ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

POTENTIAL OF *AZOTOBACTER* IN SUSTAINABLE AGRICULTURE

Mangesh Kumar Mankar*[1], Sanjay Sahay[2], Ragini Gothawal[1]

[1]*Department of Biotechnology and Bioinformatics Center, Barkatullah University, Bhopal, Madhya Pradesh, India*

[2]*Government Postgraduate College, Biaora (Rajgarh), Madhya Pradesh, India*

**Corresponding author: mangesh.mankar1991@gmail.com*

ABSTRACT

The problem of climate change has started to hit stomach of world population through its adverse effect on crops and soil microbes. A comprehensive approach is required to deal with this situation so that continuous and enough food supply to the people can be ensured. Breeding of variously resistant crop plants may be one of the approaches, but to adopt an agricultural practice that can sustain the agriculture production at higher level should be the best one. Replacing chemical inputs with the biofertilizers, especially the highly efficient strains of *Azotobacter* constitutes one of the most important components of such agricultural practice. Since its discovery in 1901, *Azotobacter* has been seen as an important agricultural input due to its multiple beneficial effects on plant growth. Therefore, extensive works have been carried out leading to much advancement in the techniques to isolate, identify, and apply this plant growth promoting rhizospheric (PGPR) bacterium to the crops has been made. Also, huge data as to multiple agricultural roles of this bacterium have been generated. The aim of this review is to recapitulate various aspects of *Azotobacter* highlighting its importance in sustainable agriculture.

Keywords: *Azotobacter* sp., Sustainable agriculture, PGPR

1. INTRODUCTION

Climate change is currently the most important environmental issue affecting all life forms including crop plants. Agriculture production is thus facing serious threat. Agriculture on the other hand is the most crucial activity shouldering the responsibility to feed billions of current and future stomachs across the world. It has thus to be sustained.

Currently, agricultural production is highly dependent on the application of chemical fertilizers. In India and many other countries, the much talked about green revolution in its wake brought large number of fertilizer manufacturing units whose products have been applied relentlessly to the soil. The practice has further been encouraged by the short term spurt in agricultural production. But very soon in India, as in other part of world, the consequence of such non-judicious use of fertilizers has been realized [1, 2]. Consequently, a global demand to reduce dependency on chemical inputs is now well in air. This can be done by introducing suitable strains of plant growth promoting (PGP) microbes in the soil. By doing so, not only the negative effects of fertilizers can be overcome, but already damaged soil can be remediated and maintained, and considerable savings on fertilizers bill can also be achieved [3-5].

PGP microbes, especially rhizosphere occupying bacteria (PGPR) have multiple roles to play in the soil viz., enhancing root's access to nutrients in soil, fixing atmospheric nitrogen to available forms, solubilizing complex phosphates to ready-to-use form, providing chemicals/molecules for promoting plant growth and controlling soil-borne pathogens. *Azotobacter* has been universally recognized as one of the most important PGPRs (Fig. 1). In 1901, Dutch microbiologist and botanist Beijerinck discovered the bacterium, *Azotobacter*. Its species *A. chroococcum* was reported to be the first aerobic non-symbiotic nitrogen fixing microbe. This bacterium has ability to grow vigorously and establish in the rhizosphere of crop plants when applied to the latter [6]. Moreover, its own capacity to produce cyst under unfavorable condition allows it to survive in the harsh environment of the soil and its seemingly strong ability to adapt to various environmental adversaries has led to evolution of enormous pheno-genotypic variations suitable for various non leguminous crops and agro-climate regions. The latter feature has especially been important to serve as catalytic force for carrying out

researches related to their isolation, identification and application. Moreover, these special qualities make *Azotobacter* species as the most successful and widespread microbe of agriculture system. Potent strains of *Azotobacter* are very useful in improving plant growth and yield, soil health and environment. They thus hold key to sustainable and climate resilient agriculture.

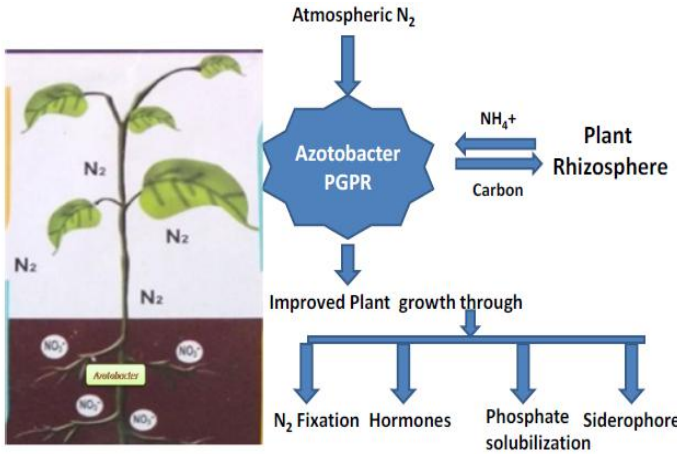

Fig. 1: Azotobacter and its beneficial effects on plants

In earlier studies, the species of *Azotobacter* have been reported from rhizosphere of several crop plants such as maize, sugarcane, rice, wheat, bajra, millets, plantation crops and vegetables [7]. The isolated cultures of *Azotobacter* have been reported to fix about 10 mg N_2/ g of carbon source like sucrose consumed under *in vitro* conditions and about 20 kg N_2/ha/year in natural soil. They constitute not only very efficient but also cost effective nitrogen fixing system [8]. Thus, the bacterium now finds approval as a component of integrated nutrient management system [9].

