



Biostimulants, Microbials, and Biological Nitrogen Fixation

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Types of Biological Products

- **Biopesticides**
- **Biostimulants**
- **Biological organisms**

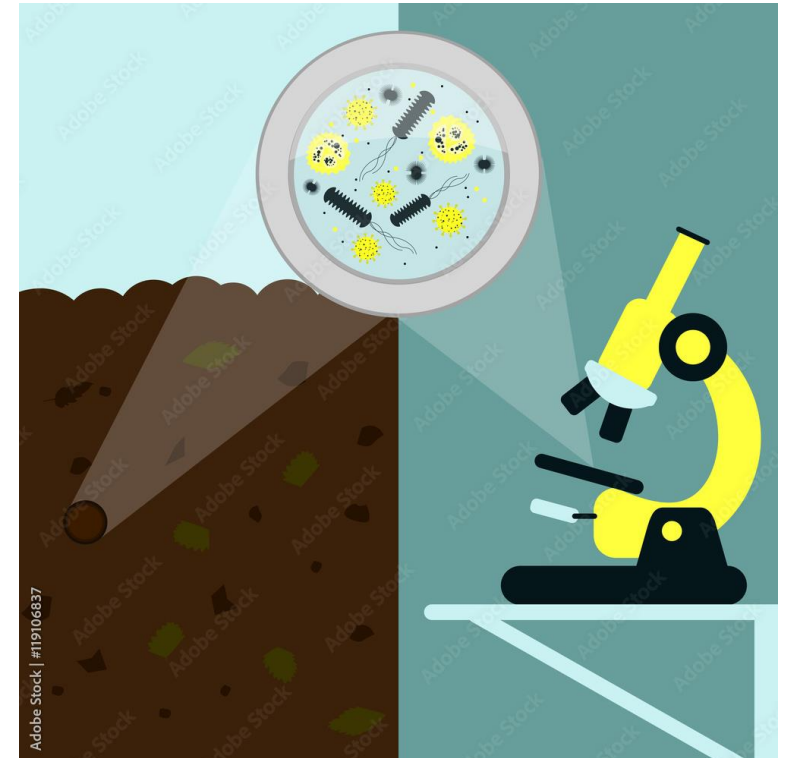


Definition of plant biostimulant:

“A plant biostimulant is a **substance(s), microorganism(s)**, or mixtures thereof, that, when applied to seeds, plants, the rhizosphere, soil or other growth media, act to **support a plant's natural processes independently of the biostimulant's nutrient content, thereby improving nutrient availability, uptake or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality, or yield.**”

Benefits of Biostimulants

- Improve soil health
- Enable plants to adapt to drought and extreme temperature
- Improve a plants ability to utilize nutrients
- Improve efficiencies of fertilizer applications(e.g. less nutrient loss to air and water)
- Increase crop yields



Categories of Biostimulants

1) **Non-microbial Biostimulants** (organic compounds):

- Plant Extracts
- Humic/Fulvic Acids
- Protein hydrolysate
- Purified molecules

2) **Microbial Biostimulants** (generally bacteria/fungi):

- Nitrogen-Fixing Bacteria
- P-Solubilizing Bacteria
- Nutrient enhancement or Plant Growth Promoting
- Mycorrhizal Fungi

Categories of Biostimulants: **Non-microbials**



Plant extracts

- Seaweed extract – biofertilizer
- Contain macro and micro nutrients, humic acids and phytohormones

Organic Acids (eg: Humic Substances)

- Humic acids and organic compounds formed from decomposition of plant and animal materials.



Categories of Biostimulants: **Non-microbials**



Protein Hydrolysate (eg: Amino Acids)

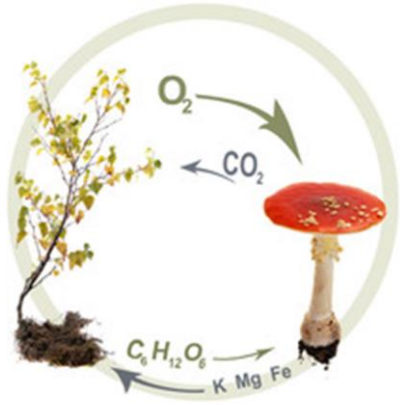
- Hydrolyzed protein: Breakdown of protein into their constituent parts such as amino acids.

Purified Molecules (eg: Chitin)

- Chitin is a component of the exoskeletons of insects and shellfish.



