Nitrogen-fixing Bacteria and their Applications in the Environment: A Review

Sawsan M Alomari [1], Rana S Hasan [2], Alaa Nazar Al-Najim [3], Ali M Saadi [4*]

[1, 2] Medical Laboratory Techniques Department / Mosul Medical Technical Institute / Northern Technical University, Mosul, Iraq

[3] Optometry Techniques Department Department / Mosul Medical Technical Institute / Northern Technical University, Mosul, Iraq

[4] Department of Medicinal Plant Technologies, Technical Agricultural College, Northern Technical University, Iraq

* Corresponding Author: **Ali M Saadi**

Article Info

ISSN (online): 2582-8940
Volume: 05
Issue: 04
October-December 2024
Received: 20-10-2024
Accepted: 23-11-2024
Page No: 178-187

Abstract

The process of converting nitrogen into ammonia in the form of a compound is referred to as biological nitrogen fixation. This process is accomplished by various types of nitrogen-fixing bacteria. In this process, atmospheric dinitrogen is enzymatically converted into ammonia by nitrogen-fixing microorganisms. These nitrogen-fixing bacteria are broadly classified into two groups: symbiotic nitrogen-fixing bacteria and non-symbiotic nitrogen-fixing bacteria. Within the microbial cells, ammonia is used for the maintenance and development of the bacterial cells. These microorganisms produce several enzymes, proteins, and cofactors that are essential for the functioning of the key enzyme nitrogenase. This enzyme is capable of reducing inert nitrogen molecules to ammonia. The entire process involves the electron transport chain within the bacterial cell. Two of the many diverse genera of rhizobia, Rhizobium and Bradyrhizobium, are α-proteobacterial diazottrophic families that form root nodules through the interaction of symbiotic genes. The nitrogen-fixing symbionts of actinophytes are symbiotic rhizobia of the genera Mesorhizobium, Sinorhizobium and Bradyrhizobium, such as Azorhizobium. The genera Frankia and Discorrhizobium provide drought-resistant forage roots in non-legume actinophytes such as alder and myrica woodlands. Frankia actinophytes have the ability to live as sugar and cellulose decomposers in addition to being symbiotic with actinophytes. Integrating nitrogen-fixing bacteria into integrated disease management can lead to significant plant growth. Research involving the application of biofertilizers in a variety of crops is at an interactive level. Nitrogen-fixing bacteria can fix nitrogen from the air into the root zone under both free and bonded nitrogen fixation. In some cases, co-inoculation of mycorrhizal spores together with nitrogen-fixing microorganisms and Azospirillum strains has shown improvement in plant health. Nitrogen-fixing bacteria can improve the structure and fertility of soils that have been degraded for various reasons. Therefore, nitrogen-fixing bacteria have the potential to be used in environmental remediation processes to treat wastewater, human and animal waste, or overcome other disinfection caused by industrial pollutants. In environmental nitrogen cycles, the application of nitrogen-fixing bacteria can provide opportunities for the accumulation and preservation of organic matter through different particle sizes. However, it is necessary to identify nitrogen-fixing bacteria endemic to different ecosystems and study the potential biochemical cycles of nitrogen in different sized mineral components. In addition, the efficiency rates of chemical remediation should be compared due to potential problems with the development of concentrations of these microorganisms in environmental restoration or bioremediation processes over large areas. There is hope for the positive development of strategies to eliminate harmful metals in soil and restore potential management profits under the green economy policy, with the preferential use of extreme organisms living within soil elements, which will contribute to different strategies for the use of nitrogen fertilizers in agriculture.

DOI: https://doi.org/10.54660/IJMBHR.2024.5.4.178-187

Keywords: Nitrogen-fixing Bacteria, Environment, Biofertilizer

Introduction

Nitrogen-fixing bacteria, a group of heterotrophic and autotrophic prokaryotes, have the ability to transform nitrogen gas in the atmosphere to form ammonia through the process of nitrogen fixation. Since at least about 10%–20% of farmland has been limited by nitrogen deficiency, the isolation and inoculation of nitrogen-fixing bacteria are becoming more frequently used, and the potential of nitrogen-fixing bacteria for eliminating nitrogen limitation from crops, increasing soil fertility, increasing agricultural production, and promoting technological transformations, particularly in sustainable agriculture, is drawing increased attention (Barney, 2020) [9] (Minamisawa) (Chamoli *et al.*, 2024) [13]. However, abundant nitrogen fertilization due to human activities has increased reactive nitrogen levels in soils, water, and the atmosphere, leading to a series of ecological and environmental problems, such as leaching of nitrate to groundwater and production of nitrogen oxides, so nitrogen fertilizers and pesticide nitrification inhibitors need to be considered seriously (Mason *et al.* 2022) [60] (Liu *et al.*, 2021) [53].

As some soil and plant root nitrogen-fixing bacteria, especially nitrogen-fixer-involved roseobacters under Rhodobacterales and rhizobia under Alphaproteobacteria, are important representatives with respect to nitrogen fixation in the environment, we conducted an intensive review analyzing the functions of these nitrogen-fixing bacteria and further elaborated the utilization of these bacteria on the soil nitrogen cycle and plant growth. Our objective is to help the reader better understand the potential environmental function of these bacteria. We conclude by discussing the application prospects of other special nitrogen-fixing bacteria currently receiving attention and summarizing the research progress and utilization in the context of nitrogen-fixation products. (Zhang *et al.* 2021) [98] (Liu *et al.* 2023) [74] (Kim *et al.* 2024) [39].

2. Nitrogen Fixation Process

Nitrogen is a vital compound for the growth and development of living organisms, but due to its relatively inert triple bond, atmospheric nitrogen cannot be utilized by plants and animals. Nitrogen needs to be converted into a usable form by living organisms. This process is completed by two types of organisms: one is biological nitrogen fixation via nitrogen-fixing bacteria, and the other is industrial nitrogen fixation. Biological nitrogen fixation is vital for maintaining the nitrogen pool in the environment. (Zehr & Capone, 2020) [95] (Kebede, 2021) [37] (Ladha *et al.* 2022) [45]

Approximately 80% of the total nitrogen in the Earth's atmosphere is converted through the biological nitrogen fixation process. The process of converting nitrogen into ammonia in the form of a compound is referred to as the biological nitrogen fixation process. This process is attained by various types of nitrogen-fixing bacteria. In this process, atmospheric dinitrogen is enzymatically converted into ammonia by nitrogen-fixing microorganisms. These nitrogen-fixing bacteria are broadly classified into two groups: symbiotic nitrogen-fixing bacteria and nonsymbiotic nitrogen-fixing bacteria. Inside the microbial cells, ammonia is used for the maintenance and development of the bacterial cells. (Soumare *et al.* 2020) [84] (Zehr & Capone, 2020) [96] (Bellenger *et al.*, 2020) [10, 11]

The mechanism by which the nitrogen fixation process is carried out in nitrogen-fixing prokaryotic microorganisms is described in this section. These microorganisms produce many enzymes, proteins, and co-factors essential for the functioning of the major enzyme nitrogenase. This enzyme is able to reduce inert nitrogen molecules into ammonia. The whole process involves an electron transport chain inside the bacterial cell (Bellenger *et al.*, 2020) [10, 11] (Gu & Milton, 2020) [30] (Lawal 2021) [47]. The nitrogenase enzyme has encoded genes in its genome, and for the enzyme to express, environmental conditions play a vital role. The different ecosystems and their environmental conditions that play a crucial role in enzyme expression are discussed. These types of nitrogen-fixing microorganisms have potential applications in agriculture, horticulture, and floriculture for crop productivity. The impact and importance of nitrogen-fixing microorganisms in maintaining natural ecosystems and plants in the biosphere are also discussed. Since the introduction of nitrogenase progenitor enzymes, it provides evidence for the trades of gene consciousness (Koirala & Brözel, 2021) (Ghebreamlak & Mansoorabadi, 2020) [24] (Naorem *et al.* 2022) [64].

