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Review

Azotobacter: A potential bio-fertilizer for soil and plant health management

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ABSTRACT

Stressor (biotic as well as abiotic) generally hijack the plant growth and yield characters in hostile environment leading to poor germination of the plants and yield. Among the plant growth promoting rhizobacteria, *Azotobacter* spp. (Gram-negative prokaryote) are considered to improve the plant health. Various mechanisms are implicated behind improved plant health in *Azotobacter* spp. inoculated plants. For example, acceleration of phytohormone like Indole-3-Acetic Acid production, obviation of various stressors, nitrogen fixation, pesticides and oil globules degradation, heavy metals metabolization, etc. are the key characteristics of *Azotobacter* spp. action. In addition, application of this bacteria has also become helpful in the reclamation of soil suggesting to be a putative agent which can be used in the transformation of virgin land to fertile one. Application of pesticides of chemical origin are being put on suspension mode as the related awareness program is still on. As far as the limitations of this microbe is concerned, commercial level formulations availability is still a great menace. Present review has been aimed to appraise the researchers pertaining to utility of *Azotobacter* spp. in the amelioration of plant health in sustainable agroecosystem. The article has been written with the target to gather maximum information into single pot so that it could reach to the dedicated researchers.

Contents

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1. Introduction

The utilization of plant growth promoting rhizobacteria (PGPR) in agriculture is continuously increasing as it offers an effective tool to replace the use of chemical fertilizers, pesticides and other harmful supplements (Ansari et al., 2017; Ansari and Mahmood, 2019ab). Growth promoting substances are produced in huge quantities by the action of these rhizosphere microorganisms that directly or indirectly influence the overall morphology and physiology of the crops. Recent advances in the field of sustainable development relies on the use and diversity of PGPR, their colonizing capability and the mechanism of action that may be used to facilitate their application as a dependable element in the management of sustainable agricultural system (Bhattacharyya and Jha, 2012; Di Benedetto et al., 2017; Ansari and Mahmood 2019a,b).

Azotobacter is a group of Gram negative, free-living, nitrogen fixing aerobic bacteria inhabiting in the soil. They are oval or spherical in shape and form thick-walled cysts (dormant cells resistant to deleterious conditions) under unfavorable environmental conditions. Around six species in the genus *Azotobacter* have been reported, some of which are motile by means of peritrichous flagella while others are immotile (Martyniuk and Martyniuk, 2003). They are typically polymorphic having size ranging from 2 to 10 μm long and 1 to 2 μm wide. The genus *Azotobacter* was recognized in 1901 by Dutch microbiologist, botanist and founder of environmental microbiology-Beijerinck and his co-workers as the first aerobic free-living nitrogen fixer. These bacteria are known to exploit atmospheric nitrogen for their cellular protein synthesis which is mineralized in the soil, imparting the crop plants a considerable part of nitrogen available from the soil source. *Azotobacter* spp. is sensitive to acidic pH, high salt concentration and temperature (Aquilanti et al., 2004). They pose advantageous impacts on the crop growth and yield through the biosynthesis of biologically active substances, instigation of rhizospheric microbes, production of phytopathogenic inhibitors, alteration of nutrient uptake and eventually magnifying the biological nitrogen fixation (Lenart, 2012). Research on *Azotobacter chroococcum* in crop production has shown its importance in improving plant nutrition and amelioration of soil fertility (Kurrey et al., 2018). Several strains of *Azotobacter* are found to be able to produce amino acids when grown in culture media supplemented with various carbon and nitrogen sources (González-López et al., 2005). Such substances produced by these rhizobacteria are implicated in several processes thus leading to plant-grown promotion (Jnawali et al., 2015). The scope of utilizing *Azotobacter chroococcum* in research experiments as microbial inoculant through release of growth substances and their impact on the plant has markedly improved crop production in agriculture (Gothandapani et al., 2017).

2. Plant growth promotion activities of *Azotobacter*

Despite a very rich literature regarding the use of *Azotobacter* in plant growth promotion is available yet, the exact mode of action behind the growth promoting activity of this bacterium is not fully explored. Several possible mechanisms have been proposed that include nitrogen fixation; growth hormone production as well as release of siderophores (Ansari et al 2017).

