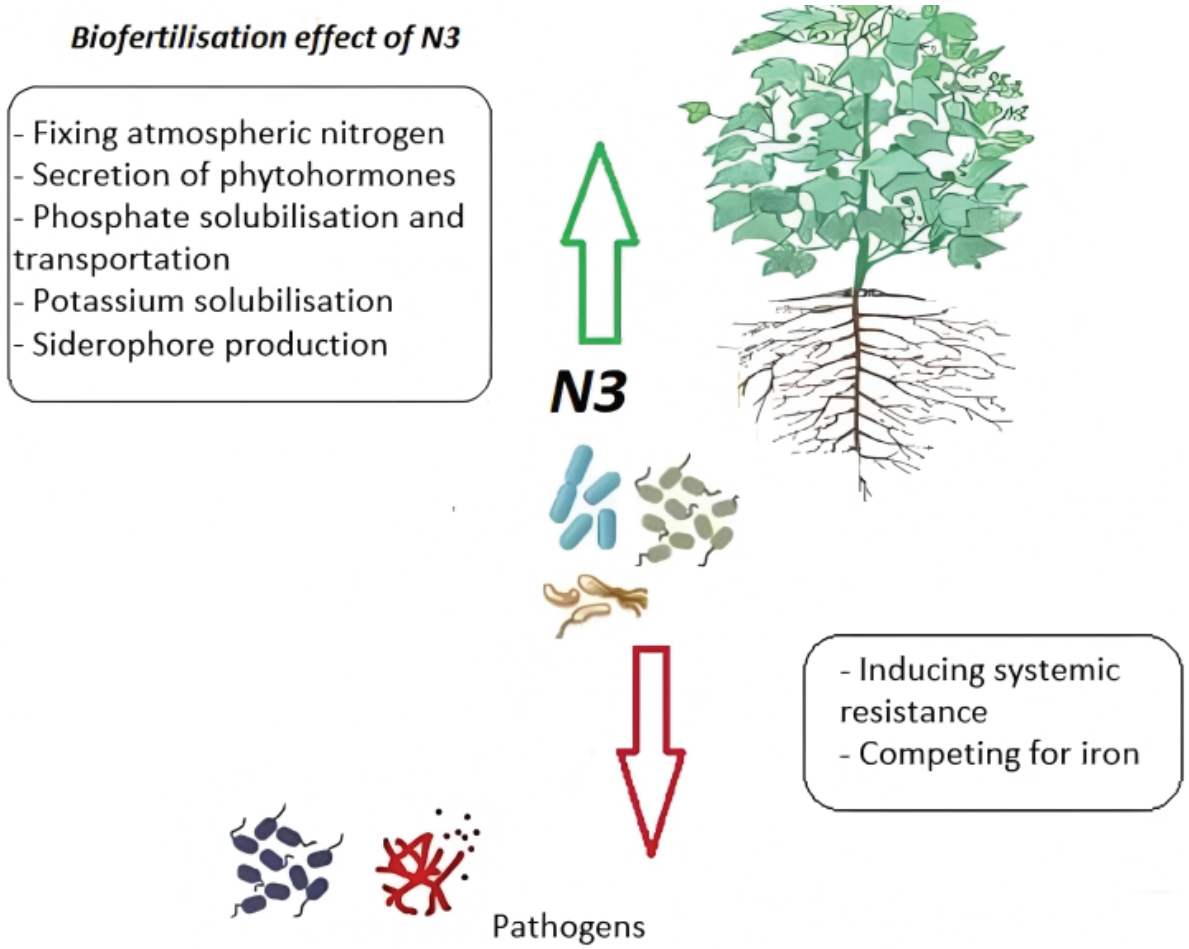
Mechanisms and Mode of Action – N3 Strains

N3 (brand name Agnyte or Enrich N3) contains plant-growth-promoting rhizobacteria (PGPR) that enhances plant growth and protects plants from disease and abiotic stresses (Souza et al. 2015). Its unique combination of PGPR—Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium —encourages the growth of a wide range of plant species (e.g., cotton, wheat, carrot, tomato and rice).

The principal intention of this literature review is to answer the most frequently asked questions about N3 in relation to its mode of action, pathogenicity, and the fate of its constituent bacteria after usage. N3 bacteria have been isolated from the soil of the United States of America (USA). N3 bacteria are neither mutants nor genetically modified organisms.



