WORLD FERTILIZER®

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Nitrogen fixing bacteria

Dr. Thomas Williams, BioConsortia Inc., examines new seed-borne approaches to optimise seasonal fertilizer performance in cereals and other agricultural crops.

Nitrogen is essential for life, directly influencing the growth and yield of agricultural crops. Plants rely on nitrogen to produce grain, fruit, and biomass. However, nitrogen availability in the soil often does not align with critical growth stages, especially later in the growing season. Nitrogen-fixing bacteria address this gap by providing a steady supply of

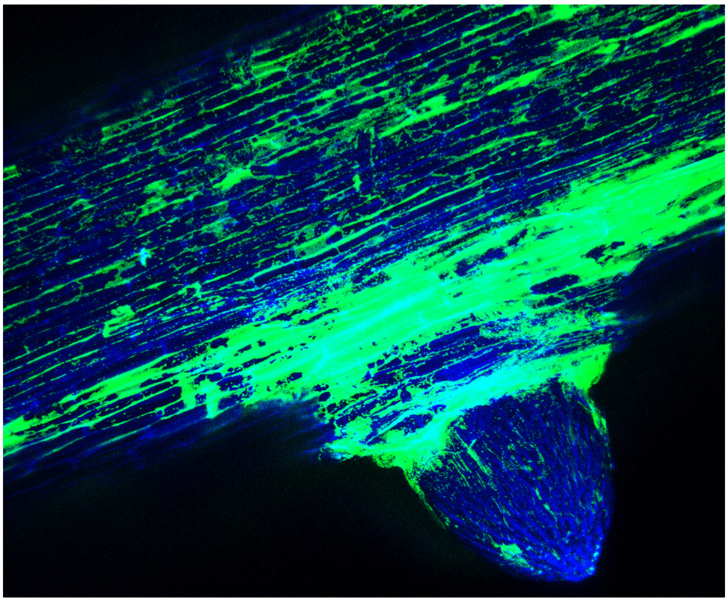

Figure 1. A first generation Always N™ nitrogen fixing seed treatment product (green) colonising the rhizosphere. (Copyright BioConsortia Inc. 2025.)

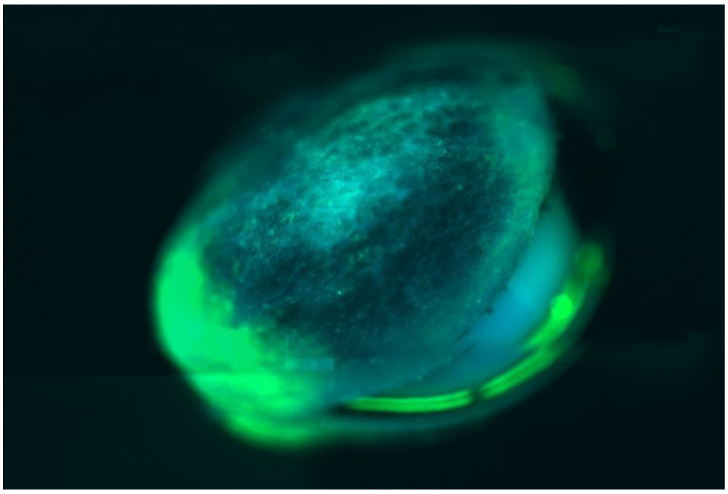

Figure 2. Fluorescent microscopy image showing Always N nitrogen fixing product colonising the surface of a seed. (Copyright BioConsortia Inc. 2025.)

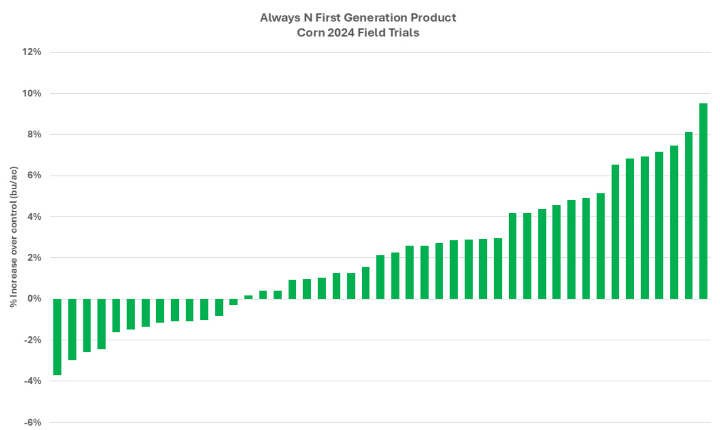

Figure 3. First generation Always N nitrogen fixing seed treatment product performance in corn in 2024 field trials over a formulation blank (control). (Copyright BioConsortia Inc. 2025.)

nitrogen in the plant's root zone throughout the growing season.

Environmental impacts of fertilizer production and use

Fertilizers have revolutionised agriculture, boosting US corn yields from 25 to nearly 160 bushels per acre in just 70 years. This progress is largely credited to the Haber-Bosch process, which converts atmospheric nitrogen into ammonia. However, this process is energy-intensive, requiring about 0.65 t of natural gas per t of urea.

