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Microbial Inoculants for Agriculture under Changing Climate

SNEHA S. NAIR, PRAMOD KUMAR SAHU AND G. P. BRAHMAPRAKASH
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
E-mail : gpbrahma@gmail.com

ABSTRACT

Risk in agriculture has been extended manifolds due to climate change. Small and medium scale farming are much affected by unpredictable weather conditions arising from climate change. Various strategies are being developed to face this challenge such as developing varieties with flexible sowing time, short duration crops, anti-transpirants, new tolerant varieties, etc. Among these, microorganisms too have a significant role in mitigating stresses arising out of climatic change. Upon inoculation, these microbes confer benefits to the plants for with standing the adverse climatic conditions. The benefit can be easily extended to small and large scale farmers by inoculation. Microorganisms are known to confer protection against draught, heat, flood, frost, salinity, *etc*. Inoculation of effective micro organisms in sufficient quantity with good survival and rhizo competence maximizes the crop success in adverse climate. Several formulations of microorganisms are reported to confer protection against adverse conditions of storage and field. This review deals with such biofertilizer formulations which are having potential to contribute to climate smart agriculture along with various stress alleviation by microorganisms. Liquid and alginate based inoculant formulations have been discussed in detail with its ability to perform in adverse climate. This review also covers novel inoculant formulations which can perform under unpredictable weather conditions.

AGRICULTURE is one of the most vulnerable sectors to climate change as it may accentuate the vulnerability in food security. Alterations of atmospheric carbon dioxide concentration, temperature, water scarcity, salinity and other biotic stresses have led to altered plant growth rates, yields and productivity. Most studies fail to address the ability of associated soil micro-organisms to shift their range to maintain their relationship with plant (Van Der Putten, 2012). Relative to above ground plant structures, soils are buffered to changes in climate which invariantly affects the soil biota. For this reason, the direct stress plants are facing may be different from what their associated soil community is experiencing. Over the due course of time plants have developed several mechanisms to combat abiotic stresses, but it eventually leads to crop loss. One globally available adaptive opportunity found in the soil is its microbial component. Microorganisms often have close associations with plant roots (Bais *et al*., 2006). Microbial mutualists influence its host performance.

Microbial communities respond to climate change through resistance or resilience (Allison and Martiny, 2008). The direct effects of climate change includes altering microbial soil respiration rates (Bradford, 2013), increased bacterial to fungal ratio of the community (Deangelis *et al*., 2015) *etc*. One such example is the second year mortality of subterranean clover in western Australia (Chatel *et al*., 1968) which resulted primarily from the number of *Rhizobium trifolii* TA1 falling off in the second and subsequent years. The major cause of this die off was traced to a water soluble microbial toxin found in soils drying out after a light rain. The problem was solved by re inoculating the fields with survived strains of *R. trifoli.*

Nearly all tissues within a plant are inhabited by a variety of microorganisms. They not only deploy many mechanisms to survive stress conditions but also confer the same benefits to crop productivity and host stress resistance. These associations can alter the expression of plant traits such as leaf area and nutrient content (Harris *et al*., 1985; Bishop *et al*., 2011; Friesen *et al*., 2011). Root symbionts such as rhizobia (De Bello *et al*., 2010) and mycorrhizal fungi (Johnson *et al*., 1997) also affect plant productivity by altering plant nutrient status.

Eventhough, the direct effects of climate change on soil community call for concern, the indirect effect mediated by plant community shifts are considered more important as they may cause the soil communities to change their distribution in the soil

profile which can ultimately lead to change in ecosystem functions such as nitrification, denitrification *etc.* (Isobe *et al*., 2011; Bakken *et al*., 2012). Changes in the relative abundance of organisms that regulate specific processes can have direct impact on rate of that process. Therefore it is essential to monitor and maintain the microbial properties of soil to enhance host stress tolerance.

The rhizosphere, with its high microbial diversity, is a vital source of beneficial plant growth-promoting rhizobacteria that could be screened and developed into potential microbial inoculants for sustainable agriculture. One of the most important problems however is the inconsistency in the field performance of Plant Growth Promoting Rhizobacteria (PGPR) inoculants under stress conditions. Therefore it is essential that we develop novel strain specific microbial inoculants that can withstand extremes of climate change, thereby contributing to mitigation of stress in host plants.

Microbes in mitigating climate stress

Microorganisms are known to survive in extremes of temperature, drought, pH, salinity, heavy metal toxicity *etc*. by deploying various adaptive features through complex regulatory processes. These microorganisms may exist as free-living in soils or attached to the surface of roots or phyllosphere, and may establish symbiotic relations with plants (endophytes), wherein, they colonize various plant tissues.

Wellstudied of these symbionts include the mycorrhizal fungi and root-nodulating bacteria and plant growth-promoting microorganisms (PGPM). These organisms confer stress resistance via diverse mechanisms. These may include production of osmoprotectants (glutamate, trehalose, proline) to modulate cytoplasmic osmolarity; production of exopolysaccharides, production of heat shock proteins and cryoprotective protectants. Investigations have shown that certain microbial species and / or strains specifically, rhizospheric microorganisms enhance plant tolerance to abiotic stresses by triggering some mechanisms that help the plant to tolerate stress (Yang *et al*., 2009).

Mechanisms of bacteria-mediated stress tolerance in plants

Stress limits crop growth and productivity. Microorganisms may deploy certain mechanisms to alleviate plant stress. Table 1 summarizes the studies published, to date, on bacterial effects on plants under abiotic stress in relation to stress type, bacteria involved and the plant species to which they were applied.