Schematic diagram of N3 mode of action on plants

Azotobacter chroococcum

Azotobacter chroococcum is a Gram negative, free-living, nitrogen fixing aerobic bacteria. Azotobacter chroococcum synthesizes auxins, cytokinins, gibberellic acid and siderophores like substances. These growth materials are the important substances regulating plants (Wani, 2016).

Azotobacter chroococcum can compete with soil fungi such as Fusarium, Pythium, and Rhizoctonia (Chauhan, 2012) and suppress their growth. It was proven that Azotobacter chroococcum improves plant resistance to higher salt conditions (Abdel Latef, 2021) and proven that foliar application of Azotobacter sp. can be used to promote plant growth and reduce nitrate accumulation in plants (Razmjooei, 2022).

The N3 constituent bacteria Azotobacter chroococcum strain was named TRIBAC AC1D. Please check the DNA sequence information for more details.

Taxonomy

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pseudomonadales
Family: Pseudomonadaceae
Genus: Azotobacter
Species: A. chroococcum

Toxicity

There is no evidence to suggest that Azatobacter chroococcum is pathogenic to humans, animals, or other non-target organisms. It is not known to cause diseases or infections in these organisms.

Environmental fate

Azotobacter chroococcum can be affected by environmental conditions, including temperature, pH, moisture, and the presence of other microorganisms. The persistence and survival of Azotobacter chroococcum in the environment will depend on a variety of factors, including the specific strain and the local environmental conditions (Aasfar 2021). Specific strains of Azotobacter chroococcum were approved to be used as a biofertilisers (Agri Life NITROFIX ™ – AC).

Azospirillum brasilense

Azospirillum brasilense is a gram gram-negative, rod-shaped, nitrogen-fixing bacterium found in the rhizosphere of various grass species and cereals, and is able to affect the growth and yield of numerous plant species (Bashan et al. 2004). Azospirillum brasilense can establish colonies on plants that have not been previously colonised (Arora 2013), making it a perfect choice to be a component of N3.

Azospirillum strains have been reported to increase plant growth by secreting various metabolites into the rhizosphere (Rodrigues et al. 2015) like phytohormones (principally auxins, gibberellins (GAs), cytokinins (CKs), nitric oxide, fix atmospheric nitrogen, polyamines), which encourages plant growth (Bashan and de-Bashan 2010).

Morphological plant-root changes such as increased root growth and/or enhanced formation of lateral roots and root hairs have been observed on Azospirillum spp. inoculation (Dimkpa et al. 2009). The presence of Azospirillum spp. in the rhizosphere has been reported to activate the hydrolysis of conjugated phytohormones and flavonoids in the root tissue, thus bringing about the release of compounds in their active forms (Saikia et al. 2010).

It was shown that Azospirillum on seeds or by leaf spray, as well as leaf spraying of Azospirillum metabolites, are strongly correlated with phytohormone production and stress tolerance gene activation (Fukami, 2017).

The N3 constituent bacteria Azospirillum brasilense strain was named TRIBAC AB1D. Please check the DNA sequence information for more details.

Taxonomy

Domain: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rhizobiales
Family: Rhizobiaceae
Genus: Azospirillum
Species: Azospirillum brasilense

Toxicity

Azospirillum spp. have been considered a low-risk microbe with no or low risk of adverse human health effects. Azospirillum spp. have been used in human food (production systems) and research applications (e.g., wheat, corn, rice, alfalfa, legumes) for many years, which means it can be deduced these organisms are safe and provide minimal risk to human health. Many biological products from Azospirillum spp. have been internationally registered as organic products by organisations such as the US Organic Materials Review Institute (OMRI 2017).

Environmental fate

Azospirillum brasilense has not been found to cause ecological damage or plant disease (Bashan 1998). The presence of Azospirillum brasilense in the rhizosphere can minimise the susceptibility of plants to diseases caused by plant pathogens (Rodrigues et al. 2015). Azospirillum brasilense is deemed to be safe to use in agricultural practice.

Bacillus megaterium

Bacillus megaterium is a gram-positive, rod-shaped bacterium that is found in soil and other environments. It can fix nitrogen from the air, making it available to plants as a nutrient (Ding, 2005) and used in phosphate fixing and potassium-fixing fertilizers (Han, 2006). The principal mechanism of phosphate solubilization by Bacillus megaterium is by producing organic acids and acid phosphatases (Shankar, 2013). It was shown that the application of Bacillus megaterium enhanced bioavailability of phosphorus and potassium in plants (Zhao, 2021).

The N3 constituent bacteria Bacillus megaterium strain was named TRIBAC PM2D. Please check the DNA sequence information for more details.