Azotobacter belongs to the bacterial family Azotobacteraceae or Pseudomonadaceae which consists of, in addition to this, another genus *Azomonas* [10]. As per DNA-RNA hybridization data, *Azotobacter* and *Azomonas* have been found closely related. Both of these genera can fix nitrogen in aerobic condition without any precondition to go for symbiotic relationship with higher plants, thus called non symbiotic nitrogen fixing organisms. Although both share nitrogen fixing mechanism, they differ in a. cyst forming ability (*Azotobacte*r can but *Azomonas* cannot), and b. fraction of guanine + cytosine pair which is 63-67.5 mol % (Tm) in *Azotobacter* whereas 52-59 mol % (Tm) in *Azomonas* [11]. Earlier, two more genera viz., *Beijerinckia,* and *Derxia* were included in the family Azotobacteraceae, but with the revelation of very different rRNA nucleotide sequence data of these genera, they were excluded from this family [12]. The first species of *Azotobacter* viz., *A. chroococcum* was isolated from the soil of Holland. Later, this species has been isolated from the soils of many other locations across the world. Another species, *A. paspali* has been isolated from the rhizosphere of grasses and so far found to be restricted to grass-rhizosphere [6]. The genus, *Azotobacter* comprises seven species viz., *A .chroococcum, A. vinelandii, A beijerinckii, A. nigricans, A. paspali, A. armeniacus,* and *A. salinestris* based on 16S rRNA gene sequence characteristics [11]. Chronology of discovery of various species of *Azotobacter* is as follows: *A. chrococcum* [6], *A. vinelandii* [13], *A. beijerinckii* [13], *A. nigricans* [14], *A. paspali* [15], *A. armeniacus* [16] and *A. salinestris* [17]. The species *A. nigricans* was then split into two subspecies, *Azotobacter nigricans* subsp. *nigricans and Azotobacter nigricans* subsp. *achromogenes* [16]. *A. salinestris* was reported as a microaerophilic species that shares many physiological traits with *A. chroococcum*, but its absolute dependence on sodium ions for its growth formed the basis for awarding it the status of separate species. For the same reason, *A. salinestris* has been considered as a suitable nitrogen fixer for salt affected soil or even as potentially useful tool for bioremediation [17].

The genus, *Azotobacter* has seen many changes as to its taxonomic position since its discovery. At the beginning it was assigned to the family Azotobacteraceae [18], but later studies on its 16S rRNA nucleotide sequences prompted its inclusion in Pseudomonadaceae. Phylogenetic data have also substantiated the grouping of *A. vinelandii* along with *Pseudomonas aeruginosa* sharing same clad [19], and subsequently it was suggested that the genera of *Azotobacter, Azomonas* and *Pseudomonas* are closely related to each other or they even might be synonyms [20]. Although, for identification 16S rRNA gene is important, *Nif* genes especially *NifH* have also served as markers for detection and study of the genetic diversity of *Azotobacter* present in the soil [21, 22]. The species and strains of *Azotobacter* have been described on the basis of *nif*H gene, which is also useful to analyze their genetic potential for the nitrogen fixation [23].

2. HABITAT AND CHARACTERISTIC OF THE SPECIES OF *AZOTOBACTER*

Azotobacter species are distributed in alkaline soil, rhizosphere, and philosopher regions of plants, most of their species however occur in the rhizosphere region of

higher plant [11]. It is primarily characterized as a free-living bacterium that can fix atmospheric N_2 in the soil with consumption of organic carbon compounds as an energy sources and that has distinctive respiratory activity. Cells of *Azotobacter* form thick-walled cysts and may produce large quantities of capsular slime but not endospore. Most species are motile, gram negative, and pleomorphic in nature (bluntly rod, oval or coccus-shaped) which produce catalase-oxidase. The cell-shape changes spontaneously or with changes in growth conditions [24, 25] and that it is impossible to obtain a culture of *Azotobacter* containing only one morphological form [25]. The genus *Azotobacter* typically is mesophilic in nature but minimum temperature of growth approaches a little above 0^oC. It does not tolerate high temperature but in the encysted form it can survive at 40-45 °C temperature [24]. The species of *Azotobacter* like alkaline pH, in acidic condition (<6) they occur in very low density or do not occur at all. The bacterium exhibits optimum physiological activities at pH 7-7.5 [11].

3. BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation (BNF) was discovered by Beijerinck in 1901. The enzyme responsible for catalyzing reduction of free atmospheric nitrogen to ammonia is nitrogenase. The overall reaction is as follow- $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$. The microbes harboring this enzyme are called diazotrophs. Nitrogenase holds key to drive the global nitrogen cycle, but itself is vulnerable to oxygen. Diazotrophs thus have to create micro-aerobic or anaerobic condition to express the activity of this enzyme [6].

Azotobacter fixes nitrogen under aerobic condition; a fixation rate of 10 mg of N_2/ g of glucose consumed has been reported. *A. vinelandii* possesses three different versions of nitrogenase, each with a unique metal associated with Fe-S cofactor. Based on the associated metals, the enzymes are termed molybdenum-iron type (MoFe), vanadium-iron type (VFe) and iron only type (Fe) [26]. The associated metals are molybdenum (MoFe), or vanadium (VFe) or none (Fe only). They all convert N_2 to NH_3 but have minor differences in respect of catalytic rates and substrate specificity, for example at low temperature (5°C) vanadium-iron type nitrogenase is more (as much as ten times) active [27].