Synthetic fertilizer use contributes to greenhouse gas (GHG) emissions through nitrous oxide (N_2O) produced by volatilisation and by nitrogen fertilizer breakdown in the soil. Up to 50% of applied nitrogen escapes into the air, soil, and waterways, exacerbating environmental harm.

Additionally, poor synchronisation between nitrogen application and plant demand limits efficiency, leaving substantial room for improvement. For corn and most row crops, fertilizer is applied pre-plant or early in the growth phase when the grower can access the field, whereas the crop has greatest need for nitrogen much later in the season. To mitigate these issues, BioConsortia Inc. has developed Always N™, a portfolio of nitrogen-fixing seed treatments that provide season-long nitrogen supply.

Free living nitrogen fixing bacteria (FLNRB)

The soil nitrogen cycle is driven by microbes that convert atmospheric nitrogen into plant-usable forms such as ammonia and ammonium. Associations between symbiotic nitrogen-fixing bacteria and plants have been most well studied in the legume model, where strains of rhizobium have been characterised and commercialised, and their use as inoculants have become best practice in agriculture in many countries. Symbiotic nitrogen-fixing bacteria have evolved to elicit nodule formation in legume root systems, protecting their nitrogen-fixing activity from degradation by oxygen.

While both symbiotic and free-living microbes perform nitrogen fixation, free-living nitrogen fixers are increasingly valuable for non-legume crops. Unlike symbiotic bacteria that form root nodules, FLNFB can persist in the environment and interact with various plant species.

Key FLNFB species, such as Azospirillum and Klebsiella, have been studied extensively. However, as gram-negative bacteria, their thin cell walls limit their commercial viability due to poor shelf-life and instability when mixed with seed treatment chemicals. To address this, BioConsortia focuses on gram-positive, spore-forming bacteria like Paenibacillus. These microbes are thick-walled, robust, long-lasting, and better suited for agricultural applications.

Unique research and development platform enables a novel approach

Academic researchers characterised the collection of genes that enable nitrogen fixing in gram-negative microbes many years ago, but only recently has the genetic nitrogen fixing potential of Paenibacillus been identified. A proprietary Advanced Microbial Selection (AMS) process has identified a collection of high-performing Paenibacillus strains capable of nitrogen fixation.

Modern seed treatments have transformed the seed industry, ensuring each seed is protected from pathogens and supported in the early stages of development. Root colonising nitrogen-fixing microbes are deployed as seed treatments to provide nitrogen directly to the root zone, remaining active on roots, converting atmospheric nitrogen into plant-accessible forms even after synthetic fertilizers dissipate. Successful products must colonise plant surfaces. Fluorescent protein markers enable researchers to track colonisation and optimise microbial performance under field-like conditions.

Some available nitrogen fixing products may claim that their microbes can be applied as seed treatments. However, once applied, the microbes do not persist on the seed coating – either due to incompatibility with other seed treatment components or to the microbe's inherently short life.

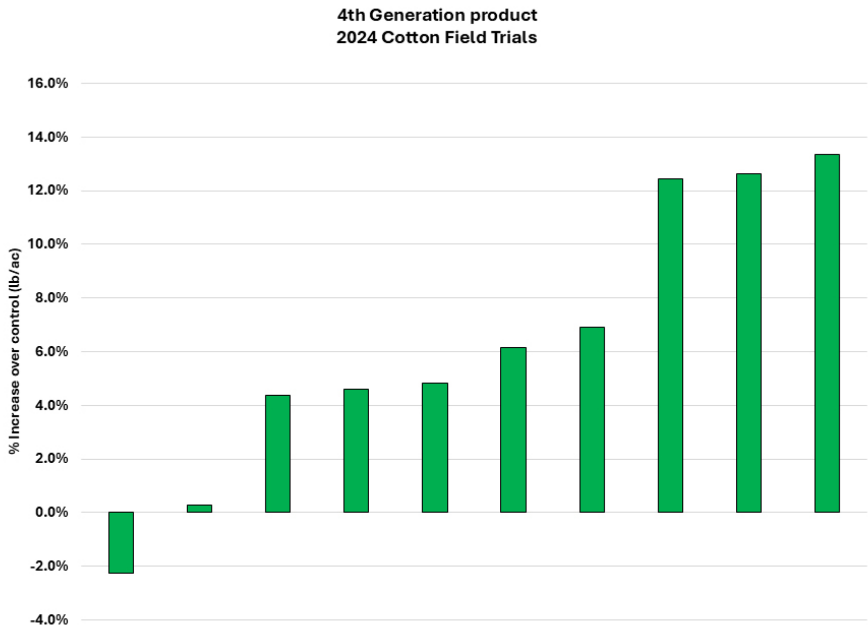

Figure 4. Always N nitrogen fixing product applied in-furrow at planting in multi-year field testing in potato increased yields equivalent to theoretical yield production from 70 lbs/acre added nitrogen fertilizer. (Copyright BioConsortia Inc. 2025.)