Taxonomy

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: Bacillus megaterium

Toxicity

It is generally considered to be non-toxic to humans and animals, and it is not known to be a human pathogen. It is known to produce a variety of enzymes and other compounds that can be used industrially, including proteases, amylases, and lipases. These enzymes are used in a variety of applications, including the production of detergents, food and feed additives, and pharmaceuticals (Vary, 2007). Bacillus megaterium is not known to be a significant environmental contaminant, and it is not listed as a hazardous substance by regulatory agencies.

Environmental fate

The fate of Bacillus megaterium in the environment depends on a variety of factors, including the presence of suitable nutrients, the availability of water, and the presence of other microorganisms. In general, Bacillus megaterium is able to thrive in a variety of environments and can survive for extended periods of time (Goswami, 2018).

References:

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., & Meftah Kadmiri, I. (2021). Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. Frontiers in microbiology, 12, 628379.

Abdel Latef, A. A. H., Omer, A. M., Badawy, A. A., Osman, M. S., & Ragaey, M. M. (2021). Strategy of salt tolerance and interactive impact of

Azotobacter chroococcum and/or Alcaligenes faecalis inoculation on canola (Brassica napus L.) plants grown in saline soil. Plants, 10(1), 110.

Agri life, biosolutions. Agri Life NITROFIX TM-AC Azotobacter chroococcum BioFertilizer APPROVED FOR USE IN ORGANIC AGRICULTURE (Regd in Fertilizer Control Order)

Arora, N. K., Tewari, S., & Singh, R. (2013). Multifaceted plant-associated microbes and their mechanisms diminish the concept of direct and indirect PGPRs. In Plant microbe symbiosis: Fundamentals and advances (pp. 411449). Springer, New Delhi.

Bashan, Y. (1998). Azospirillum plant growth-promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat. Canadian Journal of Microbiology, 44(2), 168-174.

Bashan, Y., Holguin, G., & De-Bashan, L. E. (2004). Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Canadian journal of microbiology, 50(8), 521-577.

Bashan, Y., & De-Bashan, L. E. (2010). How the plant growth-promoting bacterium Azospirillum promotes plant growth—a critical assessment. Advances in agronomy, 108, 77-136.

Ding, Y., Wang, J., Liu, Y., & Chen, S. (2005). Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. Journal of applied microbiology, 99(5), 1271-1281.

Dimkpa, C., Weinand, T., & Asch, F. (2009). Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant, cell & environment, 32(12), 16821694.

Fukami, J., Ollero, F. J., Megías, M., & Hungria, M. (2017). Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with Azospirillum brasilense cells and metabolites promote maize growth. AMB Express, 7(1), 153. https://doi.org/10.1186/s13568-017-0453-7

Goswami, G., Panda, D., Samanta, R., Boro, R. C., Modi, M. K., Bujarbaruah, K. M., & Barooah, M. (2018). Bacillus megaterium adapts to acid stress condition through a network of genes: Insight from a genome-wide transcriptome analysis. Scientific reports, 8(1), 1-12.

Han, H. S., & Lee, K. D. (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant soil and Environment, 52(3), 130.

Saikia, S. P., Dutta, S. P., Goswami, A., Bhau, B. S., & Kanjilal, P. B. (2010). Role of Azospirillum in the Improvement of Legumes. Microbes for legume improvement, 389-408.

Shankar, T., Sivakumar, T., Asha, G., Sankaralingam, S., & Sundaram, V. M. (2013). Effect of PSB on growth and development of chilli and maize plants. World Appl. Sci. J, 26(5), 610-617.

Souza, R. D., Ambrosini, A., & Passaglia, L. M. (2015). Plant growthpromoting bacteria as inoculants in agricultural soils. Genetics and molecular biology, 38, 401-419.

Vary, P. S., Biedendieck, R., Fuerch, T., Meinhardt, F., Rohde, M., Deckwer, W. D., & Jahn, D. (2007). Bacillus megaterium—from simple soil bacterium to industrial protein production host. Applied microbiology and biotechnology, 76(5), 957-967.

Wani, S. A., Chand, S., Wani, M. A., Ramzan, M., & Hakeem, K. R. (2016). Azotobacter chroococcum–a potential biofertilizer in agriculture: an overview. Soil science: agricultural and environmental prospectives, 333-348.

Zhao, Y., Mao, X., Zhang, M., Yang, W., Di, H. J., Ma, L., & Li, B. (2021). The application of Bacillus Megaterium alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. Agriculture, Ecosystems & Environment, 307, 107236.