Plants exposed to environmental stresses, show an altered change in root morphology which may be due to the production of phytohormones. Low concentration of these hormones may promote root growth but in excess lead to inhibitory effect. For example under stress conditions, the plant hormone ethylene endogenously regulates plant homoeostasis and results in reduced root and shoot growth. In the presence of ACC deaminase producing bacteria, plant ACC (1-aminocyclopropane-1-carboxylate), the immediate precursor of ethylene, is sequestered and degraded by rhizospheric bacterial cells to supply nitrogen and energy. Thereby the bacteria reduce the deleterious effect of ethylene, ameliorating plant stress and promote plant growth (Glick *et al*., 2007).

Yet, another mechanism of bacterial stress tolerance is by altering the cell envelope composition. Microbial polysaccharides (EPS) can bind soil particles to form aggregates. Plants treated with EPS producing bacteria have shown to display an increased resistance to water stress due to improved soil structure (Sandhya *et al*., 2009). EPS can also bind to cations including sodium thus making it unavailable to plants under saline conditions.

It is reported that proline produced by PGPR also protects higher plants against salt / osmotic stresses, not only by adjusting osmotic pressure but also by stabilising many functional units such as complex II electron transport and enzymes (Makela *et al*., 2000). Proline also helps the plant cell by stabilising subcellular structures such as membranes and proteins, scavenging free radicals and buffering cellular redox potential under salt stress to alleviate salt stress (Ashraf and Foolad, 2007; Kohler *et al*., 2009).

PGPR also mitigate the impact of stress on plants through the production of cytokinins, which causes the

TABLE I

Microorganisms involved in stress mitigation

Type of stress	Crop	Microorganism	Mechanism involved	Reference
Drought	Sunflower	*Pseudomonas putida* P45	Improved soil aggregation due to EPS production	Sandhya *et al*. (2009)
	Clover	*Bacillus megaterium* and *Glomus* sp.	Production of indole acetic acid and proline	Grover *et al*. (2010)
	Maize	*Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17	IAA production,	Naveed *et al*. (2014)
	Tomato and pepper	*Pseudomonas putida* KT2440	Trehalose biosynthesis	Juan *et al*. (2016)
Salinity	Maize	*Rhizobium*, *Pseudomonas*	Decreased electrolyte leakage, increase in proline production, maintenance of relative water content of leaves, and selective uptake of K ion	Bano and Fatima (2009)
	Lens esculenta Var. masoor 93	*Oceanobacillus profundus* (Pmt2) and *Staphylococcus saprophyticus* (ST1)	biofilm formation, exopolysaccharide production and endogenous osmolyte (proline and glycine betaine)	Qurashi and Sabri (2011)
	Barley and oats	*Acinetobacter spp. and Pseudomonas sp.*	Production of ACC deaminase and IAA	Chang *et al*., (2014)
Temperature	Wheat	*Pseudomonas putida* AKMP7	Reduced membrane injury and the activity of several antioxidant enzymes such as SOD, APX and CAT	Shaik *et al*. (2011)
	Grape wine	*Burkholderia phytofirmans* PSJN	Increase in the levels of starch, proline and phenols.	Grover *et al*. (2010)

accumulation of abscisic acid (ABA) in leaves, which in turn results in the closing of stomata (Figueiredo *et al*., 2008). Similarly, trehalose metabolism in rhizobia is also important for improving plant growth, yield and adaptation to abiotic stress of leguminous plants (Suarez *et al*., 2008).

Fungal endophytes

Fungal symbionts have been found to be associated with most plants in the ecosystem, where they colonize and reside entirely or partially in the internal tissues of their host plant. Collectively, mutualistic fungi including Arbuscular Mycorrhiza (AM), may confer tolerance to drought, metals, disease, heat and / or promote growth and nutrient acquisition. Thus mycorrhizae-plants symbiosis can be harnessed for climate smart agriculture as it provides plant nutrients and improves soil properties (Mukhongo *et al*., 2016). It is also known to improve phosphate nutrition by mobilizing it from distant parts to the roots. Similarly, it enhances zinc, ammonium, calcium, iron, sulfur, manganese and copper availability to the plants (Harikumar and Potty, 2007; Hu and Rufty, 2007).

Drought affected areas are especially benefitted from mycorrhizal symbiosis as it improves water absorption from soil and mitigates negative effects of draught in plants growth (Smith *et al*., 2010; Jayne and Quigley, 2014). It also improves soil structure by particle binding (Rilling and Mummey, 2006) and thus, very important in stabilizing degraded soils in both subsistence and commercial farming. Arbuscular mycorrhizal (AM) symbiosis provides excellent biocontrol of many plant pathogens (Elsen *et al*., 2001; Forge *et al*., 2001; Harrier and Watson, 2004). AM fungi inoculation were also shown to decrease the leaf content of malondialdehyde and soluble protein and enhance activities of superoxide dismutases (SOD), peroxidasse (POD) and catalase (CAT) resulting in improved osmotic adjustment and drought tolerance of mycorrhizal citrus grafting seedlings (Wu and Xia, 2005).

AM colonization by *Glomus intraradices* has been shown to contribute substantially to the flood tolerance of *Pterocarpus officinalis* seedlings by improving plant growth and phosphorus acquisition in leaves. Salt resistance was improved by AM colonization in maize (Feng *et al*., 2002), mung bean (Jindal *et al*., 1993) and clover (Ben Khaled *et al*., 2003) due to improved osmoregulation or proline accumulation. AM inoculation has also shown to improve NaCl resistance in tomato, with extent of improvement related to salt sensitivity of the cultivar (Al-Karaki *et al*., 2001).