Categories of Biostimulants: **Microbials - Fungi**



Asymbiotic

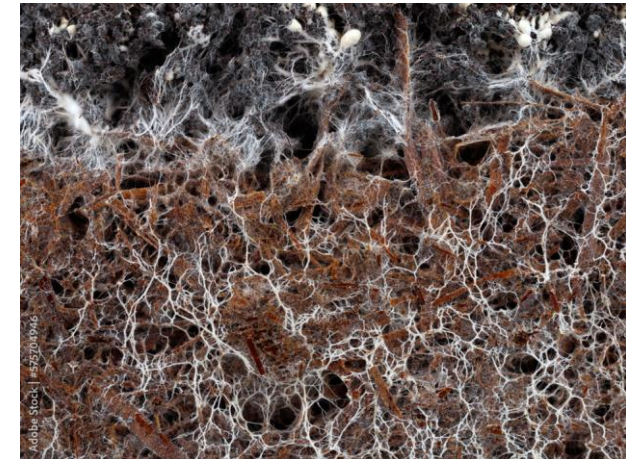
- live on decaying materials
- live independent of plants
- free up nutrients
- subject to environmental interactions

Symbiotic fungi (mycorrhizae)

- form a mutually beneficial relationship with living plants

Fungi are a group of spore producing organisms that feed on organic matter.

- Mycorrhizae: form a symbiotic relationship with plants where they exchange absorbed nutrients from the soil such as phosphorous in exchange for carbohydrates.



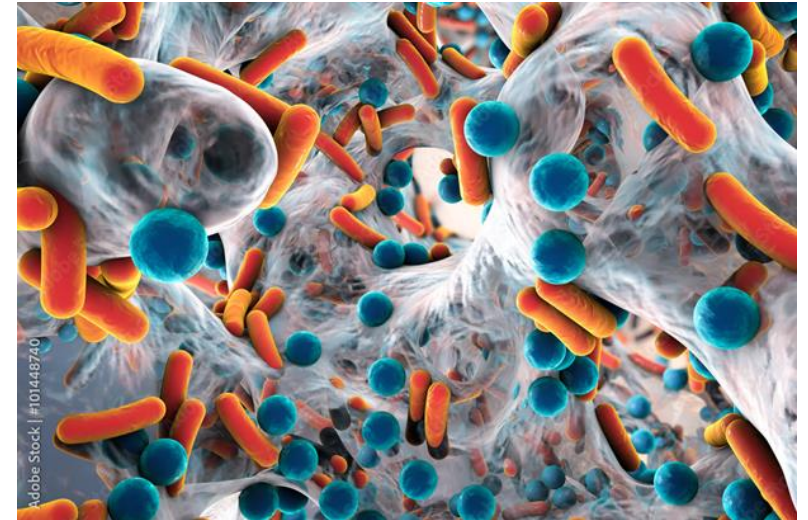
Categories of Biostimulants: **Microbials - Bacteria**

Asymbiotic

- live on decaying materials and other elements
- live independent of plants
- may free up nutrients
- are subject to environmental interactions both beneficial and competitive

Symbiotic bacteria

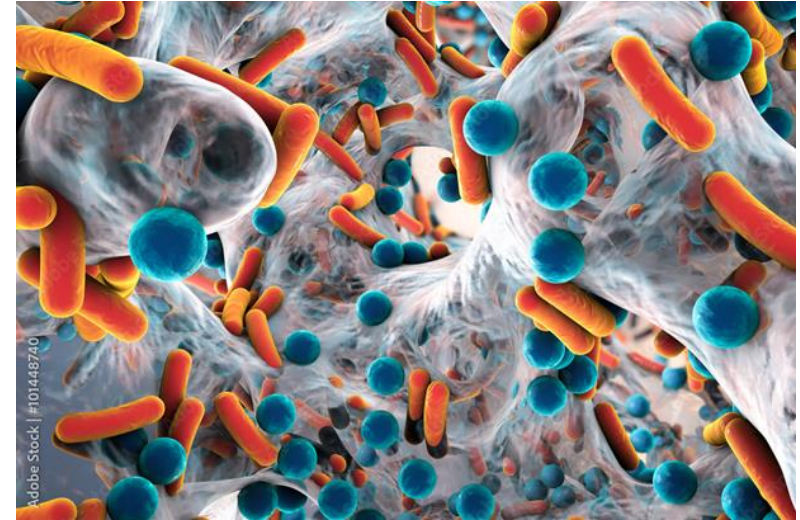
- form a mutually beneficial relationship with living plants
- often close in proximity to plant
- can even live within the plant (**endophytic**)
 - protected by plant when endophytic



