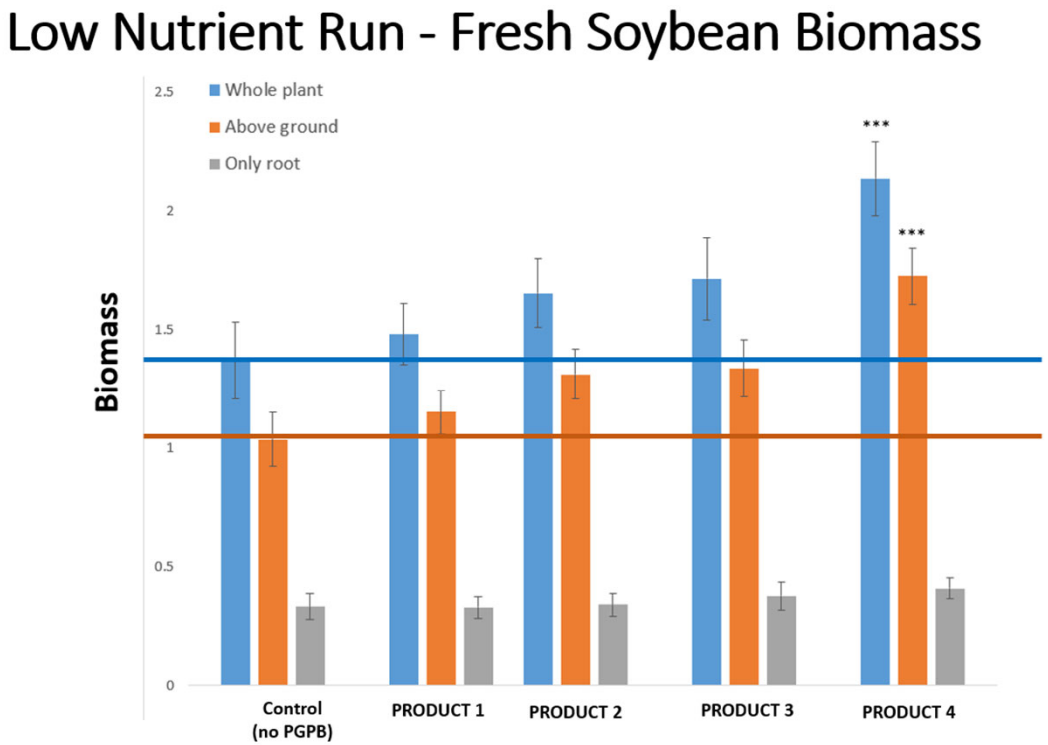
www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

TLC Laboratory Data – Low Nutrient Profile Soil

This run was performed with the low nutrient profile soil

- Five total treatments including control, plus 4 product variations
- 8 trays per treatment type, 5 treatments, a total of 40 soybean trays in the study, and 8 x 32 = 256 individual soybean seeds planted per treatment type
- This was a 15-day run and covers from planting through end of seedling growth
- **Study objective: determine yield differences between control trays and 4 different product trays**

TLC Laboratory Data – Low Nutrient Profile Soil



Remember that this study was in a low nutrient profile soil.