Besides AMF, endophytic symbiont dark septate fungi (DSF) are also found in plants growing under stressed environments. *Piriformospora indica,* a biotrophic mutualistic root endosymbiont has been reported to mimic capabilities of typical arbuscular mycorrhizal (AM) fungi. This fungus can colonize roots of a wide range of higher plants and help plants in nutrient uptake, disease resistance, stress tolerance and growth-promotion (Unnikumar et al., 2013). *P. indica* has been reported to modulate major antioxidant defense enzymes monodehydroascorbate reductase and dehydroascorbate reductase (Hamilton *et al*., 2012) and the other components of ROS-scavenging system (Waller *et al*., 2005; Sun *et al*., 2010) Another rhizosphere fungus *Paraphaeosphaeria quadrisept ata* was shown to enhance plant heat stress tolerance of *Arabidopsis thaliana* through induction of HSP101 and HSP70 (McLellan *et al*., 2007).

B. Drought tolerance

Drought stress limits crop growth and productivity, especially in semi arid regions. As a response to water deficit, plants increase the synthesis of osmolytes (proline), thus increasing the osmotic potential within cells (Farooq *et al*., 2009). Similarly, compounds exudated by root zone bacteria also include such osmolyte which can act synergistically with plant produced osmolytes in response to the stress, and this way, increase drought tolerance. Elevated proline levels have been reported to confer drought in plants. Sandhya*et al*. (2011) screened *Bacillus* sp. (*B amyloliquefaciens, B. licheniformis, B. thuringiensis, Paenibacillus favisporus, B. subtilis*) for drought tolerance and plants inoculated with these bacteria showed reduced activity of antioxidant enzymes concluding that *Bacillus* spp.inoculated maize could alleviate drought stress negative effects.

Pseudomonas sp, a very common PGPR has also been extensively used for mitigating stress in plants. Physiological modifications in soybean plants inoculated by the gibberellins secreting rhizobacterium *Pseudomonas putida H*-2–3 was shown to improve plant growth under drought conditions (Kang *et al*., 2014). The stress hormonal analysis revealed a lower level of abscisic acid and salicylic acid and a higher level of jasmonic acid content in plants with microbial application. Under stress condition the bio-inoculant, *P. putida* H-2-3 was also shown to modulate plant antioxidants by declining superoxide dismutase, flavonoids and radical scavengingactivity. *P. putida* H-2-3 induced tolerance against abiotic stress was confirmed by a reduction of sodium content in abiotic stressed plants.

AM fungi are yet another important candidates of plant growth promotion under stress conditions. Inoculation of *Glomus versiforme* in citrus plants were shown to improve the osmotic adjustment of the plant under drought stress through enhanced levels of non-structural carbohydrates, K, Ca and Mg ions resulting in the enhancement of drought tolerance (Wu and Xia, 2006).

C. Tolerance to high soil salinity

Plant exposure to salinity stress causes increase in water stress, ionic influx, oxidant imbalance, membrane disintegration, cell division impairment, and fruit development.

Endophytic symbiosis with host plants especially in roots can regulate and change the uptake of mineral nutrients, balance of plant hormones, exudation of defensive metabolites from root (Khan *et al*.,2013; Bashan *et al*., 2014). Nadeem *et al*. (2007) found that inoculation of salt-stressed maize with ACC deaminase containing *Pseudomonas syringae, Enterobacter aerogenes* and *P. fluorescens* resulted in higher K+ / Na+ ratios in combination with high relative water, chlorophyll and low proline contents. Increased total soluble sugar (TSS) content of plants under salinity stress is another important defence strategy to cope with salinity stress, and Upadhyay *et al*. (2012) showed that an increased proline and total soluble sugar in the PGPR-treated wheat plants significantly contributed to their osmotolerance.

Salt stress has also been shown to affect nodulation during *Phaseolus– Rhizobium* interaction. However, secondary inoculation of the salt-stressed plants with *Azospirillum* caused an extended exudation of plant flavonoids compared to *Rhizobium* alone, implying an induction of flavonoid genes in the presence of *Azospirillum* (Dardanelli *et al*., 2008). Thus, the co-inoculation of plants with different bacterial species may contribute to relieving abiotic stress.

D. Tolerance to extreme temperatures

Temperature extremes present a stress condition for plants. Some bacterial species and strains affect plant tolerance to high temperature. *Pseudomonas* sp. strain NBRI0987 has shown to cause thermotolerance in sorghum seedlings, through synthesis of high molecular weight proteins in leaves thus increasing the plant biomass (Grover *et al*., 2010). Inoculation of wheat seeds with *Serratia marscescens*, strain SRM, and *Pantoeadispesa*, strain 1A increased the seedlings biomass and nutrients uptake at low temperatures.

Chilling temperatures are equally hazardous to the plant community as crop may develop frost injuries leading to poor yield and productivity. When plants are exposed to below-freezing temperatures (-2 and -5^{o}C), the majority of frost-sensitive plants usually suffer from damage. When water gets this cold, water turns into ice inter and intracellularly. *Pseudomonas syringae* expresses a particular type of surface protein, ice-nucleation protein (INP), which increases temperatures at which water freezes (Burke *et al*., 1976). The introduction of an ice-minus strain of *P. syringae* to the surface of plants would reduce the amount of ice nucleate present and thereby protect plants from frost injury up to a certain extent.