A. vinelandii has not only provided information about the existence of three types of nitrogenases but also the structural details of MoFe nitrogenase. *A. vinelandii* MoFe nitrogenase is composed of two separable components; a dimeric (γ_2-dimer) Fe protein (NifH) also called ditrogenase reductase (DNR) of 220-240 kDa and a tetrameric ($\alpha_2\beta_2$-tetramer) MoFe protein (NifDK) of about 60 kDa also called dinitrogenase (DN) [28]. Each Fe protein possesses an ATP binding site, and shares a (Fe_4S_4) cluster physically enclosed between two Fe protein monomers. The Mo Fe protein is composed of two similar $\alpha_2\beta_2$-dimers, with each $\alpha\beta$- subunit consists of a P-cluster (Fe_8S_7) at α/β polypeptides interface and an M-cluster ($MoFe_7S_9C$- homocitrate) also called iron molybdenum cofactor (FeMo-co) within α-polypeptide [26]. The Fe protein is responsible for catalyzing active (with ATP consumption) electron transfer reaction via (4Fe-4S) cluster, the electrons then is routed to FeMo-co through P-cluster, the last two reactions are catalyzed by MoFe component. During this active electron transfer process, nitrogen molecule is converted to ammonia molecule. The VFe and Fe nitrogenases, apart from the α and β polypeptides, consists of δ polypeptide as well [29].

At genetic level, the coded products of at least genes viz., *nifH*, *nifD* and *nifK* form enzymatic components of nitrogenases, and there are many other *nif* genes whose coded products are involved in assembly and maturation of the enzyme. For example, *NifS* codes for a cysteine desulfurase that catalyses release of sulfur from the substrate L-cysteine for use in [Fe-S] cluster formation [30], whereas *nifU* codes for protein that acts as scaffold for [Fe-S] cluster assembly [31]. In the next stage, *nifB*-coded enzyme catalyzes NifB- cofactor formation [32]. The proteins NifB, NifEN, and NifH have been reported to be critical for the formation of FeMo-co [33]. NifEN here is known to serve as scaffold; the FeMo-co subsequently is complexed with preformed MoFe apo-protein [34]. A protein NifX seems to serve as temporary reservoir of FeMo-co precursors, and control its availability during FeMo-co synthesis [32]. An Rnf protein complex (products of genes *rnf1* and *rnf2*) does involve in the rate of expression and maturation of nitrogenase. The coded products of *rnf* genes have been reported to be required for the rapid accumulation of the matured DNR complexed with [4Fe-4S] cluster [33]. Another gene *nifL* codes for flavoprotein that negatively modulates expression of nitrogenase at transcription level by redox-dependent switching [35]. The coded product of *nifA* amplifies the expression of nitrogense by redox-dependent inhibition of *NifL* product [36]. In an interaction study it was revealed that after P- cluster

formation, the maturing MoFe protein enters into interaction with the products of *NafH*, *NifW*, and *NifZ* in that order, and that interaction of *NafY*/*NifY* occurs before the insertion of cofactors in FeMo subunit [37]. NifW and NifZ are deemed to form a complex to exert common effect i.e. protection of MoFe nitrogenase from oxygen [38].

4. NITROGENASE REGULATION

How different types of nitrogenases are regulated, has been a subject of interest. It has been found that presence of Mo activates Mo nitrogenase but is repressive for V and Fe types of nitrogenases. In absence of Mo, Fe nitrogenase is repressed by V. Interestingly, if none of the three metals is present, Fe nitrogenase is used for growth as in case of *A. vinelandii* [39]. A type of product inhibition has been reported in *A. vinelandii*, where in ammonium salt above 25 μM concentration inhibits all types of nitrogenase activities [40]. The mechanism suggested for this inhibition is a 'decrease in proton motive force' that in turn dissociates nitrogenases and their reducing equivalents thus shutting off enzyme activity [41]. The inhibition is moderated by external factors including the amount of oxygen (too little or too much without concurrent increase in respiration rate) [42, 43] and high pH [42]. In case of *A. vinelandii*, the nitrogenous compounds such as nitrate salts [43, 44] and urea [45] have also been reported to repress nitrogen fixation fully while some other nitrogen-containing organic compounds such as adenine, aspartate, casamino acids or yeast extract were found to repress nitrogenase partially [46, 47]. It is however suggested that in *A. vinelandii* fixed nitrogen-inhibited enzyme is degraded [43, 48, 49] and not stored via ADP-ribosylation for future use as found in *Rhodospirillum rubrum* [50].

5. GENETIC IMPROVEMENT

In a study with *A. chroococcum*, an engineered strain CBD 15 was created by partially knocking out gene *nifL* that regulate the process negatively and tailoring *nifA* that regulate the process positively to make it constitutively expressed. The engineered strain *A. chroococcum* CBD15 when used to inoculate wheat seeds, about 60% of improvement in yield even in the absence of urea application has been reported [51].