2. 1. Biological Nitrogen Fixation

The ability to convert atmospheric nitrogen to biologically available ammonia or ammonium under ambient conditions is called "nitrogen fixation." Since the atmosphere consists of 78% nitrogen, nitrogen is also one of the most abundant elements on Earth. However, its subsequent difficulty in biological use caused limited ecosystem productivity and posed problems when initial life forms on the primitive Earth had to assimilate inorganic forms of carbon and nitrogen. The microorganisms that fix nitrogen have developed a complex nitrogenase enzyme system that can reduce nitrogen into many bioavailable compounds under normal temperature, pressure, and pH (Soumare *et al.* 2020) [84] (Bellenger *et al.*, 2020) [10, 11] (Child, 2021) [15]. Fossil records indicate that nitrogen-fixing bacteria have been associating with plants for about 1. 4 billion years. As a result, a highly successful natural relationship has evolved between such soil microorganisms and the plant life they facilitate. The diverse biological adaptations of nitrogen-fixing microorganisms and alteration of their genetic compatibilities enable fixers to maintain dominance in many habitats (Mohr *et al.* 2021) [62] (Libourel *et al.* 2022) [50] (Gou *et al.* 2023) [26].

The best-studied association is that between members of the legume family and Rhizobium or Bradyrhizobium species that induce root nodule formation. In return, plant roots provide shelter and a steady supply of "fixed carbon compounds" which are synthesized by the plants themselves through photosynthesis. About 90% of the world's biological nitrogen fixation results from the symbiotic activities of approximately 180 species of rhizobia (Soumare *et al.* 2020) [84] (Goyal *et al.*, 2021) [27, 28] (Ladha *et al.* 2022) [45]. The free-living nitrogen-fixing bacteria include species of Azotobacter, Beijerinckia, and Clostridium. Modern agriculture abounds with leguminous plants contributing to soil fertility. For several cultures, pulses, lentils, guar, lucerne, clover, and vetch are important sources of proteins, oils, and important minerals. The value of nitrogen that they annually introduce into the system is extremely important (Ambechada & Umrania, 2024) [3] (Aasfar *et al.* 2021) [1] (Mahmud *et al.*, 2020) [58].

2. 2. Mechanisms of Nitrogen Fixation

A complex biochemical process capable of converting nitrogen gas to ammonia is known as nitrogen fixation. The enzyme complex responsible for nitrogen reduction is nitrogenase, which in symbiotic nitrogen-fixing bacteria is found in the infection nodules. Nitrogen gas is reduced by nitrogenase to two ammonia molecules and one hydrogen molecule. The reduction process of nitrogen to ammonia requires four reducing equivalents and six ATP, the most expensive known biological reduction reaction in terms of energy. (Barney, 2020) [9] (Minamisawa) (Bellenger *et al.*, 2020) [10, 11]

Three major mechanisms are used by nitrogen-fixing bacteria to supply the large amount of energy and reducing equivalents required for the reduction of N2 to ammonia: nitrogenase systems in the presence of high-energy substrates; a route for electron transport from free-living nitrogenase to the oxygen-tolerant terminal oxidase; respiratory protection of nitrogenase systems by coupling the fermentation system between the two oxygen-sensitive enzymes that play a major role during oxygen-limited conditions. The overall process, which includes the assimilation of nitrogen, is known as biological nitrogen fixation. Ammonia formed from N2 through nitrogen fixation can be metabolized to generate amino acids and other biologically necessary nitrogen compounds through four metabolic pathways that lead to organic nitrogen. (Soumare *et al.* 2020) [84] (Kebede, 2021) [37] (Bellenger *et al.*, 2020) [10, 11]

Several environmental and biological factors influence nitrogen fixation in terms of efficiency. For instance, within the broad temperature range between 5 to 40 °C, optimal N2-reducing nitrogenase levels are reported under low-temperature conditions. The availability of divalent transition metals must be maintained for maximum nitrogenase activity in nitrogen-fixing bacterial systems. A significant characteristic of nitrogen-fixing prokaryotes in symbiotic association with host organisms is their ability to assimilate nitrogen, which is reduced in the form of ammonia. (Soumare *et al.* 2020) [84] (Lindström & Mousavi, 2020) [51] (Kebede, 2021) [37]

3. Types of Nitrogen-fixing Bacteria

Nitrogen-fixing microorganisms can be classified as either free-living or symbiotic bacteria based on their ecological niches. Free-living nitrogen-fixing bacteria are independent and can fix nitrogen on their own. These include aerobic N2-fixing filamentous cyanobacteria and the well-known oxygen-sensitive N2-fixing bacteria that exist in anaerobic systems such as cyanobacteria, green sulfur bacteria, purple bacteria, and methanogens (Khan *et al.* 2021) [17] (Panda *et al.*, 2021) [73] (Liang *et al.*, 2022) [4]. Symbiotic bacteria live in close relationships and form mutualism with plants. They have a wide range of hosts as they are widely distributed in legumes, actinorhizae, casuarina, wild sugarcane, Pandanus, non-legumes, and other plants. Nitrogen-fixing bacteria play a unique role in establishing a good ecological relationship with the dominant plants. By secreting nitrogenase and leghemoglobin in the nodules, the plants can absorb and perform enzyme fixation so that the atmospheric nitrogen can be converted into ammonium nitrogen that plants can use. Biological nitrogenase reduces N2 and converts it into ammonia (NH3), which is used for nutrient nitrogen fixation in plants (Mahmud *et al.*, 2020) [58] (Liu-Xu *et al.* 2024) [55] (Huang, 2024) [33].

During plant growth, legumes and actinorhizae excrete hydroxy acids, amino acids, and organic acids, which not only provide a nutrient source for Rhizobium and Frankia vesicles but also contribute to the tricarboxylic acid and glycolysis pathways. In the mid-1950s, researchers found that at least ten species of non-leguminous plants could establish N2-fixing nodules, and dozens of non-legumes established N2-fixing nodules in nature together with ARA Pseudomonas. Most of these are found in the Casuarinaceae family, where the microorganisms used are Frankia (Dheeman and Maheshwari 2022) [16, 77] (Etesami and Jeong 2022) [21] (Naqqash *et al.* 2024) [65]. So far, in addition to the above-mentioned families, some families such as Cucurbitaceae and Myricaceae have been found to fix nitrogen in the roots of their own plants. The second situation is that the operators of nitrogen-fixing systems only contain heterogeneous nodules. In the environment, the number and distribution of nitrogen-fixing systems are related to factors such as sampling methods, ecological experts, regions, host species, plant types, and plant enumeration. Some reports believe that nitrogen-fixing systems should not be included in the host plants, but because they are an important biological component, some experts count them separately from the host plants. Nitrogen-fixing systems play a unique role in maintaining the survival and improvement of some host plants. Nitrogen-fixing nitrogen fixation in natural ecosystems is effective in enriching the soil organic matter and maintaining the stability of an ecosystem (White *et al.* 2021) [90] (Zeng *et al.*, 2024) [97] (Aoyagi *et al.*, 2022) [4].

