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REVIEW ARTICLE

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Enhancing Nitrogen Fixating Species as Potential for Crop Nutrition and Yield Stability in Agriculture

Shweta Bhodiwal[1]* and Tansukh Barupal[2]

[1]Department of Botany, IIS University, Jaipur, Rajasthan, India

[2]SBK Government PG College, Jaisalmer

ABSTRACT

Biological nitrogen fixation (BNF) alludes to a microbial intervened process based on an enzymatic "Nitrogenase" change of atmospheric nitrogen (N2) into ammonium promptly absorbable by roots. N2-fixing microorganisms altogether named as "diazotrophs" can fix organically N2 in associate with plant roots. A large part of the interest for nitrogen (N) in cereal cropping systems is met by utilizing N composts, yet the expense of production is expanding and there are likewise ecological concerns. This has prompted a developing interest in exploring different sources of N, for example, organic N2 fixatation. Biofertilizers supplement supplements primarily by fixation of atmospheric nitrogen, by phosphorus solubilization, and by synthesizing plant growth-promoting substances. The nitrogen fixing microbes of the rhizobia and different groups are utilized for growth and development advancement of vegetables and additional crops. In particular, the symbiotic rhizobacteria induce physiological and structural alterations of bacterial cells and plant roots into specific designs called as nodules. Other N2-fixing microbes are free-living fixers that are profoundly assorted and globally widespread in cropland. They address key source of nitrogen (N) in regular and farming environments lacking symbiotic N fixation (SNF). Azotobacter chroococcum, Azospirillum lipoferum, Azospirillum brasilense, Gluconacetobacter Diazotropicus, Rhizobium strains are some illustration of nitrogen fixatation which might assist the plants with developing and can make the climate to get freed off from synthetic substances.

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Introduction

Composts are known as regular or synthetic man made materials that can upgrade typical soil supplements and extend the development and improvement of harvest alongside richness of soil when it is applied to the soil, on the plant or by the fertigation (spreading or applying by the course of water system) [1]. A huge manufacture awareness of standard manures is seen for those that are mostly known as Nitrogen – Phosphorous – Potassium (NPK) fertilizers and consequently gives nitrogen (soluble base, urea, ammonium sulfate, calcium ammonium nitrate, ammonium nitrate), potassium (potassium chloride, potassium sulfate, sulfate of potash, magnesia kieserite, Epsom salt, potassium nitrate) and phosphorus (ground rock phosphates, di-ammonium phosphate and superphosphates) [2]. Thus, one limit for the plant development is non-accessibility of supplement especially phosphorus and nitrogen to plants disregarding their sufficient activity in the soil because utmost nitrogen is accessible in the natural matter of soil and to acquire it plant necessities to compete with soil micro-organisms [3,4]. These potential organic manures can assume a vital part in proficiency and legitimacy of the soil and besides shield the eco- friendly environment as well as it will likewise play an important role in economy as it is cost effective for farmers [5]. It can be said that the fixed nitrogen is a limiting supplement in many circumstances, with the principal hold of the nitrogen in environment exist the sub-atomic nitrogen from air. Plants can't accustom the molecular nitrogen directly; but it opens up from course of the nitrogen fixation that is just evolved by prokaryotic cells. For quite a while, a foreordained number of the nitrogen fixing bacterial species were acknowledged, yet over the latest 30 years nitrogen fixation has been displayed to be a property with representatives in most of the phyla of Microorganisms and moreover in methanogenic Archaea [6]. In nodules of vascular plants the symbiotic nitrogen fixation is found in the two huge significant affairs of microbes that are not connected phylogenetically: as rhizobia (Alpha-proteobacteria) associated with the leguminous plants and thus belongs to the family angiosperms, and another is Frankia (Actino-bacteria) which associates with the broad scope of plants with 8 families [7,8]. Additional significant group is cyanobacteria for the nitrogen-fixing microbes that is present in the relationship with a wide assortment of advanced and inferior plants, parasites, green growth and thus many more [9]. Helpful beneficial interaction is normally a wide assortment that colonization with the root surface of non-leguminous plants, without the advancement of separated structures [10]. A relative portrayal is displayed in table 1 for natural, inorganic and biofertilizer.