Function of Biostimulants: Microbials - Bacteria

General function

- Nutrient availability
 - mineralize/solubilize (make available)
 - help translocate to or within the plant
 - some fix nitrogen
- Elicit (trigger) plant response
 - enhance defense
 - abiotic stress tolerance
 - pathogen resistance
 - elicit offense
 - plant morphology (roots, leaves...)
 - yield
 - quality



Biological Nitrogen Fixation with Bacteria

Until about 6 years ago, biological N fixation (BNF) was with legumes

Now there are various options and conditions for BNF

Let's dive deeper into BNF

- soil or free living
- symbiotic (mutually beneficial)
- categorized as diazotrophs

Biological Nitrogen Fixation - Soil

Factors affecting nitrogen fixation in **soil living bacteria**:

- All nitrogenases are inhibited by high $[\text{NH}_4^+]$, $[\text{NO}_3^-]$ and $[\text{O}_2]$.

Diazotrophs fix nitrogen if:

Carbon is not limiting.

- Preferred source of C (sugar) must be abundantly available.

Nitrogen is limiting.

- Most diazotrophs have a nitrate reductase enzymes and will assimilate nitrogen instead of fixing N

Micronutrients are not limiting.

- Primarily molybdenum, iron, sulfur and magnesium

Oxygen is limiting

Biological Nitrogen Fixation – Symbiotic



Conditions for N-fixation **inside plant** tissues and cells

Carbon is not limiting

- There is a constant supply of simple C compounds (sugars, organic acids) provided by the host plant.
- Competition for C by other microbes is limited.

Nitrogen is limiting

- Ammonium and/or nitrate is low

Micronutrients are not limited (Mo, P, Fe, S, Mg)

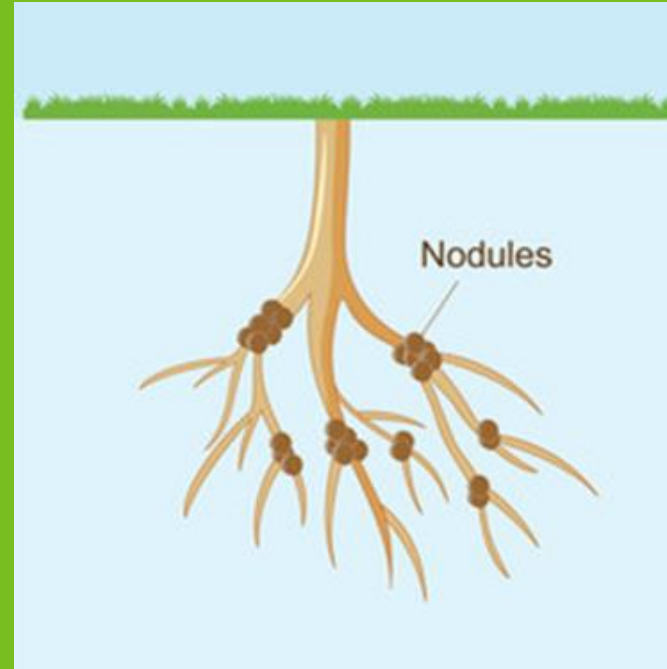
- Delivered by the plant so long as plant has access to them

Oxygen is limiting

- Adaptions in some cases

Nitrogen Fixing Bacteria

Example: Rhizobia



Commonly understood symbiotic bacteria

Bacteria that convert atmospheric nitrogen into ammonia

Bacteria are specific to a species

Significance of Location of N Fixation

Soil

- Subject to competition by other micro-organisms
- Subject to loss
- Move to or intercepted by roots
- Cross root membrane, is translocated

Rhizosphere

- Subject to fates in soil and competition by other microbes
- Cross root membrane, is translocated

Nodular

- Protected by tissue or the nodule
- Inside plant root membrane, but requires transportation