2.1. Growth hormone production

Growth substances, also known as plant hormones include natural substances produced by both the microorganisms as well as plants similarly. They impose either stimulatory or inhibitory impacts on some physiological and biochemical processes in microorganisms and plants also (Ansari and Mahmood 2019a,b). In-vitro studies by Brakel and Hilger (1965) exhibited that azotobacteria release indol-3-acetic acid (IAA) on the addition of tryptophan in to the medium whereas Hennequin and Blachère (1966) found that only small amounts of IAA were present in old cultures of Azotobacteria having no added tryptophan. In addition to auxin, gibberellins like compounds are also reported to be present in the culture of *A. chroococcum*. Brown et al. (1968) demonstrated the presence of three gibberellins like substances in a single strain of *A. chroococcum*. The quantity present in the 14-days old bacterial cultures ranged between 0.01 and 0.1 μg GA3 equivalent/ml. Moreover, Nieto and Frankenberger, 1989 identified five cytokinins in an *Azotobacter chroococcum* culture filtrate. *In vitro* presence of these plant growth promoting substances is further consolidated by the field experiments performed with various crops. Bacterial genus *Azotobacter* is reported to synthesize auxins, cytokinins, and GA-like substances that have been found to be directly associated with improved plant growth (Wani et al., 2013). Such hormones stem from the rhizosphere or root surface and impose positive effects on the growth of the higher plants growing in the nearby areas. Barakat and Gabr (1998), Puertas and Gonzales (1999), Baral and Adhikari (2013) and Akram et al. (2016) observed that plant dry weight of different crops like tomato, maize and chickpea was considerably greater on the application of *Azotobacter chroococcum* as compared to un-inoculated plants.

2.2. Nitrogen fixation

Nitrogen fixation comes among the most important biological processes and is considered as an interesting microbial activity on the earth's surface as it provides a way of recycling the nitrogen and plays an important role in nitrogen homeostasis in the biosphere (Wani et al., 2016). Moreover, biological nitrogen fixation also helps in maintaining soil fertility and improving crop productivity (Vance and Graham 1995). Azotobacteria are found to be useful organisms to be used as bioinoculants and for studying nitrogen fixation process by virtue of its ability to grow rapidly and fixing large amounts of nitrogen quickly. *Azotobacter* is able to convert atmospheric nitrogen to ammonia, which in turn is taken up and utilized by the plants (Prajapati et al., 2008). Such bacteria are immensely resistant to oxygen during nitrogen fixation due to respiration protection of nitrogenase (Hakeem et al., 2016). In addition to the respiratory protection there also exist hydrogenase uptake as well as switch on–off mechanisms for the protection of nitrogenase enzyme from oxygen (Chhonkar et al., 2009). Uptake of hydrogenase is involved in the metabolism of hydrogen (H_2) released during the process of nitrogen fixation (Partridge and Yates, 1982). The presence of optimum levels of calcium nutrient is necessary for the enhanced growth of *Azotobacter* and its ability to fix nitrogen (Iswaran and Sen, 1960) whereas, increased levels of nitrogen adversely affected the activity of Azotobacter (Soleimanzadeh and Gooshchi, 2013). Some reports suggest that *Azotobacter* has the efficiency of fixing about 20 kg N/ha/per year and thus can be applied successfully in crop production as an alternative for at least some part of mineral nitrogen fertilizers (Kizilkaya, 2009; Esmailpour et al., 2013). Various reports of reduced need of nitrogen fertilizers in crop plants inoculated with *Azotobacter* are available. Romero-Perdomo et al. (2017) reported that the application of mixed culture of *Azotobacter* starains could reduce the need of N-fertilizers up-to 50%.