Under chilling temperatures bacteria produce cold shock proteins (CSP) which has nucleic acid binding activity, sufficient for their function as RNA chaperones. The expression of these bacterial CSPs (Csp A and Csp B) were shown to improve tolerance of transgenic rice, maize and arabidopsis plants to a number of abiotic stresses including cold, heat and water deficit resulting in improved yields under field conditions (Castiglioni *et al*., 2008).

Bacteria can also survive under low temperatures by the production of antioxidant enzymes and proline. Subramanian *et al*. (2016) selectively isolated 40 psychrotrophic bacterial isolates belonging to the genera *Arthrobacter, Flavimonas, Flavobacterium, Massilia, Pedobacter* and *Pseudomonas* and treated tomato plants with the selected isolates which exhibited significant tolerance to chilling as observed through reduction in membrane damage and activation of antioxidant enzymes along with proline synthesis in the leaves when exposed to chilling temperature conditions (15°C). They concluded that psychrotolerant physiology of the isolated bacteria combined with their ability to improve germination, plant growth and induce antioxidant capacity in tomato plants could be employed to protect plants against chilling stress.

D. Other stresses

Growth and development of a plant requires uptake of inorganic ions into their systems as they play an important role in their physiological and metabolic functions. However, accumulation of heavy metals in an undesirable proportion has shown to cause cytotoxic, genotoxic and mutagenic effects on plants as well as microbes.

Studies show that some rhizobateria can exude a class of rhizobateria secretion, such as antibiotics (including the antifungals), phosphate solubilization, hydrocyanic acid, indoleacetic acid (IAA), siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which increase bioavailability and facilitate root absorption of heavy metals, such as Fe (Crowley *et al*., 1991) and Mn (Barber and Lee, 1974), enhance tolerance of host plants by improving the P absorption (Davies *et al*., 2001) and promote plant growth.

The rhizosphere, with its high microbial diversity, is a vital source of beneficial plant growth-promoting rhizobacteria that could be screened and developed into potential microbial inoculants for sustainable agriculture. However, one of the most important problems is the inconsistency in the field performance of PGPR inoculants under stress conditions. Therefore, it is essential that we develop novel strain specific microbial inoculants that can withstand extremes of climate change, thereby contributing to mitigation of stress in host plants.

2. Inoculants of microorganisms

Erratic changes in climate have led to loss of soil nutrients which call for some essential amendments in soil with regard to soil health. Microorganisms respond to climate change through a variety of mechanisms, but, most importantly, they can positively interact with the plants and help them in mitigating stress. The major concern here lies in the population of these beneficial microbes in soil. Soil enrichment with beneficial organisms paves the way to a cost effective and eco-friendly approach in conserving soil health.

The success of inoculation technology depends on two factors such as the microbial strain and inoculants formulation. In practical terms, formulation determines potential success of inoculants (Fages, 1992). Formulation should essentially consist of viable bacterial population in a suitable carrier stabilized with additives for longer shelf life (Xavier *et al*., 2004). Initially carrier based inocula were prepared using solid carrier of choice, such as, peat, lignite, talc *etc*. Though, there are reviews of successful field results using carrier based inoculum, a higher cost of production, increased labor, necessity for a sterilizing unit, and aseptic procedures during packaging *etc.* continue to be major drawbacks of carrier based inocula. Moreover microbial population in carrier based inocula show less tolerance to stress during storage due to absence of stabilizing agents, which ultimately leads to short shelf life. Now-a-days new inoculants technologies such as polymer entrapped inoculants and liquid inoculants are gaining popularity due to their longer shelf life and are being replaced as an alternative to carrier based inoculants especially in this climate changing scenario.

A. Polymer entrapped inoculants

The concept behind polymer entrapped inoculant is encapsulating microbial cells in a polymer matrix. This provides protection to microbial cells from external stresses. Polymer entrapped inoculants are slow releasing, which provides slow but continuous supply of microbial cells to the environment (Bashan, 1986; Kitamikado *et al*., 1990). The microbial cells entrapped in polymer matrix are released in soil after degradation by the soil microbes in presence of water. These polymers have been demonstrated as potential carriers of bacterial cells (Deaker *et al*., 2004). This technique has been used for many plant growth promoters like *Aspergillus brazilens* and *Pseudomonas fluorescens* (Bashan, 1986) for field inoculation. These formulations encapsulate the living cells and protect it against many environmental stresses. Different inert materials were evaluated as carriers like polyacrylamide gel, alginate *etc*. (Singleton *et al*., 2002).

Alginate is one of the most commonly used polymers for microbial encapsulation. It is commercially extracted from seaweeds like giant kelp (*Macrocystis pyrifera*), *Ascophyllum nodosum*, *Laminaria, etc*. (Yabur *et al*., 2007). It is also produced by bacteria like *Pseudomonas* and *Azotobacter* (Remminghorst and Rehm, 2009). Alginate is polymer of â-1,4-linked D-mannuronic acid and L-glucoronic acid. It is extracted in form of sodium alginate (sodium salt of alginic acid). Qualities like slow releasing, bio-degradable and non-toxic nature makes it advantageous to be used for climate smart inoculant formulation (Fages, 1992; Kitamikado *et al*., 1990). Alginate formulation containing plant growth promoting

bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* (Fages, 1992) was successfully used in wheat plants under field conditions and found comparable with other carrier based inoculants (Bashan *et al*., 1987).

