March, 2018

Azospirillum brasilense

The rhizosphere is an intricate ecosystem full of diversity and life and is supported by numerous forms of chemical transformations. Its composition is essential to the many biological processes taking place and involving: soil, a complex element providing a physical environment, organic components (both live and decomposing organisms), inorganic matter, including vital nutrients, and water, the solvent and medium allowing all chemical reactions to take place (1). Among the many organisms inhabiting the rhizosphere, are the plant growth promoting rhizobacteria (PGPR), considered to be the most abundant microorganisms in the rhizosphere, with a range of metabolic diversity. *Azospirillum* is genus of free living PGPR, capable of affecting growth and yield of numerous plant species, many of agronomic and ecological significance (2). *Azospirillum brasilense* is a particular strain that has shown to induce consistent benefits to a plant's development and growth (3). Over the last 20 years crop yield has not increased proportionately with increasing fertilizer inputs, leading to an inefficient use of nutrients and an increase in environmental risks. It has been considered that plant-beneficial rhizobacteria may decrease the global dependence on hazardous agricultural chemicals which destabilize the agro-ecosystems (4). New focus has been placed on biologicals that can safely and sufficiently contribute to the acquisition of nutrient availability for the plant. In the last couple of decades, *A. brasilense* has become one of the most studied agronomic bacterial strains due to its numerous proposed mechanisms enhancing plant nutrition and growth, including: biological nitrogen fixation, production of important plant-growth regulatory phytohormones (indole3-acedic-acid (IAA), gibberellins, cytokinins, ethylene and inhibitors), osmotic stress response in plants, phosphate solubilization and extensive crop compatibility (5,6).

Nitrogen Fixation

Biological nitrogen fixation is an important source of nitrogen in agriculture and represents a promising substitute for nitrogen fertilizers (7). Nitrogen is one of the most indispensable nutrients required for plant growth, acting as a limiting nutrient for many important biological processes occurring in a cell, including: protein synthesis, cell division, signal transduction and nutrient transport. Nitrogen is the most abundant gas in the atmosphere making up about 80% of its composition; however, it exists in a form not available to plants. The atmospheric nitrogen must be converted to ammonia through the process of biological nitrogen fixation by nitrogen fixing organisms, such as bacteria. *A. brasilense* can convert atmospheric nitrogen into

ammonium under microaerobic conditions, at low nitrogen levels, increasing a plant's nitrogen intake (8). The effects of *A. brasilense* on inoculated plants have been vastly tested in cereal plants and grasses (3). In a particular study published in the *International Journal of Plant and Soil*, the effects of inoculating wheat with a highly efficient *A. brasilense* strain under three nitrogen regimes (0, 3 or 16 mM NO_3^-, 50 ml/pot once or twice-a -week), revealed that inoculation stimulated plant growth, as well as nitrogen and nitrate accumulation in the tissues. At maturity, inoculated plants showed higher biomass, grain yield, and nitrogen content than the non-inoculated plants as well as higher grain protein concentration. The study concluded that *A. brasilense* increased plant growth by stimulating nitrogen uptake by roots (9). Although, mechanisms are still not fully understood, extensive literature affirms the capabilities of nitrogen fixation by *A. brasilense* and its effects on plants (2,7,8,10,11).

Production of Plant Growth Regulatory Substances

A review published in the *Journal of Applied Microbial and Cell Physiology* assessing research conducted on *A. brasilense*, concluded some strains of *A. brasilense* have the capacity to promote plant growth through the production and release of various, important plant growth-promoting hormones: indole-3-acetic acid (IAA), gibberellins, cytokinins, ethylene, and inhibitors (5). These hormones are essential to plant growth and development and can increase growth rates and improve host plant yields (6). IAA is considered the most important phytohormone produced by *A. brasilense*, with significant effects to the plant, such as: root elongation, root proliferation, increased root development, and increased root surface area, all leading to an increase in the absorption of water and nutrients. The effects of elongation of primary roots and increased number and length of lateral roots in plants inoculated with the genus *Azospirillum*, including *A. brasilense*, have been shown to correlate with levels of IAA (12,13). Giberellins are highly studied phytohormones capable of inducing many chemical processes in a plant that contribute to: seed germination, seedling emergence, stem and leaf growth, floral induction, flower and fruit growth, promotion of root growth, and root hair abundance (14,15,16). Similarly to IAA, cytokinins influence both cell division and cell enlargement and also affect seed dormancy, flowering, fruiting and plant aging (17). Ethylene is a plant hormone which under normal conditions regulates many physiological processes, such as: seed germination, root hair development and root elongation, leaf and organ aging, and fruit ripening (18). Inhibitors are able to terminate metabolic processes occurring in a cell during times of stress, enabling a plant to go into dormancy and increase its chances of survival. Abscisic acid is one of the strong inhibitors of growth and germination and promotes seed

dormancy (19). It can be concluded that the combined production by *A. brasilense* of all these hormones, which have multiple and similar effects, has an overall favorable effect on plant growth and morphology.

