Nitrogen Fixing Bacteria in Agriculture
Now a Real Option

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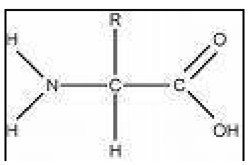
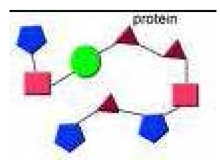
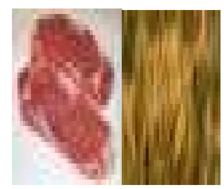
The Pursuit of Protein and Profit

All agricultural enterprises, in essence, are based on the pursuit of protein production. As agriculturalists we are using soil nutrients combined with water and sunlight to produce a protein product of commercial value, whether it is meat, grain, milk, eggs or a fibre, fruit or vegetables. The more protein we can produce per hectare, the greater the farm profitability….a simple equation.

N_2







Nitrogen **Amino Acids** **Proteins** **Protein Products**

Plant protein production is reliant upon an adequate supply of water and essential elements, in particular the building blocks of protein, **plant available nitrogen**. Nitrogen is the raw Ingredient for **amino acids**, which build **proteins**. Plants require nitrogen in greater quantity than any other element. The emphasis is on *plant available* nitrogen. All essential elements are critical, however it is nitrogen we need the most of by weight. The atmosphere comprises of **~78% nitrogen** as an inert gas, N2, which is unavailable to plants. Above every hectare of ground there is **70-80000 tonnes** of this *unavailable* nitrogen. Our crops are literally bathing in unavailable nitrogen. "Water, water everywhere, but not a drop to drink". How do we take advantage of all this free nitrogen?

In high production agriculture, the game is to convert atmospheric nitrogen into a plant available form, (NH2, NH4 or N03), so that we may then in turn apply it to our paddocks to produce large quantities of saleable protein products. Excluding legume rotations (valuable in there own right), we can get nitrogen to our crops in three main ways;

1) *The energy expensive manufacture and application of synthetic nitrogen (urea, ammonium nitrate, anhydrous ammonia etc).*
2) *The application of animal manure, composts and animal/fish products containing nitrogen*
3) *The use of specific **free living** (non symbiotic) nitrogen fixing bacteria*

These three methods are compared at the end of this article in Table 1

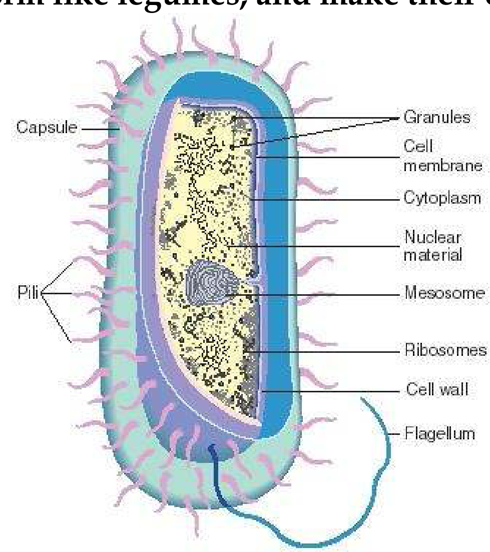
Nitrogen Fixing Bacteria Explained

Nitrogen fixing bacteria are single celled organisms that are essentially **miniature urea factories**, turning N2 gas from the atmosphere into plant available amines and ammonium via a specific and unique enzyme they possess called **nitrogenase**. Although there are many bacteria in the soil that 'cycle' nitrogen from organic material, it is only this small group of specialized nitrogen fixing bacteria that can 'fix' atmospheric nitrogen in the soil. Without the production of the nitrogenase enzyme by these specific bacteria, nitrogen fixation can not happen. *Nitrogen fixing bacteria are generally* ***endemic to most soil types*** *(both symbiotic and free living species), however in the natural state they generally only comprise a very small percentage of the total microbial population* are often strains with low performance regarding the quantity of nitrogen they can fix.