It has also been reported to enhance colonization of wheat roots by beneficial cells than that of direct soil inoculation. This proves that slow releasing microbial inoculants from alginate beads are more efficient as the microbial cells are in protective environment and doesn't get killed quickly upon application. Alginate based formulation also have been prepared for encapsulating arbuscular mycorrhizal (AM) fungi (Vassilev *et al*., 2005) ectomycorrhizal fungi (Le Tacon *et al*., 1985), *Frankia* inoculation (Sougoufara *et al*., 1989), phosphate solubilizing bacteria (Bashan *et al*., 2002), *Azospirillum* sp. (Fages, 1990), bacterial biocontrol agents (Aino *et al*., 1997) and fungi (Fravel *et al*., 1985).

Polymer entrapped formulation and climate change

In tropical, low input agriculture there is always uncertainty of rainfall and prolonged dryness prevails after sowing and microbial inoculation. Rainfed areas are suffering from erratic rainfall due to climate change. The conventional agricultural practice is not matching with rainfall and moisture availability. Prolonged dry spells after sowing and application of microbial inoculants are one of the major causes of ineffective microbial inoculation. In such conditions, alginate beads can be a formulation of choice as compared to peat, lignite or other powder formulation.

Alginate based formulation found supporting higher populations and prolonged survival of microbial inoculants even at elevated temperature of 40°C during storage (Viveganandan and Jauhri, 2000). Alginate entrapped inoculant formulations are desiccated and due to reduced water activity microorganisms will be on slow metabolism rate. It protects inoculant microbes from harsh environmental condition and releases them slowly into environment upon degradation. The degradation of beads requires water, which coincides with germination of seeds. The perfect timing of release of microbes to emerging root zone is always beneficial for an inoculant formulation. At lower temperatures also, alginate formulation were found superior over other formulations like liquid and charcoal based inoculant in maize plants (Trivedi *et al*., 2005).

Survival of inoculant in polymer entrapped beads depends on water activity (a_w) of the product. Mugnier and Jung (1985) had shown that water activity is one of the key factor on survival of bacteria, fungi and yeast in polymer matrix. They investigated that formulation shows a constant survival for a period of more than three years if water activity of product is below 0.069. Similarly, survival decreases if water activity rises above 0.069. This result clearly indicates that reduced water availability in the polymer metrics provides protective effects to the microbial cells. Use of high molecular weight compounds in growth media which doesn't affect osmolarity of the cell gives protective effects.

Dry beads of alginate are excellent in protecting microbial inoculants in dry weather. It gives an excellent survival of inocula over a long period. In a long term experiment, alginate beads containing *Azospirillum brasilense* and *Pseudomonas fluorescens* were found live even after 14 years of ambient temperature storage. A significant number of cells (10^5-10^6 CFU g^{-1} beads) survived after 14 years (Bashan and Gonzalez, 1999). This makes a perfect choice for using it for microbial inoculation in changing climate where rainfall is uncertain. It can survive for longer period and release inocula to the plant when it germinates.

Notable advantages and disadvantages of polymer entrapped formulation (Sahu and Brahmaprakash, 2016)

Advantages

- It releases microbes gradually (Digat, 1991)
- Can be stored at ambient temperatures for long periods (Bashan, 1998)
- Easy to produce and handle (Bashan, 1998)
- Non-toxic in nature (Fages, 1992)
- It provides consistent batch quality (Bashan and Gonzalez, 1999)
- It can be manipulated easily according to the need (Bashan and Gonzalez, 1999)

- can be amended with nutrients to improve the survival of the bacteria upon inoculation (Bashan, 1998)
- It temporarily protects the encapsulated microorganisms in the harsh soil environment and microbial competition (Bashan and Gonzalez, 1999)

Disadvantages

- Expensive as compared to peat based formulation (Bashan, 1998)
- It needs more industrial handling (Fages, 1992)
- Labour intensive (Bashan and Gonzalez, 1999)
- The low oxygen transfer inside bead may limit the survival of inoculum.

Like every other inoculant formulation, polymer entrapped formulation also has its own pros and cons. There is incessant research going on for improving performance of formulations with lower contamination, higher shelf life, higher effectiveness, economic production process, etc.

Amendments in alginate based polymer formulation

Several amendments have been used with alginate for enhancing the effectiveness of alginate beads and reducing the cost of mass production. Addition of clay and skim milk were tested and found augmenting bacterial survival than un-amended alginate beads. Mixing of alginate with perlite for entrapping *Rhizobium* was also useful. Two rhizobial strains of groundnut were encapsulated by alginate-perlite beads. This dry granular inoculant can be stored for longer periods without losing its viability in normal temperature. The effects of this formulation were similar to that of peat (Hegde and Brahmaprakash, 1992). Jung *et al.* (1982) used alginate formulation of *Rhizobium* with a mixture of xanthan and carobgum for legumes. Mixtures like of 5 per cent arabic gum, 20 per cent pero - dextrin, 10 per cent starch granules or 20 per cent gelatine were used for impregnating N_2 fixing and plant growth promoting bacteria like *Azotobacter chroococcum*, *Enterobacter agglomerans*, *Klebsiella pneuomoniae*, *Azospirillum brasilense*, *Bacillus polymyxa* and *Pseudomonas putida*. It had improved cell survival for longer time and nitrogenase activity. Polymer entrapped cells of *B. polymyxa* were tested for viability till 160 days and found promising (Ali *et al*., 2005). Apart from increasing performance of alginate formulation, some cheaply available materials were also used for reducing the total formulation cost. Materials like rock phosphate, bentonite clay, talc, gypsum, lignite, cement, granite powder, etc. which adds to bulkiness of formulation (Fages, 1990). Apart from adding bulkiness to the formulation amendments can improve chemical, physical and nutritional aspects of formulation in prolonged storage period (Schisler *et al*., 2004). Addition of enriching material like trehalose, maltose and sucrose help in enhancing the viability of inoculant (Brar *et al*., 2006).

