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ROLE OF BIOFERTILIZERS IN INTEGRATED PLANT NUTRIENT SYSTEM (IPNS)

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ABSTRACT

In India, framers doing intensive agriculture which led to rapid depletion of nutrient in agricultural land which is balanced by use of chemical based fertilizers. Chemical fertilizers not only disturb ecological balance but also pose health hazards besides these are quite expensive, bringing the cost of production very high. Farmers use to indulge money in purchasing costly fertilizers. So been an agriculturist we have to focus on alternative of fertilization which maintain ecological balance as well as reduce farmers expenses. Biofertilizers are low cost and eco-friendly inputs and have tremendous potential for supplying nutrients, especially N and P, and can reduce the chemical fertilizer dose by 25-50%. This review helps towards use of biofertlizers in sustainable agriculture and reducing problems associated with the use of chemicals fertilizers.

Keywords: Biofertilizer, Chemical fertilizer, Integrated nutrient management, Sustainable agriculture

Introduction

In the last century, with the concept of intensive agriculture the use chemical fertilizers is more and this made farmers to be happy of getting increased yield in agriculture in the beginning. But slowly chemical fertilizer started displaying their ill effects such as leaching, polluting water basins, destroying microorganisms and friendly insects, making the crop more susceptible to the attack of diseases, reducing the soil fertility, pose health hazards besides these are quite expensive, bringing the cost of production much higher and thus causing irrepairable damage to the overall system. Chemical fertilizers are industrially manipulated, substances composed of known quantities of nitrogen, phosphorus and potassium, and their exploitation causes air and ground water pollution by eutrophication of water bodies (Youssef *et al*; 2014). In general, 60% to 90% of the total applied fertilizer is lost and the remaining 10% to 40% is taken up by plants. The results of long-term fertility experiments have also clearly demonstrated that chemical fertilizers alone cannot sustain the productivity at current level under intensive cropping systems (Swarup *et al*. 1998).

International Plant Nutrition Institute has confirmed that Indian Soils are under nutrient crisis and also concluded that in the absence of nutrient balance sheet of Indian Agriculture will continue to be negative (TNAU, 2009). This necessitated the scope to find out suitable alternative nutrient combinations for different crops to overcome the burden of chemical fertilizers and for achieving the higher productivity. Currently, the biological approaches for improving crop production are gaining strong status among agronomists and environmentalists following integrated plant nutrient management system (Ahemad and Kibret, 2014). So, there is urgent need to encourage alternate means of soil fertilization relies on organic inputs to improve nutrient supply and conserve the field management (Araujo et al., 2008). Minerals, organic components and microorganisms are three major solid components of the soil. They profoundly affect the physical, chemical, and biological properties and processes of terrestrial systems. Organic manures are helpful to improve the physical and chemical properties which results into increasing soil fertility and productivity (Patil et al., 2013). Organic manure greatly reduces leaching of fertilizer, pesticide and herbicides into the ground water. Organic manure improves the activity of earthworm and other soil micro flora and it increases soil infiltration rate and reduces soil evaporation there by it increases soil water storage. The organic manure influences

agricultural sustainability by enhancing productivity (Singh et al., 2016). Another important component of soil is beneficial microorganism which are abundant in soil as well as in biofertilizers. A major focus in the coming decades would be on safe and eco-friendly methods by exploiting the beneficial micro-organisms in sustainable crop production (Nina, 2014). Biofertilizers are low cost and eco-friendly inputs and have tremendous potential for supplying nutrients, especially N and P, and can reduce the chemical fertilizer dose by 25-50% (Vance 1997; Rana et al., 2012).

According to the definition proposed by Vessey [2003], biofertilizers are substances which contain living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant, and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubalisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha, 2014). Through the use of bio-fertliizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. It can also provide protection against drought and some soil-borne diseases. It act as eco-friendly and cost effective inputs for the farmers ((Nilabja Ghosh, 2004, Mohammadi, K., and Yousef, S. 2012). The role and importance of biofertilizers in sustainable crop production has been reviewed by several authors (Biswas et al. 1985; Wani and Lee, 1995; Katyal et al. 1994). Thus, biofertilizers can be important components of integrated nutrients management. Integrate nutrient management plays a key role in modern agriculture in increasing the productivity of crops and sustained management of soil fertility (Singh, G. et al. 2016). They hold vast potential in meeting plant nutrient requirements while minimizing the use of chemical fertilizers. It is important to realise the useful aspects of biofertilizers and implement its application to modern agricultural practices. The new technology developed using the powerful tool of molecular biotechnology.