2.3. Siderophore production

Siderophores constitute a group of iron (Fe) chelating molecules that alter the availability of Fe in the extracellular medium through its ability to outcompete other natural ligands (Wichard et al., 2009). Microbes utilize siderophores to reach the important iron resources in the environment. More than 500 siderophores are reported however, they use only a limited set of common moieties to hold iron. Bacteria belonging to genus *Azotobacter* express iron-rich nitrogenases, through which they reduce nitrogen (Baars et al., 2016). *Azotobacter* spp. gain access to the sparingly soluble Fe in the environment by making Fe-siderophore complex and then this complex is absorbed by membrane bound receptors (Palanché et al., 2004). Such Fe-siderophore complexes may not be available to other competing microorganisms thereby they may show anti-phytopathogenic activities and can directly improve plant growth by protecting plants from the pathogens attack (Hayat et al., 2010). Various other studies have demonstrated that the siderophores produced by *A. vinelandii* also consists the ability to bind metals other than Fe and allow the uptake of additional metals like molybdenum (Mo) or vanadium (V) that are needed in nitrogenases (Bellenger et al., 2008) and also to take up toxic heavy metals like W and Zn (Huyer and Page, 1988; Kraepiel et al., 2009). Moreover, siderophores of *A. vinelandii* have also been reported to help to flourish some freshwater algae in co-culture when a significant source of nitrogen is supplied to these microorganisms (Villa et al., 2014). Baars et al., 2016 carried out an elaborated characterization of siderophore metabolome and found over 35 metal binding secondary metabolites that pointed towards the large chelome of *A. vinelandii* that included vibrioferrin, previously known to occur only in marine bacteria. *A. chroococcum* is also reported to produce vibrioferrin and amphibactins in addition to a novel family of siderophores, the crochelins. Regardless of its value in agriculture, secondary metabolome of *A. chroococcum* is not completely known. Also, structures of siderophores as well as the mechanism by which *A. chroococcum* gains access to Fe which is needed to generate high levels of nitrogenases have not yet been determined (McRose et al., 2018).

3. Potentiality of *Azotobacter* in bioremediation

Bioremediation is an effective method for reducing anthropogenic pollution from the environment. Generally utilized methods for bioremediation primarily include the activation of native soil microflora that are able to consume contaminants or introducing efficient isolates of microorganisms into the contaminated soil. Free living nitrogen-fixing bacteria belonging to the genus *Azotobacter* constitute a major proportion of soil biota (Gradova et al., 2003).

3.1. Oil-contamination removal

Bacteria related to *Azotobacter* genus are reported to exploit a broad range of organic substrates like mannitol, various organic acids, benzoic acid, phenolic compounds of soil, etc. as a source of carbon and energy and form several biologically active compounds that instigate the proliferation of rhizospheric microorganisms (Onwurah and Nwuke, 2004). Thus, it's logical to consider that such bacteria may be useful in stimulation of bioremediation of oil-contaminated soils. Introduction of *Azotobacter* into oil-contaminated soil accelerate the rate of self-purification as the bacteria is able to assimilate oil hydrocarbons both in the presence of fixed nitrogen as well as during nitrogen fixation. *Azotobacter chroococcum* is found to activate proliferation of hydrocarbon-oxidizing bacteria existing in the microbial preparation like Devoroil (Gradova et al., 2003). Piperidou et al. (2000) studied an eco-friendly bioremediation system of olive oil mill wastewater (OMWW) by *Azotobacter vinelandii* in terms of its effect on physicochemical characteristics of OMWW and also the degradation capability of the bacterium on the characteristic constituents. The results obtained demonstrated the ability of *A. vinelandii* to proliferate in OMWW by using its own constituents hence transforming OMWW into an organic liquid fertilizer. Moreover, the system removed the phytotoxic principles from OMWW along with the stimulated growth of agriculturally important microbial communities.