A. Micro alginate beads

The efficiency of alginate beads has been improved further by preparing micro alginate beads (John *et al*., 2011). It is powder like formulation containing small beads encapsulating a sufficient number of bacteria in it. Seed coating with these microbeads result in a uniform coating of inocula close to seed surface. It is especially beneficial for small seeded crops and reduces off-site drift during application (Cassidy *et al*., 1996). Micro alginate beads are produced by mixing alginate solution with rich bacterial broth and its spray into slowly stirred $CaCl_2$ solution. Spray is done by low-pressure nozzle which form mist of alginate-bacterial suspension and form small diameter alginate beads. Chemical solidification result in microbeads of diameters ranging from 50 to 200 μm. Micro alginate beads entrap sufficiently large number of bacteria ranging from ~10^8 to 10^{10} CFU per gram (Bashan *et al*., 2002).

Liquid formulation

Liquid formulations use liquid materials as carrier, which is usually water, oil or some solvents in form of suspension, concentrates or emulsions. Solid based inoculants are too cumbersome for large-scale field application and tend to plug precision air seeders used on large farms. Liquid formulations can be applied easily to seed as it passes through seed augers on the way to the planting machinery.

In the past, commercial liquid inoculants have been marketed only sporadically, basically because of the difficulties which arise in maintaining biological control after the cultures leave the manufacturer (Brockwell, 1982). Manufacturers seem to have overcome the problem of deterioration by concentrating the broth inoculant with centrifugation, addition of additives to increase the shelf life etc.

Several compounds have been studied for their protective function and added to liquid inoculants for promoting the survival of microorganisms in the formulation. Most popular liquid inoculant formulations contain particular organism's broth 10-40 per cent, suspender ingredient 1-3 per cent, dispersant 1-5 per cent, surfactant 3-8 per cent and carrier liquid (oil and / or water) 35-65 per cent by weight (Table II).

Shelf life is the first and foremost problem of biofertilizers. Carrier based bio fertilizers are not so tolerant to stress which is mostly unpredictable and uncertain in the crop fields, whereas, temperature tolerance is an advantage of the liquid biofertilizers (Mahdi *et al*., 2010). Liquid inoculants facilitate the long survival of the organism, improve quality of inoculants by increasing the population density and enhance the shelf life by use of additives.

C. Additives in Liquid inoculants

Selection of additives is based on their ability to protect bacterial cells in storage and on seeds at extremes of temperature, dessication and toxic conditions. High molecular weight polymers with good water solubility, nontoxicity and complex chemical nature are good additives (Deaker *et al*., 2004). Some commonly used additives in formulations include polyvinyl pyrrolidone (PVP), methyl cellulose, polyvinyl alcohol, polyethylene glycol, gum Arabica, trehalose, glycerol, Fe-EDTA, sodium alginate, tapioca flour *etc*. (Singleton *et al*., 2002) (Table II).

The nature and concentration of additives affect the performance of the inocula. Vendan and Thangaraju (2007) reported that the carrier based inoculants generally suffer from shortage shelf life, poor quality, high contamination and low field performance. The liquid formulations of *Azospirillum* with the amendments *viz.,* Trehalose, Polyvinyl pyrollidine and Glycerol enhanced and maintained the population upto 10 months of storage. The liquid formulation showed better adherence and survival on seeds, roots of seedlings and in the rhizosphere soil than the solid carrier based *Azospirillum* inoculants.

TABLE II

Additives and their functions

Additive	Function	Reference
PVP	high water-binding bioadhesive capacity	Tittabutr *et al*. (2007)
Glycerol	flow characteristics appear to promote rapid and even coating on seeds	Singleton *et al*. (2002)
Trehalose	stabilizing both enzymes and cell membranes, is a compatible osmoticum	Singleton *et al*. (2002)
FeEDTA	supplement iron	Singleton *et al*. (2002)
Hydroxypropyl methyl cellulose-HPMC	Soluble in water, controlled release	Fernandes junior *et al*. (2012)
PEG	suspension agents, adhesive in nature	Denardin and Freire (2000)
Glucose	enhances exopolysaccharide production, which could protect cells during the rapid drying they experience at inoculation	Singleton *et al*. (2002)

Velineni and Brahmaprakash (2011) conducted a preliminary study to determine the survival of *Bacillus megaterium* in liquid formulations supplemented with osmo / cell-protectants under the influence of high temperature, desiccation stress and their subsequent influence on P-uptake by cowpea plants. Liquid inoculants 2 containing osmoprotectants *viz.,* polyvinyl pyrrolidone (PVP), high quantity of glycerol (12 ml L-1) and glucose was shown to support higher viable population up to a storage period of four weeks at 48ºC (log10 10.62 CFU ml-1) and desiccation stress (log10 10.04 CFU ml-1) as compared to liquid inoculant-1 containing osmoprotectants *viz.,* PVP, low quantity of glycerol (1 ml L-1), trehalose, arabinose and FeEDTA; and nutrient glucose broth without any osmoprotectants.

Lee *et al*. (2016) evaluated the effects of different liquid inoculant formulations on the survival and plant-growth-promoting efficiency of *Rhodopseudomonas palustris* strain PS3, wherein, six additives (alginate, polyethylene glycol [PEG], polyvinylpyrrolidone-40 [PVP], glycerol, glucose, and horticultural oil) were used in liquid-based formulations, and their capacities for maintaining PS3 cell viability during storage in low, medium, and high temperature ranges were studied. With horticultural oil (0.5 =%) they observed that the formulated PS3 (PS3–0.5% H.o.) inoculants produced higher levels of EPS than those without formulation at any storage temperature. Therefore, it was chosen as a potential additive as it could maintain a relatively high population and conferred greater microbial vitality under various storage conditions.