In order to meet the food requirements of ever increasing population, the fertilizer requirement for crop production is very high. Biological nitrogen fixation can be the key to fill up this gap because of high cost and several other demerits of chemical fertilizers. For production of a good and efficient biofertilizer, first of all an efficient strain is required, then its inoculums (the form in which the strain is to be applied in fields) is produced. Several

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microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function **Table-1**. While producing bio-fertilizers the standards laid down by BIS have also to be kept in mind for making the product authentic. Inoculation of *Rhizobium* and application of fertilizer improved the yield attributes grain and straw yield (Balchandran and Nagarajan, 2002). Several reports have indicated that biofertilizers like *Azotobacter* and PSB alone or in combination with chemical fertilizers have great prospect in increasing productivity of wheat (Kumar and Ahlawat 2004).

Groups	Examples		
N fixing biofertilizers			
Free living	Azotobacter, Beijerinkia, Clostridium, Klebsiella,		
	Anabaena and Nostoc		
Symbiotic	Rhizobium, Frankia and Anabaena azollae		
Associative symbiotic	Azospirillum		
P solubilising biofertilizers			
Bacteria	Bacillus megaterium, phosphaticum, Bacillus subtilis,		
	B. circulans and Pseudomonas striata		
Fungi	Penicillium sp. and Aspergillus awamori		
P mobilizing biofertilizers			
Arbuscular mycorhiza	Glomus sp., Gigaspora sp., Acaulospora sp.,		
	Scutellospora sp. and Sclerocystis sp.		
Ectomycorrhiza	Laccaria sp., Pisolithus sp., Boletus sp. And Amanita		
	sp.		
Ericoid mycorrhiza	Pezizella ericae		
Orchid mycorrhiza	Rhizoctonia solani		
Biofertilizers for micro nutrients			
Silicate and Zinc solubilizers	Bacillus sp.		
Plant growth promoting rhizobacteria			
Pseudomonas	Pseudomonas fluorescens		

Table 1: Table showing different groups of microorganism and their example

N-fixing Biofertilizers (NBF): Bio-fertilizers are the formulations of living microorganisms which are able to fix atmospheric nitrogen in the available form for plants (nitrate form) either by living freely in the soil or associated symbiotically with plants. Although nitrogen fixers are present in the soil, enrichment of soil with effective microbial strains (**Table 2**) is much beneficial for the crop yields.

Table 2: Some major nitrogen fixing microorganisms and beneficiaries plant

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1	Rhizobium spp. living symbiotically in root nodules	All grain legumes (pulses), some oil
		yielding (soybean, groundnut), some
		fodder legumes (rizka and berseem)
2	Nostoc, Anabaena, Aulosira and others (free living blue green	Rice
	algae)	
3	Anabaena azollae living symbiotically with the waterfern	Rice, Azolla spp.
4	Azotobacter chroococcum (free living bacterium)	Rice, maize, cotton and others
5	Frankia spp. (actinomycete) living symbiotically in nonlegume	Alnus, Casuarina and others
	root nodules	
6	Azospirillum spp. (associate symbiont) Bacillus polymyxa,	Maize, sorghum, pearl-millet, finger
	Clostridium spp., Rhodospirillum spp.	millet and others non-specific hosts

(i) Azotobacter (Family: Azotobacteriaceae)

It is the important and well known free living nitrogen fixing aerobic bacterium, belongs to family Azotobacteriaceae. It is used as a biofertilizer for all non leguminous plants especially wheat, rice, sugarcane, cotton, vegetables, oilseeds such as mustard, linseed and for the millets such as pearl millet, finger millets, and kodomillets etc. The Azotobacter colonizing the roots not only remains on the root surface but also a sizable proportion of it penetrates into the root tissues and lives in harmony with the plants. Azotobacters are present in neutral or alkaline soils and A. chroococcum is the most commonly occurring species in arable soils. A. vinelandii, A. beijerinckii, A. insignis and A. macrocytogenes are other reported species. The lack of organic matter in the soil is a limiting factor for the proliferation of Azotobacter in the soil. The numbers of A. chroococcum in Indian soils rarely exceeds 10^{5} /g soil due to lack of organic matter and the presence of antagonistic microorganisms in soil. They can fix N up to 25 kg/ha under optimum conditions and increase yield up to 50%. The bacterium produces abundant slime which helps in soil aggregation. They also produces certain substances good for the growth of plants and antibodies that suppress many root pathogens. They improve seed germination and plant growth by producing B-vitamins, NAA, GA and other chemicals (plant hormones) that are inhibitory to certain root pathogens (Mazid et al., 2011f). Azotobacter have been found to produce some antifungal substance which inhibits the growth of some soil fungi like Aspergillus, Fusarium, Curvularia, Alternaria, Helminthosporium, Fusarium etc.