Contact Shweta Bhodiwal shwetabhodiwal.20@gmail.com Department of Botany, IIS University, Jaipur, Rajasthan, India

Table 1: Different fertilizers

Sr.No.	Types	Defination + Examples
1.	Organic fertilizer	Fertilizers derived from living or formerly living materials. e.g., animal wastes, plant wastes from agriculture, compost, and treated sewage sludge. Beyond manures, animal sources can include products from the slaughter of animals-blood meal and bone meal.
2.	Inorganic fertilizer	These include industrially synthesized fertilizers. e.g., CO (NH2)2 (Urea) 45-46% nitrogen, chile saltpetre with 15% nitrogen.
3.	Biofertilizer	Fuentes-Ramirez and Caballero-Mellado (2005) defined a biofertilizer as "a product that contains living microorganisms, which exert direct or indirect beneficial effect on plant growth and crop yield through different mechanisms". E.g., AM fungi, N-fixer, P solubilizer and K solubilizer.

The Nitrogen Fixation Process

Enzymatic transformation of ammonia from sub-molecular nitrogen is catalyzed by nitrogenase, an oxygen labile catalyst complex profoundly preserved in free living and advantageous diazotrophs [11]. The most well-known type of nitrogenase, known to as Mo-nitrogenase or traditional nitrogenase, contains a prosthetic group with molybdenum, FeMoCo (Iron-Molybdenum-Cobalt bunch). A few microorganisms, like Azatobacter and a few photosynthetic nitrogen fixers (counting some cyanobacteria), convey extra types of nitrogenase whose cofactor contains vanadium (V-nitrogenase) or just iron (Fe-nitrogenase) [12]. The nitrogenase protein, which has been isolated from various sources, is made out of two metalloproteins. Part 1, likewise assigned Mo-Fe protein, is a tetramer of 220,000 Da made out of two non-indistinguishable subunits α and β, while part 2, additionally assigned Fe protein, is a dimer of 68,000 Da shaped by indistinguishable subunits (Figure 1). Two FeMoCo are bound to α subunits of the MoFe protein. Furthermore, there are two other prosthetic groups containing 4Fe-4S bunches. 'P-groups' are covalently bound to cysteine buildups of MoFe protein crossing over α and β subunits. The third kind of Fe-S bunch is connected to the Fe protein [13]. Reduction of nitrogen is an exceptionally intricate process. The consequence of net reduction of the sub-atomic nitrogen to ammonia is normally represented by the accompanying condition: $N_2 + 16\ Mg\text{-}ATP + 8\ e^- + 8H^+ = 2NH_3 + H_2 + 16\ Mg\text{-}ADP + 16\ P_i$ Two metalloproteins for example bigger Mo-Feprotein and smaller Fe-protein parts are associated with N2 fixation. Fe-protein cooperates with ATP and Mg++, and get electron from ferredoxin or flavodoxin when it is oxidized. Mo-Fe-protein of nitrogenase complex joins with the reducible substrates for example N2 and yields two particles of NH. Apparently N2 is diminished step-wise without breaking N bond until the last reduction and production of ammonia takes place. At last two particles of NH3 are set free from the compound. At last, electron is moved to oxidize Mo-Fe-protein which becomes reduced and Fe-protein is oxidized. It is reduced form of Mo-Fe-protein which joins with N2 and different substrates to bring about NH3 and other different products regarding substrate. H2 created during this response is additionally used by certain microorganisms which have hydrogenase [14]. Reutilization of H2 increments the nitrogenase action by shielding it from hindrance of H2. Ammonia is additionally synthesized into various metabolic items in microbial cells, nonetheless, Ammonia isn't collected in the cell, albeit a couple of animal varieties might make it; rather it is integrated into natural structures by consolidating with a organic acid (a - keto-glutaric corrosive) to lead to amino acid for example glutamic acid. The alkali may likewise consolidate with natural molecules to yield alanine or glutamine [15].