6. *AZOTOBACTER* IN THE SERVICE OF AGRICULTURE

Azotobacters populate on or around the root surface, and secrete growth promoting substances in the neighbor of rhizosphere. The use of such plant-microbes interaction may overcome the global dependence on unhealthy and costly agro chemicals which destabilize the agro-ecosystems and decrease plant health and soil fertility [52, 53]. *Azotobacter* enhances plant growth and health through beneficial mechanisms that may be direct or indirect. Direct beneficial mechanisms involve fixation of nitrogen, production and regulation of phytohormones, and solubilization of complex phosphates and indirect mechanism includes production of inhibitory compounds such as HCN, hydrolytic enzymes, siderophore and antibiotics that manage pathogenic microbes in the rhizosphere. The isolated culture of *Azotobacter* fixes nitrogen in nature with an average rate of 20 kg N_2/ha/year [8].

Phytohormones are the chemical messengers that affect expression of such genes as related to seed growth, time of flowering, sex of flowers, senescence of leaves, and fruits [54]. *Azotobacter* synthesizes plant growth regulators *viz.,* indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) [55].

Azotobacter genus is capable of converting insoluble inorganic phosphorus into soluble form that can be direct absorbed by the plants as a nutrient [56]. Phosphorus solubilizing activity is determined by the ability of bacteria to release metabolites such as organic acids (gluconic and keto-gluconic acids and phosphatases), which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms [57].

Siderophore, synthesized by *Azotobacter* under iron limited conditions is low molecular weight (<10 KD) iron chelating compounds that increases iron availability through solubilization of precipitated environmental iron sources [58]. *A. chroococcum* synthesizes three classes of siderophore: a. vibrioferrin (a low- affinity α-hydroxy carboxylate), b. amphibactins (high-affinity tris-hydroxamates), and c. crochelin A (a high-affinity siderophore with mixed Fe-chelating groups) [59].

Indirect benefit is obtained from the *Azotobacter* interaction with other soil microbes such as *Rhizobium*, *Azospirillum* and Arbuscular Mycorrhizal Fungus (AMF). Many studies showed that combined application of co-inoculants is more effective than single one.

Azotobacter and *Rhizobium* are well known for their atmosphere nitrogen fixation characteristic and fix N_2 non-symbiotically and symbiotically respectively [60]. Consortium of *Azotobacter* sp. and *Rhizobium* sp. has been found to reduce water stress and improve

nutrient uptake under sub-irrigation conditions and increase chlorophyll and carbohydrates content that subsequently improve the plant growth and productivity [61].

Azospirillum is a non-symbiotic N_2-fixing organism, which lives in close association with plants in the rhizosphere. The synergetic effects of *Azotobacter* and *Azospirillum* interaction on plant's growth are mainly attributed to improvements in root architecture, an increase in the rate of water and mineral uptake by roots, the displacement of fungi and plant pathogenic bacteria and to a lesser extent, biological nitrogen fixation [62].

Soil fungi that develop beneficial symbiotic associations with plants roots and contribute to plant growth are called mycorrhizal fungi which increase the absorptive surface area of the root facilitating water and nutrients absorption [63]. Inoculation of *A. chroococcum* and AM fungi under the soil and climatic condition of arid region, characterized by fluctuation in precipitation, has been reported to increase the yield as well as root colonization of mycorrhizal population [64, 65]. These types of synergetic interaction make plants healthy and diseases free. The co-inoculation of a nitrogen-fixing and phosphate solubilizing organisms viz., *Azotobacter*, *Azospirillum* and mycorrhiza has been reported to increase net assimilation and crop growth-rate in *Nigella sativa* [66]. The interaction of *Azotobacter* and mycorrhiza has been reported to facilitate aggregation of organic material in soil and plant root development and its access to nutrient elements [67, 68].

7. METHODOLOGIES TO STUDY *AZOTOBACTER*

Azotobacter from the rhizospheric soil (depth of 10–15 cm) is isolated on nitrogen free medium following four general procedures. Isolates cultivated at pH 7.2, temperature 30°C and grow after 3-4 days of incubation. Bacterial isolates purified by streaking method on Jensen agar plate and long terms storage in TSB with 50 % glycerol at -80 °C and short term storage in TSA plate at 4 °C for further characterization [25, 69]. Preliminary identification bacterium gram negative, colonies are gummy, raised and with or without striations, viscous, with pigmentation vary from very light brown to black, and with copious capsular slime. The bacterium has the ability to form cysts under unfavorable conditions and presence of cyst is one of the criteria for the identification of *Azotobacter* genus. Nitrogenase assay (by acetylene reduction assay) and species level identification of the most promising isolates of *Azotobacter* by studying through 16S rRNA sequence characteristics [69, 70].

8. METHODS FOR ISOLATION OF *AZOTOBACTER*

8.1. Spreading method

Brown N free medium or Jensen medium is used for the isolation of nitrogen fixing bacterium. Soil sample is serially diluted with D/W and soil suspension (1:9) made up to 10^{-6}. A 0.1ml aliquot from 10^{-4}-10^{-6} dilutions series is spread over the agar plates. Slimy, glistening and brown colonies appear on plate after the period of 4-7 days is sign of *Azotobacter* species [25, 71, 72].

8.2. Direct isolation

Sample of soil air dried cleaned and sieved to fine particles before the inoculation. Lumps of soil (1gm) particles are spread on Ashbys Mannitol agar medium. After 3 to 7 days *Azotobacter* sp. appear as slimy and glistening colonies turning brown with age in case of the species *A chroococcum* [73].