All of the 4 product treatments performed better than the control

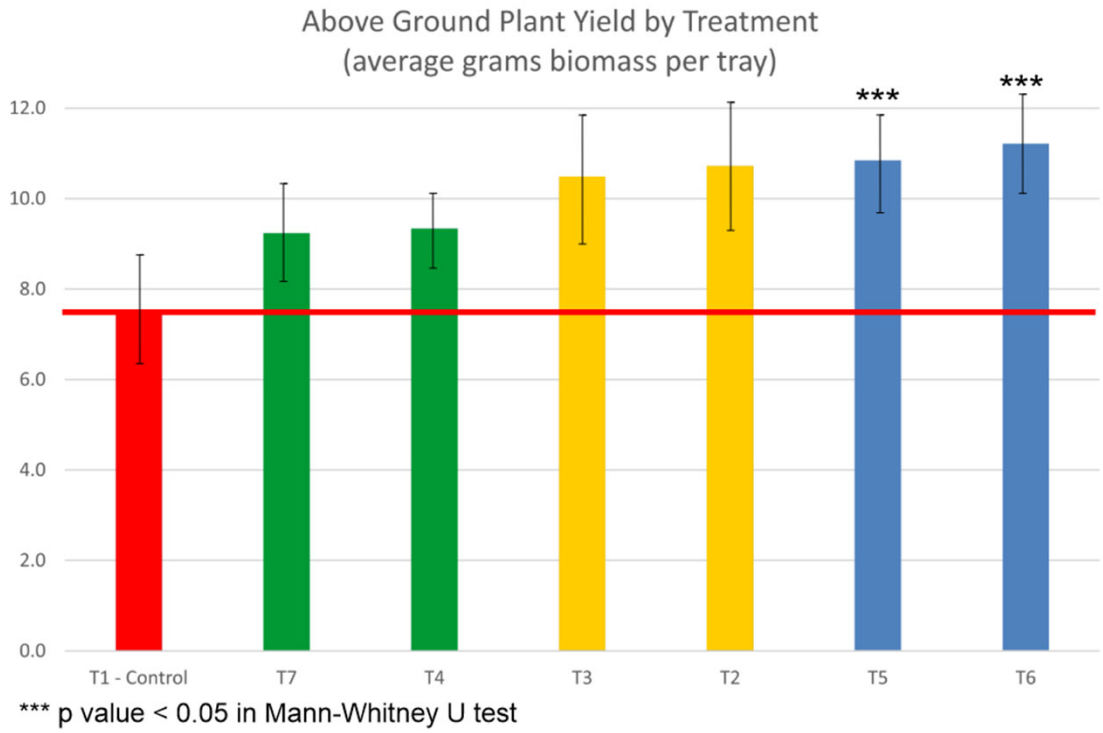
The best advantage was shown by Product 4, with about 70% increase in total biomass over 15 day run

TLC Laboratory Data – High Nutrient Profile Soil

This run was performed with the high nutrient profile soil. See soil analysis from Spectrum Analytics, slide 34, for actual values

- 8 total treatments including control, meaning seven product variations
- 8 trays per treatment type, 7 treatments, a total of 56 soybean trays in the study, and 8 x 32 = 256 individual soybean seeds planted per treatment type
- This was a planned as a 15-day run and covers from planting through end of seedling growth
- **Study objective: determine yield differences between control trays and 6 different product trays, but this time in high nutrient profile soil**

TLC Laboratory Data – High Nutrient Profile Soil



Remember that this study was in a HIGH nutrient profile soil.

All of the 6 product treatments performed better than the control

The best advantage was shown by Product 6, with 11.2 grams per tray yield vs 7.5 grams per tray yield in the control, or a 49% increase.

The yield boosts were consistently better with every treatment version, but larger percentage increases were obtained in the low nutrient profile soil

TLC Laboratory Data – Timetable for Dosing

Considerations for Applying Microbial Inputs Based on Crop Cycle

- **Seeding.** Seed treatment (including microbials) are available. Recall that the initial microbiome is derived from the seed contents
- During the **seedling stage**, the plants need biofertilization and growth hormones, so choose an input that supplies both. Apply at seeding or 1 to 2 weeks post germination
- **Mid to late vegetative stage**, a balanced product containing biofertilizer, growth hormone synthesis, and enzyme synthesis can cover the plant's needs.
- In **early reproductive stage**, the rhizosphere is sometimes limited in available nutrients, particularly P. P is less mobile, and P deficiencies are often responsible for excess biomass but little fruit or seed yield. Use of an effective input with P solubilization capability is highly recommended in this situation

Eurofins Agrosciences Services Study

Study Type

Field Efficacy Trial -Yield

Report Title

TLC-Product Yield Evaluation of AgKit275 and AgKit275-P in Soybeans

Sponsor

John Wong
TLC-Products
P.O. Box 45301, Westlake, OH 44145 US
Street Address: 26100 First Street, Westlake, OH 44145

Author

Jacob Wallbrown
Research Scientist
Eurofins Agroscience Services, LLC
3481 Concord Rd
Lafayette, IN 47909
Phone: 330-402-8736
Email: Jacob.wallbrown@as.eurofinsus.com

Report Date

November 9, 2023

Who is Eurofins Agroscience Services?

Eurofins Agroscience Services is particularly known for its expertise in agrochemical testing and regulatory consultancy. Capabilities include:

- ❑ **Extensive Experience:** Eurofins Agroscience Services has over 30 years of experience in crop protection. This long-standing history in the field signifies a deep understanding of agricultural practices and challenges.
- ❑ **Wide Range of Services:** They provide analytical, regulatory, and field support to a variety of sectors within agriculture, including agrochemical, bio-pesticide, biocide, and fine chemical manufacturers, as well as plant breeders.
- ❑ **Global Reach and Expertise:** Eurofins Agroscience Services operates on a global scale, indicating their capability to handle diverse agricultural conditions and regulations in different regions.