Inter cellular

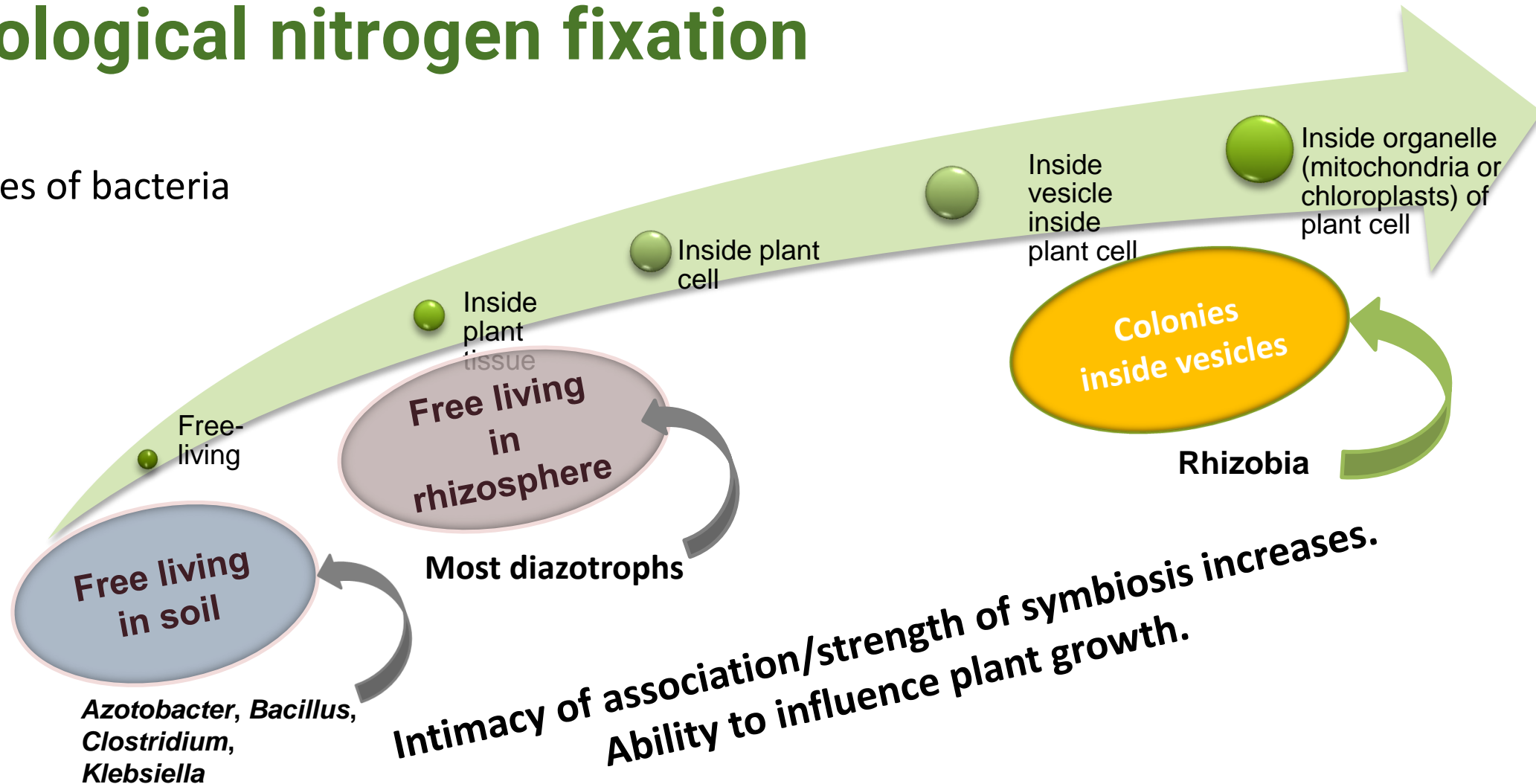
- Inside plant - between cells: Translocation across cell wall

Intra cellular (inside)