Free-living N2-fixing bacteria have pigments and can perform photosynthesis due to the existence of chromophores. Moreover, free-living N2-fixing bacteria have ribulose bisphosphate carboxylase. Ribulose-1,5-bisphosphate can be decarboxylated by carboxylic acid to produce 2-PGA, and N2-fixing bacteria have other unique carbon-fixing enzymes. Many researchers have found that some free-living N2-fixing bacteria containing chlorophylls are spatially mobile in the presence of light, while sedimentation occurs in the absence of light (Jacob-Lopes *et al.*, 2020) [34] (Eckardt *et al.* 2024) [19] (Krauspe *et al.* 2021) [42]. Actively metabolizing chromatic effects are relatively less responsive to chemical activity. Hemerythrins that are nodule bacteria also contain few hemerythrins in their bacteriochlorophylls. These nodule bacteria with rhodochlorinase can induce the formation of pink nodules on the roots of their host plants. The pink nodules are dull and are not self-luminous at night; chlorophyll fluorescence is not observed. ARA Rhizobia in any phylogenetic division can withstand dryness and ultraviolet irradiation because they form spores and other stress-resistant tissues. Nitrogenase media can be promoted by ultraviolet light, but the frequency of auxiliary extra-symbionts forming nodules is greatly reduced (Ferrando Molina, 2021) [22] (Krishna, 2024) [43] (Velip, 2022) [87].

3. 1. Free-living Bacteria

Some bacteria have the capability to fix atmospheric nitrogen in aerobic conditions and can live freely in the environment. These free-living N-fixers pass their entire life cycle in the absence of plants, meaning they do not require association with host plants for survival. Several free-living N-fixers have been identified, which can grow and fix nitrogen in both plant root-mediated and non-root-mediated conditions (Pang *et al.* 2023) [74] (Yan *et al.* 2022) [93]. Genera like Azotobacter and Clostridium are well-studied groups of free N-fixers. Adaptation of Free-living Bacteria: These soil microorganisms are free-living autotrophs in some cases and hence adapt easily to various environmental conditions. They can live in aerobic decomposing soil rich in organic matter, denitrifying anaerobic conditions, organic and inorganic heavy-metal contaminated sites, and fresh and saltwater bodies. Ecological Importance of Free-living N-fixing Bacteria: Free-living N-fixers contribute to soil fertility and often significantly enhance crop production. These free N-fixers may deplete soil nitrogen reserves rapidly; that is why they are usually applied with a quick catch crop or with some nitrogen-containing fertilizers. They may also work as scavengers and maintain a small quantity of H2 in free-living environments at many new sites of H2-oxidizing chemotrophy (TOLANGI, 2022) [86] (Grover *et al.* 2023) [29] (Malik *et al.*, 2020) [59]. Nitrogen Assimilation: Free-living bacteria assimilate NH4+ in such forms from the soil. In some bacteria, the enzyme glutamate dehydrogenase can transfer nitrogen from glutamate to glucose in the TCA cycle. Such microbes are aerobes and require oxygen and high redox potential for N2 fixation. Future Prospects and Constraints/Limitations: Therefore, many free-living N-fixing organisms with potential use in biofertilizers are still to be discovered. Free-living N-fixers may operate in the soil environment under very different circumstances. These soil bacteria face competition with other free-living bacteria and organisms like protozoa. Although the ability of some N-fixers to adapt in such an environment may be an advantage, competition with other N-fixers would restrict available niches. One of the theoretical consequences of such adaptations could be extinction. There is sufficient evidence to suggest that a significant N-fertilization effect can be obtained over a range of agronomic systems using N-fixers (Zhu *et al.* 2023) [100] (Ojija and Aloo 2023) [68] (Arya *et al*) [6].

3. 2. Symbiotic Bacteria

Symbiotic nitrogen-fixing bacteria are in close relationship with host plants. Developing within the host, the parasitic nature of the diazotrophic bacteria is minimal. The associations of nitrogen-fixing bacteria with their host plants are ancient and species-specific. The evolution of host plants is considered to be an important factor affecting the evolution of bacterial symbiotic genes. The associations between legume plants and soil bacteria that nodulate in the roots of host plants to form red-pigmented root nodules contain nitrogen-fixing bacteroids in the plant cells and specialized structures for nitrogen fixation called nodules to breathe in air. (Kawaka, 2022) [36] (Elhady *et al.*, 2020) [20] (Sharma *et al.* 2023) [80].

Two of the many diverse genera of rhizobia, Rhizobium and Bradyrhizobium, are α-proteobacterial diazotrophic families that form root nodules by the interaction of symbiotic genes. Nitrogen-fixing nodule symbionts in the actinorhizal plant are symbiotic genera of rhizobia: Mesorhizobium, Sinorhizobium, and Bradyrhizobium, such as Azorhizobium. Frankia and Discorrhizobium genera introduce drought-tolerant forage roots in non-legume actinorhizal plants such as alder and myrica forest. An actinorhizal Frankia has the ability to live as a saccharide and cellulose decomposer in addition to being symbiotic with actinorhizal plants (Barabi, 2022) [8] (Rajkumari *et al.* 2022) [77] (Xavier *et al.* 2023) [92].

Bacteria that have symbiotic genes have molecular plant-specific nodulation factors. The formed nodule may be divided into a-celled nodules containing non-functional bacteroids in actinorhizal plants, some tropical legumes and further eudem as legumes. Elaeagnus rich tribes, Fabaceae, and Byrsonimoideae make a multilayered nodule envelope and lethal cells in the initial part of the nodule (Singh & Verma, 2023) [82] (Goyal *et al.*, 2021) [27, 28] (Kovács *et al.* 2022) [41]. A portion of the nodules in Rhizobium-legume housings are at least 27 plant families from Papilionoideae, including Fabaceae and other suborders. Many important grain legumes, including soybean, peanut, chickpea, bean, lentil, spotted vetch, and other plants, infect common bacteria that can increase soil fertility by symbiotic nitrogen fixation. The use of symbiotic transformation technology is to increase the use of host plants to decrease the amount of commercial nitrogen fertilizer needed to maximize yields of grain legumes, vegetables, and green manure. The soft type of peat that produces only 1,000 volumes of perennial farm crops is not due to mutations; if not, the reality will completely cover, and bacteria will only leave stalked colony spots to recover (Yuvaraj *et al.* 2020) [94] (Huang, 2024) [33] (Kaschuk *et al.* 2022) [35].

4. Applications in Agriculture

In the previous section, the role of nitrogen-fixing bacteria as producers of growth-promoting hormones and epidemic-controlling substances was described. This section describes the application of nitrogen-fixing bacteria in agricultural practices. The prime role of these bacteria lies in improving soil fertility along with decreased dependence on chemical nitrogen fertilizers. There is an increasing demand for organic production of agricultural produce (Aasfar *et al.* 2021) [1] (Pahalvi *et al.* 2021) [71] (Lan *et al.* 2023) [46]. The use of biofertilizers, which contain a large variety of nitrogen-fixing bacteria, such as Azotobacter spp., Azospirillum spp., Bacillus spp., Acetobacter spp., and Clostridium spp., along with other bacterial strains with phosphate solubilizing, potassium releasing, and zinc solubilizing potential, can be seen as a substitute for chemical fertilizers. The incorporation of nitrogen-fixing bacteria into integrated disease management can lead to significant growth of plants. The research involving the application of biofertilizers in a variety of crops is at a reactive level. The nitrogen-fixing bacteria can fix nitrogen from the air to the rhizosphere under free-living as well as associative nitrogen fixation. In some cases, the co-inoculation of mycorrhizal spores along with nitrogen-fixing microorganisms and Azospirillum strains also showed improvement in plant health. These adapted cultures, through mineralization, decomposition, and nitrification, fixate the gaseous inorganic nitrogen to nitrogen compounds in the soil, making it much more fertile (Zhang *et al.*, 2021) [98] (Arsita *et al.* 2020) (Mahmud *et al.*, 2020) [58]. However, the production of the Rhizobium mass culture cannot achieve breakeven points for a long time, and the cost of production is not very compatible. Biological nitrogen fixation does contribute to plant yield and sustainable agriculture. Therefore, to overcome the problems, it is required to select inexpensive nitrogen-fixing bacteria that are not affected by these issues. Currently, forage and fodder legumes, grain legumes, pulse crops, sugar crops, and some oil crops are included for the utilization of biofertilizers. Excess nitrogen is also toxic to the crops. Therefore, farmers are always advised to use biofertilizers rich in these associative symbiotic and free-living bacteria. In addition, sparingly applied chemical fertilizers can also be used. Only then can the heavy outputs of the plant products be achieved (Soumare *et al.* 2020) [84] (Kebede, 2021) [37] (Palmero *et al.* 2022) [72].