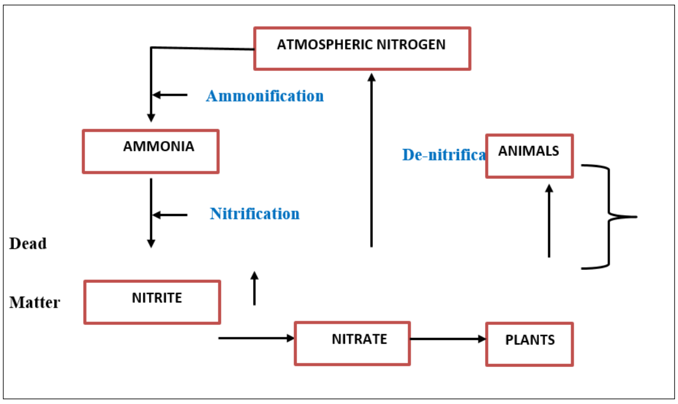

Figure 1: Nitrogen Fixation Cycle

Microbial Biofertilizers

A biofertilizer of useful living microbial cultures, when applied to the plant surfaces, soil or seed then it colonize the rhizosphere or within the host plant and a while later advances the plant development by growing the accessibility, supply, or take-up of fundamental enhancements to the host. Likewise, in contrast to the chemical composts, biofertilizers are more accessible to the farmers. cyanobacteria, bacteria and fungi are the main gathering of microorganisms that are mostly utilized in the microbial biofertilizer what's more greater part of these groups is additionally have the symbiotic relationship with plants. In table 2 a few microbial manures are referenced in light of their tendency and capability.

Table 2: Important Groups of Microbial Fertilizers

Sr. No	Group of Biofertilizers	Sub-group	Examples
1.	Nitrogenfixing	Symbiotic	Rhizobia (Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium Mesorhizobium Allorhizobium), Frankia, Anabaena azollae, and Trichodesmium
		Free-living	Anabaena, Azotobacter, Beijerinkia, Derxia, Aulosira, Tolypothrix, Cylindrospermum, Stigonema, Clostridium, Klebsiella, Nostoc, Rhodopseudomonas, Rhodospirillum, Desulfovibrio, Chromatium, and Bacillus polymyxa
		Associative	Azospirillum spp. (A. brasilense, A. lipoferum, A. amazonense, A. halopraeferens, and A. irakense), Acetobacter diazotrophicus, Herbaspirillum spp., Azoarcus spp., Alcaligenes, Bacillus, Enterobacter, Klebsiella, and Pseudomonas

Nitrogen-Fixing Microbes

Nitrogen is the most abundant as well as ubiquitous in the air, yet turns into a limiting supplement because of trouble of its fixation and take-up by the plants. Thus, certain microorganisms, some of which can shape different relationship with plants also, are fit for impressive nitrogen fixation. This property considers the proficient plant uptake of the fixed nitrogen and lessens loses by denitrification, draining, and volatilization. These organisms can be:

Free-living in the soil: The evaluation of nitrogen fixation by free-living bacterias is troublesome, yet in certain plants like Medicago sativa, it has been assessed to range from 3 kg N ha_1 to 10 kg N ha_1 [16]. Azotobacter chroococcum in arable soils can fix 2-15 mg N g_1 of carbon source in culture media, and it further delivers plentiful slime which totals soil. Notwithstanding, free-living cultures of nodulating bacterial symbionts (e.g., Frankia) have been found to fix environmental nitrogen in the rhizosphere of their host and even sometimes also in non-host plants [17]. For Beijerinckia mobilis and Clostridium spp., inoculation strategies for leaf splash and seed absorbing stimulated growth and development in cucumber and grain plants by critical nitrogen fixation and different components of bacterial plant development hormone synthesis [18]. Free-living cyanobacteria (blue green growth) have been harnessed in rice cultivation in India which can give up to 20-30 kg N ha_1 under ideal circumstances [19].

Having symbiotic associations (of rhizobia, Frankia, and cyanobacteria) with plants: For rhizobium bacteria the nitrogen-fixing effectiveness is the significant group of biofertilizers that contains microorganisms as like Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium, and Allorhizobium which can shift till 450 kg N ha_ among various strains and host vegetable species, in which root nodules are framed [20-25]. The rhizobial biofertilizers can be in powder, fluid, and granular plans, with various disinfected carriers like peat, perlite, mineral soil, and charcoal [26]. Frankia, a nitrogen-fixing actinomycete just as like rhizobia, can likewise frame root nodules in the several woody plants [27-30]. This mycelial bacterium structures symbioses with the underlying foundations of a few non-leguminous plants like Casuarina, Alnus (Birch) Myrica, Rubus, and so on. These actinorhizal plants are utilized for the production of timber and fuelwood, windbreaks, and shelterbelts, revegetation, mix plantation and land recovery [31,32]. The Frankia innoculation is viewed as significant in nurseries and in dis-turbed or arid conditions [33]. Except these, leaves of a couple of plants (e.g., Ardisia) foster extraordinary internal cavities holding symbiotic nitrogen-fixing micro-organisms like Xanthomonas and Mycobacterium, and thusly, these leaves behaves as nitrogen manure for the soil [34]. Another biologically significant group is that of cyanobacteria — blue green growth (BGA) — some of which like Nostoc, Trichodesmium, and Anabaena add to around 36% of the worldwide nitrogen fixation and have been accounted for to be useful in upgrading rice-field richness for the development of rice in many areas of the world [35-37]. Aquatic BGA can additionally give natural development of hormones, proteins, nutrients, and minerals for the dirt [38].