- Most intimate
- Less translocation cost
- Not subject to loss or competition

Biological nitrogen fixation

Types of bacteria



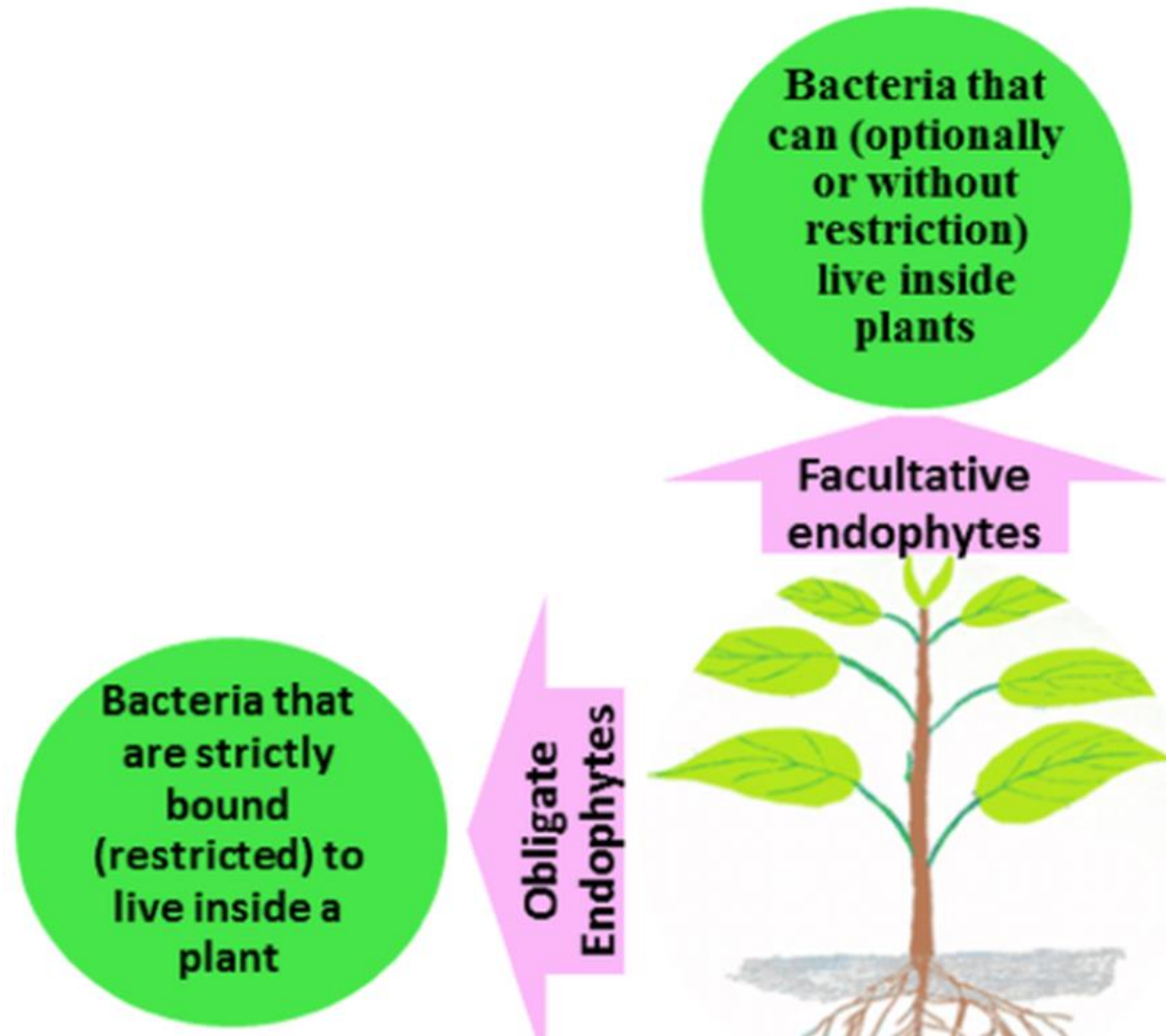
Bacteria

Obligate Endophyte:

- Dependent on host plant for their growth, survival and propagation.
- Consistently inside plant and exerting influence.

Facultative Endophyte:

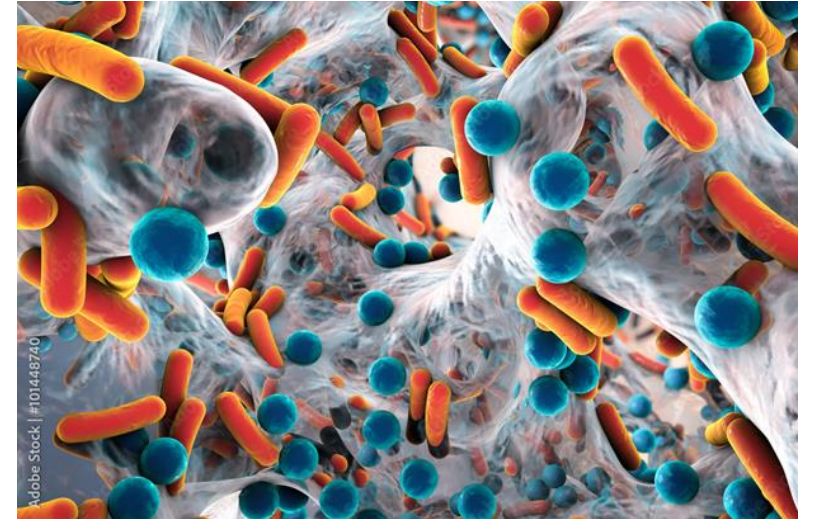
- Reside inside and outside the plant.
- Only inside of the plant some of the time exerting influence.