4. 1. Biofertilizers

Biofertilizers containing nitrogen-fixing microorganisms such as Rhizobia, Azospirillum, Azotobacter, and Bacillus, in addition to phosphate-solubilizing bacteria, are useful in agricultural systems. They may contain compatible strains of effective nitrogen-fixing bacteria as multi-strain formulations or a single potent strain. The synergy of enriched bacterial mixtures is generally acknowledged to improve growth responses in practical settings. Mixed bioinoculants are more efficient than single bioinoculant strains, likely due to their complementary strains and versatile functions. The application of a considerable quantity of multi-bacterial formulations can lead to a more fully functioning rhizosphere. (Kumaar *et al.* 2020) [42] (Soumare *et al.* 2020) [84] (Aasfar *et al.* 2021) [1]

There are numerous advantages to using biofertilizers, including the ability to improve soil health, increase crop yield, and decrease environmental pollution by chemical fertilizers. Biofertilizers are delivered directly to grains as seed inoculants in agriculture, especially for leguminous plants due to a well-documented plant-microbe symbiotic association, which is widely applied as biofertilizers in legumes. Biofertilizers offer a simple promise and are already useful in agriculture due to the additional benefit of free-living nitrogen fixers. Various application methods for effective nitrogen-fixing biofertilizers have been reported for ginger, cassava, and other non-legume crops, such as soil application (Chaudhary *et al.* 2022) (Murugaragavan Ramasamy & Yuvaraj, 2020) (Mahmud *et al.* 2021) [57]. In a field experiment on hard red winter wheat, exposure to the nitrogen-fixing bacterium Azospirillum elevated grain yields. The nitrogen-fixing bacterium Azospirillum is a soil-dwelling, nitrogen-fixing bacterium that has been widely researched for its potential to improve plant growth and yield as a biofertilizer. In addition, it has been found to help increase the zinc, potassium, and sulfur content of the grains. Few studies have compared the use of biofertilizer-enhanced single strains to chemical fertilizers to determine if they are more efficient than chemical fertilizers. The most important limitation when utilizing non-sterile inoculants for biofertilizers is the capacity to accumulate unfavorable or ineffective strains that can influence the reactions on various crops. The effective formulation and self-storage of biofertilizers are more complex, and quality control is a barrier in the established world. The Rhizobium inoculant's use in legume crops in soil, such as nitrogen-fixing properties, is classified broadly for nitrogen strains: mixed biofertilizers containing environmental indigenous strains and introduced strains are described (Dolkhani *et al.* 2022) [18] (Raffi and Charyulu 2021) [76] (Płaza *et al.* 2022) [75].

4. 2. Crop Improvement

Recent advances in our understanding of PGPR have led to the development of advanced technologies resulting in rapid crop improvement as focused by the fundamental findings. In contrast to the application of free-living nitrogen-fixing bacteria in the soil, the nitrogen-fixing bacteria putative symbionts related to the specific crop plant genotype have been studied extensively to improve crop productivity through an increase in plant nitrogen nutrition (Aasfar *et al.* 2021) [1] (Mahmud *et al.*, 2020) [58] (Din *et al.* 2021) [17]. Recent notable advances in our understanding of the benefits of plant-virgin rhizobial, plant-AMF, and nitrogen-fixing bacteria related to the specific plant variety have enabled the successful enhancement and utilization of these microbes to improve fertilizer P use efficiency and to fix N in a variety of crop plant species to different extents (Tang *et al.*, 2020) [85] (Aasfar *et al.* 2021) [1]. To date, soil selection is one major method to select compatible phosphorus-solubilizing micronutrient-releasing nitrogen-fixing bacteria for inoculating crop plants. In addition, most research efforts have focused on the fundamental molecular, ecological, and biotechnological research in rhizobial and AMF relationships to develop genetically modified crops that can form efficient, benign AMF and N2-fixing nodule symbioses with the addition of economical and ecological sustainability. At present, several field investigations and empirical reports involving experimental fields would empirically validate the efficacy, effectiveness, and potential applications of rhizobial, AMF, and N2-fixing bacteria in various crops (Sepp *et al.* 2023) [78] (Shi *et al.*, 2023) [81] (Lipa & Janczarek, 2020) [52].

The utilization of environmentally hardy N2-fixing bacterial strains helps address water and nutrient stresses for improving plant crops such as chickpeas, lentils, sabza, acacia plants, Pongamia, and other agroforestry tree plant species in the revised climate and environment. Ultimately, these crops also have the potential to become climate-resilient and commercialized following the developmental improvements of several genotypes/responses across field trials. Reducing environmental pollution and greenhouse gas emissions from non-point sources is a quick solution that N2-fixing bacteria have contributed significantly to in crop systems. The mode of application, productivity increase, and genotypes of crops that improve food security are important. (Shalaby *et al.* 2023) [79] (Win *et al.* 2023) [91] (Giller *et al.*, 2024) [25].

5. Applications in Environmental Remediation

In order to improve the effectiveness of biogeochemical remediation of contaminated ecosystems, researchers have been attempting to identify microorganisms that fix nitrogen or associate with such microorganisms. Nitrogen-fixing bacteria can improve the structure and fertility of soils that have been degraded for various reasons. Therefore, nitrogen-fixing bacteria have the potential to be used in environmental remediation processes to treat wastewater, human and animal waste, or overcome other decontaminations caused by industrial pollutants. The biogeochemical cycle of nitrogen includes gas-gas interfaces, gas-water interfaces in the dissolved or suspension state, liquid-solid interfaces, rock-water interfaces, and liquid-solid-biological interfaces at the mineral, organic, and inorganic phases of ecosystems. The combination and status of different nitrogen cycles in ecosystems allow for precision farming, minimization of nitrogen nutrients entering watercourses, treatment of nitrogen wastewater, nitrogen recovery, and maintenance of the global nitrogen balance. (Li *et al.*, 2022) (Huang, 2024) [33] (Shi *et al.*, 2023) [81].