3.2. Pesticide degradation

Microorganisms are effective degraders of pesticides in contaminated soils. Lindane, also known as Hexachlorocyclohexane (HCH) is among the most extensively utilized broad-spectrum organochlorine pesticides in India. It is reported to be a possible carcinogen (Walker and Morey, 1999). Pesticides applied to soil may be used as substrates by microorganisms and undergo degradation (Abo-Amer, 2011). The capability of *Azotobacter* sp. to use aromatic compounds has been known for several years. It is able to degrade the derivatives of aromatic compounds like benzoate, p-hydroxy benzoate, protocatechuic acid, 2,4-D,2,4,6-Trichlorophenol, etc. (Gahlot and Narula, 1996; Moreno et al., 1999). *Azotobacter* sp. has also been reported to degrade a range of other chlorinated phenols like 2-Chlorophenol, 4-Chlorophenol, 2,6-Dichlorophenol and 2,4-6-Trichlorophenol by *Azotobacter* sp. (Gaofeng et al., 2004). *A. chroococcum* significantly metabolized 2,4-dichlorophenoxyacetic acid (2,4-D) as a sole carbon source (Balajee and Mahadevan, 1993; Kumar et al., 2016). *A. vinelandii* growth rate was found similar in 2,4-D amended medium when compared with non-amended media (Ferrer et al., 1986). Selected strains of *A. chroococcum* have proven to be effective in lindane degradation, both *ex situ* and *in situ* at lower concentration like 10 ppm (Anupama and Paul, 2009). However, at higher concentration of lindane, efficiency of bacteria to degrade it was found reduced. This may be due to the fact that at higher concentrations lindane exert inhibitory impact on bacterial growth (Ergüder et al., 2003). Kole et al. (1994) demonstrated that *A. chroococcum* is able to transform a popular herbicide, pendimethalin into non-toxic products, thereby establishing the fact that the bacterium is essential not only for vigorous crop production but also for the environmental harmony.

3.3. Heavy metal tolerance

The soil microbial community faces extremely high pressure due to adulteration of soil by a range of toxic materials including heavy metals along with other organic contaminants of wastewater, sewage sludge etc. The addition of heavy metals in several forms in the environment results in significant alterations of the microbial diversity and activities, thus directly affecting the soil fertility (Smith and Giller, 1992). Some of the heavy metals are necessary for microbial growth and biochemical reactions in very low concentrations in the cell. However, as the heavy metal concentration increase it becomes largely toxic to microorganisms thereby leading to disturbance in vital ecological processes (Afef et al., 2011). Contamination of the environment by heavy metals has resulted in the manifestation of heavy metal tolerant microorganisms in the soil polluted with metals (Piotrowska-Seget et al., 2005). In addition, such heavy metals, once in the soil, accumulate preferentially in the parts where the plant roots are aggregated and in the forms that are easily available to plants. These heavy metals are then absorbed by the plants thus, ultimately entering the food chain. Microorganisms use different kinds of mechanisms related to resistance and detoxification of heavy metals (Nies, 2003) thus play prominent part in biogeochemical cycling of harmful heavy metals leading to the remediation of metal-contaminated environments (Jing et al., 2007; Abo-Amer et al., 2013; Mohamed and Abo-Amer, 2012). Abo-Amer et al. (2014) demonstrated that among *Azotobacter* isolates extracted from the soil contaminated with wastewater, 10 strains exhibited considerable degree of resistance to the heavy metals like Co^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+}. The study thereby highlighted the possible utilization of such bacterial isolates for the bioremediation of metal-contaminated system. Studies by Joshi and Juwarkar (2009) revealed that a heavy metal-resistant strain of *Azotobacter* spp. possess a high tendency of binding with Cd and Cr both under in vitro as well as in vivo conditions, and thereby consists of significant control of their uptake by wheat plants raised in heavy metal polluted soils. Resistance to heavy metals in *Azotobcter* spp. is demonstrated to be provided by plasmids (Robson et al., 1984). But, in case of *Azotobacter* species particularly, prior to the entry of heavy metal into the cell, they face an encounter with extracellular polymeric substances, which are reported to be produced in large amounts by this bacterium (Gorin et al., 1961). Extracellular polymeric substances thus clearly play the role of first barrier by chelating the metal ions and restricting their access into the bacterial cells.

3.4. Azotobacter and saline environment

Among the various abiotic stress's salinity is considered to be major abiotic stressor which undermines the plant health and wellbeing (Yang et al., 2009). Salinity causes great interruption in water and ionic movement of plant cells that hampers the plant growth, morphology, physiology and other activity of plant life leading to death of plant's life (Maggio et al., 2007). There are various activities including anthropogenic that cause the soil salinization, however, primary cause is natural processes which offers marked amount of salt accumulation in soil and groundwater (Pitman and Lauchli, 2002; Rengasamy, 2002).