Field trials carried out in different locations have demonstrated that under certain environmental and soil conditions inoculation with azotobacteria has beneficial effects on plant yields (Mazid et al., 2011d). For sugarcane, *Azotobacter indicum* is suitable in acidic sols in which it forms *rhizo bacteriocoenotic* association with roots and application in soil is economical but a large amount of organic C- and Mo is needed for stimulating nitrogenase enzyme activity

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during N fixation (Mazid et al., 2012b; Khan et al., 2012a). Bahadur *et al.* 2013 reported similar increases in grain and straw yield of wheat due to *Azotobacter* and PSB application in combination with chemical fertilizers indicating increases in N and P uptake by wheat due to *Azotobacter* and PSB biofertilizers due to N_2 fixation and P solubilization in soil.

ii Symbiotic: Rhizobium (Family: Rhizobiaceae)

They belong to family Rhizobiaceae, symbiotic in nature, fix nitrogen 50-100 kg/ ha with legumes only. The morphology and physiology of Rhizobium will vary from free-living condition to the bacteroid of nodules. Rhizobium has the ability to fix atmospheric N- in symbiotic association with legumes and certain non legumes like, Parasponia (Saikia and Jain, 2007). The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of Rhizobium.

Pulse crops have unique properties of nodulation through *Rhizobium* bacteria. In recent years use of Rhizobium culture has been routinely recommended as an input in pulse cultivation. In India about 30 million hectares of land is under pulses cultivation. It is useful for pulse legumes like chickpea, red-gram, pea, lentil, black gram, etc., oil-seed legumes like soybean and groundnut and forage legumes like berseem and lucerne. It colonizes the roots of specific legumes to form tumour like growths called root nodules, which act as factories of ammonia production. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group, detailed information was given in **Table 3**. The appropriate strain can increase the crop yield up to 10-35% since N is fixed at 40-200 kg/ha which is able to meet up to 80-90% of N need of the crop (Verma 1993). Also, residual N is beneficial for the next crops grown in the same field. Jain *et al.* (2007) reported that *Rhizobium* along with micronutrients significantly enhanced the P and N uptake as compared to control in mungbean.

Table 3: Cross inoculation group of different rhizobia species.

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Rhizobium sp.	Cross inoculation groups	Legume types
R. leguminosarum	Pea group	Pisum, Vicia, Lens
R. phaseoli	Bean group	Phaseolus
R. trifoli	Clover group	Trifolium
R. lupini	Lupini group	Lupinus, Orinthopus
R. japonicum	Soyabean group	Glycine
R. meliloti	Alfalfa group	Melilotus, Medicago, Trigonella
Rhizobium sp.	Cowpea group	Vigna, Arachis

(iii) Associative symbiotic: Azospirillum

Azospirillum is an associative symbiotic nitrogen fixing bacteria. The bacteria of Genus Azospirillum are N_2 fixing organisms isolated from the root and above ground parts of a variety of crop plants. Five species of Azospirillum have been described to date A. brasilense, A.lipoferum, A.amazonense, A.halopraeferens and A.irakense. It fixes the considerable quantity of nitrogen in the range of 20- 40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton etc. Azospirillum lipoferum and A. brasilense are primary inhabitants of soil, the rhizosphere and intercellular spaces of root cortex of graminaceous plants. Apart from nitrogen fixation, growth promoting substance production (IAA), vitamins, nicotinic acid, gibberllins, disease resistance and drought tolerance are some of the additional benefits such as better germination, early emergence and better root development.

(iv) Azolla Family: Azollaceae

Azolla is a free floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green algae *Anabaena azollae* a cyanobacterium. Azolla fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. *Azolla* is considered to be a potential biofertilizer for wetland rice and it is known to contribute 40-60 kg N/ha per rice crop. Rice growing areas in South East Asia and other third World countries have recently been evincing increased interest in the use of the symbiotic N_2 fixing water fern *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. For green manuring, Azolla is sown in the field or in a separate shallow pond. Water is drained off the field and Azolla is incorporated into the soil before transplanting of paddy. Dried inoculum of Azolla is also presoaked in 50 ppm of superphosphate solution for 12 h and inoculated in the paddy filed (Kannaiyan, 2002). One kg of

it fixes 40-55 kg N/ha, 15-20 P/ha and 20-25 kg K/ha in a month, thus increasing yield of flooded paddy by 10-20% (Ghosh, 2004).