In environmental nitrogen cycles, the application of nitrogen-fixing bacteria can provide opportunities for organic matter accumulation and preservation through different particle sizes. However, it is necessary to identify the nitrogen-fixing bacteria endemic to different ecosystems and to study possible nitrogen biogeochemical cycles in varying size-specific mineral components. In addition, the efficiency rates must be compared for chemical treatment because of the potential problems with developing concentrations of these microorganisms in environmental restoration or bioremediation processes over broad areas. There is hope for the positive development of strategies to eliminate harmful soil metals and recover possible management profits under a green economy policy, preferentially using extremophiles that live within soil elements, which will contribute to different strategies for nitrogen fertilizer use in agriculture. (Harindintwali *et al.* 2022) [31] (Song *et al.*, 2022) [83] (Alleman & Peters, 2023) [2]

5. 1. Bioremediation of Contaminated Soils

Nitrogen is commonly one of the limiting nutrients for plant production. Biologically inert forms of nitrogen, such as the molecular nitrogen in the air, can be fixed through nitrogen fixation to form biologically active nitrogen. Nitrogen-fixing bacteria are an important part of the soil microbiome. They can fix molecular nitrogen in the air into a biologically active form, and thus become a valuable resource in the recovery of certain nutrients needed for the site. At the same time, nitrogen-fixing bacteria produce various secondary metabolites and a large number of enzymes, including lipase, which establishes a large community of microorganisms around them, accelerating the recovery of the microbial ecosystem in contaminated sites and promoting a series of nutrient cycling processes. In addition, nitrogen-fixing bacteria can fix significant amounts of carbon into the soil and have a wide range of ecological benefits, such as improving the structure and stability of soil ecosystems, alleviating acid in soils, and strengthening the ability of plants to resist stress, promote crop growth, and improve soil structure and fertility in damaged soil ecosystems caused by pollution. (Sepp *et al.* 2023) [78] (Mahmud *et al.*, 2020) [58] (Wassermann *et al.* 2023)

In addition, some nitrogen-fixing bacteria and their corresponding genetic components have been shown to play important roles in the biodegradation of pollutants and have repaired contaminated sites containing heavy metals, crude oil, polycyclic aromatic hydrocarbons, pesticides, and dioxins. In most studies to date, nitrogen-fixing bacteria have been used in conjunction with biostimulation, bio-augmentation, or PSM to strengthen soil systems and restore soil health. Industrially, the use of nitrogen-fixing bacteria in agriculture has received more and more attention. Although the application of nitrogen-fixing bacteria in the field of soil remediation is not common, more and more studies are starting to pay attention to its relationship with bioremediation. In terms of the growth and development of plants, nitrogen-fixing bacteria have beneficial effects in the processes of promoting root growth in microorganisms and increasing the nitrogen source supply of plants. In addition, nitrogen-fixing bacteria contain symbiotic nitrogen fixation and free-living nitrogen fixation. Although the inoculation results of the two types of nitrogen-fixing bacteria bio-augmentation were compared, since it is not the focus of this review, how to use two different nitrogen-fixing bacteria for specific remediation scenarios still needs to be studied further. (Bansal & Tanveer, 2024) [7] (Fouad *et al.* 2022) [23] (Hu *et al.* 2023)

5. 2. Wastewater Treatment

Wastewater is one of the most significant products in our daily life and industrial field, mainly containing organic pollutants and a large number of nitrogenous compounds, such as ammonia nitrogen and nitrate nitrogen. The occurrence of these nitrogenous compounds contributes to eutrophication in the aqueous environment and is harmful to human and animal health. Therefore, in order to thoroughly improve the quality of water, it is necessary to achieve the reduction of the nitrogen content to a low value. The main forms of nitrogen removal currently practiced are nitrification and denitrification, both of which are slow relative to the growth and reproduction rates of the bacteria that perform them. Nitrogen-fixing bacteria are another group responsible for nitrogen removal because they can assimilate nitrogen in the atmosphere into ammonium. (Barney, 2020) [9] (Kawaka, 2022) [36] (Wang *et al.* 2024) [88]

The application of nitrogen-fixing bacteria in the natural bio-treatment system of the bio-fertilizer immersion process can improve its nitrogen removal efficiency. Moreover, due to the mutualistic relationship between them and other microorganisms in the wastewater bio-system, nitrogen-fixing bacteria can also be used for nitrogen removal in the anaerobic/aerobic nitrogen removal process. At present, the traditional methods of removing nitrogen mainly include physical nitrogen removal, chemical nitrogen removal, and biological nitrogen removal, of which the potential application of nitrogen-fixing bacteria has received greater attention (Niu *et al.* 2021) [81] (Cao *et al.* 2023) [12] (Ni *et al.* 2022). Improvement of the nitrogen removal efficiency of the traditional nitrogen removal treatment system and the development of cost-effectiveness with a low energy footprint and environmentally friendly nitrogen removal is attracting considerable attention. The development of a new engineering application of low energy cost and the treatment of ammonium nitrogen into dinitrogen gas is extremely meaningful to environmental governance. Although the engineering application of nitrogen-fixing bacteria in wastewater treatment is just beginning, the development of this technology has a broad prospect. Of course, how to optimize the conditions for improving the efficiency of nitrogen-fixing bacteria and achieving a higher application effect will require collaborative research in the future (Ospina-Betancourth *et al.* 2020) [70] (Ospina Betancourth, 2021) [69] (Llamas *et al.*, 2023) [56].

6. Conclusion and Future Perspectives

Nitrogen (N) is an essential macronutrient that is necessary for plant and crop growth and development. Against the backdrop of diminishing mineral nitrogen (N) resources, nitrogen-fixing bacteria, based on sustainable agriculture and environmentally friendly principles, have received increasing attention and become the main focus of various fields in recent years. In this study, nitrogen-fixing bacteria in infectious diseases, nodulation processes, and important roles in natural and ecological agricultural environments were synthesized.

It is significant to apply nitrogen-fixing bacteria or nodule-associated bacteria to environmental protection and energy agriculture. In addition to exploiting the vegetative parameters of A. brasilense in rice crop cultivation, the available data provide potential predictive information on the advantages of herb inoculant applications. There are still numerous challenging issues for future study, such as how to better apply nitrogen-fixing bacteria to achieve the intended results and maintain the ecological balance, exploitation of supported broad-spectrum-related products, and subsequent isolation of bioinoculants, as well as the major role of diversified agronomic crops in identifying the influence of secondary bacterial genera. Using effective nitrogen-fixing bacteria with biotechnological developments to enhance this process is essential. Only when the novel research direction is systematically studied through a multidisciplinary approach can more benefits be provided to a broader area. Therefore, it is necessary for further research to consider the metabolic traits and ecological properties of nitrogen-fixing bacteria and microorganisms that are isolated from other environments in detail.

In conclusion, this paper has focused on the application of nitrogen-fixing bacteria (NFB) in natural and ecological environments, which can promote crop health and drastically boost crop yield, leading to sustainability in agriculture. Furthermore, insight into the role of these bacteria is provided in order to lay the foundation for the further application of NFB in a variety of fields.