To obviate the abiotic stresses, scientists are working enthusiastically to find out some conducive solution. Among, beneficial microorganisms are surmised to be the putative agent to be used for the purpose. They influence the growth and biochemical markers and also help them to accelerate the production of some organic molecules that immune the plants against various abiotic stresses. In addition, plant growth promoting beneficial bacteria (PGPBB) have been found to improve the plant health status by obviating various biotic and abiotic stresses (Ansari and Mahmood 2019a,b). There is an implication of various mechanisms occurring at the soil-plant-microbe interfaces that regulate the plant growth and yield performance. Application of PGPBB are considered very important in the plant health improvement through bypassing the stresses in hostile environments (Yang et al., 2009). *Azotobacter* spp. (nitrogen-fixing bacterial strains) are currently being used successfully in the sustainable agriculture at large scale (Islam et al., 2013). *Azotobacter* spp. are characterized by nitrogen fixation, siderophore production, IAA and exopolysaccharide production that improve the plant health and indol-3-acetic acid and exopolysaccharides (EPS) production (Gauri et al., 2012). There are various other facets of *Azotobacter* spp. in addition to prominent characteristics that enhance the tolerance index of the plant in hostile environment (Ruzzi and Aroca, 2015).

4. Role of *Azotobacter* in plant disease management

In addition to its beneficial impact on plant growth promotion, *Azotobacter* is also known to be associated with the suppression of pathogenic diseases of plants. Several examples are present in the literature advocating the importance of disease suppression by different species of *Azotobacter*. Maheshwari et al. (2012) demonstrated that the strain TRA2 of *A. chroococcum* which is an isolate of wheat rhizosphere showed strong antagonistic activity against root rot fungus *Macrophomina phaseolina* and *Fusarium oxysporum*, in addition to improving plant growth of wheat which might be due to ameliorated plant health. *Azotobacter* provided good protection to the plants by aggressively colonizing the roots of wheat crops. Akram et al. (2016) found that disease incidence by root-knot nematode *Meloidogyne incognita* was significantly reduced when *A. chroococcum* was applied to chickpea plants. Several mechanisms can be implicated behind the management strategies used by the bacteria for the control of plant diseases. These may include the production of siderophores, antimicrobial substances, toxins and also the growth hormones like auxins, gibberellins and cytokinins. However, no single mechanism can be held completely responsible for the disease suppression and more than one way could be used by the bacteria depending upon the bacterial strain, environmental conditions, pathogen involved and also the target. Such strategies used by the bacteria have been demonstrated to impart major resistance towards the attack of the plant pathogens. Verma et al. (2001) demonstrated the in vitro production of antimicrobial/antifungal substances by different strains of *A. chroococcum*. They found that only 37% of the total strains were able to inhibit the growth of *Rhizoctonia solani* and about 25% against *Xanthomonas campestris*. Moreover, regarding the nature of the antimicrobial substances, it was revealed that most of the antimicrobial substances were extracellular and only few were found to be bound to the cell wall. *Azotobacter* spp. have the ability to produce siderophores that bind to the available form of iron Fe^{+3} in the rhizosphere, thereby depriving the phytopathogens from iron availability and protecting the plant health. *Azotobacter* is reported to produce an antibiotic having similar structure as that of anisomycin, which is well established fungicidal antibiotic. Some examples of the pathogens that have been managed by the use of *Azotobacter* as a bioinoculant includes *Alternaria, Fusarium, Rhizoctonia, Macrophomina, Curvularia, Helminthosporium* and *Aspergillus* (Jnawali et al., 2015).

5. Current trend in utilization of *Azotobacter* as potent biofertilizer

As the *Azotobacter* is a non-symbiotic microbe, its maximum potential to enhance plant productivity can be exhausted by co-inoculating it with some other biofertilizers as compared to its single application. In addition to directly benefitting the plants through enhanced mineral uptake, *Azotobacter* also accelerate beneficial activities of other biofertilizers, if used in consortium. Moreover, reports of other microorganisms enhancing the plant growth activity of *Azotobacter* are also available. Currently, several reports of *Azotobacter* being utilized along with other microbes are found to be highly preferable among researchers as well as farmers.