8.3. Enrichment method

8.3.1. Method A

Soil sample process air dried and cleaned to fine particles. Two gm of soil poured into 500 ml Erlenmeyer flask containing 18 ml of Burk's medium and incubated for 48 hrs. A 0.1 ml aliquot of bacterial suspension spread on the Burk's agar plates. Bacterial colonies thus appeared produce pigmentation which is sign of *Azotobacter,* are then sub cultured onto agar plates five times and finally single colonies are selected and restreaked on Burk's agar plate [36].

8.3.2. Method B

Sample process same as but enrichment in Winogradsky solution for 7-14 days followed by streaking onto Ashby's medium and incubated for 48 hrs. Bacterial colonies produce pigmentation and turning brown to black color with age sign of *Azotobacter* [74, 75].

8.4. Soil paste method

Soil sample process and prepare soil paste by 30–50 g soil mixed with 20% (v/w) of DDW and 1% (w/w) of Mannitol in a porcelain mortar. Soil paste is rushed inside a petridish containing Mannitol medium by the use of sterile spatula to acquire a smooth and leveled surface. After 3-7 days of incubation, characteristic brown colonies of the bacterium developed on the medium which are then purified by repeated streaking [76].

9. MASS PROPAGATION AND APPLICATION

Suitable strains of the bacterium are designated for applying as biofertilizer. This necessitates their commercial level production, processing and distribution. Broth culture is raised, blended with carrier, cured, packaged and then stored at 20 °C before being distributed to farmer (Fig. 2) described according to Bureau of Indian standard (BIS, 1985) manual [77]. The commercial preparation is applied to the crop-land by different methods. Culture may be applied to seed, seedling, soil or leaf as diluted suspension in water or after mixing it with jaggary or compost.

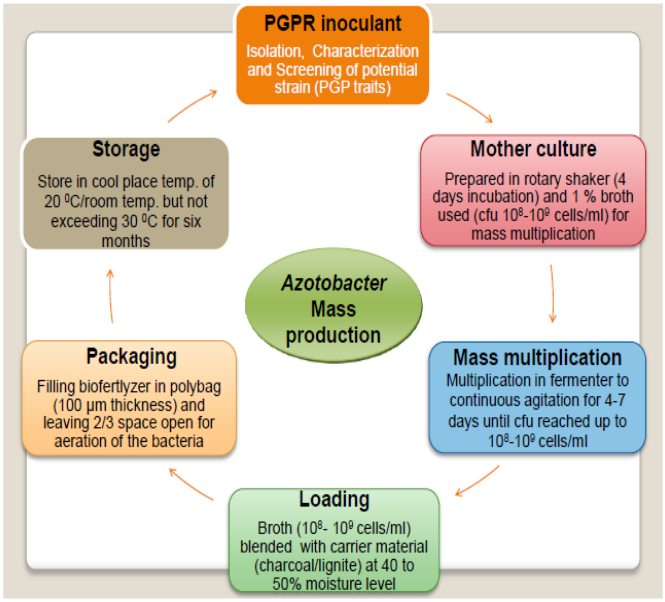

Fig. 2: Steps for the isolation to mass production of *Azotobacter* strains [77]

10. DIFFERENT METHODS OF APPLICATION WITH THEIR DOSES PROCEDURE AND CROPS BENEFITED

10.1. Seed treatment/seed inoculation

The seed coating method is most common practice of applying *Azotobacter* biofertilizer and it used for the all non leguminous crops which are sown through seeds. N fixing bacteria at recommended rate [Culture (200 gm) + 10%Jaggary solution in ratio 1:2] mix well and prepare slurry of culture and sprinkle on to heap of 10-12 kg seeds. Seed are than treated with this slurry thoroughly in a way that thin layer is formed around the seed. Culture coated seeds kept in shade for some time for drying and sown immediately [78].

10.2. Root/seedling treatment

This method has been found very much suitable for crops such as paddy, tomato, chilly, onion, tobacco, cabbage, cauliflower and flower crops. The seedling roots of transplanted crops are treated in a biofertilizer solution before transplantation in the field condition. Small bundle of seedling roots dipped in culture slurry [Culture (1kg) + water in ratio 1:10] and leave for 30 minutes than transplant immediately. Before the planting so that root are well drenched with the culture slurry [79].

10.3. Soil treatment

All non leguminous crops are benefitted. Twenty five kg of culture mix with 40-60 kg of compost or soil and broadcasted onto the field at the time of sowing or at the time of irrigation in standing crops. The culture mix has to be broadcasted uniformly in the soil and water well [80, 81].

10.4. Set treatment

Planting material like sugarcane and banana benefited. Pieces and sets of planting material immersed in suspension [Culture (2 kg) + water in ration 2:50] and to leave for 30 minutes. After drying in a shade it immediate planting and field is irrigated within 24 hrs [79].

10.5. Foliar Spraying

The liquid formulation @ 4 liters diluted in 500 liter water and pooled into a sterile container and applied as foliar spray in intervals accordingly crops like Mulberry, Cotton and Sisal [81, 82].

10.6. Through irrigation

All non leguminous crops benefited where drip irrigation is in a practices. The liquid formulation can be used @ 4 liters/hac diluted in 500 liter water is delivered to individual plant via piping [81].

