Summary of Regional Studies in Corn on Selected Commercial Asymbiotic N-fixing Organisms and Suggestions for Companies Developing Similar Products

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Evidence for asymbiotic N-fixing organisms finds that these organisms were active 1.5 billion years ago—some of the oldest organisms found in the fossil record.
(Boyd & Peters, 2013, Frontiers in Microbiology)

Compared with about 59 million years ago for symbiotic N-fixers (Sprent and James 2007, Plant Physiology)

Point #1- Asymbiotic N-fixing organisms are a natural part of soils

Asymbiotic N-fixing organisms

Organisms, usually a species of bacteria, that have the ability to fix atmospheric N (N₂), transforming it into NH₃, which is immediately attached to a ‘carbon-skeleton’, safening it.

The fixation requires energy, which when conducted in soil comes from organic matter.

N-fixation is an energy-expensive process.
The enzyme that serves as ‘fixation facilitator’ in bacteria is nitrogenase.
To convert 1 N₂ to 1 NH₃ requires 16 ATP molecules (produced during photosynthesis) and 8 electrons.

Energy limits N fixation.
(Smercina et al., 2019, Applied Environmental Microbiology)

For comparison, production of 1 peptide bond in protein synthesis requires only 5 ATP (still considered ‘high energy requirement’).
**Nitrogenase enzyme**

\[ C_{7}H_{19}Fe_{7}MoNO_{7}S_{9-12} \]

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**Substrates to meet energy requirement**

Energy sources for asymbiotic N-fixing organisms close to plant roots are the root exudates that surround many roots, and also includes components of soil organic matter and residue decay intermediary compounds in the bulk soil.

N-fixing organisms that exist inside the plant tissue have to receive substantial energy from the plant within which they reside.

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**Images of root/exudate/soil particle interfaces**

RP 1 images (SEM) of fine root (1), root exudates/biofilms (2), fine roots (3), clay mineral grains (4), clay mineral aggregates (5), root exudates/biofilms (6), organic filament (7), fine clay aggregates (8), fine root (9), and RP 2 images (SEM) of aggregates with abundant root remnants (10), pollen (11), root tap protruding through clay aggregate coated by exudates (12), dominant network of fine roots (13), and organic filament, primary mineral, clay minerals coating organic filament (14, 15, 16).

From Razzaghi et al., 2017.

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**Diversity of Asymbiotic bacteria types**


Genera include species from *Azotobacter, Ocrhobactrum, Sphingomonas, Opitutus, Clostridium, Pseudomonas* and ‘a host of others’.

There are many species, some more efficient than others, Their activity is linked to substrate and soil condition.
Asymbiotic N-fixing bacteria are in most soils. Their activity increases when tillage decreases. *(Food & Housing)*

Lamb, Doran and Peterson, 1987
Nonsymbiotic dinitrogen fixation in no-till and conventional tillage SSSAJ 51:356-361

Recorded greater activity with no-till, but concluded that it was not great enough to contribute to any N credit. They considered the values from incubation to be values that might be experienced in the field. But the disturbance of soil probably killed billions of N-fixing critters, so values are index.

In North Dakota, there is an N credit for 6 or more years of continuous no-till, one-pass shallow tillage, shank strip-till, amounting to 40-50 pounds N per acre.

Part of this credit probably comes from the increased microbial biomass under no-till that protects N from loss. But a part of the credit, perhaps 25-33% may come from greater asymbiotic activity in long-term no-till.

Franzen et al. 2019, SSSAJ

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**Turns out the ‘conventional till’ site across the fence was one-pass shallow tillage, so the same tillage category**

From 2019 thru 2021, 6 sites in eastern North Dakota were sampled each month for asymbiotic N fixing activity. Change in activity was related to rainfall within 30 days before sampling and mean air temperature. Franzen et al., SSSAJ, 2023
The relationship of N-fixing activity to soil condition made a large impact on the trend of activity over a season.

Jamestown, 2021. Period from late May to early July moist, then very dry.

Gardner, 2020. Period for July sampling, soil was saturated in a high clay soil.

Summary of what we know about native activity-

**Greater in long-term no-till.** Their activity increases when their ‘homes’ are not destroyed and there is sufficient food to support their N-fixation.

Moist soil and warm conditions favor N-fixation. Dry soil conditions, saturated soil conditions, and cold soil temperatures inhibit their activity.

Regional studies on commercial asymbiotic N-fixation products

Products tested-
- **Envita**, Azotic North America  *Gluconacetobacter diazotrophicus*
- **Utrisha**, Corteva Agriscience  *Methylobacterium symbioticum*
- **ProveN**, PivotBio  *Klebsiella variicola*
- **ProveN 40**, PivotBio  *Kosakonia sacchari & Klebsiella variicola*

Summary of results from 10 states.

No means no difference between same N rate with or without additive
Yes means a yield increase present at least 1 N rate

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<th>Utrisha</th>
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Growers should be skeptical about new products

Point #2-
Try them on replicated strips on the farm.

Refer to L. Thompson, 2022 from Proceedings of the North Central Extension- Industry Soil Fertility Conference for ideas regarding on-farm testing and data analysis.

Example from Thompson paper:
Farmers have GPS
Farmers have GPS yield monitors
Farmers have everything they need to replicate treatments and test product effectiveness.

Point 3-

There should be a quick method of analysis developed to determine whether the organism is alive and functioning in the container, the field or the plant.
**Point 4**

Organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer and on the farm awaiting application.

**Storage and Disposal**

*Storage Conditions:* Keep the product in a well-ventilated place. Store at room temperature, do not subject to temperatures below 39 °F (4 °C). Keep the product hermetically closed.

*Storage and Handling:* Do not freeze. This product contains live non-pathogenic organisms. Store between 39°F to 68°F. Store in well ventilated buildings, away from foodstuffs and animal feed. Keep out of reach of children. Keep out of direct sunlight. Do not open product container until ready for use.

**Point 5**

Organisms should be able to compete and ‘win the war’ with native microorganisms in order to survive and perform its function.

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**Point 6**

Organisms should be adapted to variable moisture, variable soil pH and variable soil salts in order to perform its function.