Living in rhizosphere (associative/associated) without endophytic symbioses: In contrast with endophytic symbionts, these nitrogen-fixing microorganisms have less symbiotic association with roots. These incorporate Herbaspirillum spp. And Acetobacter diazotrophicus with sugarcane, sorghum, and maize [39,40]. Azoarcus spp. with Leptochloa fusca (kallar grass); types of Alcaligenes, Azospirillum, Bacillus, Enterobacter, Herbaspirillum, Klebsiella, and Pseudomonas with rice and maize and Azospirillum with host specificity involves the different perennial as well as annual plants [41,42]. A few research have shown that because of nitrogen fixation and growth- promoting hormones, Azospirillum expanded the development and harvest yield of wheat, rice, sunflower, sugar beet, carrot, oak, tomato, pepper, eggplant, and cotton [43,44].

The inoculum of Azospirillum can be reasonably created and applied by a basic peat formulation [45]. The biofertilizer of Acetobacter diazotrophicus was found to fix and make accessible up to 70% of sugarcane crop with nitrogen requirement of around 150 kg N ha_1 yearly [46]. In this way, the capacity of nitrogen fixation in significant amount of these microorganisms makes them attractive for their application as biofertilizers.

Other Mineral-Solubilizing Biofertilizers

Soil-dwelling microorganisms can additionally be utilized as biofertilizers to give different supplements other than nitrogen and phosphorus like potassium, iron, zinc, and copper. Certain rhizobacteria can solubilize insoluble potassium structures, which is one more fundamental supplement that is important for plant development [47]. The higher biomass yields because of expanded potassium take-up have been seen with Paenibacillus glucanolyticus (for dark pepper), Bacillus edaphicus (for wheat), and Bacillus mucilaginosus in co-vaccination with the phosphate-solubilizing Bacillus megaterium (for eggplant, pepper, and cucumber) [48,49]. Another significant mineral is zinc, which is available at a very low concentration in the Earth's crust, because of which it is remotely applied as the costlier solvent zinc sulfate to overcome its deficiency in the plants. Notwithstanding, a few microorganisms, for example, Thiobacillus thiooxidans, Bacillus subtilis, and Saccharomyces spp. can solubilize insoluble less expensive zinc compounds like zinc oxide, zinc carbonate, and zinc sulfide in soil [50]. Likewise, microorganisms can hydrolyze silicates and aluminum silicates by providing protons (that causes hydrolysis) and natural acids (that structure buildings with cations and hold them in a broke up state) to the medium while using, which can be valuable to the plants. For instance, an expansion in rice development and grain yield because of expanded disintegration of silica and supplements from the soil was noticed utilizing a silicate-solubilizing Bacillus sp. associated with siliceous residues of rice straw, black ash and rice husk [51].

Application Practices of Microbial Biofertilizers

Biofertilizers are generally supplied as carrier-based inoculants with an importance of being easier and cheap to produce. The large scale manufacturing of biofertilizers includes microorganism cultures, handling of transporter material, with broth culture mixing of carrier material, and then packing. The ideal carrier materials utilized in the readiness of biofertilizers should be less expensive, locally accessible, and more straightforward to process; should be non-poisonous and natural in structure (so they stay biodegradable) with high water-holding limit; and ought to convey higher bacterial cells and support their endurance for longer time. A portion of the generally involved transporter materials in the development of good-quality biofertilizers are killed peat soil/lignite, vermiculite, charcoal, press mud, and soil combination. Thus, these can have disadvantage of having lower shelf line, temperature sensitive, and becomes less powerful by low cell counts. Thus, fluid formulation have been produced for Azospirillum, Rhizo-bium, Azotobacter, and Acetobacter which is costlier and have the benefits of having easier production, higher cell counts, longer shelf line, no pollution, stockpiling up to 45oC, and more greater competence in soil [52]. In any case, the application practices of microbial biofertilizers incorporate seed treatment, seedling root plunging, and soil application.