11. EFFECT OF *AZOTOBACTER* ON PLANT GROWTH AND DEVELOPMENT

Kyaw co-workers and Dubey co-workers concluded their study report that *Azotobacter* sp. has to potential to fix atmospheric nitrogen, siderophore production, phosphate solubilization, hydrolytic enzymes, and synthesize growth regulator indole compound and it can used as bio-inoculant for the sustainable and profitable agriculture [83, 84]. Several reports of the study state that seed inoculation with efficient strain of *Azotobacter* sp. significantly increased the plant growth, biomass, uptake of nutrients, grain and stover yield (Table 1).

Table 1: PGP traits of *Azotobacter* species and their effect on crops

PGP bacteria	PGP traits	Host plant	Effect on plant	Reference
Azotobacter chroococcum	Phosphate and potassium solubilization, IAA and siderophore	Maize	PGPR inoculation showed positive impact on plant height dry shoot weight N, P, Fe concentration and chlorophyll content compared to control under drought condition at 40% field capacity	[85]
Azotobacter chroococcum	N_2 fixation, phosphate solubilization, IAA and hydrolytic enzymes	Cotton	Co-inoculation significantly enhanced growth of plant and reduces 50% dose of inorganic N (urea)	[86]
Azotobacter chroococcum	IAA phosphate solubilization, HCN and Ammonia production	Turmeric	Significantly increased 6 % curcumin content in inoculated plant compared to un-inoculated plant with positive impact on shoot height and fresh biomass and root fresh biomass	[87]
A salinestris, A armeniacus and *A chroococcum*	N_2 fixation, GA3, IAA and zeatin synthesis, siderophore, HCN, salicylic acid, NH_3, ACCD enzymes and Fe tolerance	Wheat	Seed coating with bio-inoculants positively increased root hairs and seminal root in seedling of wheat	[52]
Azotobacter	Salinity tolerant	Wheat	Significantly increased grain yield total nitrogen and biomass of plant	[88]
Azotobacter chroococcum mutant strain	Siderophore	Cotton	Effective bio-pesticide against *R solani cotton* and *R solani rice* disease index 167% and 25% respectively. In crop guar disease index 25 % and 133 % against *R solani cotton* and *R solani* respectively	[89]
Azotobacter chroococcum	N_2 fixation, phosphate solubilization, IAA, siderophore and hydrolytic enzymes	Sesame	Effective plant growth promoters with reduction of 50% dose of inorganic N (urea) and biocontrol agent against *Macrophomina phaseolina*	[84]
Native *Azotobacter chroococcum*	N fixer, P solubilizer, IAA, HCN, and Siderophore production	Wheat	Significantly increased grain yield, protein % of seed, 1000 seed weight, P, N, Zn and Fe uptake	[90]

12. CONCLUSION

Plants in their innate habitats are co-habited by different kinds of microbes and some microbes directly interact with plants for mutually beneficial purposes. Understanding of such a complex nature of plant–microbe interactions can potentially offer new strategies to enhance the plant productivity. *Rhizobium* takes more time for nodule formation and then only symbiotic nitrogen fixation starts but *Azotobacter* starts nitrogen fixation asymbiotically in the soil as soon as it establishes there, and thus help better plant growth right from the initial stage of seedling growth. *Azotobacter* has thus become one of the important components of integrated nutrient management (IPM). Further, study on the importance of application of native strains and genetically improved strain of this bacterium and improvement of conditions for plant-microbe-*Azotobacter* interaction for sustainable agriculture and food security is very important.

Conflict of interest

The authors declare that they have no conflict of interest.

13. REFERENCES

1. Wartiainen I, Eriksson T, Zheng W, Rasmussen U. *App Soil Ecol,* 2008; **39(1):**65-75.
2. Adesemoye AO, Torbert HA, Kloepper, JW. *Microbial Ecology*, 2009; **58(4):**921-929.