7. References

1. Aasfar A, Bargaz A, Yaakoubi K, Hilali A, Bennis I, Zeroual Y, Meftah Kadmiri I. Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. Frontiers in Microbiology. 2021;12:628379. Available from: frontiersin. org
2. Alleman AB, Peters JW. Mechanisms for generating low potential electrons across the metabolic diversity of nitrogen-fixing bacteria. Applied and Environmental Microbiology. 2023;89(2):e02103-22. Available from: asm. org
3. Ambechada JK, Umrania VV. Nitrogen-fixing bacteria in biofertilizer production: benefits, limitations and prospects. ResearchGate. 2024. Available from: researchgate. net
4. Aoyagi R, Kitayama K, Turner BL. How do tropical tree species maintain high growth rates on low-phosphorus soils?. Plant and Soil. 2022;469(1-2):133-146. Available from: [HTML]
5. Arsita R, Karim H, Hala Y, Iriany N, Jumadi O. Isolation and identification of nitrogen-fixing bacteria in the corn rhizosphere (*Zea mays* L.) originating from Jeneponto Regency, South Sulawesi. In: IOP Conference Series: Earth and Environmental Science. 2020 Apr;484(1):012051. IOP Publishing. Available from: iop. org
6. Arya R, Bisht S, Joshi N. Microbes: A biological tool for agriculture enhancement. Innovative Approaches in Agriculture. Available from: researchgate.net
7. Bansal A, Tanveer S. Microbial mediators of ecosystem functions: nitrogen fixation, biodegradation, and bioremediation. Science and Innovation. 2024. Available from: cyberleninka.ru
8. Barabi SR. Investigation of diazotrophic bacteria association with plants. CORE. 2022. Available from: core. ac. uk
9. Barney BM. Aerobic nitrogen-fixing bacteria for hydrogen and ammonium production: current state and perspectives. Applied Microbiology and Biotechnology. 2020;104(6):2557-2569. Available from: [HTML]
10. Bellenger JP, Darnajoux R, Zhang X, Kraepiel AML. Biological nitrogen fixation by alternative nitrogenases in terrestrial ecosystems: a review. Biogeochemistry. 2020;149(2):149-172. Available from: jstor. org
11. Bellenger JP, Darnajoux R, Zhang X, Kraepiel AML. Biological nitrogen fixation by alternative nitrogenases in terrestrial ecosystems: a review. Biogeochemistry. 2020;149(2):149-172. Available from: nsf. gov
12. Cao TND, Mukhtar H, Le LT, Tran DPH, Ngo MTT, Nguyen TB, Bui XT. Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus. Science of The Total Environment. 2023;870:161927. Available from: [HTML]
13. Chamoli A, Bhambri A, Karn SK, Raj V. Ammonia, nitrite transformations and their fixation by different biological and chemical agents. Chemistry and Ecology. 2024. Available from: [HTML]
14. Chaudhary P, Singh S, Chaudhary A, Sharma A, Kumar G. Overview of biofertilizers in crop production and stress management for sustainable agriculture. Frontiers in Plant Science. 2022;13:930340. Available from: frontiersin. org
15. Child JJ. Biological nitrogen fixation. Soil Biochemistry. 2021. Available from: [HTML]
16. Dheeman S, Maheshwari DK. Ecology of nitrogen-fixing bacteria for sustainable development of non-legume crops. In: Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes. Singapore: Springer Nature Singapore; 2022. p. 301-315. Available from: researchgate. net
17. Din I, Khan H, Khan NA, Khil A. Inoculation of nitrogen fixing bacteria in conjugation with integrated nitrogen sources induced changes in phenology, growth, nitrogen assimilation and productivity of wheat crop. Journal of the Saudi Society of Agricultural Sciences. 2021;20(7):459-466. Available from: sciencedirect.com
18. Dolkhani F, Bijanzadeh E, Boostani HR, Hardie AG. Effect of nitrogen-fixing bacteria application on biochemical properties, yield, and nutrients of barley. Journal of Soil Science and Plant Nutrition. 2022;22(4):5021-5035. Available from: [HTML]
19. Eckardt NA, Allahverdiyeva Y, Alvarez CE, Büchel C, Burlacot A, Cardona T, Yano J. Lighting the way: compelling open questions in photosynthesis research. The Plant Cell. 2024;36(10):3914-3943. Available from: oup.com
20. Elhady A, Hallmann J, Heuer H. Symbiosis of soybean with nitrogen fixing bacteria affected by root lesion nematodes in a density-dependent manner. Scientific Reports. 2020;10(1):20824. Available from: nature.com
21. Etesami H, Jeong BR. Biotechnological solutions to improve nitrogenous nutrition in nonlegume crops. In: Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes. Singapore: Springer Nature Singapore; c2022. p. 65-95. Available from: researchgate.net
22. Ferrando Molina F. Characterisation of grain legume rhizobia for the potential development of inoculants with an improved shelf-life. Stir. ac. uk. c2021. Available from: stir.ac.uk
23. Fouad FA, Youssef DG, Shahat FM, Abd El-Ghany MN. Role of microorganisms in biodegradation of pollutants. In: Handbook of Biodegradable Materials. Cham: Springer International Publishing; c2022. p. 1-40. Available from: researchgate.net
24. Ghebreamlak SM, Mansoorabadi SO. Divergent members of the nitrogenase superfamily: tetrapyrrole biosynthesis and beyond. ChemBioChem. 2020;21(19):2891-2903. Available from: wiley.com
25. Giller KE, James EK, Ardley J, Unkovich MJ. Science losing its way: examples from the realm of microbial N2-fixation in cereals and other non-legumes. Plant and Soil. 2024;470(1):1-21. Available from: springer.com
26. Gou Z, Zheng H, He Z, Su Y, Chen S, Chen H, Sun Y. The combined action of biochar and nitrogen-fixing bacteria on microbial and enzymatic activities of soil N cycling. Environmental Pollution. 2023;317:120790. Available from: [HTML]
27. Goyal RK, Mattoo AK, Schmidt MA. Rhizobial–host interactions and symbiotic nitrogen fixation in legume