Osmotic Stress Response in Plants

Plants can undergo osmotic stress due to a number of causes, including drought or adverse soil conditions, disrupting an array of chemical processes occurring inside a plant cell. One of the many benefits of *A. brasilense* is its ability to reduce osmotic stress in plants through the accumulation of large amounts of osmolytes in the presence of stress factors. Osmolytes are compounds affecting osmosis and can help a cell with water retention by preserving its volume and balance (6). Another proposed mechanism for reducing osmotic stress, is this bacterium's ability to morphologically change during adverse conditions. Naturally, *A. brasilense* occurs as a vibroid (rod-shaped), but under water-stressed conditions, it has the ability to change into a cyst-like form, improving its chances of survival. This assumed, enlarged spherical form develops an outer layer of polysaccharides, which may help in its resistance to desiccation. Additionally, it accumulates an abundance of poly-β-hydroxybutyric acid (PHB) granules, which are polymers assisting in energy storage and metabolism (20). When water-stressed conditions are eliminated, the bacterial cells revert back to vibroid form (6). Inoculation with *Azospirillum*, particularly *A. brasilense*, and its effects on improved plant growth under water-stressed conditions have been largely tested and demonstrated since the 1980s (6). Although exact mechanisms remain unclear, it is one of the many contributions this organism has on plant fitness and endurance.

Phosphate Solubilization

Phosphorus (P) is an important element of plant nutrition enabling important biological processes in plants including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and cellular respiration to take place (21). Although phosphate is readily available in soils, it exists in an unusable and inorganic form, requiring organisms to convert it into a form useable by the plant. This often leads to the addition of fertilizer additives that introduce phosphate in a soluble form. The implications of using these fertilizers such as high costs and negative environmental effects, create a focus on eco-friendly and more economically feasible alternatives. The use of microbial inoculants (bio-fertilizers) possessing P-solubilizing capabilities in agricultural soils is considered an environmentally friendly alternative to further applications of chemically based P fertilizers (22). Although mechanisms remain unclear, it has been concluded that *A. brasilense* is capable of phosphate solubilization (5). One proposed

mechanism is the ability of *A. brasilense* to produce and release siderophores. Siderophores are complexing agents (a compound able to form associations with a metal) that have a high affinity for iron and are produced by almost all microorganisms in response to iron deficiency. They act as solubilizing agents by enabling the transport of molecules across cell membranes. Biological phosphorus solubilization involves the release of biological compounds like siderophores, which enable the release of enzymes, which consequently enable the release of phosphorus from a substrate (typically a metal) and make it available for uptake by a plant (4). Run-off, ocean eutrophication, increasing costs of mining and transporting phosphorus, as well as concerns of its entity as a finite source, are reasons why alternative options to phosphate solubilization are so important and why increased focus leading to research and production of these phosphate solubilizing micro-organisms is of great importance.

Host Versatility

There has been extensive research and documentation focusing on the inoculation of *A. brasilense* in non-legumes (3); however, emerging research supports the assertion that this beneficial soil bacterium is not crop specific and can perform associations with a large range of hosts. It has been reported that *Azospirillum* strains have no preference for crop plants or weeds, or for annual or perennial plants, and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots (6). It can be concluded that bacteria belonging to the genus *Azospirillum,* which include the species *A. brasilense,* are general root colonizers and are not plant specific bacteria.

Our Company's Focus

Extensive genetic, biochemical and ecological studies have ranked *Azospirillium*, as one of the best characterized genera among associative plant growth promoting bacteria inhabiting the rhizosphere (8). Currently, Reforestation Technologies International (RTI) is a main supplier of *A. brasilense* inoculants in North America. Through much accumulated data, on-site testing, and development of our proprietary trademarked *A. brasilense* inoculants, Azos®, RTI has acquired a reputation as an innovator and leader in the biotechnology industry. Our proprietary formulations have focused on various elements to increase product efficacy as we continue to make substantial contributions to modern agriculture. Our continued research will allow for further manipulation of this and other PGPR to assist growers in their practices of sustainable agriculture in a world with a dramatically increasing population.

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