3. Alves BJ, Boddey RM, Urquiaga S. *Plant Soil*, 2003; **252(1):**1-9.
4. Hungria M, Nogueira MA, Araujo RS. *Biol Fertil Soils*, 2013; **49(7):**791-801.
5. Hungria M, Campo RJ, Souza EM, Pedrosa FO. *Plant Soil,* 2010; **331(1-2):**413-425.
6. Beijerinck MW. Ueber Oligonitophile Mikroben, Zentralblattfiir Bakteriologie, Parasitenkunde, In: Fektionskrankheiten and Hygiene, Abteilung; 1901. p. 561-582.
7. Arun KS. Bio-fertilizers for sustainable agriculture. Sixth ed. Jodhpur (India): Agribios publishers; 2007.
8. Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N. *Protoplasma*, 2014; **251(3)**:511-523.
9. Jnawali AD, Ojha RB, Marahatta S. *Adv Plants Agric Res,* 2015; **2(6):**1-5.
10. Winogradsky S. *Ann Inst Pasteur,* 1938; **60(1):**351-400.
11. Becking JH. The Family Azotobacteraceae. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. The Prokaryotes. New York: Springer; 2006. p. 759-783.
12. De Smedt J, Bauwens M, Tytgat R, De Ley J. *Int J Syst Evol Microbiol*, 1980; **30(1):**106-122.
13. Lipman JG. New Jersey State Agriculture Experiment Station Annual 24th Report New Jersey. 1903.
14. Krasilnikov NA. SSSR Moskva-Leningrad USSR, 1949.
15. Dobereiner J. *Pesqui Agropecu Bras,* 1966; **1(1):**357-365.
16. Thompson JP, Skerman VBD. *IJSB,* 1981; **31(1):**215-218.
17. Page WJ, Shivprasad S. *Int J* Syst *Evol Microbiol,* 1991; **41(3):**369-376.
18. Pribram E. Klassifikation der Schizomyceten (Bakterien). Franz Deuticke: Leipzig; 1933.
19. Rediers H, Vanderleyden J, De Mot R. *Microbiol,* 2004; **150(5):**1117-1119.
20. Young JM, Park DC. *Int J Syst Evol Microbiol,* 2007; **57:**2894-2901.
21. Ueda T, Suga Y, Yahiro N, Matsuguchi T. *J Bacteriol,* 1995; **177(5):**1414-1417.
22. Widmer F, Shaffer BT, Porteous LA, Seidler RJ. *App Environ Microbiol,* 1999; **65(2):**374-380.
23. Zehr JP, Capone DG. *Microbial Ecoly,* 1996; **32(3):**263-281.
24. Saribay G. MSc Thesis, The Middle East Technical University Ankara Turkia, 2003.
25. Jensen HL. The Azotobacteraceae" Bacteriological Reviews XVIII. 1954: 195-214.
26. Mohanraj S, Pandey A, Mohan SV, Anbalagan K, Kodhaiyolii S, Pugalenthi V. In *Biohydrogen.* Elsevier; 2019. p. 413-434.
27. Miller RW, Eady RR. *Biochem J,* 1988; **256(2):**429-432.
28. Seefeldt LC, Hoffman BM, Peters JW, Raugei S, Beratan DN, Antony E, Dean DR. *Acc Chem Res,* 2018; **51(9):**2179-2186.
29. Chatterjee R, Ludden PW, Shah VK. *J Biol Chem*, 1997; **272(6):**3758-3765.
30. Zheng L, White RH, Cash VL, Dean DR. *Biochemistry,* 1994; **33(15):**4714-4720.
31. Johnson D, Dean DR, Smith AD, Johnson MK. *Annu Rev Biochem*, 2005; **74(1):**247-281.
32. Hernandez JA, Igarashi RY, Soboh B, Curatti L, Dean DR, Ludden PW, Rubio LM. *Mol Microbiol*, 2007; **63(1):**177-192.
33. Curatti L, Brown CS, Ludden PW, Rubio LM. *PNAS USA*, 2005; **102(18):**6291-6296.
34. Fay AW, Blank MA, Rebelein JG, Lee CC, Ribbe MW, Hedman B, Hodgson KO, Hu Y. *PNAS USA,* 2016; **113(34):**9504-9508.
35. Hill S, Austin S, Eydmann T, Jones T, Dixon R. *PNAS USA*, 1996; **93(5):**2143-2148.
36. Tejera NLCMMGJ, Lluch C, Martinez-Toledo MV, Gonzalez-Lopez, J. *Plant soil*, 2005; **270(1):**223-232.
37. Jimenez-Vicente E, Yang ZY, Ray WK, Echavarri-Erasun C, Cash VL, Rubio LM, Seefeldt LC, Dean DR. *J Biol Chem,* 2018; **293(25):**9812-9823.
38. Lee SH, Pulakat L,Parker KC, Gavini N. *Biochem Biophys* Res *Commun,* 1998; **244(2):**498-504.
39. Bellenger JP, Wichard T, Xu Y, Kraepiel AM. *Environ Microbiol*, 2011; **13(6):**1395-1411.
40. Kleiner D. *Arch Microbiol,* 1974; **101(1):**153-159.
41. Laane C, Krone W, Konings W, Haaker H, Veeger C. *Euro J Biochem,* 1980; **103(1):**39-46.
42. Klugkist J, Haaker H. *J Bacteriol*, 1984; **157(1):**148-151.
43. Buhler T, Sann R, Monter U, Dingler C, Kuhla J, Oelze J. *Arch Microbiol*, 1987; **148(3):**247-251.
44. Kleinschmidt JA, Kleiner D. *Arch Microbiol*, 1981; **128(4):** 412-415.
45. Burns RC, Bulen WA. *Biochim Biophys Acta-Enzymol Biol Oxi*, 1965; **105(3):**437-445.
46. Gadkari D, Stolp H. *Arch Microbiol*, 1974; **96(1):**135-144.
47. Sorger GJ. *J Bacteriol,* 1968; **95(5):**1721-1726.
48. Klugkist J, Haaker H, Wassink H, Veeger C. *Euro J Biochem*, 1985; **146(3):**509-515.
49. Dingler C, Kuhla J, Wassink H, Oelze J. *J Bacteriol*, 1988; **170(5):**2148-2152.
50. Ponnuraj RK, Rubio LM, Grunwald SK, Ludden PW. *FEBS Lett,* 2005; **579(25):**5751-5758.
51. Bageshwar UK, Srivastava M, Pardha-Saradhi P, Paul S, Gothandapani S, Jaat RS, et al. *Appl Microbiol*, 2017; **83(15):**e00590-17.
52. Rubio EJ, Montecchia MS, Tosi M, Cassán FD, Perticari A, Correa OS. *Sci World J*, 2013; **1:**1-12.