Most growers are familiar with legume nitrogen fixing bacteria called Rhizobium and the colonies they form inside nodules, visible white lumps on the roots of legumes. It is well established that specifically selected, high performance strains of these symbiotic legume bacteria can fix between **50 to 200 units of nitrogen per season**, depending on soil moisture. To do this job, these specific strains need to be inoculated into the legume seed to ensure their numbers are high enough to colonize each plant and to bring about a significant influence on the total nitrogen production.

Lesser known N fixing species, however just as important to agriculture, are the **free living (non symbiotic) nitrogen fixing bacteria** that can fix nitrogen for legume ***and*** non-legume crops alike.

These organisms, like the legume Rhizobium bacteria, need to undergo a rigorous selection process to choose the highest performing strains. Also like legume inoculums, they need to be *inoculated into the crop to achieve 'threshold levels'* where their numbers are great enough to effect significant nitrogen production. Thanks to recent developments in microbiology technology, this can now be achieved with a great degree of confidence. **Non legumes can now be made to perform like legumes, and make their own nitrogen from the sky.**



N2

Nitrogen gas
Not plant available

Nitrogen fixation bacteria

NH4

Plant available
ammonium

Types of Nitrogen Fixers

There are two main types of *free living* nitrogen fixing bacteria that are useful to agriculture and can now be sourced as a commercial inoculum;

1) Soil dwelling species (such as *azotobacter* and *pseudomonas* spp) that live on the **surface of plant roots** in the soil, consuming the sugary exudates from the plant, using this energy source to fuel the conversion of nitrogen gas into plant available nitrogen.

2) Nitrogen fixing species that ***live inside*** the plant known as **endophytes** (such as *azospirillum* spp). These organisms live in the intracellular spaces in the plants vascular system and take **dissolved nitrogen gas** from the sap flow and convert it into **amines and ammonium nitrogen** for plant use. Some of these strains of endophytic bacteria are very efficient and can fix large quantities of nitrogen in a crop cycle. Much selection and trial work has been carried out over the years on a number of these high performing strains.

The Plant – Bacteria Communication / Feedback System

N fixing bacteria use plant carbon as a high calorie energy source to fuel the biological reaction that converts N2 gas into plant available N compounds. Whether it be the soil dwelling species or the endophyte N fixing species, the plant is *controlling* the amount of energy (plant carbon) the N fixing bacteria receive to perform their N fixation function. As such the ***quantity of nitrogen being fixed for plant use is controlled by the plant itself***.

For example, when there is **limited soil moisture nitrogen fixation slows down**, dictated by the plants diminishing nitrogen requirements and subsequent frugal supply of carbon to the bacteria.

When **soil conditions are optimum, nitrogen fixation is maximized** by the plant's increasing supply of carbon to the bacterial colony, in turn increasing N fixation to meet the increasing nitrogen requirements. It works like a **feedback system** and assures the plant receives just the right amount of nitrogen it requires based on the growing conditions at the time.

This is in stark contrast to physically applying nitrogen products where the grower must guess how much nitrogen to apply at the start of the season. ***If we get this wrong we can oversupply nitrogen in a dry year, dramatically reducing water use efficiency and plant health, 'cooking' the crop.*** In a wet year we may not be applying enough to capitalize on the good moisture conditions and subsequent N requirement. Nitrogen fixation solves this problem via this natural feedback system.

It has been demonstrated that by utilizing the *correct nitrogen fixing species, and applying a known number of these bacteria* to a crop to achieve effective colonization, that these organisms will supply up to **110 units and more of nitrogen per hectare** per season **with one inoculation**, depending on seasonal soil moisture conditions.

Reliable Colonization – the CFU Count

Effective colonization is measured in '**Colony Forming Units'** or CFU.
Two CFU per mm2 of leaf surface or root surface early in the crop cycle is all that is required to inoculate endophytic and non endophytic N fixing bacteria into and onto the plant. Below this critical threshold level, reliability of response dwindles. To achieve this threshold level, the bacteria being applied *must have consistent and high viability* and contain a high concentration of the correct bacterial colonies.