5.1. Consortium of eukaryotes and prokaryotes (Azotobacter plus various biocontrol fungi)

Among the fungal biofertilizers, phosphate solubilizing mycorrhizal fungi has reported to make best consortium with *Azotobacter* in enhancing plant growth attributes (Behl et al., 2003). Synergistic interaction between the free-living nitrogen fixing bacteria, *Azotobacter* and AM fungus, *Glomus* are reported by several workers (Ishac et al., 1986; Akram et al., 2016). Bagyaraj and Menge (1978) studied the impact of inoculating tomato plants with *Glomus fasciculatum* and *Azotobacter chroococcum* either individually or concomitantly on the population of rhizospheric bacteria. They recovered highest populations of bacteria (including actinomycetes) from the rhizosphere of tomato plants inoculated with both *G. fasciculatum* and *A. chroococcum* as compared to the plants inoculated with either *G. fasciculatum* or *A. chroococcum* alone. The inoculation of tomato plants with *G. fasciculatum* enhanced *A. chroococcum* population in the rhizosphere which remained maintained at a high level for a longer time. On the other hand, inoculating tomato roots with *A. chroococcum* increased the infection and spore production by *G. fasciculatum*. The dry weight of tomato plants was found to be significantly increased in the plants inoculated with both *G. fasciculatum* and *A. chroococcum* when compared to non-inoculated plants. Behl et al. (2003) observed similar effects of the dual inoculation of AM fungus and *Azotobacter* in wheat. Aseri et al. (2008) observed that pomegranate (*Punica granatum*) plants were better able to survive under stressed environmental conditions when applied with a mixture of *A. chroococcum* and *Glomus mosseae*. Study conducted by Arora et al. (2018) indicated that AM fungus *Piriformospora indica* and *A. chroococcum* combinedly formed a mutualistic symbiosis in *Artiemisia annua* L. resulting in an improved plant physiological and biochemical attributes resulting in enhanced artemisinin content.

5.2. Development of bacterial consortium

Positive responses from the crops co-inoculated with *Azotobacter* and *Rhizobium* has been recorded from various crop plants under laboratory, greenhouse as well as field conditions (Wani and Gopalakrishnan, 2019). While *Azotobacter* is able to produce growth hormones like auxins and gibberellins and thus enhancing root growth, it in turn could make available more root area to rhizobia for infection. This would result in increased nodulation, nitrogen fixation and ultimately crop yield improvement (Verma et al., 2014). Synergistic effect of *A. chroococcum* and *Bradyrhizobium* on mung bean (*Vigna radiate*) has been observed by Yadav and Vashishat (1991) while that on chickpea was observed by Siddiqui et al. (2014). Another bacterium that is commonly reported to be symbiotically related to *Azotobacter is Azospirillum*. Positive reports on the inoculation of *Azotobacter + Azospirillum* on the yield of chick pea (*Cicer arietinum*) (Parmar and Dadarwal 1999), mustard (*Brassica juncea*) (Tilak and Sharma 2007), rapeseed (*Brassica napus* L) (Yasari et al., 2009) and chilli (*Capsicum annum* L.) (Khan et al., 2012) are available**.** In addition to being useful in improving plant growth and yield attributes, coinoculation of *Azotobacter* and *Azospirillum* have also been found to alleviate the adverse effect of salinity stress on some plants. Yousefi et al. (2017) observed that seeds of hopbush shrub (*Dodonaea viscosa* L.) inoculated with *Azospirillum + Azotobacter* and exposed to salinity stress, showed enhanced germination percentage and improved plant growth parameters. Thus the advantages of co-inoculating *Azotobacter* and *Azospirillum* to a crop mainly depends on their capacity to improve root development, rate of water and mineral uptake, biological nitrogen fixation, antagonistic impact on plant pathogens like fungi bacteria and nematodes and to a lesser extent by the alleviation of abiotic stress on plants (Okon and Itzigsohn, 1995).