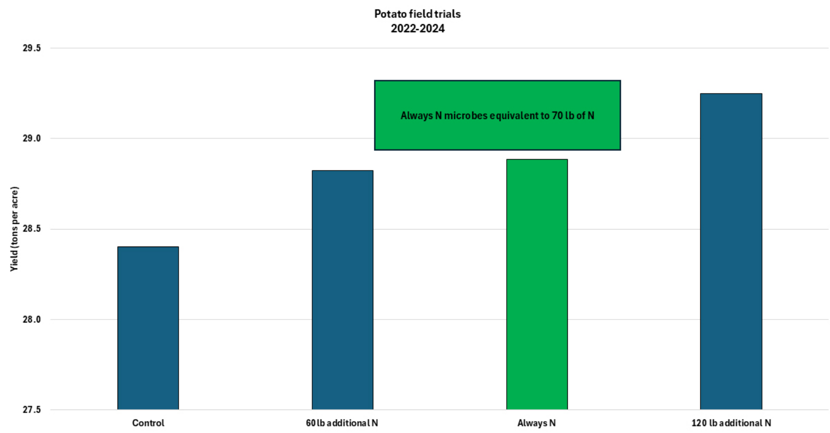

Figure 5. Initial field testing in 2024 demonstrated Always N seed treatment provided 6.3% yield increase (lb lint/ac) in cotton. (Copyright BioConsortia Inc. 2025.)

Overcoming limitations of wild-type FLNFB

Wild-type (naturally occurring) FLNFB strains are abundant in the soil of agricultural systems but fix nitrogen only when necessary, limiting their contribution as tools for plant nutrition. Nitrogen fixation is energy-intensive, and microbes typically prioritise other metabolic processes when ambient nitrogen is available.
This limitation can be addressed through advanced gene editing; engineering microbes to fix nitrogen consistently, even in nitrogen-rich environments.

Demonstrating nitrogen-fixation activity: a simple overview

Demonstrating nitrogen-fixation activity involves several key scientific validations. Genomics analysis confirms that these microbes possess the nif gene cluster, essential for microbial nitrogen fixation. Genetic analysis of strains with gene deletions validates this gene cluster's function by creating versions without the key nif genes, demonstrating that the nitrogenase enzyme – responsible for fixing nitrogen – is no longer functional when the gene is removed. Laboratory and in planta tests, such as the acetylene reduction assay (ARA) that detects nitrogenase conversion of acetylene (C_2H_2) to ethylene (C_2H_4) that can be very sensitively detected by gas chromatography, further prove that nitrogenase is active. Plants treated with Always N products exhibit significant improvements in nitrogen-related traits, including greater growth and biomass, higher nitrogen content in shoots, better grain protein levels, and healthier canopies observed through normalised difference vegetation index (NDVI) and chlorophyll readings. Impact data highlights these benefits, with microbial treatments increasing biomass by 63% (308 lb/acre vs 192 lb/acre), grain protein by 3% (98 lb/acre vs 95 lb/acre), and shoot nitrogen content by 5%.

This comprehensive validation demonstrates how these microbes enhance nitrogen fixation and promote sustainable farming practices.

Case study: field performance and yield benefits

First-generation Always N products have demonstrated robust performance in multi-year field trials: it has been demonstrated through laboratory, greenhouse, and field trials over more than five years of research that these products provide nitrogen to the plants through their clear ability to fix atmospheric nitrogen. In the field, they have been shown to increase crop yields.

Results from field trials include:

- Corn: field trials have demonstrated increased yields by 5.5 bushels per acre, with an 85% win rate in trials.
- Wheat, potatoes, cotton and rice: field trials have validated significant yield improvements across diverse crops and environments. For example, potato field trials show greater than 3% yield increase across three-years testing, equivalent to the theoretical yield created by 70 added lbs of synthetic nitrogen fertilizer.
- Vegetables: field trials have demonstrated enhanced productivity in tomatoes, peppers, and lettuce via in-furrow applications. In tomato trials conducted over three years, 12.1% yield increases were seen, which is equivalent to the yield delivered by 57 added lbs of nitrogen fertilizer.

Future innovations

Researchers continue to enhance the nitrogen fixation potential of the company's patented spore-forming bacteria. The genomic analysis platform has further revealed the complex regulation of nitrogen fixation in Paenibacillus, allowing the new discovery of novel gene targets that enhance the mode of action.

Next-generation products show promise for even greater yield improvements, with some strains delivering threefold higher nitrogen-fixation rates than first-generation products and additional research is already underway. Future embodiments show enhanced performance over first and second generation products, offering growers increasingly optimised fertilizer alternatives that leverage microbially produced nitrogen to maximise yield and reduce ecological impacts.

Sustainability and agricultural impact

Free living nitrogen fixing bacteria are promising tools for more sustainable agriculture as they have the potential to reduce modern agriculture's heavy reliance on synthetic nitrogen. Many FLNFB products on the market display promising results yet have major limitations on their stability. Gram-positive microbes show the nitrogen fixation capability and shelf-stability needed to fuel wide industry adoption through innovative seed treatment technology. WF