(v) Blue Green Algae (BGA)

Most N fixing BGA are filamentous, consisting of chain of vegetative cells including specialized cells called heterocyst which function as micro nodule for synthesis and N fixing machinery. BGA forms symbiotic association capable of fixing N- with fungi, liverworts, ferns and flowering plants, but the most common symbiotic association has been found between a free floating aquatic fern, the Azolla and Anabaena azollae (BGA). These phototropic prokaryotic bacteria are effective only in submerged paddy in presence of bright sunlight by forming a bluish-green algae on standing water and by converting the insoluble P into soluble forms, fixing N to the tune of 2-30 kg/ha thereby raising the crop yield by 10-15% when applied at 10kg/ha/BGA biomass. They also produce indole acetic acid (auxin) and gibberllic acid. They too add growth-promoting substances including vitamin B12, improve the soil's aeration and water holding capacity and add to bio mass when decomposed after life cycle.

Phosphate Solubilizing Microorganisms

Phosphorus (P), the second important plant growth limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms (Khan et al., 2009). Despite of large reservoir of P, the amount of available forms to plants is generally low (Ezawa et al., 2002). This low availability of phosphorous to plants is because the majority of soil P is found in insoluble forms, while the plants absorb it only in two soluble forms, the monobasic (H2PO⁻⁴) and the diabasic (HPO₂⁻⁴) ions (Bhattacharyya and Jha, 2012). To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil (Mckenzie and Roberts, 1990). Likewise, phosphate solubilizing bacteria have the capability to solubilize the residual or fixed soil P, increase the availability of P in the soil produce growth promoting substances (Selvakumar *et al.*, 2009), and thereby increase the overall P- use efficiency of the crops. Phosphate to plants by solubilizing inorganic as well as organic phosphates (Saxena and Sharma, 2007). This process of phosphates solubilization is

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associated to the production and release of organic acids of low molecular weight. It is accepted fact that their carboxyl and hydroxyl groups inter-chelate in the phosphate bound cations, resulting in their conversion to soluble forms (Alam *et al.*, 2002). Phosphate solubilizing bacteria are also produce plant growth hormones, participate in bio control activities and effect the process of nitrogen fixation (Neelam and Meenu., 2003). *Bacillus, Achromobacter, Agrobacterium Micrococcus, Burkholderia, Erwinia, Flavobacterium, Pseudomonas*, and *Rhizobium*, are some well-known phosphate-solubilizing bacterial strains which are used as biofertilizers and bio-control agents for agriculture improvement (Tamilarasi et al., 2008; Srivastava and Shalini 2009).

Sundara et al. (2002) applied rock phosphate with a PSB (Bacillus megaterium var. phosphaticum) in lignite-based culture medium in a field experiment. They found that without P application PSB amendment could increase sugarcane yield by 12.6 percent. PSB and P fertilizer together reduced the P requirement by 25 percent. PSB also improved the sugar yield and juice quality. In conclusion, PSB may be of greatest value in allowing use of cheaper P sources (e.g., rock phosphate instead of superphosphate). Singh and Pareek (2003) reported that combined inoculation of *Rhizobium* + PSB significantly increased the nitrogen and phosphorus content in grain and stover, N and P uptake kg ha-1 over control. Yadav et al. (2007) observed that highest grain yield (12.49 q ha⁻¹) grains pod⁻¹ (13), test weight (42 g) and maximum number of nodules (36) were recorded with application of *Rhizobium* and PSB + P₂O₅ @ 75 kg ha⁻¹ + poultry manure at 5 t ha⁻¹ in green gram. Singh *et al.* (2004) conducted a field experiment on sandy loam soil at Jobner and revealed that the both *Rhizobium* and PSB inoculation significantly increased the N, P content and its uptake in grain and Stover of greengram over single inoculation.

Mycorrhizae

Symbiosis between plant roots and certain soil fungi e.g. Vesicular Arbuscular Mycorrhiza (VAM) plays an important role in phosphorus cycling and its uptake by plants (Biswas et al., 2001). These symbiotic micro-organisms have extensive mycelial network and can increase the transport of other mineral elements such as zinc and copper. VAM fungi can play an important role in enhancing P availability to plants in deficient soils and can save P-fertilizer by 25-30% (Somani *et al.*, 1990). Additional benefits from the mycorrhizal symbiosis include increased tolerance of heavy metal contamination or drought, as well as lesser

susceptibility to root pathogens or herbivory. Mycorrhizal fungi may also improve soil quality by having a direct influence on soil aggregation (Rillig et al., 2002) and therefore aeration and water dynamicsThis is of two types, the ectomycorrihae, is found in trees and is beneficial for forest tress whereas, the second one is endomycoorhizae which is common in crop plants.