6. Molecular approaches to improve bio-fertilization properties of *Azotobacter*

Azotobacter spp. have been recommended to be used as biofertilizers to replenish the nitrogen level (Gauri et al., 2012). While improving nutritional properties of *Azotobacter* as bio-fertilizer, it is essential to consider cost effective technique that can provide cheaper source of biofertilizer to agriculture industry. When considering the large-scale production of *Azotobacter*, it is necessary to optimize cultural and nutritional parameters in order to enhance its growth in fermentation as well as to enhance its ability as biofertilizer (Gomare et al., 2013). Biotechnological and industrial interest in bacterial inoculants and polymers produced by them has been amplified due to their useful properties and scope to make new substances that can be utilized as much effective tonic for soil and plant health management. *A. vinelandii* have great importance in biotechnological applications due to their ability to produce important biological molecules namely poly-β-hydroxybutyrate (PHB), the exopolysaccharides (EPS) and siderophores (Diaz-Barrera and Soto, 2010). With the help of genome editing either addition or deletion of targeted gene(s), the nitrogen fixation ability of *A. vinelandii* can be dramatically enhanced. The targeted gene manipulation is carried out in such a way that the urea from common metabolites is converted into terminal products (Barney et al., 2015). There are various cogent methods which can be executed to enhance the ammonium levels excreted by *A. vinelandii* by disrupting the *nifL* gene from the *nifLA* operon system (Bali et al., 1992; Brewin et al., 1999; Ortiz-Marquez et al., 2012). In addition, there is much variation in soil ecology of different regions. This leads to the inability of a single strain of *Azotobacter* to be most effective in all the regions and could not be applied universally as a biofertilizer. Keeping in mind the importance of EPS and other compounds produced in the establishment of the bacterium in the agricultural soil, *Azotobacter* strain with characters like highest ability to fix nitrogen and better production of such compounds should be taken into account. Besides, further research to advance the understandings by manipulation of these properties according to the needs of human kinds may be of much consideration in next generation agriculture (Gauri et al., 2012).

7. Future prospects and possibilities in commercialization of *Azotobacter*

Owing to its ability to improve plant health through nitrogen fixation, growth hormone production, phosphate solubilization, plant disease management and reclamation of better soil health, *Azotobacter* is one of the best options to be used as biofertilizer for eco-friendly and sustainable crop production. Understanding and manipulating all these beneficial properties of *Azotobacter* may prove to be a key interest for the future endeavors of crop improvement (Kyaw et al., 2019). However, there is an urgent need to carry out more studies related to improving screening techniques, isolation and characterization of plant growth promoting and antimicrobial compounds from the bacterial isolates and elucidation of the molecular basis of mechanisms involved (Verma et al., 2010). Moreover, further research related to the exploration of the potential of *Azotobacter* in improving soil fertility is also essential by utilizing modern technology of soil genomics etc. (Wani et al., 2016**).** To ensure the extraction of maximum benefits from the bio-fertilizer, a challenge to research community is to find out compatible partners i.e. a particular strain of Azotobacteria will form a good association with a particular plant genotype (Wani et al., 2013). In future, these free-living nitrogen fixing bacteria are supposed to supplant the agrochemicals which impose a variety of side effects to sustainable agriculture.

8. Conclusion and future research

Application of *Azotobacter* spp. can be very beneficial in the removal of various stresses. Introduction of putative strains is also carried out to improve the soil physical and chemical properties. The microbiome of rhizosphere is also manipulated in the presence of suitable strains which is considered to be very beneficial in the plant health improvement. Use of *Azotobacter* spp. in various field crops has advocated and justified that it has obviated the plant stressors of various origins. Acceleration of biosynthesis of various beneficial organic molecules in plant body has strengthened the plants and enabled them to fight against the stressors. However, extensive research is still needed to elucidate the exact mechanisms implicated into how *Azotobacter* spp. obviate the stressors and ameliorate the plant health. In capsule, *Azotobacter* spp. could ameliorate the stresses of various agricultural crops which are developed due to the biotic and abiotic agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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