Besides the various additives used to improve the shelf life of the product, specific compounds can be introduced into the formulation to enhance the efficacy of biofertilizer. Legume biofertilizers containing elicitors of nodulation are already marketed. Mabood *et al*. (2006) conducted field experiments to study the effect of preinducing *Bradyrhizobium japonicum* strains with methyl jasmonate (MeJA), alone or in combination with genistein (Ge), on nodulation and N fixation of Soybean under field conditions. Genistein and MeJA were shown to increase nodule number, nodule dry weight per plant, and seasonal N fixation, as compared with the control treatment, inoculated with un induced *B. japonicum*.

Advantage of liquid formulation (Girisha *et al*., 2006)

- Achieve complete sterilization of medium
- Sterilization of liquid medium is easier compared to solid carriers
- Any contamination occurring during storage can be easily noticed
- Does not require any sticker material, unlike carrier based biofertilizer.
- Offers protection to cells against high temperature
- Easy to apply and can be effectively integrated with mechanized farming.
- The amount of inoculant needed for seed inoculation is less

Field response of liquid inoculants

Researchers have shown that the performances of liquid formulations are comparable to that of carrier based inocula. Sridhar *et al*. (2004) developed a liquid inoculant using osmoprotectants for phosphate solubilizing bacterium (*Bacillus megaterium*) and studied the effect of application of Mussoorie Rock Phosphate (MRP) and inoculation with different formulations of *B. megaterium* on P- uptake of cowpea. They observed a significantly higher total-P (8.14 mg/plant) and maximum total biomass (4.94 g/plant) in plants treated with MRP and liquid inoculant-2 (containing osmoprotectants *viz.,* Polyvinyl Pyrrolidone (PVP), glycerol and glucose) and concluded that the increased P- uptake by cowpea when inoculated with liquid inoculant-2 + MRP was mainly due to efficient solubilization of insoluble soil-P as well as added MRP which attributed to higher population of *B. megaterium* that was maintained in liquid inoculant-2 (log 10 10.50 CFU/ml).

Brahmaprakash *et al*. (2007) evaluated the performance of liquid *Rhizobium* inoculants over carrier based *Rhizobium* inoculants through national level on farm trials.These trials were performed during kharif and rabi seasons of two successive years (2001 and 2002) in groundnut, soybean, redgram and chickpea. Trials were conducted on National Level covering 14 districts of 7 states which come under different agro climatic zones. It was found that in all the crops tested, the liquid *Rhizobium* inoculant gave

better yield than carrier based inoculant (Plate 1). The increase in the yield of groundnut, soybean, pigeon pea and chickpea treated with liquid inoculants ranged from 4.0-27.0, 26.0 - 42.0, 2.0-19.6 and 8.1-24.5 per cent, respectively (Graph 1).

C. Novel inoculant formulations for climate resilient agriculture

Fluid bed dried inoculant formulation

This novel formulation has many benefits in climate resilient agriculture. This is prepared by drying the formulation in fluidized condition in a dryer. Reducing water activity makes it more stable and resulted in low contamination rate, increased survival and enhanced plant growth even if some dry spell prevails after sowing (Sahu, 2012; Brahmaprakash and Sahu, 2012; Sahu *et al*., 2013; Lavanya *et al*., 2015; Sahu and Brahmaprakash, 2016; Sahu *et al*., 2016a). This new approach of making bioformulation has some obvious benefits over other formulations. The instability of performance and contamination are major drawbacks in bioinoculant industry. The technique, however, is in its primitive stage and much research is required for its successful implementation at field level (Sahu *et al*., 2016b).