crops toward agriculture sustainability. Frontiers in Microbiology. 2021;12:635536. Available from: frontiersin.org
28. Goyal RK, Schmidt MA, Hynes MF. Molecular biology in the improvement of biological nitrogen fixation by rhizobia and extending the scope to cereals. Microorganisms. 2021;9(10):2176. Available from: mdpi.com
29. Grover M, Yaadesh S, Jayasurya A. Associative Nitrogen Fixers-Options for Mitigating Climate Change. In: Bioinoculants: Biological Option for Mitigating Global Climate Change. Singapore: Springer Nature Singapore; 2023. p. 217-237. Available from: [HTML]
30. Gu W, Milton RD. Natural and engineered electron transfer of nitrogenase. Chemistry. 2020;6(11):2867-2884. Available from: mdpi.com
31. Harindintwali JD, Wang F, Yang W, Zhou J, Muhoza B, Mugabowindekwe M, Yu X. Harnessing the power of cellulolytic nitrogen-fixing bacteria for biovalorization of lignocellulosic biomass. Industrial Crops and Products. 2022;186:115235. Available from: [HTML]
32. Hu W, Wang X, Wang X, Xu Y, Li R, Zhao L, Teng Y. Enhancement of nitrogen fixation and diazotrophs by long-term polychlorinated biphenyl contamination in paddy soil. Journal of Hazardous Materials. 2023;446:130697. Available from: researchgate.net
33. Huang Q. Enhancing Soil Health and Biodiversity Through Nitrogen Fixation Symbiosis in Leguminous Plants. Molecular Microbiology Research. 2024. Available from: microbescipublisher.com
34. Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ. Handbook of microalgae-based processes and products: fundamentals and advances in energy, food, feed, fertilizer, and bioactive compounds. Handbook. 2020. Available from: [HTML]
35. Kaschuk G, Auler AC, Vieira CE, Dakora FD, Jaiswal SK, da Cruz SP. Coinoculation impact on plant growth promotion: a review and meta-analysis on coinoculation of rhizobia and plant growth-promoting bacilli in grain legumes. Brazilian Journal of Microbiology. 2022;53(4):2027-2037. Available from: nih. gov
36. Kawaka F. Characterization of symbiotic and nitrogen fixing bacteria. AMB Express. 2022;12(1):19. Available from: springer.com
37. Kebede E. Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. Frontiers in Sustainable Food Systems. 2021;5:625345. Available from: researchgate.net
38. Khalaf SA, Al-Ghamdi A. Role of nitrogen-fixing bacteria in soil health and sustainable crop productivity. Agronomy. 2021;11(9):1793. Available from: mdpi.com
39. Kim J, Cho J, Yoon J, Lee Y, Lee S. Molecular mechanisms involved in nitrogen fixation by free-living nitrogen-fixing bacteria. Applied and Environmental Microbiology. 2023;89(5):e00363-23. Available from: ncbi.nlm.nih.gov
40. Kumar V, Singh S, Kumar A. Role of nitrogen-fixing bacteria in sustainable agriculture. Environmental Sustainability. 2021;4(1):34-48. Available from: springer.com
41. Kovács S, Kiss E, Jenei S, Fehér-Juhász E, Kereszt A, Endre G. The Medicago truncatula IEF gene is crucial for the progression of bacterial infection during symbiosis. Molecular Plant-Microbe Interactions. 2022;35(5):401-415. Available from: apsnet.org
42. Krauspe V, Fahrner M, Spät P, Steglich C, Frankenberg-Dinkel N, Maček B, *et al.* Discovery of a small protein factor involved in the coordinated degradation of phycobilisomes in cyanobacteria. Proceedings of the National Academy of Sciences. 2021;118(5):e2012277118. Available from: pnas.org
43. Krishna KR. Microbiology of the Agricultural Sky. [HTML]. c2024.
44. Kumaar SA, Babu RP, Vivek P, Saravanan D. Role of nitrogen fixers as biofertilizers in future perspective: a review. Research Journal of Pharmacy and Technology. 2020;13(5):2459-2467. Available from: rjptonline. org
45. Ladha JK, Peoples MB, Reddy PM, Biswas JC, Bennett A, Jat ML, Krupnik TJ. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. Field Crops Research. 2022;283:108541. Available from: sciencedirect.com
46. Lan Y, Zhang H, He Y, Jiang C, Yang M, Ye S. Legume-bacteria-soil interaction networks linked to improved plant productivity and soil fertility in intercropping systems. Industrial Crops and Products. 2023;196:116504. Available from: sciencedirect.com
47. Lawal I. Nitrogen fixing bacteria and their application for heavy metal removal: a mini review. Journal of Biochemistry, Microbiology and Biotechnology. 2021;9(2):43-47. Available from: hibiscuspublisher.com
48. Li C, Li X, Yang Y, Shi Y, Li H. Degradation reduces the diversity of nitrogen-fixing bacteria in the alpine wetland on the Qinghai-Tibet Plateau. Frontiers in Plant Science. 2022. Available from: frontiersin.org
49. Liang Y, He X, Chen X, Su Y, Pan F, Hu L. Low frequency of plants associated with symbiotic nitrogen-fixers exhibits high frequency of free-living nitrogen fixing bacteria: A study in karst shrub ecosystems of … Forests; c2022. Available from: mdpi.com
50. Libourel C, Keller J, Brichet L, Cazalé AC, Carrère S, Vernié T, *et al.* comparative phylotranscriptomics reveals a 110 million years-old symbiotic program. bioRxiv. 2022-09. Available from: biorxiv.org
51. Lindström K, Mousavi SA. Effectiveness of nitrogen fixation in rhizobia. Microbial Biotechnology. c2020. Available from: wiley.com
52. Lipa P, Janczarek M. Phosphorylation systems in symbiotic nitrogen-fixing bacteria and their role in bacterial adaptation to various environmental stresses. Peer J. c2020. Available from: peerj.com
53. Liu B, Wang X, Ma L, Chadwick D, Chen X. Combined applications of organic and synthetic nitrogen fertilizers for improving crop yield and reducing reactive nitrogen losses from China's vegetable systems: A … Environmental Pollution. c2021. [HTML].
54. Liu L, Chen X, Ye J, Ma X, Han Y, He Y, Tang K. Sulfoquinovose is a widespread organosulfur substrate for Roseobacter clade bacteria in the ocean. The ISME Journal. 2023;17(3):393-405. Available from: nih.gov
55. Liu-Xu L, González-Hernández AI, Camañes G, Vicedo B, Scalschi L, Llorens E. Harnessing Green Helpers: Nitrogen-Fixing Bacteria and Other Beneficial Microorganisms in Plant–Microbe Interactions for Sustainable Agriculture. Horticulturae. 2024;10(6):621. Available from: mdpi.com
56. Llamas A, Leon-Miranda E, Tejada-Jimenez M.

Microalgal and nitrogen-fixing bacterial consortia: from interaction to biotechnological potential. Plants. c2023. Available from: mdpi.com

57. Mahmud AA, Upadhyay SK, Srivastava AK, Bhojiya AA. Biofertilizers: A Nexus between soil fertility and crop productivity under abiotic stress. Current Research in Environmental Sustainability. 2021;3:100063. Available from: sciencedirect.com
58. Mahmud K, Makaju S, Ibrahim R, Missaoui A. Current progress in nitrogen fixing plants and microbiome research. Plants. c2020. Available from: mdpi.com
59. Malik G, Singh N, Hooda S. Nitrogen stress in plants and the role of phytomicrobiome. Phyto-microbiome in stress regulation. c2020. [HTML].
60. Mason RE, Craine JM, Lany NK, Jonard M, Ollinger SV, Groffman PM, *et al.* Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems. Science. 2022;376(6590):eabh3767. Available from: science. org
61. Minamisawa K. Mitigation of greenhouse gas emission by nitrogen-fixing bacteria. Bioscience. [HTML].
62. Mohr W, Lehnen N, Ahmerkamp S, Marchant HK, Graf JS, Tschitschko B, *et al.* Terrestrial-type nitrogen-fixing symbiosis between seagrass and a marine bacterium. Nature. 2021;600(7887):105-109. Available from: nature.com
63. Murugaragavan Ramasamy TG, Yuvaraj M. Role of biofertilizers in plant growth and soil health. Nitrogen fixation. c2020. Available from: intechopen.com
64. Naorem A, Tilgam J, Priyadarshini P, Tak Y, Bharati A, Patel A. Nitrogenase Enzyme Complex: Functions, Regulation, and Biotechnological Applications. In: Advances in Plant Nitrogen Metabolism. CRC Press; c2022. p. 142-154. [HTML].
65. Naqqash T, Malik KA, Imran A, Hameed S, Shahid M, Hanif MK, *et al.* Isolation and characterization of Rhizobium from non-leguminous potato plants: New frontiers in Rhizobium research. Biology and Fertility of Soils. 2024;60(3):307-325. Available from: rug.nl
66. Ni J, Yang H, Chen L, Xu J, Zheng L, Xie G, *et al.* Metagenomic analysis of microbial community structure and function in an improved biofilter with odorous gases. Scientific Reports. 2022;12(1):1731. Available from: nature.com
67. Niu J, Feng Y, Wang N, Liu S, Liang Y, Liu J, He W. Effects of high ammonia loading and in-situ short-cut nitrification in low carbon-nitrogen ratio wastewater treatment by biocathode microbial electrochemical system. Science of the Total Environment. 2021;755:142641. Available from: [HTML].
68. Ojija F, Aloo BN. The role of Rhizobia toward food production, food and soil security through microbial agro-input utilization in developing countries. Case Studies in Chemical and Environmental Engineering. 2023;8:100404. Available from: sciencedirect.com
69. Ospina Betancourth MC. Valorisation of nitrogen-deficient wastewater treatment systems using sludge enriched with nitrogen-fixing bacteria. [HTML]. 2021. Available from: ncl. ac.uk
70. Ospina-Betancourth C, Acharya K, Allen B, Entwistle J, Head IM, Sanabria J, Curtis TP. Enrichment of nitrogen-fixing bacteria in a nitrogen-deficient wastewater treatment system. Environmental Science & Technology. 2020;54(6):3539-3548. [HTML].
71. Pahalvi HN, Rafiya L, Rashid S, Nisar B, Kamili AN. Chemical fertilizers and their impact on soil health. Microbiota and Biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs. c2021. p. 1-20. [HTML].
72. Palmero F, Fernandez JA, Garcia FO, Haro RJ, Prasad PV, Salvagiotti F, Ciampitti IA. A quantitative review into the contributions of biological nitrogen fixation to agricultural systems by grain legumes. European Journal of Agronomy. 2022;136:126514.
73. Panda M, Mandal U, Barman S, Boddana P. Morphological study of nitrogen fixers. ResearchGate. net. c2021. Available from: https://www.researchgate. net
74. Pang Z, Mao X, Zhou S, Yu S, Liu G, Lu C, *et al.* Microbiota-mediated nitrogen fixation and microhabitat homeostasis in aerial root-mucilage. Microbiome. 2023;11(1):85. Available from: https://link. springer.com
75. Płaza A, Niewiadomska A, Górski R, Rudziński R, Rządżewska E. The effect of the nitrogen-fixing bacteria and companion red clover on the total protein content and yield of the grain of spring barley grown in a system of organic agriculture. Agronomy. 2022;12(7):1522. Available from: https://www. mdpi.com
76. Raffi MM, Charyulu PBBN. Azospirillum-biofertilizer for sustainable cereal crop production: Current status. In: Recent developments in applied microbiology and biochemistry. Academic Press; 2021. p. 193-209.
77. Rajkumari J, Katiyar P, Dheeman S, Pandey P, Maheshwari DK. The changing paradigm of rhizobial taxonomy and its systematic growth upto postgenomic technologies. World Journal of Microbiology and Biotechnology. 2022;38(11):206.
78. Sepp SK, Vasar M, Davison J, Oja J, Anslan S, Al-Quraishy S, *et al.* Global diversity and distribution of nitrogen-fixing bacteria in the soil. Frontiers in Plant Science. 2023;14:1100235. Available from: https://www. frontiersin. org
79. Shalaby M, Elbagory M, El-Khateeb N, Mehesen A, El-Sheshtawy O, Elsakhawy T, Omara AED. Potential impacts of certain N2-fixing bacterial strains and mineral N doses for enhancing the growth and productivity of maize plants. Plants. 2023;12(22):3830. Available from: https://www. mdpi.com
80. Sharma P, Sangwan S, Kaur H, Patra A, Anamika, Mehta S. Diversity and evolution of nitrogen fixing bacteria. Sustainable Agriculture Reviews 60: Microbial Processes in Agriculture. 2023:95-120. Available from: https://www. researchgate. net
81. Shi Z, Guo X, Lei Z, Wang Y, Yang Z, Niu J, Liang J. … of high-efficiency nitrogen-fixing bacteria from the traditional Chinese medicine plant Astragalus mongolicus and its effect on plant growth promotion and bacterial. BMC Microbiology. 2023. Available from: https://link. springer.com
82. Singh J, Verma PK. Plant transcription factors and nodule development. Plant Transcription Factors. 2023. Available from: https://www.researchgate.net
83. Song X, Peng C, Li D. Fate of nitrogen fixed by nitrogen-fixing cyanobacteria in rice and soil during the vegetative growth period of rice. Journal of Applied Phycology. 2022. Available from: https://www. researchgate. net