Eurofins Agrosciences Services Study

Study Design

- Seed: Glycine max (GLXMA) as was used in the TLC laboratory studies discussed in this presentation
- Evaluated yield of 4 different TLC formulations vs a control
- With 5 treatments including control, 4 replications performed per treatment, thus a 20 treatment randomized complete block design
- Each plot was a 10 x 20 ft zone, and the harvested area was the two inner rows
- The soil was 39% sand, 40% silt, 21% clay, with good fertility level and good drainage.

- Planting was performed on Jun5, 2023.
- Product dosing was performed on Jun 16, July 14, and August 4

A drone view of the 20 plots is shown on the right.

Drone view, 20 blocks, Aug 9, 2023

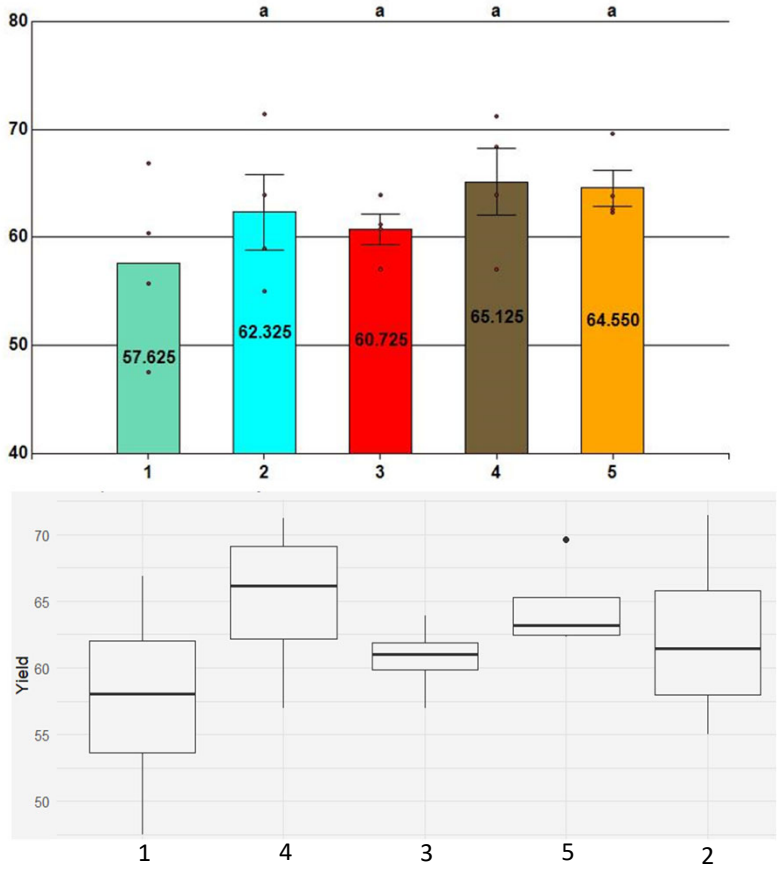
Eurofins Agrosciences Services Study

Products Tested

- 4 different product formulas were used in this study.
- Each was applied at the same time as noted, seedling, mid-vegetative, and early reproductive
- Each of the products was prepared the same way. In large commercial applications, a simple aerated prep is used before the products are applied on a large scale. This procedure was used in the study
- Compared to control, every treatment showed increased yield of 5% to 15%
- The 15% improvement was seen from Treatment 4, known as ACF-SRP, which provides a mix of biofertilizer activity, enzyme production, but is specialized for P solubilization

Note: the Principle Investigator Jacob Wallbrown commented that the TLC formulas performed as well as anything Eurofins tested this year.

While confidence interval was about 92.5% chance that differences were not coincidences, the results were conclusive enough for our decision making.



www.ultraclear.com ABI Inc. 5350 Transportation Blvd., Suite 18 B Garfield Hts., OH 44125 800-847-8950

In a nutshell:

Statistically Robust Greenhouse Experiments, Efficacy Data from Eurofins Agrosciences, and Many Field Trials on Many Crops Confirm the Efficacy of PGPB from UltraClear.com.

Click for MORE INFORMATION