53. Kakraliya SK, Jat RD, Kumar S, Choudhary KK, Prakash J, Singh LK. *Inter. J Curr Microbiol App Sci,* 2017; **6(3):**152-163.
54. de Garcia, Salamone IE, Hynes RK, Nelson LM. Role of Cytokinins in Plant Growth Promotion by Rhizosphere Bacteria In: Siddiqui ZA, editors. PGPR: Biocontrol and Biofertilization. Dordrecht: Springer; 2005. P. 173-195.
55. El-Fattah DAA, Eweda WE, Zayed MS, Hassanein MK. *Ann Agric Sci*, 2013; **58(2):**111-118.
56. Rivas R, Peix A, Mateos PF, Trujillo ME, Martínez-Molina E, Velázquez E. *Plant Soil,* 2006; **287(1-2):**23-33.
57. Sagoe CI, Ando T, Kouno K, Nagaoka T. *Soil Sci Plant Nutr,* 1998; **44(4):**617-625.
58. Schwyn B, Neilands JB. *Ann Biochem*, 1987; **160(1):**47-56.
59. Zhang X, Baars O, Morel FM. *Metallomics*, 2019; **11(1):**201-212.
60. Brown ME, Walker N. *Plant Soil*, 1970; **32(1-3):**250-253.
61. Dashadi M, Khosravi H, Moezzi A, Nadian H, Heidari M, Radjabi R. *Adv. Stud Biol*, 2011; **3(8):**373-385.
62. Okon Y, Itzigsohn R. *Biotechnol Adv,* 1995; **13(3):**415-424.
63. Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C. *J Exp Bot*, 2005; **56(417):**1761-1778.
64. Solanki AS, Kumar V, Sharma S. *J Agri Technol,* 2011; **7(4):**983-991.
65. Shishehbor M, Madani H, Ardakani MR. *Ann Biol Res,* 2013; **4(2):**174-180.
66. Khorramdel S, Kuchaki A, Mahallati MN, Ghorbani R. *Iran J Agro Res*. 2008; **6:**285-294.
67. Hosamani PA, Lakshman HC, Sandeepkumar K, Channabasava A, MA K, SB G. *J Sustain Agric*, 2011; **5(4):**428-433.
68. Amraei Bf, Ardakani MR, Rafiei M, Paknejad F, Rejali F. *In Biol Forum*, 2015; **7(2):**895-900.
69. Aquilanti L, Favilli F, Clementi F. *Soil Biol Biochem,* 2004; **36(9):**1475-1483.
70. Cappuccino JC, Sherman N. *Microbiology: A Laboratory Manual*, 3rd ed. New York: Benjamin/Cummings Pub. Co; 1992.
71. Brown ME, Burlingham SK, Jackson RM. *Plant Soil,* 1962; **17:**309-319.
72. Knowles R. Free living dinitrogen-fixing bacteria. In: Black CA, editor. Methods of Soil Analysis, USA: ASA-SSA, Madison; 1982. p. 10071-11077.
73. Mishustin EN, Shilnikova VK. In soil Biology Reviews of Research. Paris: UNESCO Publication; 1969.
74. Augier J. A propos de la numeration des Azotobacter en milieu liquide. Paris: Annales de l'Institut Pasteur; 1956.
75. Pochon J, Tardieux P. Techniques danalyse en microbiologie du sol. France; 1962.
76. Becking JH. The family Azotobacteraceae. In: Ballows A, Truper HG, Dworkin M, Harder W, Schleifer KH, editors. The prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Berlin, Heidelberg: Springer; 1981. p. 795-817.
77. BIS. Biofertilizers and Organic Fertilizers in Fertilizer (Control) Order. Ghaziabad, (UP): 1985. p.1-68.
78. Singh R. Handbook of agriculture. 6th ed. New Delhi: Indian Council of Agriculture Research publishers; 2015.
79. Dhandar DG, Prabhudesai HR, Sundaram RNS. Extn Folder No 17. 1999.
80. Chandra Shekara P, Balasubramani N, Sharma R, Shukla C, Kumar A, Chaudhary BC, Baumann M. Farmer's Handbook on Basic Agriculture. Navsari (Gujrat): Desai Fruits & Vegetables Pvt Ltd; 2016.
81. Maity A, Marathe RA, Dhinesh Babu, K, Singh NV. Extension Bulletin No, 2016; **6:**1-22.
82. Sudhakar P, Chattopadhyay GN, Gangwar SK, Ghosh JK. *J Agri Sci,* 2000; **134(2):**227-234.
83. Kyaw EP, Soe MM, San San Yu ZKL, Lynn TM. *J Biotech Biores,* 2019; *1*.
84. Dubey RC, Maheshwari DK, Kumar V, Pandey RR. *Arch Phytopathol Plant Prot,* 2012; **45:**437-454.
85. Shirinbayan S, Khosravi H, Malakouti MJ. *Appl Soil Ecol,* 2019; **133:**138-145.
86. Romero-Perdomo F, Abril J, CameloMMoreno-Galván A, Pastrana I, Rojas-Tapias D, Bonilla R. *Rev Argent Microbiol*, 2017; **49:**377-383.
87. Kumar S, Bauddh K, Barman SC, Singh RP. *Ecol Eng,* 2014; **71:**432-437.
88. Chaudhary D, Narula N, Sindhu S S, Behl R K. *Physiol Mol Biol Plants,* 2013; **19(4):**515-519.
89. Chauhan S, Wadhwa K, Vasudeva M, Narula N. *Arch Agron Soil Sci,* 2012; **58:**1365-1385.
90. Rajaee S, Alikhani HA, Raiesi F. *Agris records,* 2007; **11(41):**285-297.