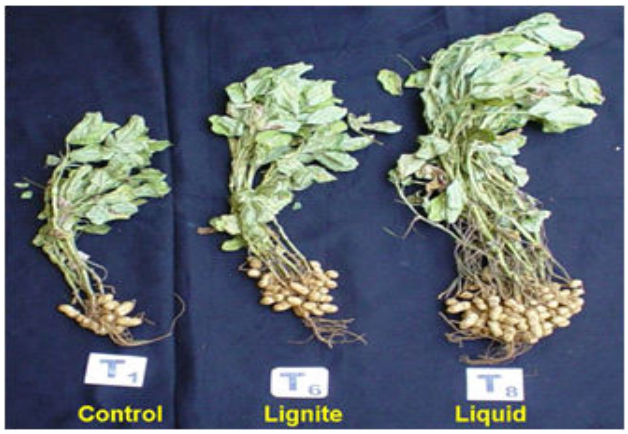
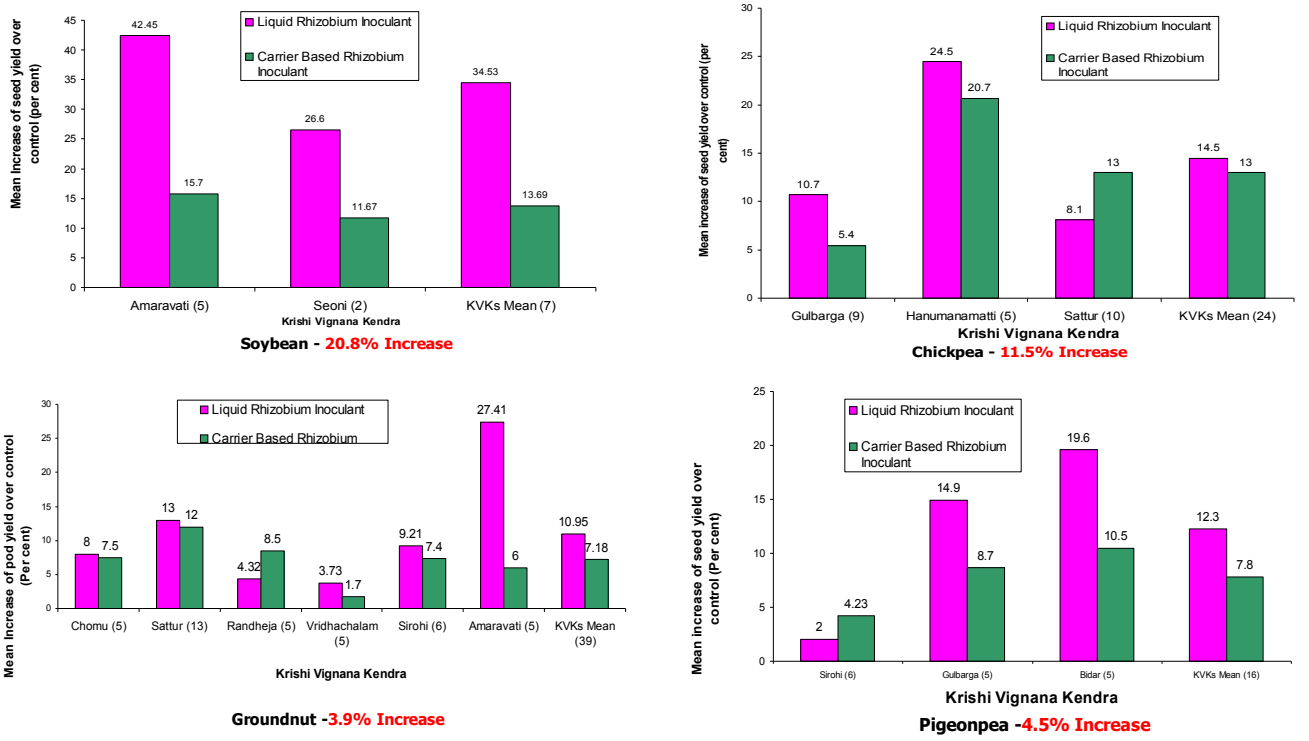


Plate 1: Effect of liquid inoculant application. (Source : Brahmaprakash *et al.*, 2007)

In fluid bed dryer (FBD), substrate to be dried is suspended against gravity by an upward flowing air stream at terminal velocity. Suspended particles provide higher surface area for drying which causes high rate of moisture transfer. This machine was before used in food and pharmaceuticals industries different drying operations and have tremendous potential to be used in biofertilizer industry (Srivastava and Mishra 2010; Brahmaprakash and Sahu, 2012; Sahu *et al*., 2013).



Graph 1: Performance of Liquid Rhizobium inoculants over Carrier based Rhizobium inoculants in farmer's field. (Source : Brahmaprakash *et al.,* 2007)

Bioflocs based bioformulation

Bioflocs of *Azotobacter* and *Paenibacillus* were tested in various environmental stresses like higher temperature, desiccation and salinity under *in-vitro* condition. Higher tolerance to these environmental stresses was conferred by both natural and artificial bioflocs as compared to their vegetative cell formulations. The natural biofloc formulation found exhibiting higher stress tolerance as compared to formulation made from artificial biofloc (Kalaiarasi and Dinakar, 2015). The exopolysachharides mediated biofloc formulation of PGPR exhibited higher tolerance to various environmental stresses.

A. Hydrogel based bioformulation

Hydrogel based microbial inoculant. Hydrogel represents a group of polymeric materials of hydrophilic nature which can hold large amounts of water in their 3D metrics (Narjary *et al.,* 2012). It is frequently used in sandy soils for improved water availability by increasing water retention and reducing drainage pores. Increased activity of microorganisms and mycorrhiza using such super absorbent hydrogels was reported by Degiorgi *et al.* (2002).

Suman *et al*. (2016) has reported a consortium of *Azotobacter chroococcum, Pseudomonas fluorescence* and *Trichoderma viride* in hydrogel based formulation. This formulation supported good microbial growth, higher shelf life and improved bio efficacy as compared to lignite and liquid formulations in wheat. Apart from increasing shelf life of microbes, hydrogel based bioinoculants are useful in reducing soil erosion and mitigating effects of frequent droughts. Therefore, hydrogel based bioformulation can be fruitful in application of microbial inoculants in changing climate.

Future potential of microbial inoculants in mitigating climate change

The upcoming challenge in bioformulation industry is to produce climate smart inoculants with higher microbial count, extended shelf life in storage, improved survival and effectiveness in changing field conditions, improved resistant towards adverse soil conditions like salinity, draught, heavy metal contamination and competition from native flora etc. Microbial agents like endophytes, AM fungi, plant growth promoting bacteria, biocontrol agents, *etc*. are having huge potential to serve the need of agricultural productivity in changing climate. Bio-prospecting for potential microbes and characterizing existing ones for alleviating abiotic stress will provide successful strategy to mitigate climate change. Various complementing consortium of microbial inoculants can be of great success in biotic, abiotic stresses and nutrient management for sustainable agriculture. The efficiency of formulations also needs to be reconsidered for different abiotic stresses. Novel formulations and amendments in existing techniques are necessary for bringing inoculant formulation more competitive and successful in natural environment.

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