Commercially available non host specific nitrogen fixing bacteria

<i>Azoarcus indigenus</i>	Soil
<i>Azorhizobium caulinodans</i>	Endophyte
<i>Azosporillum brasiliense</i>	Soil
<i>Curtobacterium salicis</i>	Endophyte
<i>Gluconacetobacter diazotrophicus</i>	Endophyte
<i>Klebsiella variicola</i>	Soil
<i>Kosakonia sacchari</i>	Endophyte
<i>Methilobacterium symbioticum</i>	Endophyte
<i>Paenibacillus polymyxa</i>	Endophyte

- likely others



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Azoarcus indigenus

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Gluconacetobacter diazotrophicus

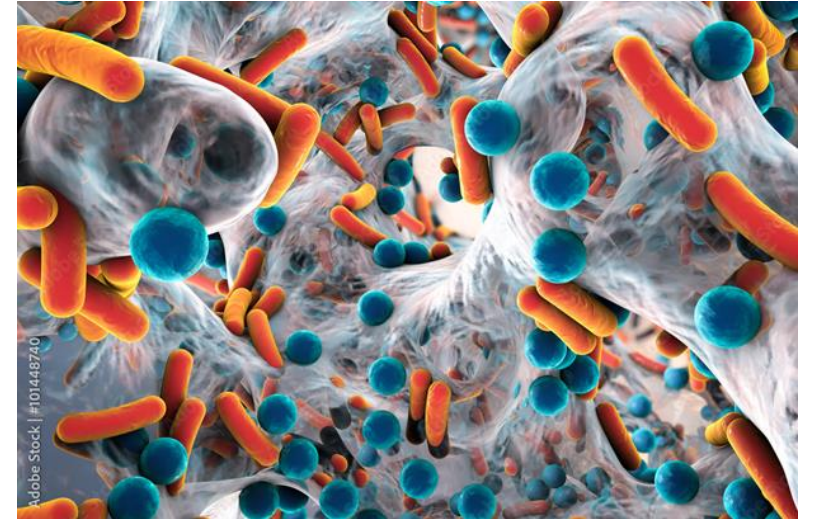
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	Rhizobia	<i>G. diazotrophicus</i>
Crops	Crop specific	Multiple crops
Establishment & Crop Response	Weeks	Days
Total Amount of Nitrogen Supplied	Significant and majority of need	Supporting, yet significant
Location of Nitrogen Fixation	Outside plant cells – in nodule (Intercellular)	Inside plant cells (Intracellular & intercellular)
Life in soil without a host	Able to survive for extended time as free living	Cannot exist extended time as free living (requires host)
Plant segment of Nitrogen Fixation	Roots: Nodules	Systemic (shoots & roots):
N Fixation in presence of extra Nitrogen	Nitrate Slows or stops fixation (plant becomes lazy)	Nitrate does not inhibit Nitrogen fixation

Observed Crop Responses to *G. diazotrophicus*

- Improved nitrogen use efficiency
Supplemental N
- Increased chlorophyll content
- Water use efficiency
- Yield increase
- Quality



Envita Std Practice Envita



Std Practice

Envita



Envita

Std Practice

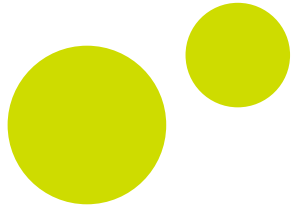
Potato Agronomist Quote:

Excerpt from an email communication in 2022:

“We’ve looked at Envita (*Gluconacetobacter diazotrophicus*) ... for 2 years in MI. [Gd] helps with drought stress (delayed each irrigation by 1/2 to 1 day on [Var]), and also helps plants use Nitrogen more efficiently ...”

Observation from same agronomist: later in the season, potatoes didn’t wilt so much during the day when treated with Gd. This was noted at the later foliar fungicide timing. (paraphrase)

Gluconacetobacter diazotrophicus Offers



- Systemic, season-long nitrogen fixation
 - Endophytic (inside) and throughout the plant
 - Such N is plant available and protected
 - Fills in the gaps
- Crop flexibility from alfalfa to zucchini
- Flexibility in application method and timing
- Offers productivity and sustainability

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