In India, Tata Energy Research Institute (TERI), New Delhi and Forest Research Institute, Dehradun have established mycorrhizae banks. Inocula of these can be procured as needed and used in horticulture and forestry programmes.

Silicate solubilizing bacteria (SSB)

Microorganisms are capable of degrading silicates and aluminum silicates. During the metabolism of microbes several organic acids are produced and these have a dual role in silicate weathering. The studies conducted with a *Bacillus* sp. isolated from the soil of granite crusher yard showed that the bacterium is capable of dissolving several silicate minerals under *in vitro* condition. The examination of anthrpogenic materials like cement, agro inputs like super phosphate and rock phosphate exhibited silicate solubilizing bacteria to a varying degree. The bacterial isolates made from different locations had varying degree of silicate solubilizing potential. Rice responded well to application of organic sliceous residue like rice straw, rice husk and black ash @ 5 t/ha. Combining SSB with these residues further resulted in increased plant growth and grain yield. This enhancement is due to increased dissolution of silica and nutrients from the soil.

Zinc solubilizers

Zinc being utmost important is found in the earth's crust to the tune of 0.008 per cent but more than 50 per cent of Indian soils exhibit deficiency of zinc with content must below the critical level of 1.5 ppm of available zinc (Katyal and Rattan, 1993). The plant constraints in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate (ZnSO₄). But the fate of applied zinc in the submerged soil conditions is pathetic and only 1-4% of total available zinc is utilized by the crop and 75% of applied zinc is transformed into different mineral fractions (Zn-fixation) which are not available for plant absorption (crystalline iron oxide bound and residual zinc) (Alloway, 2008). The zinc can be solubilized by

microorganisms viz., *B. subtilis, Thiobacillus thioxidans* and *Saccharomyces* sp. These microorganisms can be used as bio-fertilizers for solubilization of fixed micronutrients like zinc (Raj, 2007). The results have shown that a *Bacillus sp.* (Zn solubilizing bacteria) can be used as bio-fertilizer for zinc or in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate (ZnCO₃) and zinc sulphide (ZnS) instead of costly zinc sulphate (Mahdi et al. 2010).

Plant Growth Promoting Rhizobacteria (PGPR)

The group of bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR) (Subbarao 1999; Wu et al., 2005; Heidari et al., 2011). Alternatively, Somers et al. (2004) classified PGPR based on their functional activities as (i) biofertilizers (increasing the availability of nutrients to plant), (ii) phytostimulators (plant growth promotion, generally through phytohormones), (iii) rhizoremediators (degrading organic pollutants) and (iv) biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites) (Antoun and Pre'vost, 2005). Furthermore, in most studied cases, a single PGPR will often reveal multiple modes of action including biological control (Kloepper, 2003; Vessey, 2003). However, in accordance with their degree of association with the plant root cells, PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR). The ePGPRs may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex. For eg. Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas and Serratia etc. iPGPRs locates generally inside the specialized nodular structures of root cells. For eg. Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium and Rhizobium of the family Rhizobiaceae. The potentiality of PGPR in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Several PGPR formulations are currently available as commercial products for agricultural production of beneficial crops. Bertrand et al. (2000) showed that a rhizobacterium belonging to the genus Achromobacter could enhance root hair number and length in oilseed rape (Brassica napus). Achromobacter increased NO3 and K uptake and, consequently, shoot and root dry weights by 22 to 33 percent and 6 to 21 percent respectively.

K-Solubilizing Bacteria

Bacteria such as *Frateuria aurantia* are capable of mobilizing mixture of K into a usable form to the plants known as K solubilizing bacteria, applied to all crops in association with other Biofertilizers without any antagonistic effect. However, while positive responses have been observed in a wide range of field trials, there is remarkable inconsistency in responses across crops, regions and other conditions (Ghosh, 2004).

Conclusion

With excessive use of chemical fertilizer, flora and fauna is adversely effected as well as it disturb the ecological balance. Indian soil fertility is diminishing gradually. In some way biofertilizer can help to provide nutritional requirement of agricultural crop. The major challenge in this area of research lies in the fact that identification of local and efficient strain of particular area that can survive better in competition with other microorganism. The integration of different microbial capabilities into combined biofertilizers with numerous potential yield-promoting effects is also desirable in integrated nutrient management.

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