Twin N™ Inoculum – Species Selection and Freeze Drying

To date, the Twin N™ product is the only commercial N fixing inoculum that can claim high viability and high numbers to achieve reliability. This particular inoculum has been developed and manufactured to a pharmaceutical standard that guarantees a very high and very stable CFU count. Added to this, the **Twin N inoculum contains of the worlds most researched and most highly productive nitrogen fixing bacteria species**. The species selection criteria is based on**quantity of nitrogen** able to be fixed by the organism, the suitability to a wide **variety of soil** types, climates and ability to **colonize all plant types**.

Twin N™ is also the only free living nitrogen fixing bacteria product developed to date that contains both *azotobacter* (soil borne species), **azospirilum** (endophytic species), and free living nitrogen fixing bacteria, together in a freeze dried form. This means the **plant is inoculated and colonized both inside and out** with highly efficient forms of nitrogen fixing bacteria, meaning two systems of N fixation are at work … hence the name *Twin* N. It is the **species selection** and the proprietary **freeze drying process** that enables an extremely high and guaranteed bacterial count in every vial. Once fermented and then freeze dried, bacterial numbers and viability remain unchanged indefinitely, until water is added to rehydrate them in order to apply them to a crop.
This is critical to achieving successful and reliable colonization of the bacteria into the crop. These factors sponsor the products demonstrative efficacy and reliability in the field.

Form of Nitrogen and Plant Efficiency -Amine and ammonium vs. Nitrate

Solid nitrogen fertilizers such as urea or animal manures have their nitrogen in the amine and ammonium form. However, once added to the soil, native & ubiquitous *nitrosomonas* and *nitrosococcus* bacteria quickly turn this form of N into nitrates in the process of nitrification. **Nitrate** is very mobile in the soil and is easily leached. (Only 50% of applied urea is actually used by the plant, the other 50% is lost, and sometimes more). Nitrate is also easily taken up by the plant and serves, in moderation, as a good source of N for protein production. However, nitrate nitrogen is so easily taken up by the plant and so it is easy to overload the plants ability to metabolize the compound into protein. Over supply leads to the production of shorter chain, incomplete protein compounds that weaken the plant. High leaf nitrates result in a watery, softer plant, much more prone to insect and disease attack and actually poisonous to animals, including humans (nitrate poisoning). Nitrate is also a well known and documented carcinogen.

Conversely, nitrogen supplied **by N fixing bacteria** is delivered to the plant directly in the **amine and ammonium** forms. The bacteria excrete nitrogen as urea, the same form that is contained in animal urine. In essence the bacteria are miniature bio-urea factories. It is

metabolically inexpensive for the plant to turn this bacterial form of N into plant amino acids and true proteins.
The result is a much healthier, more efficient and stronger plant.

Nitrogen Fixation – Now a Genuine Option for Nitrogen Management

Until the recent advent of Twin N™ onto the market, commercial free living nitrogen fixing bacteria inoculums have been very unreliable because of wide variations in CFU analysis, achieving excellent results in some situations, and no results in many other situations. As farmers can not rely on "a chance that it may work" approach, particularly with nitrogen applications, free living nitrogen fixing bacteria technology did not enter main stream farming as a viable alternative to other forms of N fertilizers. Twin N™ is quickly changing this world wide, and significantly reducing the cost of production where medium to high nitrogen applications are essential for crop yield.

TABLE 1 – Comparison of Nitrogen Products

	SYNTHETIC N	COMPOSTED ANIMAL MANURES	Twin N™ N FIXING BACTERIA
N efficiency to plant (stability in soil)	50%	65- 75%	100%
Reliability of N Supply	Good	Good	Good
Cost effectiveness for N supply	Medium	Low	High
Impact on soil health	Negative	Positive	Positive
Cost of application	Medium- high	High	Low
Loss of N through Leaching	High	Low	No issue
Long term Sustainability	Poor	Good	Very Good