Mode of Biofertilizer Fiormulation

There are various ways for applying formulated biofertilizer into soil and these are

I. Seed vaccination with powder details,

II. Mix the dry biofertilizers with seeds in the seed container,

III. Sprinkle technique - Mix a modest quantity of water along with the seeds before peat powder is added,

IV. Slurry technique - In this the biofertilizer is suspended in the water then, at that point, add it to the seeds and mix properly,

V. Biofertilizer and cement are applied as slurry to the seeds and hence covered with the ground material as like lime,

VI. pelleting of seeds,

VII. Seedling root plunge

VIII. Peat suspension in the water i.e., showered into the wrinkle during planting (Bashan 1998).

Available Microbial Biofertilizers

There are a few microbial biofertilizers accessible as dried or fluid cultures under various business trademarks available in market, which are utilized for different purposes including improvement of plant development and soil ripeness. For example, the rhizobia biofertilizers can fix 50-300 kg N ha_1 that increments yield by 10-35%, keep up with soil fertility, and leaves residual nitrogen for succeeding harvests [53,54]. The Azatobacter biofertilizer utilized for practically all crops and can fix 20-40 mg N g_1 of carbon source that causes up to 15% expansion in yield; keeps maintained fertility of soil; produces development advancing substances like complex of vitamin B, giberellic acid and indole acetic acid; and is further useful in biocontrol of plant sicknesses by suppressing a portion of the plant microorganisms [55,56]. Some phosphorus-solubilizing bacterial biofertilizers as AU7 which is generally reasonable and suitable for all crops that produce catalysts which mineralize the insoluble natural phosphorus into the soluble form, subsequently expanding crop yield by 10-30% [57]. Different microbial biofertilizers that are accessible in market alongside application are portrayed in table 3.

Table 3: Different Microbial Biofertilizers Available In Market Along With Their Application

Sr. No.	Microbial biofertilizers	Trade names	Applications
1.	Azotobacter chroococcum, different strains of Azotobacter (non-symbiotic)	Bioazoto, Bhoomi Rakshak, Kisaan Azotobacter culture, and Azonik	For all crops like wheat, sorghum, barley, maize, paddy, mustard, sunflower, sesamum, cotton, sugarcane, banana, grapes, papaya, watermelon, onion, potato, tomato, cauliflower, chilly, lady finger, rapeseed, linseed, tobacco, mulberry, coconut, spices, fruits, flowers, plantation crops, and forest plants
2.	Azospirillum lipoferum, Azospirillum brasilense, and different strains of Azospirillum	Biospirillum, Green Plus, Bio-N, Azo-S, ROM, and Spironik	(1) For normal and acidic soils and dry soils (2) For paddy and other crops
3.	Gluconacetobacter Diazotropicus	Sugar-Plus	For sugarcane
4.	Rhizobium strains (symbiotic, nitrogen fixing)	Biobium, Rhizo-Enrich, Kisaan Rhizobium culture, Rhizoteeka, Green Earth Reap N4, and Rhizonik	Pulses (gram, peas, lentil, moong, urd, cowpea, and arhar), oil legumes (groundnut and soyabeans), fodder legumes (barseem and lucerne), and forest tree legumes (subabul, shisam, and shinsh)

Conclusion

Different nitrogen-fixing microorganisms have been isolated from grain roots by culture-subordinate techniques, and when utilized as plant inoculants they have changing degrees and systems for plant development advancement [58-62]. A few past endeavors to increment nitrogen fixatation in cereals by advancing pseudonodules with phytohormones get failed. Remarkably, as of late acquired ammonium discharging mutants of some plant associated diazotrophs were powerful for advancing plant growth and development proposing that they became equipped for providing nitrogen to their hosts. Organic nitrogen fixatation in plants can be a sustainable source of nitrogen and may redirect our ongoing reliance on modern industrial nitrogen production. This is particularly applied for food production in the globe where farming creation is as yet based on higher-yielding assortments as well as hybrids however with a simultaneous expansion in inorganic nitrogen application. Despite the fact that accomplishing hereditarily changed nitrogen-fixing grain crops is a complicated cycle, the methodologies that are being pursued at present are making excellent opportunities for producing such plants within a reasonable time-frame. If this happens, the worldwide natural advantages of decreased substance manure utilization will be enormous, and we guess that negative environmental results of nitrogen fixing cereals will minimize. Other than nitrogen, other agrarian sources of info, like phosphorus and water, may limit crop efficiency. Artificial symbioses, cooperative nitrogen fixation in non-legume plants, particularly in cereals as like rice, wheat, maize, focused on or biased rhizosphere, and comprehension of endosymbiotic and endophytic nitrogen fixation with non-legume plants are a portion of the methodologies that ought to be examined at a great extent.

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