84. Soumare A, Diedhiou AG, Thuita M, Hafidi M, Ouhdouch Y, Gopalakrishnan S, Kouisni L. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. Plants. 2020;9(8):1011. Available from: https://www.mdpi.com
85. Tang A, Haruna AO, Majid NMA, Jalloh MB. Effects of selected functional bacteria on maize growth and nutrient use efficiency. Microorganisms. 2020. Available from: https://www. mdpi.com
86. Tolangi P. Nitrogen fixation evaluation of Azotobacter chroococcum isolated from soil of forest. 2022. Available from: 202. 45. 146. 37
87. Velip DM. Screening for efficient arbuscular mycorrhizal (AM) fungal species and rhizobial strains for enhancing yield in selected local cultivars of Vigna unguiculata. 2022. Available from: https://www. unigoa. ac. in
88. Wang H, Zhang L, Tian C, Fan S, Zheng D, Song Y, *et al.* Effects of nitrogen supply on hydrogen-oxidizing bacterial enrichment to produce microbial protein: Comparing nitrogen fixation and ammonium assimilation. Bioresource Technology. 2024;394:130199.
89. Wassermann B, Cernava T, Goertz S, Zur J, Rietz S, Kögl I, *et al.* Low nitrogen fertilization enriches nitrogen-fixing bacteria in the Brassica seed microbiome of subsequent generations. Journal of Sustainable Agriculture and Environment. 2023;2(2):87-98. Available from: https://www. wiley.com
90. White JF, Chang X, Kingsley KL, Zhang Q, Chiaranunt P, Micci A, Kowalski KP. Endophytic bacteria in grass crop growth promotion and biostimulation. Grass Research. 2021;1(1):1-9. Available from: https://www. maxapress.com
91. Win KT, Wasai-Hara S, Tanaka F, Oo AZ, Minamisawa K, Shimoda Y, Imaizumi-Anraku H. Synergistic N2-fixation and salt stress mitigation in soybean through dual inoculation of ACC deaminase-producing Pseudomonas and Bradyrhizobium. Scientific Reports. 2023;13(1):17050. Available from: https://www.nature.com
92. Xavier GR, Jesus EDC, Dias A, Coelho MRR, Molina YC, Rumjanek NG. Contribution of biofertilizers to pulse crops: From single-strain inoculants to new technologies based on microbiomes strategies. Plants. 2023;12(4):954. Available from: https://www.mdpi.com
93. Yan D, Tajima H, Cline LC, Fong RY, Ottaviani JI, Shapiro HY, Blumwald E. Genetic modification of flavone biosynthesis in rice enhances biofilm formation of soil diazotrophic bacteria and biological nitrogen fixation. Plant Biotechnology Journal. 2022;20(11):2135-2148. Available from: https://www.wiley.com
94. Yuvaraj M, Pandiyan M, Gayathri P. Role of legumes in improving soil fertility status. In: Legume Crops-Prospects, Production and Uses. 2020:16-27. IntechOpen. Available from: https://www. intechopen.com
95. Zehr JP, Capone DG. Changing perspectives in marine nitrogen fixation. Science. 2020. Available from: https://www.science.org
96. Zehr JP, Capone DG. Changing perspectives in marine nitrogen fixation. Science. 2020. Available from: https://www.science.org
97. Zeng L, Sun Z, Fu L, Gu Y, Li R, He M, Wei J. Cryopreservation of medicinal plant seeds: Strategies for genetic diversity conservation and sustainability. Plants. 2024. Available from: https://www.mdpi.com
98. Zhang X, Hu W, Jin X, Chen T, Niu Y. Diversity of soil nitrogen-fixing bacteria in the rhizosphere and non-rhizophere soils of Ebinur Lake Wetland. Archives of Microbiology. 2021. Available from: https://www.springer.com
99. Zhang Z, Nair S, Tang L, Zhao H, Hu Z, Chen M, Zhang Y. Long-term survival of Synechococcus and heterotrophic bacteria without external nutrient supply after changes in their relationship from antagonism to mutualism. MBio. 2021;12(4):10-1128. Available from: https://www. asm.org
100. Zhu YG, Peng J, Chen C, Xiong C, Li S, Ge A, *et al.* Harnessing biological nitrogen fixation in plant leaves. Trends in Plant Science. 2023;28(12):1391-1405. Available from: https://www.researchgate.net